TECHNICAL MYCOLOGY

THE UTILIZATION OF MICRO-ORGANISMS IN THE ARTS AND MANUFACTURES

A PRACTICAL HANDBOOK ON

FERMENTATION AND FERMENTATIVE PROCESSES FOR THE USE
OF BREWERS AND DISTILLERS, ANALYSTS, TECHNICAL
AND AGRICULTURAL CHEMISTS, PHARMACISTS,
AND ALL INTERESTED IN THE INDUSTRIES
DEPENDENT ON FERMENTATION

BY

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IN TWO VOLUMES

Vol. I.—SCHIZOMYCETIC FERMENTATION

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PREFACE

Dr. LAFAR has paid me the compliment of forwarding me a copy of the first volume of his "TECHNICAL MYCOLOGY," with a request that I should write a preface to the work. A perusal of the book gives me the impression that its contents will in themselves be a sufficient recommendation, and ensure the success of the work through its own inherent value; consequently, an Introduction by me is so far superfluous. Should, however, a few words of mine be the means of helping to secure for the work of my young colleague a readier introduction, here and there, than it would perhaps otherwise find, I shall be exceedingly pleased.

The First Volume treats of BACTERIA. In a series of chapters we are shown the predominant *rôles*—both useful and antagonistic—played by these organisms in Distilling and Brewing; in the preparation of Wines and the Manufacture of Vinegar; in the Dairy; in Farming; in the preparation of Agricultural Fodder; and in the manufacture of Tobacco and of Sugar. Then follows an account of the relation of Bacteria to sundry transformations occurring in Nature, particularly the important facts recently established in connection with the combination of free nitrogen by bacterial agency, with the iron and sulphur bacteria and the bacteria of nitrification.

It might be feared that, in a work aiming at objects so decidedly practical, the theoretical side of the subject would possibly be overlooked. This is, however, not the case in the present instance, as a glance at the Table of Contents will suffice to show.

That the Author possesses a grasp of the historical development of the subject has already been evidenced in his previous treatises, and the same feature often appears in the present volume.

In the majority of the Text-books and Manuals published in recent years, great confusion exists with regard to the appending of authors' names to the Illustrations. In one and the same book, for example, we meet with instances where the name of the author of the original work whence the copy has been taken is given—as it should be—and also

with other cases where the actual author is ignored, his name being replaced by that of the compiler of some text-book from which the *copy* was obtained—*i.e.*, some one who himself has done nothing more than copy. Such a mode of procedure is in a high degree calculated to produce a misty conception of the actual circumstances in the mind of the reader, the more so because, as stated, no importance is attached to the occurrence. DR. LAFAR has, however, set vigorously to work to combat this bad habit by taking all his reproductions direct from the original sources, so that they are clear and accurate representations of these originals.

The subjects included in the present work have been dealt with in a many-sided manner, the Botanical as well as the Technical and Chemical aspects having been borne in mind, although preference has throughout been accorded to the two latter. The style is flowing and clear, in many places lively and picturesque, and I have read with interest even those portions wherein I am not at one with the opinions of the author. The attention devoted to the most recent developments of the subject gives a special value to the book.

Within the last two decades the study of Microbiology has made gigantic strides, both in the pathological and the technical branches of the subject ; and just as investigations into the Physiology of the higher plants gave the first impetus to the establishment of Agricultural Experimental Stations in all countries, so, in like manner, have the Physiology of Fermentation and Technical Bacteriology called into existence, within the last few years, a number of Stations and Laboratories for the development of those branches of industry wherein micro-organisms play an important part. Formerly, Chemistry exercised an undisputed sway over the whole of this realm, but now Biology has won for itself a co-ordinate position therein—a fact which is now being recognised (although not yet to an adequate extent) in the filling of professorships at the various Technical High Schools. An army of eager workers has arisen, new technical journals have sprung into existence, and a great number of treatises and books are published on the subject every year. However cheering this may be in itself, the fact cannot be gainsaid that a portion (unhappily much too large) of these publications ought properly never to have seen the light. It is true that an intimate connection with practical conditions sets fresh tasks before the investigator, and exerts on the whole a sufficiently stimulating influence ; but, on the other hand, the same circumstance gives rise to the danger of diverging into by-paths, and neglecting the strict scientific conditions of

investigation. Since these Stations and Laboratories are, as a rule, maintained by the circle of practical men for whom they work, the investigators appointed thereto are often subjected to regrettable pressure. Even though, otherwise, a certain amount of freedom is allowed them in these institutions, they labour under the great difficulty of being obliged—whilst engaged in the task of scientific investigation—to be ready at any moment to give assistance—coupled with analyses and any wished-for disclosures—to the parties interested. Still further difficulties arise when practical men foolishly intermeddle in scientific investigations, and especially when results that shall be immediately available for practical utilisation are impatiently demanded—results which, however, are only attainable by scientific investigation, and cannot be forced on at pleasure.

Under circumstances of this nature it requires great strength of character not to give way to outside pressure, and many examples are met with in the literature of the subject where this firmness has been lacking.

The result of these vexed relations between Scientists and practical men has been to call into existence a quasi-scientific literature by which neither Science nor Practice has benefited—a result which every one who has the healthy development of this subject at heart must greatly deplore and endeavour to improve according to his ability. These conditions are, however, in existence, and we must take them into account. Amongst the chaff which occupies a large part of the aforesaid technical journals, there is, nevertheless, some really good grain to be found, and he who undertakes to write a work on Technical Mycology must not content himself with gathering from purely scientific sources alone, but must, at the same time, work through the technical journals as well. This (by no means easy) task has been accomplished by DR. LAFAR with commendable discernment and ability.
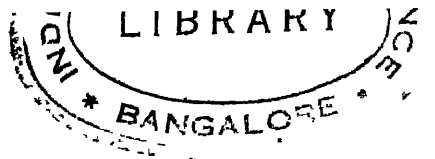
In the last few years, certainly, we have had various Text-books and Manuals giving a summary of larger or smaller sections of Technical Microbiology ; none of them, however, has treated the whole of this extensive field from so comprehensive a point of view. To prepare a work like the present requires not only many-sided discernment, but also enthusiasm for the task, combined with courage and endurance—properties with which the book shows the author to be endowed. The work will be welcomed, not only by those for whom it is primarily intended—viz., Technical Chemists, Chemists dealing with food-stuffs, fermentation, and agriculture,

Pharmacists, and Agriculturists—but many a professor also will derive benefit from its pages for his lectures and researches. In this respect the copious bibliographical references will also be of good service. In the present volume we have unfortunately only the numbers of the references, it being intended that the Bibliography shall be published as an Appendix to the second volume. This increases the desirability of the early appearance of the latter.

The Publishers have produced the work in a handsome and substantial manner, and in this respect also the impression produced is of the best.

EMIL CHR. HANSEN.

CARLSBERG LABORATORY,
        COPENHAGEN,
                September 1896.

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TECHNICAL MYCOLOGY:

THE UTILIZATION OF MICRO-ORGANISMS

IN THE ARTS AND MANUFACTURES.

INTRODUCTION.

I.

THE THEORY OF SPONTANEOUS GENERATION.

§ 1.— Technical Mycology is the Science of the Character and Activity of Fermentative Organisms.

Fermentative organism is the name given to any minute being of vegetable nature capable of exciting fermentation   Whether any given minute organism is to be considered as a "fermentative organism" or not depends, therefore, on the answer to the question . "Does it possess the power of causing fermentation ? "

In studying this subject the first task that lies before us is to obtain a definition of the term fermentation, or, in other words, to establish the common factor of all the manifold processes classified under that general title.

This is, however, as will soon be apparent, no light task , and the probability of our attempts being crowned by a successful result will be greater if we limit the scope of the question at the outset, and for the moment consider the term "fermentation" as applying merely to those phenomena with which it is associated in colloquial language, viz., the conversion of must into wine, wort into beer, wine into vinegar, and fresh milk into sour, &c.   To these may also be added the process of putrefaction.

Adhering to this restriction of the term, let us follow in imagination the path of investigation which has led to the knowledge that all the above-named phenomena are occasioned solely by the activity of minute living organisms, and constitute a manifestation of their vitality , that *fermentation and putrefaction are, in short, not purely chemical molecular transformations, but physiological processes.*

Moreover, history shall be our instructor, and lead us on further, to the comprehension of those other processes, which, for the present, we assume as standing without the pale of the term "fermentation," but which, nevertheless, should actually be included therein.   Such processes are, *inter alia*, the transformation of ammonia into nitric acid, occurring in the soil of our fields , the decomposition

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and dissolution of dead vegetable matter; the ripening of cheese, the formatio of bog (iron) ore, &c. &c.

§ 2.—Discovery of Fermentative Organisms.

The organisms taking part in the processes of fermentation are so minut that only a few can be detected, and that very imperfectly, by the unassisted ey The term microbe, introduced into the vocabulary of science by C Sedillot (I.) in 1878, belongs to them of right. Their examination could not be carried o anterior to the invention of appliances for observing minute bodies under hig powers of magnification, and therefore the inventors of the microscope deserv to be held in grateful remembrance in the domain of fermentation. These wei Hans and Zacharias Janssen, father and son, spectacle-grinders, of Middelburg in Holland, who, about the year 1590, constructed a combination of lense which, although, of course, very imperfect when compared with the instrumer of the present day, must be regarded as the first compound microscope made

Nevertheless, however great this step undoubtedly was, both from a theoretici and practical point of view, and however fruitful it proved in results, seeing tha it rendered possible *later* discoveries in the world of the "infinitely little," au especially of the fermentative organisms; still the fact remains that the *firi* fundamental observations were made, not with the compound, but with th simple microscope, which then, as now, was little more than a magnifying glas or bi-convex glass lens.

The honour of having discovered the presence of extremely small and hithert undetected organisms in putrescent and fermenting liquids belongs to anothe native of Holland, by name ANTONY VAN LEEUWENHOEK. Born at Delft in 163 he acquired during his apprenticeship to a linen or cloth merchant in Amsterdar some skill in grinding small glass lenses Of this skill he made further use afte his final return to his native town, and succeeded in producing lenses capable o magnifying from 40 to 100, and even to 150 times. With these he examine various minute objects, and frequently, amongst others, all kinds of vegetabl infusions in a state of decomposition. He discovered therein sundry extremel small creatures, many of them capable of motion, which he therefore regarded a animals, and named from their habitat infusoria. He died in 1723 The moderi world has entitled him "the father of micrography," *i.e.* that science which treat of the most minute forms of life.

This newly-discovered field of research was at first regarded by Leeuwen hoek's successors from an almost exclusively medical standpoint, as it is a natural instinct in man to try and maintain health and to prevent disease A that particular period, too, a special impetus was given to the study of medicine by the ravages of the plague, which only too frequently pursued its destructive course throughout Europe.

On the other hand, the study of the phenomena of fermentation derivec little or no benefit from Leeuwenhoek's discovery. The first investigator whom we meet with in this domain is the Viennese physician, Marcus Antonius Plenciz, who in his work "*Opera medico-physica*," issued in 1762, applied the results of Leeuwenhoek's discoveries, not only to the field of medicine, but also to that of fermentation and putrefaction In the latter connection he arrivec at the noteworthy conclusion that "a body undergoes putrefaction when the germs of vermicular creatures begin to develop and multiply; because these animals excrete numerous precipitations consisting of volatile salts, by which the liquids are rendered turbid and malodorous."

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1 The Roman numerals given in brackets after the names of investigators refer to the Bibliographical References forming an appendix to the second volume

However a■■■■■■■■ acquaintance with these minute creatures may have been to ■■■■■■■rs who succeeded Plenciz, and however useful, from a practical ■■■■■■iew, might be to observers the processes of decomposition which■■■■■■duced, these questions were nevertheless forced temporarily into the ■■■■ground by another, namely, the origin of these minute organisms. ■■■■■■

How do the minute ■■■■■es so copiously developed in infusions originate ?

Some opined that ■■■■ organisms were produced from certain unorganised (and therefore inani■■■■ substances—chemical compounds—present in the liquid in question, their ■■■ation being therefore considered as spontaneous (*generatio spontanea*), ■■■ising from elementary substances (primary generation). Or, whilst proce■■■ from elementary substances, as differing therefrom (heterogeneous), or di■■■■ thereto (equivocal), hence the name **Heterogenesis** or *generatio æquivoca ■■■■* which terms, as well as that immediately to be noted, have the same ■■■■■.

The party oppose■■■■■d, on the other hand, the possibility of a transition from a lifeless to ■■■■■dition (abiogenesis), and asserted that when "infusoria" are detect■■■■■■ion, a liquid or matter undergoing decomposition, their existence is ■■■■■■■ germs present therein.

Which view is ■■■■■■ On this point there arose, about the middle of the eighteenth century, ■■■■rmed one of the liveliest disputes agitating the domain of natural sc■■■■■ that period, and which, after occupying the most earnest attention of s■■■■■■ccessive generations of scientists, only terminated, after numerous flu■■■■■■ about the middle of the present century. From among the nu■■■■■■■ators who took part in this controversy, mention can here be ma■■■■■■■■Needham on the one side and Spallanzani on the other being entitled ■■■■■■st place.

§ 3.—Needham's Demonstration in Favour of "Generatio Æquivoca."

The most energetic champion of the theory of spontaneous generation was the English divine, N■■■■■ (I.). This theory was in existence long before his time, and had ■■■■■owned supporters—among them the chemist Van Helmont, who proposed a method for producing artificial mice—but until then had not progressed beyond the stage of indefinite assertion and unfounded hypotheses. The cause of the extraordinary support and approval accorded to the assumptions put forward by the English divine is, on the other hand, attributable to the novel manner in which he arrived at his theory (published in 1745), viz, not by untenable hypotheses, but by well-directed experiments.

He set to work, for example, in the following manner An aqueous meat extract was boiled for a short time in a flask, which was then made air-tight and left to stand for several days or weeks When opened at the end of this time, the contents proved to be plentifully infested with "infusoria," from which Needham concluded that as the "eggs" originally present in the liquid were killed by the boiling and the entry of fresh ones from the outside was precluded, therefore the living infusoria discovered in the liquid on re-opening the flask must have originated spontaneously, not from eggs (germs), but from the lifeless constituents of the liquid.

The great impression produced on his contemporaries by these statements can be appreciated by reference, for instance, to Buffon's work on the "System of Generation."

§ 4.—Spallanzani's Experiments.

Of the two hypotheses forming the basis of Needham's deduction, the accuracy of the second, *i e.* that relating to the exclusion of outside germs, was examined first   Some twenty years after the appearance of the English theory, the Abbé SPALLANZANI (I) published a dissertation in which he combated the doctrine of spontaneous generation   In this work the Italian divine detailed the experiments which had led him to the conclusion that a development of the animalculæ in question, in an infusion maintained at boiling-point for three-quarters of an hour, was only possible provided air, which had not been previously exposed to the influence of fire, had been admitted   This position was also maintained by Spallanzani in a second treatise (II)

Nevertheless, the supporters of the spontaneous generation theory were still far from regarding their cause as lost.   They characterised these experiments as inconclusive, since (so they said) " by the immoderate heat Spallanzani chose to employ, the air in the vessel is so unfavourably changed, and rendered so unsuitable for the maintenance of life, that it is no occasion for surprise that all development was lacking " This objection was curtly rejected by Spallanzani, but an experimental confutation was only arrived at much later.   The next step in this direction was accomplished in 1836 by—

§ 5.—Franz Schultze's Experiment.

In order to avoid under-estimating the value of the very short treatise (I) published by this investigator, regard must be had to the influence attained by
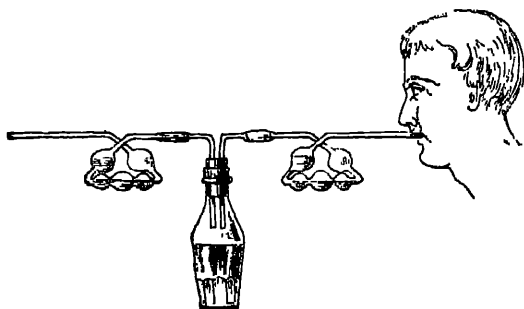
FIG 1 —Franz Schultze's Experiment

Chemistry in all branches of natural science during the sixty years that had elapsed since Spallanzani's demonstration, an influence which will be elucidated, in so far as it refers to the theory of Fermentation, in subsequent sections   The idea that ordinary air acts as an inducer of fermentation   or putrefaction by reason of its content of living germs was first called into existence by Schultze.

He described his experiment as follows " I filled a glass flask half full of distilled water (Fig 1), with which I had mixed various animal and vegetable substances, and closed it with a sound cork, through which were passed two tight-fitting glass tubes bent to elbow joints. I next placed it in a sandbath and applied heat until the water boiled briskly, so that all parts were exposed to a temperature of 100° C.   Whilst the hot water vapour was still issuing from the two tubes, I attached to the end of each an apparatus employed by chemists, in the course of organic analyses, for the absorption of carbon dioxide.   That on the left-hand side was filled with concentrated sulphuric acid, the other with a solution of potassium hydroxide " After cooling the apparatus, air was drawn through twice every day during the ensuing two months, in such a manner that it had to pass through the sulphuric acid before entering the flask.   The results confirmed the expectations of the investigator, the contents of the flask when

ed being found free from living organisms, which, however, soon made their
trance when the open flask was freely exposed to the air. This proved that
ous exposure to the influence of fire is not an essential condition for
ving air of the power of inducing fermentation or putrefaction

hree years later, THEODOR SCHWANN (II) entered the field as an opponent
e theory of spontaneous generation Of his labours in this direction, a
, modification of the Schultze experiment, consisting chiefly in the substi-
1 of a heated metal tube for the bulb tubes (see Fig. 2), occupies merely a
dary position More

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urseniate, &c." He
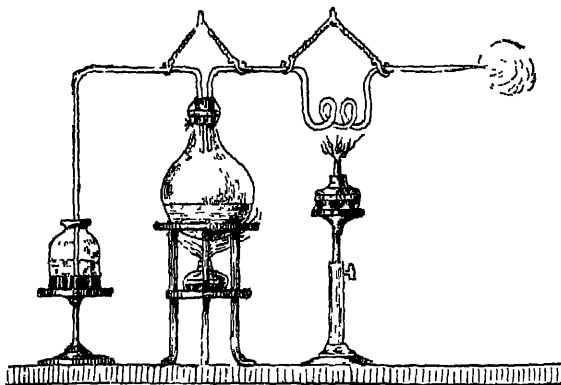
FIG. 2.—Theodor Schwann's Experiment.

herefore, the founder of the science of antiseptics. Concerning his
neutral researches in the narrower field of alcoholic fermentation, mention
made in a subsequent chapter.

o adherents of spontaneous generation applied to Schwann's method of
ing the air the same objection (referred to above) which they had previously
against Spallanzani They did not even consider themselves confuted by
ults of Schultze's experiment, but asserted that here also the treatment of
·, although by no means so violent, unfavourably modified its composition.
futation of this doubt was only accomplished after a lapse of seventeen
and that by

§ 6.—The Labours of Schröder and Dusch (I).

tigated by the researches of Loewel, who found that ordinary air could be
id of its property of inducing crystallisation in a supersaturated solution
um sulphate by filtration through cotton-wool, the two investigators
above modified, in 1853, the arrangement of Schultze's experiment, by
ig the incoming air to pass through a glass tube packed with cotton-wool
entering the flask It was found that by means of this (decidedly not
at") treatment the air also lost its power of causing decomposition and
mation of minute organisms in extracts which would remain unchanged
ir was excluded.

importance of this demonstration must not, however, be over-estimated,
nly proves the presence in the air of a "something" capable of giving
hving creatures in inanimate nutrient media, and of exciting substantive
s (fermentation and putrefaction) therein. Concerning the nature of this
'something," the experimenters could give no satisfactory account, they
ft it an open question whether the something was gaseous or not It

may be considered that they were unduly diffident, since the action of the cotton-wool filter proves that this something must necessarily be a solid body and not a gas. But, on the other hand, both investigators could point to experiments wherein the previously boiled test liquid afterwards underwent decomposition, notwithstanding the fact that all the air which was allowed access to it had been filtered through cotton-wool Milk they had, in their first treatise, recognised as such a liquid, and to this were added, in a second communication by SCHRODER (I.), yolk of egg, meat, and meat broth, in all of which cases the filtration of the air proved useless. This led Schroder to separate the phenomena of decomposition—characterised as **fermentation** and **putrefaction**—into two groups · the one, which he designated "voluntary decomposition," requiring only oxygen for its inception, whilst the other, e g. the fermentation of wort, required, in addition, the collaboration of that unknown constituent of the air, which could be destroyed by fire or arrested by a cotton-wool filter "Whether this active substance should be regarded as germs floating in the air, or as some hitherto unknown chemical substance modified by high temperature and separated and fixed by the influence of contact with the cotton fibres, must remain undecided "

Glancing back for a moment at the work of Schultze, one would be only too readily disposed to consider the results of Schroder and Dusch's experiment as a retrograde step, since they not only did not afford us any further information beyond that established by Schultze as to the nature of the germs in the air, but also called in question the accuracy of Schultze's results. And, in fact, repetitions of the Schultze experiment by many other workers, with various modifications, especially with regard to the kind of test liquid employed, confirmed the results of Schroder and Dusch. In numerous instances decomposition ensued, even in the boiled liquid, when purified air (filtered or heated to redness) alone was admitted, whilst in other cases, under precisely similar conditions, the boiled sample remained unaltered for any length of time. Thus the state of the question at the commencement of the sixth decade was just about as far advanced as at the beginning of the century, and the adherents of the spontaneous generation theory were more certain of triumph than ever.

§ 7. The Examination of this Theory by Pasteur.

However, the day of refutation was close at hand, though the proof was not obtained by the methods which had generally been favoured hitherto, but which had led to no definite issue.

Experimenters had so concentrated their attention on keeping the air admitted to the boiled liquid perfectly free from active germs, that it had not occurred to any one to ask if the sterilisation of the liquid could not be equally ensured by simply boiling it, either momentarily or continuously for a short time.

Reasoning from the fact that all known forms of created life (animal as well as vegetable) were incapable of resisting the temperature of boiling water, even when exposed thereto for merely a short time, the conclusion was arrived at that the same effect was produced on the small germs in question. It was therefore considered, humanly speaking, certain that every liquid could be rendered free from active germs by boiling for a short time. This was agreed to both by those who accepted and by those who rejected the doctrine of spontaneous generation Still such belief was based on a mere assumption, as CH. BONNET (I), a contemporary of Spallanzani's, implied when he inserted the following query in his work opposing the theory of spontaneous generation · "Is it, then, certain that there exist no animals or eggs capable of supporting a

temperature equal to that of hot ashes without losing their life or reproductive power ?"

Pasteur called to mind this doubt of Bonnet's when he began to subject the theory of spontaneous generation to experimental examination in response to the offer made in 1860 by the Paris Academy of Science of a prize for "an attempt, by means of suitable experiment, to throw new light on the question of spontaneous generation." From the report of his researches, which appeared early in the year 1862, in the form of a comprehensive treatise (I.), well deserving perusal, only the most important result can be referred to here viz., the demonstration of the possibility, by the assistance of sufficiently prolonged heating at an adequately high temperature, of **sterilising** (*i.e.* freeing from living germs) any substance whatsoever, and of the fact that a sample so sterilised will not subsequently undergo decomposition, but will remain unaltered so long as care is taken to prevent the access of germs from the external air.

The objection raised by the heterogenists, viz., that decomposition is prevented by the strong heating having rendered the sample unsuitable for the production of germs, can be easily disposed of by inoculating the liquid with a few germs, these will be found to develop rapidly and luxuriantly. The substantiality of these germs was demonstrated by Pasteur in a very beautiful experiment, for which he employed a culture vessel similar to that described by H. Hoffmann (I.) in 1860, and now generally known by the name of **Pasteur flask**, a glass flask (fitted with a tubulus at the side for facilitating inoculation) the neck of which is drawn out small and bent twice like a swan's neck The external air is obliged, in order to gain access to the contents of the bottle, to pass through this neck, and as the direction of movement is changed at the first bend, all the germs are deposited there.

Thus was laid the foundation on which the edifice of Fermentation Physiology was gradually raised. The possession of perfectly sterile culture media, and the power of protecting them from the intrusion of unauthorised germs, is a *sine quâ non* for a successful and reliable study of the organisms of fermentation.

§ 8.—Béchamp's Microzyme Theory.

Pasteur's investigation and elucidation of the causes of the tenacity of life exhibited by many germs thenceforward occupied the earnest attention of mycologists, and finally led to the acknowledgment that this power of resistance is possessed by the reproductive organs known as **spores**. The morphology and physiology of these organs forms the subject of §§ 48 to 55 At present, the only point to be emphasised is that when these life-retentive organs are once killed, no spontaneous development of germs can occur in the liquid harbouring them; hence such liquid will remain sterile until it is artificially re-inoculated.

It might be supposed that the adherents of the doctrine of spontaneous generation would have responded to these demonstrations by abandoning their previous attitude of opposition This, however, they did not do, they merely changed the field of combat without altering their opinion As they could no longer maintain that organised creatures could be spontaneously derived from unorganised substances, they contended that the dead cells had the power of liberating organised living matter capable of development into the various species

In a subsequent paragraph we shall learn that in the cell contents of most fungi, *e g* yeast, small, highly refractive bodies, known as **microsomata**, may be frequently observed. On applying pressure to the cover-glass placed on a preparation containing cells that exhibit such enclosures, the membranes are ruptured and the microsomes are liberated If, now, the latter be transferred to another

nutrient solution, there will most assuredly be a development of organisms, if we omit those precautions which are considered essential by the bacteriologist, but superfluous by those who believe in spontaneous generation. Such development is, however, due, not to the microsomes, but to the germs introduced during the transfer. Although this is so evident, it is strange that this view should have had its opponents, as, for instance, the botanists H. Karsten and A. Wigand (I. and II.), and, with still greater pertinacity, A. Béchamp. The last-mentioned designates these microsomes (" granulations moleculaires ") microzymes, and attributes to them such tenacity of life that they are able to remain dormant, not only for years, but even for entire geological periods, since, as Béchamp asserts, he has found microzymes of cells which were buried in the strata formed during the Cretaceous period still retaining their vitality and reproductive power. A full account of this microzyme theory, which many amateur bacteriologists have considered to be indisputable—communications respecting which have been incessantly intruded upon the notice of the Academy of Science at Paris—is given in a bulky volume which Béchamp (I.) laid before his sceptical contemporaries in 1883.

§ 9.—Spontaneous Generation only Unproven, not Impossible.

Omne vivum ex ovo (every living creature from an egg), *omne vivum ex vivo* (every living creature from living creatures)—was the watchword elevated to a dogma by the triumphant opponents of the theory of spontaneous generation. Were they correct? or did they encroach beyond the limits of the facts they demonstrated? Let us devote a few moments to a critical review of the question.

One thing is established beyond doubt, namely, that all the instances of supposed spontaneous generation brought forward by the adherents of the theory have been vitiated by numerous errors It is, moreover, *established* that the occurrence of spontaneous generation has not been proved, no unassailable experiment being known in which living creatures were produced from inanimate substances. Spontaneous generation is therefore *unproven*.

Whether it is also an *impossibility* is a point still to be decided. If the theory of evolution, as presented by Lamarck and Darwin, be traced towards its origin in the lowest organisms, we come to a standstill with the question "And from whence then comes the ultimate and lowest creature?—How did organic life originate on our globe?"

The reply furnished by the English physicist Thomson [1]—that our earth was fertilised in its youth by meteors bringing the germs of organisms from other heavenly bodies—affords no solution, but merely transfers the question to another scene and to a more distant period of the past, and at once suggests the further question: "How did life originate on these unknown, extra-mundane sources of creative messengers?" There are only two possible answers to these questions, viz., spontaneous generation, or a miracle

As a matter of reason, we are therefore obliged to assume that, at some definite moment in the past, organised living beings were produced from unorganised potentially organic substances, and further, that such creative power may still be operating, may perhaps be performing at present The *possibility* cannot be gainsaid.

That bacteria are the result of this primary creation of living beings is very questionable, and even improbable, since their structure is much more complicated than is consistent with their presumed origin directly from chemical elements, unmodified by changes in passing through simpler intermediate organisms.

[1] Lord Kelvin.

Many investigators, and amongst them C. Nägeli (I.), assumed that these lowest forms really exist, although undiscovered at present, and in his important and highly suggestive work on the Theory of Descent—which also contains a fine chapter on "the limits of knowledge in natural science"—this author touches upon the question under consideration. He calls these presumptive connecting links Probiën (pre-existing), on the ground of their being the predecessors of all known forms of living beings. Such a Probion resulting from spontaneous generation would be "merely a drop of homogeneous structureless plasma, devoid of any definite form and composed of albuminates, associated only with the compounds necessary for nutrition."

"We must assume"—says de Bary—"that organisms must at one time have originated from organisable but unorganised substances, without progenitors. . . . To prove such a primary creation of a living being is of the highest interest, and exercises the same fascination on the investigator as the expectation of the homunculus in the phial did on the alchemist. The experience of centuries has, however, shown that the homunculus when it actually appeared was simply a small imp which had been secretly passed into the flask by sleight of hand. . . . Therefore—admitting all imaginable possibilities—the law, based on experience, of origin from ancestors, corresponds with the enlightened state of our knowledge, and this is the starting-point that must be taken in a work which has to deal with the exact sciences."

§ 10.—Stahl's Theory of Fermentation.

WHOEVER was the first to leave the juice of sweet fruit to itself in storage for a few days had the pleasure of observing a phenomenon hitherto unknown—the incipient decomposition of the mass—which we nowadays term alcoholic fermentation. This observation was made at so early a date that we have no record of it beyond myth and tradition .The Greeks fêted the deity Bacchus as the inventor of wine, and the Egyptians ascribed to Osiris the first introduction of brewing.

Acquaintance with the nature of this phenomenon was, however, of an extremely superficial character for a very long time   Even in the later Middle Ages the word *fermentatio* (fermentation) was employed as synonymous with *digestio* (digestion), the latter word being also currently used to denote any form of chemical reaction; and the word " ferment " was applied to any body capable of producing such reaction.

At an early date it would necessarily be noticed that the " must " when in a state of fermentation became covered with a froth, and that at the end of this operation a copious deposit, viz , yeast, was left at the bottom of the vessels Fermentation was therefore looked upon as a process of purification, by which the initially turbid and discoloured liquid was so improved and freed from dirt, that the purified alcohol exhibited its true properties   For this reason the deposit was described as the *fæces vini* or *fæces cerevisiæ, i e.* the excrement of the wine or beer.   This view was held by, *e g* Basilius Valentinus, a German monk and alchemist, who lived at Erfurt early in the fifteenth century

It was also noticed that this sediment was a powerful ferment, *i e.* it was capable of rapidly exciting a brisk fermentation in still unfermented liquids, such as wine-must or beer-wort.   This idea was adopted in other branches of chemistry, so that any reaction was considered as elucidated when the body acting as " ferment " therein could be identified   Moreover, the " philosopher's stone," the goal of the labours and aspirations of the alchemists, was nothing but the much sought for, but never discovered, universal " ferment " for every possible chemical process!

Among the disciples of the alchemic school, one other, viz , STAHL (I), deserves mention, because his views on the nature of fermentation were adopted by Liebig a hundred and forty years later.   Stahl extended the definition of fermentation to all forms of decomposition, his theory being expressed *verbatim* as follows : " Putrefaction (and also fermentation) is internal movement   A body undergoing such internal movement may easily induce the same in any other body, which, though still quiescent, is susceptible of such movement."

§ 11.—Gay-Lussac's Opinion.

Stahl's view remained in vogue until the commencement of the nineteenth century, when Gay-Lussac, in 1810, enunciated a new theory to a new

age. The discovery by Lavoisier that combustion is a process of oxidation, a combination of oxygen with the combustible substance, was an event the influence whereof extended over the entire domain of chemistry. The assignment to oxygen of a part in the process of fermentation was therefore opportune; but Gay-Lussac was especially prompted by another circumstance.

A Parisian confectioner and cook, named Appert, had made practical use of the experiment devised by his contemporary Spallanzani for the refutation of the heterogenists and, after some preliminary trials, perfected his process for preserving meats, vegetables, spirituous liquors, &c. To this end he exposed them, in hermetically closed vessels, to the temperature of boiling water for some time—a process which had somewhat earlier (1782) been recommended by the Swedish chemist SCHEELE (I) for the conservation of vinegar. In this way APPERT founded a new branch of industry—the manufacture of conserves—which brought him both wealth and fame. He published a volume (I.) which comprised the results of his experience   It was widely circulated and ran into several editions, the first of which appeared in 1810, and the fourth in 1831.

It is therefore little matter for surprise that the attention of the Parisian chemist was directed (whether from the culinary or the literary side) to the productions of his enterprising fellow-citizen. GAY-LUSSAC (I) now examined conserves prepared according to Appert's process, and found them to be free from gaseous oxygen. This incited him to make fermentation experiments with wine-must, &c , the results of which led him to assert that the presence of oxygen is necessary to the *inception* of fermentation. A number of over-zealous colleagues, in expounding their master's opinion, added new features to it, and subsequently credited him with the assertion that oxygen is the actual ferment—a statement as unfounded as it is inaccurate. Gay-Lussac only claimed for the gas a single function, the *inception* of fermentation , once the process was in operation the stimulus was no longer required. With regard to the nature of this stimulating action he was, however, unable to report more definitely.

Among the observations which led Gay-Lussac to adopt this view, mention may be made of one which appeared to him particularly conclusive, namely, the *sterilisation of wine-must by sulphuring*. When wine-casks, before filling, are thoroughly sulphured—*i e.* the internal air contained in them is heavily charged with sulphur dioxide by burning sulphur in the casks—the grape juice thereafter introduced remains quiet and passive, without fermenting. This circumstance is now unanimously ascribed to the vitality of the yeast cells in the must being destroyed by the sulphurous acid. Gay-Lussac, on the other hand, viewing the matter differently from his standpoint, held the opinion that as the sulphurous acid had a strong affinity for the oxygen, the two combined, and as no oxygen was available for starting the fermentation, the must necessarily remained inert.

The experiments made by Schwann in 1838, and described in § 13, refuted the opinion of Gay-Lussac, by demonstrating that the *rôle* of exciting fermentation is set up by certain microscopic living creatures which perform their functions in the absence of oxygen. Subsequent research proved that the presence of this gas is altogether superfluous, so far as the progress of alcoholic fermentation is concerned, although it is not without influence thereon. PASTEUR (II) in 1861 established it as a fact that this progress is more satisfactorily effected when the fermenting liquid is subjected to brisk aeration.

§ 12.—Cagniard-Latour's Vitalistic Theory of Fermentation.

The French apothecary ASTIER (I. and II) has generally been credited with being the next individual, after Leeuwenhoek, who gave his attention to the nature of yeast. An examination of his published works shows, however, that

his investigations into fermentation were conducted without the aid of the microscope, so that he did not bring to light any actual facts concerning the nature of yeast, but—as was 'pointed out, though in vain, by QUEVENNE (I.) as far back as 1838—based his assumptions on hypotheses devoid of foundation.

In the same way another Frenchman, viz., Desmazières, the reputed pioneer of the founders of the vitalistic theory of fermentation, cannot permanently retain this title. Like Astier, he is said to have recognised the part played by yeast in fermentation, but, as a reference to his treatise, published in 1826 (in pages 42 to 67 of vol x. of the *Ann. des Sc. Nat*), will show, this assertion is incorrect. In these observations Desmazières viewed the matter simply as a naturalist. His investigations of the fungoid growths covering the surface of moist substrata were conducted from this point of view, and it was in the course of this study that he examined the mycelia that develop on beer, &c. These consist of masses of elongated cells, to which he gave the name *Mycoderma cerevisiæ*. As he fancied they exhibited powers of locomotion, he considered them as belonging to the animal kingdom (*animalcula monadina*), but, true to his purely descriptive inclinations, he disregarded their physiological properties, and especially their influence on the substratum. Thus the reputation attributed to Desmazières of having, in 1826, microscopically studied the morphology of the yeast-like cells, to which Persoon had definitely alluded four years earlier, is dissipated by facts.

On the other hand, a German worker, viz., EHXLEBEN (I), had already, in 1818, correctly estimated the importance of yeast, in that he asserted it to be a living organism, the vital functions of which are the cause of fermentation. Unfortunately he did not follow up this idea, which was thrown out as a mere occasional remark in his treatise on practical analytical experiments. Otherwise he would, in 1818, have anticipated what was only accomplished twenty years later, viz., the establishment of the fact that (alcoholic) fermentation is causatively connected with the life (*vita*) of certain organisms. This was determined, almost simultaneously, by three investigators working quite independently of each other Cagniard-Latour in France, and Theodor Schwann and Friedrich Kutzing in Germany.

The paths by which these three arrived at their common goal differed. The versatile French technicist is known by name to the majority of educated people on account of the siren he invented, and which is largely used in the science of acoustics. He also devoted some attention to brewing, and compiled a work on the fermentation of beer. The preliminary studies undertaken in this connection led him to more closely investigate the nature of the " yeast," of which—notwithstanding the observations of his two compatriots already mentioned—practically nothing was then known. This material he examined with the assistance of the microscope, and laid the results of his researches before the Parisian Academy on June 12, 1837, in a short paper (II.) containing the following chief points

1 Beer-yeast, instead of being an inanimate chemical substance, as previously supposed, actually consists of small globules which possess reproductive power, and are therefore living organisms.

2. These bodies *appear* to belong to the vegetable kingdom, and to reproduce themselves in two ways.

3. They *seem* to act upon sugar solution only whilst still living , wherefore it may, with great probability, be concluded that, by their vital activity, carbon dioxide is liberated, and the sugar solution transformed into an alcoholic liquid

§ 13.—The Researches of Theodor Schwann.

As the words printed in italics in the two preceding sentences show, and as a closer examination of the original treatise will more clearly reveal, Cagniard

did not indubitably establish the vegetable nature of yeast. The accomplish-
ment of this task, and the attribution of this organism to its proper position in
the system of Botany, formed the subject of a treatise published by SCHWANN (I.)
in the first half of 1837, *i.e* contemporaneously with Cagniard's paper

In following up the results of his researches on spontaneous generation,
Schwann studied beer-yeast, and found that the individual globules, of which the
mass was seen under the microscope to consist, frequently became united into
chain-like or laterally branching bands, and presented to the eye an appearance
greatly resembling that of many already well-known multicellular fungi. It
was not this discovery alone, however, but rather their mode of reproduction,
which induced Schwann to consider these bodies as of a vegetable nature   In
this process the globule pushes out from its interior a small nodule, which
Schwann was able to observe develop to its normal dimensions   " Observation
of its growth leaves no doubt as to its vegetable nature, since animals do not
reproduce themselves in this manner." The rate of reproduction of the globules
kept pace with the increasing briskness of the fermentation, so that Schwann
came to the opinion that it was highly probable that the development of the
fermentation was induced by that of the organism

"Vinous fermentation must therefore be regarded as the decomposition
occasioned by the sugar fungus extracting, from the sugar and a nitrogenous
body, the materials necessary to its nutrition and growth, whereby such elements
of these bodies (probably among other substances) as are not taken up by the
plant unite, by preference, to form alcohol "

This discovery was communicated by Schwann to his friend and colleague,
Professor Meyen, who tested and confirmed it, " stating with reference thereto,
that the only doubt arising was whether the organism in question was an alga or
a thread fungus, the latter seeming the more likely by reason of the absence of
green pigment." Thus yeast was recognised as a fungus, and, from its capacity
of fermenting sugar, was designated **sugar fungus** whence the current generic
name, *Saccharomyces* Meyen.

According as such a sugar fungus was found active in beer-wort or wine-must,
it was called by the specific name of *S cerevisiæ* or *S. vini*, which names remained
in general use in their original significance until REES (I) in 1870 proposed a
system of differentiation which will be more fully noticed in a subsequent
paragraph.

As follows from the remarks already made, the name " yeast" applied merely
to one particular group of ferments, viz., those producing alcoholic fermentation.
For a considerable period after Cagniard's discovery, however, it was used indis-
criminately for all ferments   Thus, for example, Pasteur speaks of the " yeast "
of lactic fermentation, meaning thereby *Bacteria ,* and even in 1879 Nägeli, the
investigator of the fission fungi, refers in his " Theory of Fermentation " to the
" yeast " of putrescent urine   This misuse of the term has been abandoned, and
the name " yeast " is now only employed when speaking of the budding fungi
that excite alcoholic fermentation.

§ 14.—Friedrich Kützing's General Theory of Fermentation.

The views promulgated by this German worker in the field of Vegetable
Physiology and the Algæ were in harmony with the spirit manifested in the
" Elements of Philosophic Botany "

Published almost simultaneously with the above-mentioned communications
of Cagniard-Latour and Th. Schwann—though actually compiled at a much
earlier date (before 1834)—Kützing's treatise (I) on this subject surpassed
those of his two colleagues in more than one particular   The value of his

actual determinations is not, in our opinion, lessened by the fact that he was an advocate for spontaneous generation, since at that time (in 1837) there existed no decisive and unassailable proofs to controvert this theory

Kützing did not restrict his researches solely to alcoholic fermentation, but also instituted comparisons with a number of other similar phenomena, regarding them all from the same point of view.   Even though he must share with others the credit of having discovered the organised structure of yeast, that of determining the vegetable nature of the "mother of vinegar" and recognising its mode of action belongs to him alone   With these discoveries are associated a number of others of minor importance, such, for example, as the physiological basis of the method (propounded by Scheele) of preparing gallic acid by allowing a solution of pyrogallic acid (e g. gall-nut extract) to become infested with mould.   The numerous phenomena he brings under our notice constitute so many proofs of the theory that fermentation cannot be regarded as a purely chemical process   " It is well known that chemistry explains vinous fermentation by the reaction of the so-called gluten on the amylum (starch) and sugar. I must firmly maintain that the explanation does not give me a clear idea of the process, and I am inclined to doubt whether others are more fortunate in this respect   It is, however, certain that the entire process of alcoholic fermentation is dependent on the formation of yeast, and the acid fermentation on the formation of the vinegar plant. . . . Along with the increased growth of these organisms the reproductive impulse also increases, and, concurrently, their reaction on the liquid present. . . . In so far as fermentation is synonymous with a reciprocal reaction of organic and inorganic bodies on the constituents of a given liquid which may be regarded as forming the nutrient medium of the organic product, so is it necessarily synonymous with every organic vital function   wherefore organic life = fermentation.   On the other hand, such processes as lead to the production of vinegar from alcohol by the use of platinum black or other similar methods, cannot be compared with fermentation, being purely chemical, whilst fermentation is an organo-chemical process, as is also the life process of any organic body "

One of the three members of the committee appointed by the Académie des Sciences of Paris to report on the memoir presented by Cagniard—namely, Turpin (I.)—took the opportunity thus afforded of experimentally dilating upon his compatriot's work, and of amalgamating these new " discoveries" with the revelations of Schwann and Kützing.   In this way a volume, containing more pages than Latour's communication had columns, came into existence, without, however, adding to our knowledge in the slightest degree. Turpin seems, however, to have thoroughly known his public, since he is even now regarded as one of the founders of the vitalistic theory of fermentation, not only by compilers of text-books, but also by actual investigators, from whom one might more reasonably expect a more thorough study of the original works of their predecessors.

§ 15.—Liebig's Decomposition Theory.

Two years subsequent to the publication of the works of Cagniard, Kützing, and Schwann, Liebig placed before his colleagues a new theory, according to which fermentation was a purely chemical reaction.

In order to avoid judging this chemist unreasonably, one must bear in mind the age wherein this theory was promulgated   Synthetic organic chemistry had just been founded   Eleven years previously (1828) Wöhler had succeeded in artificially preparing urea, to the astonishment of his contemporaries, who had hitherto considered as impossible the artificial production of organic

substances outsi... ...table body of whose vital functions they
are the outcome... ...stances could not be produced without the
concurrence of vit... ...till that time an established maxim. To
overthrow this do... ...that any desired organic substance *can* be
prepared without the ...of vital action, was the endeavour of the
majority of the chemi... ...age, Liebig being one of the foremost, most
industrious, and stu... ...kers in the cause. It is, therefore, small
matter for astonishment ...agniard-Latour, Kutzing, and Schwann to
the contrary, notwi... ...e could conceive a theory of fermentation
wherein the action of liv... ...nisms had no place and their vital force was
ignored.

The struggle ag... ...considered to be the objectionable theory of
these three physiologi... ...ened by Liebig in 1839 with an anonymous
treatise (I.), in whic... ...bservations of the microscopists were covered
with highly amusing s... ...the next year he returned to the charge in
earnest in his work o... ...Chemistry in Relation to Agriculture and
Physiology," on pp ... ...ich his new theory is enunciated. This is, as
already mentioned, ...put forward by Stahl more than a century
before

Liebig considered ... ...tion as molecular movement, which a body in
a state of chemical ... ...decomposition, transfers to other substances
whose elements are ... ...ly combined. Between fermentation (in its
limited sense) and put... ...re is the following difference : In the latter—
putrefaction—the de... ...is transmitted by the decomposing material—
viz., the albumino... ...efaction, once begun, is continued by inherent
movement, even ... ...al cause has been rendered inactive With
fermentation it is oth... ...In this process the body (sugar) in a state of
incipient decomposition cannot transmit the movement to the still undecom-
posed substance Consequently this function has to be performed by an extra-
neous causative agent, a ferment, which in this case is necessary not only for
commencing (as with putrefaction), but also for continuing the decomposition.

It must be admitted that, at first sight, this definition, as also the differentia-
tion between fermentation and putrefaction, is very attractive. Nevertheless
it will not bear the light of keen criticism Take, in the first place, the character
on which the distinction between fermentation and putrefaction is based, viz ,
that the former will not go on without the presence of the ferment, whereas,
on the other hand, putrefaction, when once started, continues spontaneously,
the ferment being no longer needed. The reason why Liebig was induced to
make this distinction is easy to fathom. In the case of fermenting beer-
wort—which Liebig usually had in view when speaking of fermentation
—the ferment (beer-yeast) was discernible to the naked eye, and experience
taught that without this ferment the fermentation could not be satisfactorily
carried on. On the other hand, the presence of those minute organisms which,
as we now know, insinuate themselves into all substances liable to putrefaction,
and decompose the same without, as a rule, giving rise to such a multiplication
of the deposited ferment as can be remarked by the inexpert eye, is not so
immediately apparent as in alcoholic fermentation.

Thus, even in Liebig's opinion, yeast is essential to the continuance of
fermentation , only, the ferment is degraded to a simple albuminoid substance.
To enter nowadays into a further onslaught against this theory would be
merely storming an undefended position. Moreover, as the subsequent editions
of the aforesaid work demonstrate, it was gradually modified by its author, so
that the form in which it was presented in his latest exposition (II.) in 1870
differs in many particulars from the original.

The ocular demonstration, in individual instances, of the untenable nature of the hypotheses supporting this theory, to those whom the representations of Latour, Kutzing, and Schwann had not succeeded in convincing, was the congenial task undertaken by Pasteur, and brought by him to a successful issue with great experimental skill.

§ 16.—Pasteur's Theory of Fermentation (III.).

The victorious antagonist of the theory of spontaneous generation was not content with controverting the views of Liebig, he also sought to erect a better theory in its stead. According to this doctrine, it is the *lack of free oxygen* that leads to the fermentation being set up by the organism as a means of supplying itself with the energy it requires by seizing upon the oxygen thereby obtainable. "Fermentation is life without air" A very slight experience in this matter suffices for the recognition of the fact that this theory takes no account of the several kinds of fermentation in which the presence of oxygen is a necessary condition, viz, the so-called oxidation fermentations—the best example of which is afforded by the acetic fermentation. In this respect the theory cannot be further alluded to in the present chapter, which is devoted to *general* considerations. It will be fully dealt with in a subsequent chapter. The only remark to be made now is that this theory also has proved untenable

The permanent value of the services rendered to Fermentation Physiology by Pasteur are not diminished by the disproval of his theory of fermentation, since they have their root in the successful endeavour, by means of careful and extensive experimental demonstration, to bring into universal recognition the theory originated—but only imperfectly formulated—by Cagniard, Kutzing, and Schwann, of the causative connection between fermentation and the vital activity of the microbe;

§ 17.—C. Nägeli's Physico-Molecular Theory.

Although it was by this time indubitably established that without the vital activity of micro-organisms no fermentation could occur, no clear account had been given as to *how* the activity itself was exerted. Several explanations were possible According to one which was especially advocated by Kutzing and Pasteur, a decomposition was effected within the cells of the organic ferments, which obtained their nourishment from the fermenting material (*e.g.* sugar) and discharged the fermentation products as waste matter.

According to another, the decomposing force simply emanated from the cells and became the direct cause of the decomposition of the fermentable matter around them.

The physico-molecular theory, proposed by NÄGELI (II.), expresses this view in the following words "Fermentation is, therefore, the transference of conditions of movement in the molecules, atomic groups, and atoms of the various compounds constituting the living plasma (which compounds remain chemically unchanged) to the fermentative material, whereby the equilibrium of its molecules is destroyed and their dissociation induced " The radius of the sphere of influence of the individual yeast cells is estimated by Nageli as from 20 to 50 μ.

This definition differs from that formerly given by Liebig merely in a single, though important, consideration, viz., it regards the living cell as the source of action, whereas the other definition speaks of inanimate albuminoid substances. Nägeli was, however, unable to prove the correctness of his theory, and the calculations deduced from other observations which he brought forward in support thereof have in course of time proved inapplicable.

§ 18.—The Enzymes and M. Traube's Ferment Theory.

There is still another possible explanation of the power of living cells to act at a distance. This regards the cells, not as centres of radiating molecular movements, but as forming centres of production of metabolic products which penetrate through the cell membrane into the surrounding liquid. There they become widely distributed by diffusion, and by their influence bring about the decomposition of certain constituents of the solution, but do not undergo any chemical change themselves. These active bodies are called enzymes, and their behaviour is, as will be observed, different from that of ordinary chemical agents, since the latter effect alterations in other groups of atoms by their chemical affinity, whereby the old combination is broken up, and the separated portion enters into a new atomic grouping with a part of the active agent. Accordingly, a definite weight of the agent can only displace a definite quantity (known as the "equivalent weight") of other compounds; whereas the enzymes behave differently, their activity being *practically illimitable*. They do not combine with the products of the reaction, but continue to act on the residual undecomposed substance.

The first enzyme was discovered by PAYEN and PERSOZ (I.) in 1833, who detected in malt extract a substance—which they termed diastase—capable of converting starch into sugar. They were, however, unable to isolate it in a pure condition. Three years later THEODOR SCHWANN (III.) discovered in gastric juice pepsin, subsequently also named peptase, which in faintly acid solutions resolved undiffusible albumen into assimilable dissociation products. Since that time the same enzyme has also been detected in various vegetable organisms, many varieties of bacteria in particular having the power of elaborating it. There will be ample opportunity for reference to this point along with the other known enzymes at a subsequent stage. At present we have only to consider them as the basis of a theory of fermentation, the formulation of which dates back as far as 1858, but has come to the front more of late years, and—so far as can be judged from the data at present available—will acquire still greater importance.

As we have observed in § 10, the meaning attached, under the influence of alchemical views, to the word ferment was, until the close of the eighteenth century, very comprehensive, and it was only then that the restriction of the term to bodies inciting fermentation began. Contemporaneous with the development of positive knowledge with regard to these bodies was the discovery of the enzymes, the behaviour of which resembled that of the former, in so far that they exhibited a capacity of inducing decomposition. Moreover, the obscurity in which these organisms were still enshrouded was equally mysterious in both cases , and, since the organic nature of the true instigators of fermentation was either unknown or was not considered worth attention by the chemists of the day, it happened that the name "ferment" was also applied to the newly discovered enzymes   With an increasing insight into the true state of the matter grew the conviction that two very different things had been grouped under one name, and this conviction found expression in the distinction thenceforward of the true instigators of fermentation as organised or structural ferments, whilst the enzymes were designated unorganised or structureless ferments. These terms are still current in chemical text-books, whereas in Fermentation Physiology it is customary to speak merely of fermentative organisms on the one hand and of enzymes on the other.

M. TRAUBE (I. and II) in 1858 made the origin and influence of these enzymes the basis of a new conception (Ferment Theory) of fermentation, according to which this process is not instigated by the organisms themselves, but by the enzymes formed as products of their vitality and excreted by them.

This theory was accepted by many persons, *e.g.* by Hoppe-Seyler, who considered it as being so self-evident to chemists as not to require any demonstrative evidence. Nageli, on the other hand, advocated its rejection, mainly for the reason that he was not convinced as to the existence of any fermentative enzymes. Numerous workers have, however, since investigated this point, with the result that Traube's opinion has again found general acceptance. The investigations of Miquel with regard to urase, *i e* the enzyme excreted by the bacteria of uric fermentation, merit special mention in this place, because they brought our ideas into closer harmony with the ferment theory, at least so far as regards the ammoniacal fermentation of urine. Moreover, they have greatly strengthened the position of the ferment theorists by proving that this urase cannot be placed amongst inorganic chemical substances, in the ordinary sense of the term, but is really an intermediate stage between these and living protoplasm. Miquel goes so far as to say that his urase is actually protoplasm, chiefly differing from that of the cell contents in that it dispenses with the protection of the cell wall, and remains and works on the outside.

The reader desiring fuller information on the properties of the enzymes than can be obtained from the present work is referred to the comprehensive treatise published by E. Bourquelot (I.) in 1896

§ 19.—General Definition of Fermentation.

We will now pass in review all the preceding explanations, and attempt to extract from each of them whatever can possibly afford us assistance in finally arriving at a satisfactory definition of the term fermentation. In the first place, Liebig's explanation certainly does not call for further consideration in this connection. In the results of the remaining researches we find one factor common to all, and that is the certainty that, for the inception and continuance of the process, which—in harmony with the limitation expressed in § 1—we have hitherto entitled "fermentation," the presence and active collaboration of low types of living organisms is a prime essential. Concerning the nature of the influence, whether direct or indirect, of these organisms, opinions are, however, divided

If, now, the instigators of fermentation be examined *seriatim* according to the method outlined later on, it will be found that not only are they vegetable, but also that all belong to the same class, and that, too, the lowest in the vegetable kingdom, namely, the fungi. The power of inciting fermentation is, however, restricted to comparatively few of the genera of this class. Nevertheless, these latter are so intimately connected with the others, from a botanico-morphological point of view, that it is impossible to classify the fungi into two sub-groups, characterised by the presence or absence of this faculty, without introducing serious systematic anomalies.

The limitation that can be given to the definition of fermentation may be thus expressed . " Fermentation is a decomposition effected by the vital activity of fungi " Nevertheless, as is evident from what has already been intimated, no greater precision can at present be imparted to such part of this definition as refers to the nature or mode of action of the ferment. On the other hand, as will soon be apparent, the word "decomposition" must give place to a term that is both more accurate and more comprehensive.

The phenomena of fermentation forming the starting-point from which the workers from Cagniard to Nageli began their researches, and which up to the present have been the sole subject under our consideration, possess one characteristic in common, *i e* they are always attended by the degradation of complex organic compounds into simpler ones. By regarding this characteristic by itself,

fermentation might be defined as a *decomposition of organic substances* by the agency of fungi

The researches of the past fifteen years (1880–1895) have, however, necessitated considerable modifiations of the words italicised.   The study of the bacteria of the nodules of leguminous plants has taught us that their main-function consists in bringing about the **combination of free atmospheric nitrogen:** hence their action is not a decomposing (analytical) one, but is **synthetic** or constructive, so that in respect of these organisms our definition will have to include the word "transformation."   The adjective "organic" may still remain, since the microbes in question require organic nutriment in addition to the free nitrogen.

We shall, however, find ourselves constrained to reject this latter term when we come to the study of nitrification, and make the acquaintance of another group of microbes able to dispense with organic nutriment, and indeed thriving and acting most effectively only when surrounded by inorganic substances exclusively. Consequently we arrive at the following final and conclusive definition   **Fermentation is a decomposition or transformation of substances of various kinds brought about by the vital activity of fungi.**

§ 20.—The So-called Spontaneous Fermentation of Sweet Fruits.

The point of the preceding definition lies in the concluding words, which restrict the term fermentation to such decompositions or transformations as are produced by the vital activity of *fungi*. It is, however, not impossible for similar decompositions to be effected in other ways, especially by the aid of other vegetable cells differing from fungi.   An example of such a reaction, resembling fermentation but not induced by fungi, is afforded by the so-called spontaneous fermentation of fruit.

The first reliable data with regard to this phenomenon were collected by LECHARTIER and BELLAMY (I.) in 1869, all previous observations having to be disregarded because they do not show that the activity of yeast-cells was precluded.   Starting with the notes made in 1821 by their compatriot BÉRARD (I.), these French investigators succeeded in establishing the fact that sweet fruits, *e.g.* cherries, when kept in an uninjured condition and free from yeast in an atmosphere of carbon dioxide, consume a portion of their sugar content, gaseous carbon dioxide being evolved in the process and alcohol formed   The presence of this latter substance in the cells of the fruit substance can be proved by distillation, as much as one per cent. by weight having been detected in this way.

PASTEUR (IV. and V.) also studied this phenomenon, which he employed as one of the main buttresses of his previously formed theory asserting fermentation to be a universal phenomenon, not dependent on certain organisms, but occurring in every living vegetable cell debarred from a supply of oxygen.   This spontaneous fermentation appeared to form a striking proof of the correctness of this theory, but the hopes thereby raised proved vain, since Pasteur was himself the one to discover that alcohol (though in merely minute quantities) is also formed in fruits when they are exposed to the air.

§ 21.—Decompositions Effected by Light and Air.

To the chemist—who is obliged to store in the dark and in properly stoppered receptacles various inorganic reagents and normal solutions which he desires to maintain in an unaltered condition—it will not be surprising to learn that sterile

solutions of organic substances—such, for example, as are stored for use as nutrient media for bacteria—also gradually undergo slight modifications when air and light find admittance thereto The proof that these changes are of a purely chemical nature lies in the fact that they do not occur when the causes indicated are absent.

In many instances the oxidation of the medium effected in this manner exerts a favourable influence on the development of the organism subsequently inoculated therein Such, for example, is the case with beer-yeast. In wort through which air has been blown ("roused") for some time, the yeast sown therein develops more rapidly, and deposits more quickly and satisfactorily ("breaks" better) at the conclusion of fermentation, a circumstance highly desired by the brewer Sunlight possesses an even greater decomposing power than that of atmospheric oxygen On this point we are indebted to E. DUCLAUX (I) and W. SEEKAMP (I) for exhaustive researches, the former of whom found that, in presence of air, a sterile solution of tartaric acid is split up by sunlight into formic acid, carbon dioxide, and water, according to the equation—

$$C_4H_6O_6 + 3O = 2CH_2O_2 + 2CO_2 + H_2O$$
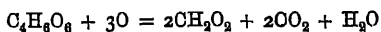
In a second communication (II.) on this subject the same worker showed that glucose and lactose in a sterile alkaline solution gradually decompose into alcohol and carbon dioxide on exposure to sunlight, even when oxygen is excluded The same products are yielded by them when fermented with yeast. When baryta (BaH_2O_2) or lime (CaH_2O_2) was substituted for the alkali, lactic acid was produced instead of alcohol. Under the same treatment maltose yields dextro-lactic acid ; levulose, levo-lactic acid ; and invert sugar the optically inactive acid A similar observation was made by WEHMER (I) with respect to a sterile solution of oxalic acid

These observations are of great interest to the bacteriologist, from a theoretical as well as a practical point of view, as they convey a special warning to protect his stores of nutrient media from the influence of sunlight G Roux, as the result of his adverse experiences, had already given the same warning as to the prejudicial influence of the changes produced by sunlight on bacterial growth, prior to the more exhaustive research by Duclaux.

These facts have a further interest, more nearly connected with our definition of fermentation, since they demonstrate the occurrence of decomposition processes by purely chemical means, apart from the intervention of micro-organisms We will therefore modify our general definition of fermentation, and, in place of stating that fermentation is a process accomplishing transformations of matter with the aid of micro-organisms *only*, will reverse the phrasing, and say that only such changes as are effected *exclusively by the vital action of ferments* come within the meaning of the term fermentation. The point of the definition, as already mentioned at the commencement of the previous paragraph, lies in the words italicised.

However equivalent the action of purely inorganic force on the one hand, and of living organisms on the other may appear, it is so in regard to quality only, the quantitative effects, the amount of substance decomposed in unit time, being widely different. Regarded from this point of view, the minute ferments appear as centres for the accumulation of high-tension energy, by the release of which force the decomposition in view can be effected, not only in a shorter time, but also in a more restricted space, than is possible by the action of purely chemical forces

III.

THE ORGANISMS OF FERMENTATION.

§ 22.—The Position of the Organisms in the Botanical System.

THE study of **Mycology**, or the science of Fungi, can be pursued from several standpoints   The purely scientific position is assumed by the botanist, who accords to each kind just as much importance as its morphological and physiological considerations warrant   If, however, the standard of interest adopted be that of the importance of the part played by the fungi in practical life—*i.e.* **Applied Mycology**—then the number of species to be studied is reduced in a very gratifying manner.   The degree of attention bestowable on those remaining is determined, not by their systematic position, but by the influence they exert on their environment, the nutrient medium.

If the object subjected to the influence of the fungi is a **living** creature, *i.e.* an animal or a plant, it is thereby brought into the condition universally known as **diseased**.   Fungi endowed with this power are designated **pathogenic**, and their study is entitled **Pathogenic Mycology**, which is subdivided into two branches, according to the natural classification of the infected organism.   In the case of human beings or animals, we have the study of **Medico-Pathological Mycology**, and in the case of plants, **Phyto-Pathological Mycology**.

On the other hand, inanimate objects, such as milk, wort, vinegar, manures, leather, indigo, &c., on which the influence of the fungi is manifested by symptoms of decomposition, constitute the subject of **Technical Mycology**, which differs from the pathological branch in another characteristic, namely, in the nature of the influence suffered by the object   Pathological Mycology is exclusively concerned with pathogenic, and therefore noxious, fungi, and its object is to bring about their exclusion and annihilation.   The aim of Technical Mycology is different, being to effect, by the aid of fungi, useful decompositions and transformations which, without the use of such living tools, could only be accomplished incompletely, or in a more roundabout and costly manner

It is, therefore, with the influence of the fungi on their environment, *i e.* the manifestations of their vitality, that technical mycology has to do   Hence it is principally the study of the vital functions of ferments, and may therefore be also termed **Fermentation Physiology**.   With botanico-physiological considerations it is concerned only in so far as they either afford assistance in the comprehension of the physiology of the organisms, or facilitate the differentiation of the various species from one another, and to this extent a knowledge of the morphology of the fungi is of essential assistance to the technical mycologist in the attainment of his objects.   Before going more minutely into this matter, it will, however, be advisable to have a general view of the position occupied by the fungi in the botanical system

As every reader will be aware, the sub-kingdom *Cryptogamia*, which comprises all non-flowering plants, is divided into three chief sections, or seven classes, viz..

CRYPTOGAMIA.	I *Thallophyta* —Thallous plants, without leaves, stems, roots, or vascular bundles.	Class 1	*Fungi.*—Fungi, devoid of chlorophyll.
		„ 2	*Algæ.*—Algæ, containing chlorophyll
	II. *Bryophyta* —Mosses, with leaves and stems, devoid of true roots and vascular bundles.	„ 3.	*Hepatinæ* —Liverworts
		„ 4.	*Musci* —Feather mosses.
	III *Pteridophyta* —Vascular cryptogams, with leaves, stems, true roots, and vascular bundles	„ 5.	*Equisetinæ.*—Horse-tails.
		„ 6	*Lycopodinæ* —Lycopodium
		„ 7.	*Filicinæ.*—Ferns.

To the first of these three main divisions belong all those plants designated **Thallophytæ** on account of the absence of any specialisation of parts, such as stem, leaves, &c , and from the comparatively simple form (**Thallus**) of the individual. This section is subdivided into the two classes fungi and algæ.

The body of all the remaining plants, from the mosses upwards, shows, on the other hand, a differentiation of parts into stem and leaf, and is generally designated **Cormus**, all the higher plants being therefore generally grouped under the title **Cormophytes.**

Of the seven cryptogamic classes, only one, the first and lowest (*Fungi*), comes under consideration in Fermentation Physiology. In accordance with the preceding scheme, these fungi are definable as · cryptogamic plants, devoid of chlorophyll, roots, stems, leaves or vascular bundles; or, expressed in a more concise form the *fungi* are thallous growths devoid of chlorophyll.

§ 23.—Classification of the Fungi.

The fungi are arranged, according to the individual mode of growth, into two main groups, namely, *Schizomycetes*, or fission fungi, and *Eumycetes*, or higher fungi The latter consist in the main of thread-like cells, grow by acrogenesis, form true branches, and reproduce by special organs called **spores.** Conversely, the multiplication of the (always unicellular) fission fungi is effected by subdivision or fission, whence their name, so that we have the following scheme .

Fungi { *Schizomycetes* (Fission fungi) —Fission
{ *Eumycetes* (Higher fungi) —Acrogenous, Branching

No doubt many readers will miss from this classification a third sub-group, viz , the *Myxomycetes*, or mucus fungi. In order to at once disarm any objection on this score, we will here mention that the organisms in question have been shown by recent researches to belong, not to the vegetable, but to the *animal* kingdom, of which they constitute the lowest type of development. For this reason modern systematology has applied to them the name bestowed by De Bary, viz , *Mycetozoa*, animal fungi, or fungoid animals.

§ 24.—Schizophytæ.

As their systematic juxtaposition would lead one to conclude, the fungi and algæ exhibit many traits in common, and, in fact, the only important character by which they can be differentiated is the *absence of chlorophyll* in the first-named.

As will be readily understood, it is especially the lower and more simply constructed species of algæ that, apart from the characteristic difference just mentioned, approximate closely to the fungi. This is particularly the case with the fission algæ or *Diatomaceæ*, which, with respect to their method of reproduction by fission, bear no slight resemblance to the fission fungi

Owing to their greater size, and consequent discernibility, the algæ formed the subject of investigation at a much earlier date than the very much smaller fission fungi, which necessitated the employment of more perfect methods of examination, so that, by the time the latter began to be studied, the green algæ had already been systematised. The temptation to include the newly-discovered bacteria among the analogous algæ was therefore great, and thus it was that the meritorious German algologist FR. KÜTZING (I) was induced to ascribe the acetic acid ferment discovered by him, and now known as *Bacterium aceti*, to the algæ, under the name of *Ulvina aceti*

This inclination to regard fission fungi and algæ as belonging to the same class was also manifested by subsequent workers, and the more so since the greater insight gained in the interim spoke more conclusively in its favour than was possible in the initial, imperfect stage of knowledge   Hence FERDINAND COHN (II.) in 1875 was obliged to discard his own (IV) term for the bacteria, viz., *Schizosporæ*, as also that (*Schizomycetes*) proposed by Nageli (V) in 1857, and to classify these organisms with the lowest of the algæ (*Nostoc*, *Chroococcus*, *Merismopedia*, *Oscillaria*, &c) into one group, which, from their common and characteristic property of reproduction by fission, he called *Schizophytæ* (fission plants), and set up as an independent division.

§ 25.—Assimilation of Carbon Dioxide without the Aid of Chlorophyll.

The classificatory basis for arranging the Thallophytes under the two groups *Algæ* and *Fungi*, viz., the presence or absence of chlorophyll, is not of a morphological, but of a physiological nature, as its connection is not with the form, but with the vital activity of the cell.   Now, it has been proved by much research that chlorophyll plays an important part in the life of green plants The chlorophyll in the cells, aided by sunlight, splits up the carbon dioxide which the plant has absorbed from the air.   The oxygen of the CO_2 is exhaled, whilst the carbon is retained and utilised in the elaboration of the various organic substances of which the body of the plant is composed   This operation is known as the assimilation of carbon dioxide   Until recently, the opinion was generally held that this process could not go on without the assistance of light and chlorophyll, and as the fungi are, without exception, devoid of the last-named substance, it has been laid down as a law that the fungi are incapable of assimilating carbon dioxide and of constructing their cells of inorganic substances like the algæ do.   The researches of Winogradsky have, however, shown that there are fission fungi capable of splitting up carbon dioxide in the dark and without chlorophyll, so that the above law has lost its universal applicability. This point will be more completely treated in the chapter on the nitrifying bacteria

§ 26.—Saprophytes and Parasites.

Plants that are unable to obtain their necessary supplies of material by drawing on the resources of inorganic nature exclusively are termed parasites. As is evident from the statements in the preceding paragraph, all the fungi (with some exceptions to be hereafter mentioned) are therefore to be characterised as parasites   Incapable of elaborating the highly complex molecule of their cell substance from the elements or the simplest atomic compounds (CO_2, H_2O, NH_3, &c), they depend for their supply of nutrient material on ready-formed organic substances, which they have then merely to rearrange according to their needs.

If this semi-prepared nutriment is obtained from a living creature (animal or

plant), such fungi are designated **parasitic**; but if, on the other hand, they utilise inanimate (or defunct) organic material, they are known as **saprophytes**.

The parasites proper may be divided into two groups strict or **obligate parasites**, which are restricted to living animals or vegetable bodies, and **facultative parasites**, which will also thrive on suitable inanimate substrata, and can therefore be cultivated on artificial nutrient media.

This latter group forms the connecting link between Pathological and Technical Mycology. The former science regards these, together with the obligate parasites, as the causes of disease, whilst conversely Technical Mycology is interested in the facultative parasites on account of the decompositions they induce in artificial media, i.e. outside the animal or vegetable economy. This interest is, however, purely scientific, since, for hygienic reasons, the use of parasitic ferments for practical technical purposes is precluded.

The true interest of the technicist in the domain of Mycology is exclusively centred in the non-parasitic ferments, and these alone form more especially the subject of the present work, the first volume being devoted to the *Schizomycetes*, and the second to the *Eumycetes*.

DIVISION I.

SCHIZOMYCETIC FERMENTATION.

SECTION I.

GENERAL MORPHOLOGY AND PHYSIOLOGY OF THE SCHIZOMYCETES.

CHAPTER I.

FORM AND DIMENSIONS.

§ 27.—Forms of Growth.

THE cell forms of all the *Schizomycetes* may be typified by a small rod; hence these organisms are generally known by the name of *bacteria*, from the Greek rendering of this descriptive term, *bakterion*, a rod

When the dimensions of a bacterial cell are equal in all directions, it is termed a coccus or micrococcus, monas or coccobacterium. On the other hand,
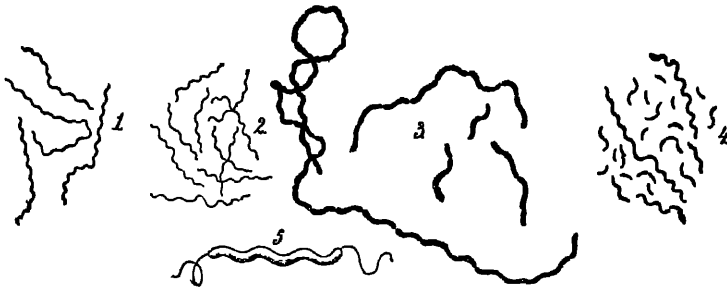
FIG 3—Spirochæta and Spirilla   Mag 950.   (*After P Baumgarten*)

1 Spirochæte Obermeieri, the cause of relapsing fever (*Febris recurrens*)
2 Spirochæta from human dental mucus
3 Denecke's Spirochæte from putrescent cheese
4 Spirochætal form of Koch's Cholera asiatica bacillus
5 Spirillum volutans, Cohn

when the cell is not iso-diametrical, but exhibits a difference between length and breadth, the name bacillus, or elongated rod, is applied, provided the length is at least double the breadth, but when the former does not attain this rela-tive size, we then speak of bacteria. This latter term has therefore a twofold application : a general one, in so far as all the fission fungi are briefly called

2₅

"bacteria;" and a special one, referring only to a particular form of growth, as just mentioned.

A bacterial cell of the bacillus type which, during a certain physiological

A

B
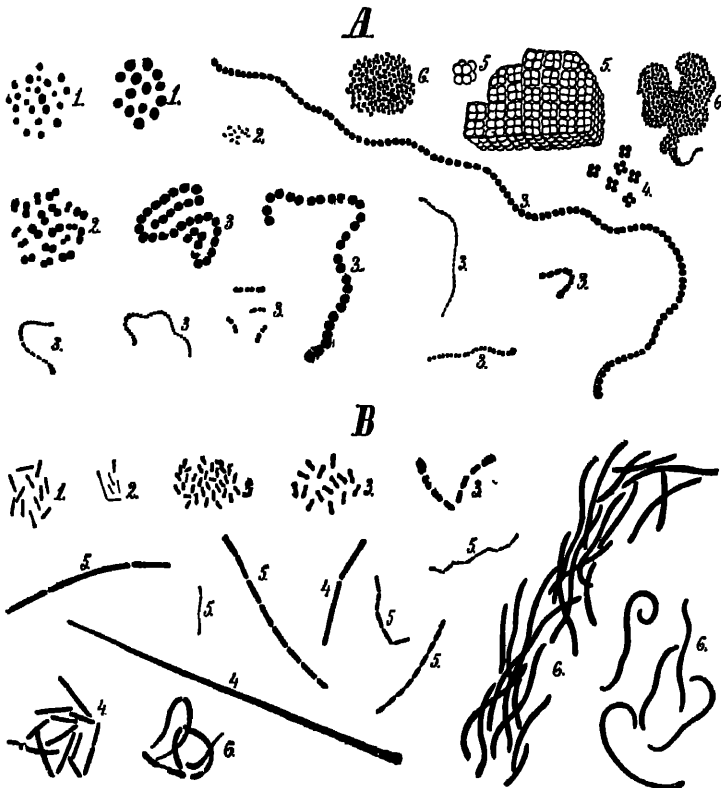
F IG. 4.—Illustrations of the manifold variety in size and form of different bacteria.

Except A_4 and A_5, all the other illustrations are representations of equally magnified bacteria from a single drop of putrescent blood   *(After P Baumgarten)*   Mag 950   Those in A are all symmetrical cells, those in B are elongated

A.—1 Cocci (micrococcus) of various sizes
     2 Diplococci of various sizes
     3 Streptococci of various sizes
     4. Micrococcus tetragonus (from a pure culture)   Mag 950.
     5 Sarcina ventriculi. Mag. 700
     6 Staphylococci.
B —1, 2, 4. Separate long rods of various lengths and breadths
     3 Short rods, partly of biscuit form.
     5. Chains, composed of either short or long rods.
     6. Long threads.

process (to be studied later on under the name of " spore formation "), swells out like a spindle or club, is generally termed a **clostridium**

If the rod is arched like a bow or bent in the form of a comma, it is spoken of as a **vibrio**, and if the bends be repeated several times, then a wavy kind of growth denoted **spirochæte** ensues. When the bend departs from the level plane and shapes itself in such a manner that it can be applied to the surface of a cone or cylinder, the type of growth becomes spiral, and is designated **spirillum**

An illustration of these forms is given in Fig. 3. According to a remark of Emmerich's, these arcuate forms of growth are more especially abundant in muciferous substances, *e.g.* in the excrement of snails, which is rich in mucin, as also among the algæ (already in a state of decomposition, and therefore abounding in vegetable mucus) frequently covering the sides of drains and watercourses

E WEIBEL (I.) has published a series of observations on these forms of growth, while fuller information concerning the *spirilla* frequently to be found in the drainings from manure heaps can be obtained from KUTSCHER's (I) treatise on this subject.

When the longitudinal development is excessive, then the name of **thread bacteria** is given to the cells, which are further distinguishable into the forms to be noticed later, *Cladothrix, Streptothrix, Crenothrix,* and *Leptothrix* being described in chapter xxxiv., and *Beggiatoa* and *Thiothrix* in chapter xxxv. More detailed notices of the *Diplococcus, Streptococcus, Pediococcus, Staphylococcus,* and *Sarcina* forms of growth will be found in chapter iv.

§ 28.—Dimensions of Bacteria.

Notwithstanding our previous statement that the bacteria are the smallest of all known living organisms, it can be established that the differences (Fig. 4) in their size hitherto observed must be characterised as very considerable. In the smallest kinds the dimensions are under 1μ, *i.e* are less than 0.001 m.m For instance, the diameter of a lactic-acid-producing *coccus* examined by P. Lindner was 0 6–1 0 μ. By way of contrast, mention may be made of *Clostridium butyricum*, this bacillus measuring from 3 to 10 μ in length, with a breadth of 1μ. The giants among the bacteria are to be found in the sub-group of chromogenic bacteria, *e g.* the genera *Chromatium* and *Ophidomonas*, which have, therefore, formed the subject of exhaustive investigation with regard to the internal construction of the bacterial cell

We may here briefly allude to a fission fungus which, although unimportant from a practical, technical point of view, forms, thanks to its large size, an especially favourable object for the exhibition of proportional dimensions, spore formation, &c., viz., the *Bacillus megatherium*, found by DE BARY (I.) on cabbage leaves. As the specific name would imply, we have here an organism which excels in size all other fission fungi, as much as the prehistoric *Megatherium* surpassed his contemporary congeners This form is further illustrated in Fig. 5 , the individuals h, r, k, l, will be described in chapter vi (treating of spore germination), so that only m and b need be considered at present These are rods 2 5 μ wide and 10 μ in length, each of which would easily hold about ninety of the previously described cocci.

FIG 5 —Bacillus megatherium

  m Two individual rods
  b Two rods at the moment of reproduction by fission   (*After De Bary*)   Mag 600

It will be useful to remember that the wave-length of light (corresponding to the spectral line D) radiating from the sodium flame is about 0 6 μ, *i e.* about equal to the diameter of the above-named lactic acid coccus Bearing this in mind, the remark already made, that the smallest of the bacteria are almost invisible, becomes comprehensible.

§ 29.—Mutability of Form.

The question as to whether any given species of bacterium assumes only one of the forms of growth already described, or has the power of appearing in various shapes, is one of wide interest. A reliable answer could, however, only be given when the necessary appliances for studying the separate species in the form of pure cultures, *i e.* cultures practised upon an isolated single cell protected from subsequent contamination by other organisms, had been invented. This possibility was first achieved early in the "eighties," since which time the investigation of this question has proceeded with energy, and the results have shown that the theory of uniformity of growth (**monomorphism**) is untenable for bacteria, and must give place to the theory of multiformity (**pleomorphism**).

The remark made in a previous paragraph that the names **bacillus, coccus,** &c., simply indicate certain forms of growth, will now first become perfectly clear. When, in future pages, one or other of the various bacteria is qualified by the generic name *Bacillus* (for instance, *Bacillus ureæ*), it is *not* meant that the said species appears *only* in the form of rods. On the contrary, it may *possibly* present itself in the form of cocci, threads, &c , and has only received the generic name of *Bacillus* on account of its *generally* having this form, and especially when the culture has attained the acme of its development.

This capacity—commonly known as mutability—of assuming a variety of shapes is not possessed in an equal degree by all bacteria, and in a few of them it even appears to be altogether lacking. Abundant pleomorphism chiefly prevails in the arthrosporic bacteria, whilst, conversely, the kinds capable of forming **endogenous** spores are mostly endowed with a smaller number of mutation forms. This difference will be reverted to in a subsequent chapter.

The inciting cause of mutability is generally external, depending on the conditions to which the culture is subjected. Since these can, to a certain extent, be arbitrarily determined and controlled, a means is thus at hand of exercising a formative interference in the existence of these organisms. Among the morphological forces thus available, two are particularly powerful, viz., the influence of temperature and the composition of the nutrient medium. The former has been elucidated by the studies of E Chr. Hansen on the acetic bacteria, which will be exhaustively discussed in chapter xxxvii. On the other hand, H. Buchner convincingly demonstrated the influence of the mode of nutrition on the cell form of the hay bacillus (reported in chapter xvii)

A large number of the species of bacteria noticed in the following paragraphs are pleomorphic, and there will therefore be ample opportunity of becoming acquainted with this phenomenon in all its details and varieties.

§ 30.—Involution Forms.

The remarks made in the preceding paragraphs with regard to the influence of the composition of the nutrient medium require a not unimportant supplementary explanation When we arbitrarily bring about an alteration in the form of a cell by modifying the conditions of the culture, a suitability of the medium to the evolution of the species of bacterium in question is presupposed, so that the vitality and reproductive power in the new form of cell thereby produced is preserved

This is, however, no longer the case in the modifications of form which result when the medium is exhausted of nutrient materials, and consequently enriched with injurious metabolic products. These degenerations of the cell have received from Nageli the name of **involution forms.** They do not enter into the series of

Involution Forms.
...teria, after Maddox
...ymyxa, after Praxmowski
...opii, after Kurth.
...lis, and
...is, after H. Buchner
...after Warming

disten... ...examples given in Fig. 6. The faculty of forming spores is no longer one of their attributes, and they must be regarded as a diseased condition preceding dissolution. In the following pages frequent occasions will arise of calling attention to similar degeneration forms.
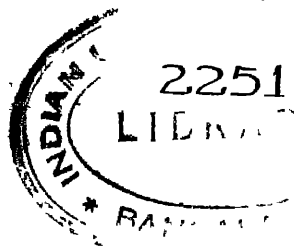
CHAPTER II.

STRUCTURE AND CONSTITUTION OF THE BACTERIAL CELL.

§ 31.—Chemical Composition of the Cell Wall.

NAKED cells, *i e* those wherein the protoplasma is unprotected by an envelope are unknown amongst *schizomycetes*, the body of the cell being in all fission fungi shut off from the outer world by a membrane or cell wall. Little is as yet known regarding its chemical composition, but the few investigations that have been made in this direction demonstrate that we have to do with a structure which not only differs in different species, but also undergoes alteration according to the dietary of the cell.

The proximate assumption that the cell wall of the fission fungi consists of cellulose cannot withstand searching criticism except in rare instances. At present only one single species, viz, the *Bacterium xylinum*, examined by O. LOEW and A. J. BROWN (I.), for which this assumption is justified, is known. The cell walls of this acetic acid bacterium exhibit, after a suitable purification, the following chemical composition, ascertained by ultimate analysis —

	Found.	Calculated for $[C_6H_{10}O_5]n$
Carbon	44 26	44 44
Hydrogen	6 25	6 17
Oxygen	49 49	49 39

The cell wall of this microbe is stained blue by aqueous or alcoholic solutions of iodine, by iodine and sulphuric acid, and with iodo-chloride of zinc. In a work from the pen of DREYFUSS (I.), dealing, however, chiefly with higher fungi, and therefore preferably to be considered only in the second volume, a few species of fission fungi were included in the scope of investigation (*e.g.* a culture of hay bacillus obtained according to Roberts' directions). These when tested for cellulose by iodine yielded affirmative results The amount was, however, only very small. According to NENCKI and SCHAFFER (I.), the cell wall of certain putrefactive bacteria contains nitrogen, but they do not give the names of the species.

§ 32.—Optical Properties of the Cell Wall.

Yet another means has been employed for obtaining an insight into the chemical nature of the cell membrane of the fission fungi, namely, its optical behaviour.

The cell walls of the higher plants are known to be anisotropic (doubly refractive), a property also employed by F v. Höhnel as a means of differentiating textile fibres. If a couple of cotton fibres be placed on the stage of a polarising microscope, the Nicol prisms of which are crossed, the plane of oscillation of the polarised daylight issuing from the lower Nicol undergoes, in its passage through the doubly refractive threads, a rotation, in consequence of which the fibres appear bright and coloured against the dark background. If the fibres are themselves coloured or dyed, they assume the corresponding complementary colours.

The attempt to utilise this means in the service of bacteriology was success-fully made by AMANN (I.). Since, under otherwise identical conditions, the size of the angle through which the plane of polarisation is rotated—and conse-quently the degree of illumination of the field of vision—is proportionate to the thickness of the anisotropic object in question, it follows that, by reason of the small dimensions of the cells under examination in this case, their double refrac-tion cannot be observed when they are in a colourless condition   Now the eye is better capable of appreciating differences of colour than degrees of brightness, and therefore Amann, calculating on this peculiarity, stained his bacteria, assum-ing that if the membranes were anisotropic and doubly refractive, an appearance similar to that mentioned above in the case of cotton fibres would be produced. The results confirmed his expectations, the stained bacteria (*Bacillus tubercu-losis* and *B anthracis*) exhibiting pleochroism, and being therefore doubly refractive.

§ 33.—Zooglœa Formation.

Similar to the behaviour of the cell membrane of higher plants is the ten-dency to swell up manifested by the membrane of many of the fission fungi, the cell wall becoming distended to such an extent, through the absorption of water, that its thickness often far exceeds the internal diameter of the cell.   If to such (previously killed) bacteria be added a solution of an aniline dye, the latter will be absorbed by the cell contents, which, when examined under the micro-
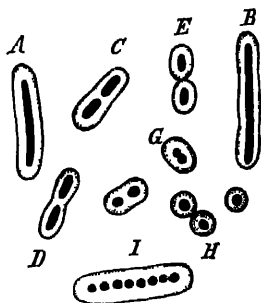
FIG. 7 —Bacillus Pneumoniæ cruposæ

A and B, elongated rods , C, D, E, short rods, G–I, cocci   All the cells exhibit highly distended membranes   Mag about 1500   (*After W Zopf*)
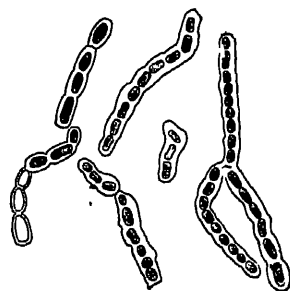
FIG 8 —Bacterium Pasteurianum

Zooglœa formation in an old film on the surface of lager beer   Fixed and stained by Lœffler's   method.   Mag. 1000 (*After Hansen*)

scope, will then be visible, enclosed in a colourless, or merely faintly coloured, slightly refractive, and, consequently, paler cell wall
  Among the pathogenic bacteria the *Pneumobacillus* (Fig. 7), recognised as the cause of croupous pneumonia, was the first in which this property was observed—by P. FRIEDLÄNDER (I.) in 1883.   He gave the name of capsule to the mucinous integument, a term still remaining in use, especially among medical bacteriologists.   Consequently we understand by capsule bacillus one wherein the cell wall is found to be in a distended condition under normal con-ditions of vitality   The *Bacillus diatrypeticus casei* (Fig. 2 of Plate I.), described in a subsequent section, may be mentioned as a second example
  As already stated, these mucinous envelopes are not affected by the ordinary method of staining.   If, however, they are previously treated with a suitable mordant, the colour will be readily absorbed and fixed   Recipes for capsule

staining are given by Friedlander, Ribbert, Lœffler, and others. The annexed illustration (Fig. 8) is drawn from a preparation stained in this way. It represents *Bacterium Pasteurianum*, an acetic acid bacterium discovered by Hansen. The chain seen at the left-hand side differs from the remaining portions of the figure. The upper half presents no special peculiarities, and shows (like the remaining chains) three darkened cells held together by the swollen membrane; whereas in the lower moiety the dark parts are wanting, the three cells formerly present therein having been accidentally crushed in making the preparation, so that only the mucinous envelope remains behind. In a more closely investigated case (not, however, with this bacterium) the substance composing the capsule was identified chemically as related to mucin, or probably identical therewith.

If the gelatinisation of the cell walls proceeds to a little greater extent, it causes the individual cells to become joined or cemented into a coherent mass, called zooglœa by COHN (V.), the size of the agglomerations being greater or smaller according to the degree of development

In a few species of bacteria, colouring matter or ferric oxide is stored in the mucinous envelope, further particulars of which will be found in chapter xxxiv

§ 34.—Plasmolysis.

The difficulty of detecting the cell envelope of bacteria can be removed by immersing the organisms in a solution of salt, which exerts a hygroscopic action on the cell contents, in consequence whereof they shrink and retreat from the cell wall, whereupon the latter is readily recognisable, even in unstained preparations.

This kind of influence, called **Plasmolysis** by H. de Vries, was first observed in bacteria by De Bary, in connection with his *Bacillus megatherium*

The experiment is simple in performance An ordinary preparation of the bacteria under examination is made, a few cotton fibres being immersed to prevent the escape of the organisms. The salt solution is then allowed to flow in at the edge of the cover-glass, its tranfusion being facilitated by absorbing the water with blotting-paper held at the opposite side. Provided the solution is not excessively concentrated, and that it contains no toxic substance, the resulting plasmolysis in no wise destroys the vitality of the cells. It can be made to disappear again by washing out the hygroscopic reagent.

The concentration of the cell contents caused by plasmolysis, which is also generally accompanied with an increased refractive power, leads very often, as BUCHNER (I.) pointed out, to agglomerations of material, which, unless more closely examined, might illusively indicate the presence of endogenous spores.

In such bacteria as are endowed with the power of locomotion, the rate of movement is retarded by increased concentration of the plasmolytic solution. WLADIMIROFF (I. and II) entitled the smallest percentage capable of arresting locomotion the **critical solution**. The values of this for the various salts—on any determined species of bacteria—show an unmistakable regularity, the chloride of any given metal (K, Na, &c) having the weakest, and the bromide and sulphate the strongest retarding effect, whilst the nitrate occupies an intermediate position. As far as the bases are concerned, potassium, sodium, and ammonium rank in the order given. E OVERTON (I.) has published several communications with regard to a number of substances which are unable to effect plasmolysis owing to their passing through the plasma as rapidly as water

The weakest solution of sodium chloride (NaCl) capable of effecting plasmolysis is, according to A. FISCHER (I), o 5-o 75 per cent. for *Cladothrix dichotoma*, and for *Crenothrix Kuhniana* 0.75-1.0 per cent. These limits are, however, not

unconditionally applicable, as the following example will show   A one per cent. solution of common salt produced no plasmolysis in *Clostridium butyricum* when the cultures were only two days old, and the cells consequently young, whereas after a further four days they were, without exception, completely plasmolysed. It will perhaps be useful to recall that a solution of o.5 gram of NaCl in 100 grams of water is usually known as **physiological salt solution**, and is frequently used under the supposition that it neither takes up nor gives up water from or to the cells,—an assumption that, in view of the preceding figures, cannot be universally justifiable.

If it is desired to render permanent ("fix") the plasmolytic condition of the cells, they must be killed, a result easily obtained by the use of sublimate or iodine solution   Ten per cent lactic acid is also an excellent fixing medium, instantaneous in action.   After fixing, staining can be effected.   More detailed information on the practice of fixing will be found in a work by A. ZIMMER-MANN (I.).

§ 35.—Structure of the Cell Contents.

This study, which presents no small difficulty on account of the minuteness of the organisms to be examined, has been closely followed up during the past several years only.   All that was previously known was that the cell contents of the bacteria consisted of a homogeneous invacuolate plasma, in which small, highly lustrous granules were frequently seen embedded

BÜTSCHLI (I.) in 1889 discovered in a few large chromogenic bacteria (*e.g. Chromatium Okenii* and *Ophidomonas jenensis*), as also in *Spirochæte serpens* and *Beggiatoa*, that their cell contents could, as a rule, be distinguishable into two parts, viz, a **central body** and a **parietal layer**, the latter being adjacent to and surrounded by the cell wall

The parietal layer may either surround the central body on all sides, so that the latter nowhere touches the cell wall, or may be restricted to one side only, in which event it is generally, in the case of rod-shaped bacteria, found at the two poles   This differentiation of the cell contents can be rendered visible (Fig 9) by suitable stains, e g. hæmat readily taken up by the central ing it easily distinguishable f...... ... . coloured parietal layer

This treatment brings out a second and much more important fact the central body appears as a complicated structure, reminding one of that seen in honeycomb   A number of granules of red-violet colour—called by Butschli "red grains"—are stored in a reticular framework which is coloured blue by the staining dye   These granules do not occur in every cell, and no cell has more than one.   These enclosures are detectable, even in the unstained preparation, on account of their high refractive power.   They—the bodies, not their enveloping framework—were first observed by V. BABES (I.), then studied by P ERNST (I. and II.), and were regarded as the starting-point of spore formation, being on that account designated **sporogenic granules**, but this assumption has with good reason, been contested by later workers.   For a more accurate examination
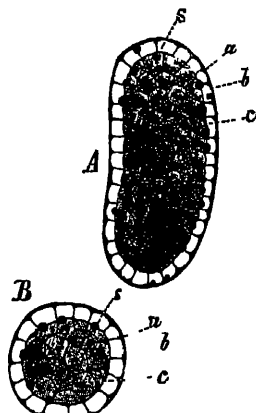
First killed, then freed from bacterio-purpurin and sulphur granules by solvents, and finally stained with hæmatoxylin The reticulated structure of the (hatched) central body (c), as also of the parietal layer (b), and the dark chromatin granules (s), Bütschli's "red grains," can then be detected   Magn 2000–2500   (*After Bütschli.*)

we are indebted to SCHEWIAKOFF (I.), according to whom each of these sma
enclosures contained in the honeycomb cells possesses a thin skin, the presenc
of which can be convincingly demonstrated by pressing the preparation unde
a cover-glass, whereupon the skin bursts, without, however, its contents bein
dispersed. The latter can therefore only be of a solid and not of a fluid natur
The chemical nature of the skin is unknown ; it does not give the cellulos
reaction. So far as the composition of the granular contents is concerned, th
last-named investigator has identified therein by micro-chemical means pota;
sium, calcium, and oxalic acid, in addition to the indeterminate organic matte
In their behaviour, more closely investigated by WAHRLICH (I.), towarc
colouring and solvent reagents, these enclosures in the central body of th
bacterium resemble those granular constituents of the cell nucleus of highe
plants known as chromatin granules, on account of their high power of absorbin
colouring matters. On the other hand, the reticular mass of the central bod
resembles in point of structure and micro-chemical reaction the lignin of th
nuclear framework of the cells of higher plants.

These observations led BÜTSCHLI (II.) to the opinion that the central body ·
bacteria should be regarded as the (comparatively large) nucleus thereof whils
on the other hand, the above-named parietal layer corresponded to the cytoplas
of the cells of higher plants—a conception which has not withstood the test
criticism.

The structure of the parietal layer has not yet been determined wi
certainty Sundry observations, however, indicate that it exhibits a radi
honeycomb appearance. It is in this layer that the colouring matter of th
chromogenic bacteria is lodged, whereas the granules of sulphur in the sulphi
bacteria are located in the central body. The individual species of the gen
Granulobacter established by Beyerinck—to which belong the instigators of t
butyric acid and butyl alcohol, &c., fermentations—under certain conditions
culture store up in their interior copious supplies of granulose, owing to whi
circumstance they are stained a deep blue by iodine. It should be mention
that W. MIGULA (I) could not detect any honeycomb structure of the cell plasi
during his researches into the structure of the *Bacillus oxalaticus*, discovered
Zopf.

It may perhaps be useful, though not exactly necessary, to remind the read
of the well-known botanical fact that the living plasma strives to prevent t
access of colouring matters. In this connection, also, the behaviour of the varic
kinds of fission fungi differs Some of them exhibit merely a slight resistan
and absorb colouring matter without their vitality being impaired, as Birc
Hirschfeld established with respect to ·phloxine red in the case of the typh
bacillus In the majority of instances, however, this resistance is so great th
the cells must be killed before they can be stained. On this account most of t
staining solutions employed for bacteriological purposes contain additions (ε
alcohol) destructive to the vitality of the cell, which being accomplished, t
plasma readily absorbs the colouring matter. According to the researches
DREYFUSS (I.), it is not the true albuminoid matter, but the nuclein (also
constituent of plasma) which fixes the colour

Thanks to the care bestowed on the subject by medical bacteriologists specie
interested therein, the art of staining bacteria has reached a high degree
perfection during the last few decades. Exhaustive directions thereon are
be found in HUEPPE'S (I.) handbook and EISENBERG'S (I.) treatise, and BERNH
(I) has issued a very cheap book highly useful for laboratory work.
Fermentation Physiology the examination of the bacteria being usually perforr
on the living, unstained organisms, the aid of staining is seldom required ,
principal occasions being when so-called cover-glass preparations have to be k

for purposes of future microscopical comparison. The preparation of such is described in all the books just mentioned.

§ 36.—Elementary Composition of the Bacterial Cell.

Determinations on this point by micro-chemical analysis were first attempted by KAPPES (I.), whose attention was principally devoted to *Micrococcus prodigiosus*, cultures of which were made on solid nutrient media, and then, when they had arrived at sufficient development and had expanded into mass cultures, examined for the amount and composition of the dry matter therein. Cultures of this fission fungus on agar-agar contained on an average   Water 85 5 per cent , and dry matter 14 5 per cent , the latter yielding ethereal extract 0 7 per cent , nitrogen 1.7 per cent , and ash 2 0 per cent.   Calculated on the dry substance, the following percentage composition was arrived at for the microbe in question

	Per Cent
Ethereal extract (fat, &c)	4 8
Albumen (N × 6 25)	71 2
Ash	13 5
Undetermined substances	10 5

An examination made by NISHIMURA (I) of a pure culture of a water bacillus gave the following as the constitution of the dry matter in this microbe

	Per Cent.
Albumen	63 5
Carbohydrates	12 2
Alcoholic extract	3 2
Ethereal extract	5 1
Ash	11 2
Lecithin	0 68
Xanthin	0 17
Guanin	0 14
Adenin	0 08

The most noticeable figures here are those relating to the content of nitrogen, which show that, in this respect, the bacteria are excelled by but few organisms, while they have no compeers in the vegetable kingdom   To demonstrate this fact more clearly the subjoined figures have been selected, showing the mean nitrogen content in the *dry substance* of .

	Lean beef	Cow's milk	Soja beans	Russian wheat.	Truffles	Micrococcus prodigiosus
Nitrogen per cent	14.3	4 4	6 0	3 5	5–6	11 4

The earliest researches on the nitrogenous constituents present in bacteria were carried out by NENCKI and SCHAFFER (I), both of whom isolated from putrefactive bacteria a nitrogenous substance to which they gave the name of mycoprotein.   The highly concordant results of a series of ultimate analyses of this substance gave the following mean values

$$C \quad 52\ 32 \qquad H \quad 7\ 55 \qquad N . \ 14\ 75 \qquad O + S \quad 25\ 38$$

The percentage (14 75) of nitrogen found is remarkable, and shows that mycoprotein must have a very different constitution from that of ordinary albumin or protein, which, as is well known, contains about 16 per cent of nitrogen   The amount of this compound in the dry substance of bacteria is at least 40, and sometimes as much as 50 per cent.   Differences are also apparent as regards their behaviour towards reagents, nitric acid, for example, converting albumin into a bright yellow compound, named, on the proposition of MULDER (I.), xanthoproteic acid, whereas mycoprotein does not give this reaction.

§ 37.—Quantitative and Qualitative Selective Power.

The proportions of the constituents present in the available nutriment or nutrient medium supplied seldom correspond to the requirements of the organism which has to make good therefrom losses of substance or energy. It will then take from the supply the substances of which it has need, and leave the residuum entirely untouched, only when the former are present in abundance. This faculty, entitled **quantitative selective capacity**, is possessed by all living organisms, and among them bacteria. Kappes, in his treatise already referred to, gives very instructive examples of this as well. He compared the composition of the bacterial crop with that of the soil (nutrient medium) in which it was grown; in this case peptonised meat extract, agar-agar. This contained, apart from the 1 5 per cent. of agar-agar, which does not come under further consideration here, altogether 2.5 per cent of actual nutrient materials, and yielded 0.3 per cent. of dry bacterial substance; that is to say, only 12 per cent of the total nutriment was extracted. The relative proportions of the individual constituents in the medium on the one hand and in the crop on the other proved very different. Thus, for instance, the ratio of CaO MgO was in the medium 0.70 : 0 44; in the crop, 0 56 . 1.05. Of nitrogenous substances (N × 6.25) the former contained 42.5 per cent, calculated on the dry substance, and the latter 71 2 per cent., and so on

The requirements of bacteria in respect of ash constituents were first investigated by NÄGELI (IV.), in 1879. SPRENGEL (I) was admittedly the first to demonstrate that higher plants (*Phanerogamia*) absolutely require for the construction of their cells a number of mineral substances, viz , K_2O, CaO, MgO, Fe_2O_3, P_2O_5, SO_3, all of which must be present, and in sufficient quantity, before the phanerogamic plant can thrive. With the *Cryptogamia* the case is, however, different. According to Nägeli, the fission fungi (tested by him in this connection) are less exacting, since potassium can be replaced by rubidium or cæsium without detriment, so far as the fungi are concerned, though not by the alkaline earths.

Of the latter it is sufficient when one of the following, CaO, BaO, SrO, MgO, is present, iron can be dispensed with. It follows therefrom that not only quantitative but also **qualitative powers of selection** are possessed by bacteria. An authoritative confirmation of Nägeli's discovery is highly desirable, and would prove a very thankworthy task if conjoined with observations on the formative influence of the individual ash constituents. A typical example of this kind of study, alike instructive, stimulating, and worthy of imitation, has been made by Winogradsky on a film yeast, and will be referred to in the second volume. A preliminary step in this direction was taken by A K FEDOROLF (I.) in 1895, who, in continuing an investigation commenced by Gamaleja, found that while lithium chloride caused abnormal cell forms in *Bacillus megatherium*, *B typhi abdominalis*, *Vibrio choleræ asiaticæ*, and *Bacterium coli commune*, it had no apparent influence on the cells of *Bacillus subtilis*, *B. anthracis*, and other microbes

The quantitative selective power referred to above must not be understood in the sense that the organisms inhabiting the nutrient medium always have the same composition, irrespective of the relative proportions of its nutrient constituents. E CRAMER (1) studied this matter exhaustively in relation to several pathogenic bacteria and *Micrococcus prodigiosus*, and arrived at the conclusion that a typical composition of any species is out of the question. According to the kind of medium, the temperature and age of the culture, and other conditions, it may happen that one crop will contain twice as much of a particular constituent as another crop. Thus, for example, the amount of dry matter varied from 15.9 to 26.0 per cent., and of ash from 1.60 to 3.21 per cent.

CHAPTER III.

POWER OF INDEPENDENT MOVEMENT IN BACTERIA.

§ 38.—Molecular Movement and Locomotion.

LIKE his predecessors, Chr Ehrenberg considered the bacteria as animalculæ, and that, chiefly, because in not a few of them he discerned active locomotive powers For the same reason Pasteur was inclined in 1861 to regard his "vibrion butyrique" as an infusorial animalcule In opposition to this was established the fact that the faculty of voluntary movement is not peculiar to the bacteria, but is also possessed by many motile spores belonging to the algæ; that is to say, by organisms unanimously admitted to be of vegetable nature.

This motile power will now be more closely considered, both as regards its nature and causes.

The movement known as the **Brownian or molecular movement**—which can frequently be observed in small particles held in suspension in liquids, whereby each particle describes a small orbit (sometimes rectilinear, sometimes circular or oval) outside its horizontal axis of rotation—is not included in this consideration. The cause of this Brownian movement has not yet been examined with sufficient accuracy , but it is a purely physical one and in no wise physiological, since it is manifested not only by living cocci, but also, as already stated, by emulsions of many inanimate substances both of inorganic and organic nature.   Ali-Cohen, in his treatise referred to below, describes a very useful means by which one can decide in doubtful cases whether the movement is **independent** or merely molecular.   According to the researches of Exner, the latter diminishes in briskness as the viscosity of the circumambient liquid increases.   If then a little of the doubtful sample be mixed with a lukewarm liquefied 5 per cent. solution of gelatin, both locomotive and molecular movement will at the outset remain unaffected, but in proportion as the surrounding medium cools and becomes more viscous, the latter movement will diminish, and finally cease altogether, whilst the voluntary and independent movement will continue

By long-continued practice and observation the faculty will be gradually acquired of distinguishing, without such aid, the so-called molecular from the true voluntary bacterial movement, which we will now describe.   This movement is of two kinds, the first of which is occasioned by alternate contraction and re-expansion of the plasma canal.   This kind of movement occurs in the case of such thread bacteria as attach themselves to a support by one of their poles, and then swing with a pendulous motion from this point of suspension, either remaining in the same plane the while or describing a cone

§ 39.—The Flagella or Cilia.

Much more frequent is the second or **roving** movement, noticeable in free unattached bacteria, and produced by special locomotive organs termed **flagella** or cilia.   These were first noticed by EHRENBERG (I.), in 1836, in a spirillum which he discovered in a brook near Jena, and named *Ophidomonas jenensis.*   Closer attention was first bestowed on these organs by COHN (I.).

PLATE I

DESCRIPTION OF THE DRAWINGS

Fig. I.

Micrococcus Sornthalii
Without cilia. Magn. 650
After *Adametz*

Fig II.

Bacillus diatrypeticus casei
Without cilia. Capsule bacillus.
Magn 1300.
After *Fr Baumann*

Fig. III

Bacillus cyanogenus
Cilia staining. Magn 1100
After *Loeffler*

Fig. IV.

Large Ciliated Bacilli
From a vegetable infusion. Accompanied
by other non-ciliated fission fungi. Magn
1000
After *Loeffler*

Fig V.

Spirillum Undula.
From a vegetable infusion. With tufted
polar cilia. Magn. 800.
After *Loeffler*.

Fig VI

Spirillum rubrum, Esmarch.
With polar cilia. Magn. 1000
After *Loeffler*
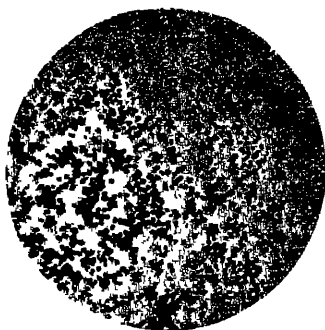
FIG I

FIG II
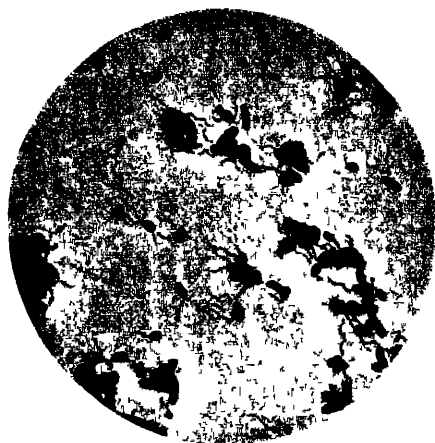
FIG III.

FIG IV

FIG V

FIG VI

VAN TIEGHEM (I.) was of opinion that these locomotive organs were peculiar to bacilli, but similar locomotive powers were at a later date observed in the cocci, such as the *Micrococcus tetragenus mobilis ventriculi*, discovered by MENDOZA (I) in 1887; the *Micrococcus agilis*, found by ALI-COHEN (I.) in 1889, a coccus (unspecified) studied by LOEFFLER (II.) in 1890, later, the *Micrococcus agilis citreus* of MENGE (I); and finally, the *Sarcina mobilis* of MAUREA (I.)

The position of these organs in the bacilli is either polar or lateral. The polar flagella are either single—as, *e g.* in *Chromatium*—or in tufts, the latter consisting, in the case of *Bacterium termo*, of three or four, and in various spirilla of eight to twelve, cilia. In **Spirillum undula** they are often plaited into the form of a *queue* The lateral cilia are, as was found by A FISCHER (II), evenly distributed over the entire surface of the bacterial cell, their number being given by Loeffler as twelve in the case of the typhus bacillus.

Starting with the assumption that the **number** of the cilia and their distributive arrangement on the cell are constant for each kind, A. MESSEA (I) endeavoured to make this character the basis of a classification of the bacteria L LUKSCH (I) proposed the same method for readily differentiating *Bacterium coli commune* from *Bacillus typhi abdominalis*, which is very important in the bacteriological examination of water He found the former microbes to be provided with at most three cilia apiece, whereas the bacillus had from eight to twelve Subsequently, however, it was ascertained by FERRIER (I) that the number, form, and length of the cilia depend on the conditions of the culture. From the bacterium in question cultures can be obtained the individual cells of which exhibit as many as ten cilia; by this determination, therefore, the system of Messea, as also the hopes of Luksch, were deprived of support. Moreover, Messea had been forestalled, as in 1864 DAVAINE (I.) proposed to separate the fission fungi into two groups, the one, forming his genus *Bacteridium*, comprised all the species in which he cov^ld ^ot ^^t^^t ^ l   ^ ^
circumstances; whilst the othe
species. In J Schroeter's work
which a notice is given in a subs
adopted, but later workers have abandoned it, and the terr
perfectly obsolete.

When a bi-polar ciliated bacillus divides in two in the
the new-formed poles are, naturally, without such locon
outset, but they quickly develop, and thenceforward eac
ciliated at both poles. That these organs are extremely
emphasised. Frequently they are undetectible by the
observation, even with objectives of the highest power and ^^^^^^ ^^^
since it is difficult to see the cilia, not only because of their extreme minu
but also because their refractive power is almost the same as that of tl
in which they are immersed. In order to make them more readily reco$
use is made of the special methods of staining devised by LOEFFLER (I).
directions relative to these will be found in UNNA's (I) historio-critical ^^
of the development of bacterium-staining, drawn up in 1888, and continueu
L. HEIM (I.) up to the year 1891. Plate I. shows four photographs of mo
bacteria taken by Loeffler from preparations stained in this way

§ 40.—Histology of the Cilia.

This subject has hitherto received little attention. Van Tieghem considered the cilia to be gelatinous elongations of the cell envelope, and their movement as merely passive, the locomotive power being ascribable to contractions of the plasma in the cell. He found that the cilia of *Clostridium butyricum* gave the

cellulose reaction with ammoniacal copper oxide. Zopf, on the other hand, explained these organs as contractile plasma-threads, which could be alternately protruded from, and withdrawn into, the central cell mass through apertures in the cell integument, which apertures, however, have hitherto been unobserved.

This assumption was combated by A. Fischer, who found that when motile bacteria were subjected to plasmolysis, and the cell contents therefore caused to contract, the cilia were not drawn in, as should be the case if they were continuations of the plasma (**pseudopodia**). For arresting the movement of the bacteria examined by him, the strength of the solution of salt had to be higher than the minimum capable of producing plasmolysis. Fischer's observations favour the view that the cilia are appendages of the cell, consisting of a membrane enveloping the protoplasmic contents, which have an immediate connection with the substance of the bacterial cell

Adverse influences stop the movement, and the cilia become motionless and torpid. According to the cause, this condition is said to be one of **torpidity through cold, heat, darkness, light, hunger, desiccation, or poison** Bearing this in mind, it must not be concluded that any species of bacteria which may not exhibit movement under ordinary microscopic examination is therefore necessarily non-motile, but it should be further examined under various conditions, and, in extreme cases, tested for the presence of cilia by staining, since it may be in the torpid condition.

§ 41.—Chemotaxis,

The extended researches of ENGELMANN (II. and III) teach us that certain roving bacteria (i e. those endowed with spontaneous movement), and, in particular, various putrefactive bacteria, have a great need for oxygen, while other species do not require it. If a drop of liquid containing a mixture of these two kinds be brought under the microscope, it will quickly be seen that the one species hastens to the edges of the cover-glass, where oxygen penetrates by diffusion and is most abundant, whilst the individuals of the other species gradually retreat, and collect at the centre, where the (to them) unwelcome or obnoxious gas does not penetrate. Repeating Engelmann's experiment by inserting a thread of green (i.e. oxygen-excreting) alga in the drop, and directing a small solar spectrum thereon, then the oxygen-loving

FIG 10—Oxygen-loving bacteria infesting a thread of alga lying in the micro spectrum The chlorophyll granules contained in the alga cells are not shown, but the spectrum lines are given to denote the position of the spectrum   Magn. 200   (*After Engelmann*)

bacteria will be seen collected around these alga threads, and surrounding those spots in the micro-spectrum (Fig. 10) where the maximum evolution of oxygen is taking place, that is to say, between the spectrum lines B and O in the red, and also at F Oxygen, therefore, exerts an attractive and stimulating action on many bacteria, and may thus be employed as an isolating and separating agent therefor. Conversely, motile bacteria may also be employed as a delicate reagent for oxygen BEYERINCK (I), to whom we are indebted for a very

useful treatise on this question, examined more closely the methods of topographical arrangement adopted in liquid media by various motile bacteria under the influence of oxygen, which arrangement he called **respiration figures.** Some of these are shown in Fig II.

From the researches of Stahl and De Bary, and especially those of W. PFEFFER (I), we learn that not only oxygen, but also various other substances,
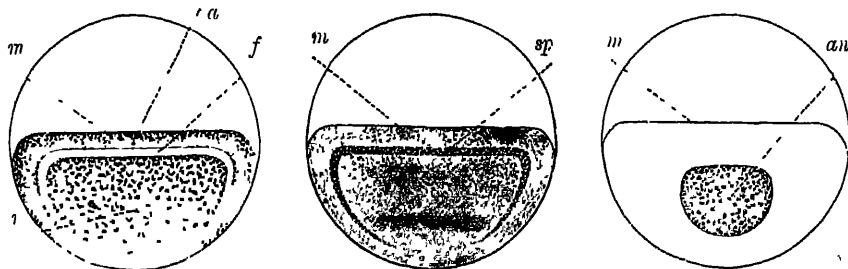
FIG II —Respiration figures of motile bacteria. *(After Beyerinck)* Natural size

The three figures are horizontal projections of bacterial preparations, each in a large drop of water The three large circular cover-glasses only are shown, the slides not being reproduced A small platinum wire (not shown in the figures) is placed at the part represented by the top of the drawing, between the cover-glass and slide, so as to form a wedge-shaped space, which is occupied by the drop of water, the base of which lies in *m* (= meniscus)

The three figures represent

I Respiration figure of the **aerobic type** The roving individuals collect in the oxygenated border zone (*a*), whilst the quiescent ones (*r*) remain in the interior, leaving a vacant space (*f*) between the two

II Respiration figure of the **spirillum type** These organisms require and tolerate only traces of oxygen They therefore collect, not at the circumference of the drop, but at a little distance therefrom (*sp*), where the tension of the penetrating gas is lower

III Respiration figure of the **anaerobic (air-shunning) type** These migrate to the centre (*an*) of the drop, as being the place of lowest oxygen content

are capable of attracting or repelling bacteria and other micro-organisms, a faculty to which the name of **positive or negative chemotaxis** has been given. Use is made of this property in order to capture the motile species in a bacterial mixture by introducing therein a capillary tube filled with a solution of a substance which exerts an attractive influence on one or other of the motile species.

Among the inorganic compounds the salts of potassium have the greatest power of attraction, and are therefore most frequently employed for this purpose. Of the organic compounds, asparagin is particularly effective. The sap or juice of raw potatoes contains both these lures, and is therefore highly efficacious More particular information on this method of isolation is given by ALI-COHEN (II).

The attractive power of such agents is not limited to motile bacteria alone, but also extends to higher sessile fungi If such an attracting agent be brought sufficiently near to a culture of the latter description, the growth and extension of the cell threads on that side of the culture will show marked exuberance This attempt on the part of sessile fungi to turn towards a point of chemical attraction is entitled **Chemotropism** Its cause is identical with that of **Chemotaxis**, the difference in the effect being due to the difference of the object influenced It may be mentioned that MIYOSHI (I.) has performed exhaustive experiments of this kind.

CHAPTER IV.

VEGETATIVE REPRODUCTION BY FISSION.

§ 42.—Division in One Direction.

In order to reproduce by fission in one direction, the cell becomes elongated, and a partition (*septum*) is developed in the interior of the cell at right angles to the length. This septum then divides into two lamellæ, thus effecting the separation of the **daughter-cell** from the **mother-cell**. If the organism is living under conditions favourable to its vitality, each of these cells will soon undergo a similar process of division   In many instances the new-formed cells of the second, third, fourth, &c , generations do not become entirely detached, but remain connected one with another; and if—as is most often the case—the division takes place continuously in the same direction, **chains** of cells are formed.   When the members composing the chain consist of cocci, the fission fungus is frequently designated a **streptococcus**, instead of merely coccus.   Hallier and Itzigsohn proposed to apply the term *Mycothrix* to these rosaries of cocci.   When the members are united chiefly or exclusively in **pairs**, the organism is termed **diplococcus**, and such of the cocci as incline to group themselves in grape-like agglomerations are frequently designated, in medico-bacteriological literature, by the name **staphylococcus**, first bestowed on them by OGSTON (I.) in 1880.

FIG 12.—Bacillus tumescens

a and b, chains of short members.   (*After A Koch.*)   Magn 1100

If the members of a chain are not iso-diametric cocci, but rods, they are mostly termed **thread** (filamentous) **cells**, of which the hay bacillus affords an excellent example.

In an iso-diametric cell, the separation of the new cells—and therefore the preliminary expansion of the mother-cell—may occur in one of two directions either lengthwise or crosswise, the former case—wherein the position of the dividing septum is **transverse**, *i.e.* perpendicular to the longitudinal direction—being the most usual.   On the other hand, only a few examples of the second or **longitudinal separation** are as yet known.   One of them is afforded by the *Bacillus tumescens*, discovered by ZOPF (I.), which will nearly always be found infesting slices of boiled carrot, when the latter are left to themselves for some time in a not too damp condition.   According to the conditions of vitality prevailing, this microbe develops either chains of *long cells* formed by transverse fission, or cell bands the members of which are *short* and joined *broadside* on, the attached sides measuring 2.1 μ each, whereas the *length* of each is only 1 3 μ. This microbe, therefore, exhibits both styles of fission.   On the other hand, *longitudinal fission* alone is manifested in a fission fungus discovered by METSCH-NIKOFF (I.), and named *Pasteuria ramosa*, which grows in the ventral cavity of certain water-fleas (*Daphnia pulex* and *D. magna*), where it produces a fatal disease   In these microbes the new septum is always longitudinal.

In the separation process now under consideration, wherein the division of the mother-cell and the casting off of the daughter-cell take place in one

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direction only, the number of mother- and daughter-cells is always equal; the *total number* (*e*) of cells obtained from the initial cell (*a*) at the end of the nth reproduction being

$$e = 2^n a$$

A much greater rate of increase is attained in a given time when the sub-division is effected in several directions *simultaneously*. Examples are known of both possible events, viz, separation in *two*, and separation in *three* directions at right angles to one another.

§ 43.—Division in Two Directions.

In this case the cell contents subdivide into four parts by the formation of two septa intersecting each other at right angles, and each splitting into two lamellæ, whereupon the mother-cell becomes four daughter-cells   The latter again undergo subdivision, whereby $4 \times 4 = 16$ cells are formed   At the end of *n* subdivisions, the total number of cells from *a* individuals will be—

$$e = 4^n a = 2^{2n} a$$

In this case, where the separation goes on continuously in two directions perpendicular to one another, and so always in the same plane, there results—provided the gradually extending cells retain their connection—a mosaic-like stratified plate which has been named **Merismopedium** (divided plate) A coccus exhibiting this method of reproduction is known as a **pediococcus** To this group belongs the lactic-acid-producing *Pediococcus acidi lactici* discovered by Paul Lindner.

§ 44.—Division in Three Directions.

Cocci, wherein the reproduction of the cell is effected by division in all the three directions of space, are designated **Sarcina**. In this case the contents of the mother-cell divide into eight equal parts by the formation of three flat septa, perpendicular to each other, which subsequently split up into two layers, whereby each of the eight daughter-cells is surrounded on all sides with cell-membrane.

When these, as they generally do, remain attached together, their appearance resembles a corded bale of goods, or a cubical packet   See Fig 13

In the course of reproduction an initial number of cells represented by *a* will increase by *n* processes of division to a total of—

$$e = 8^n a = 2^{3n} a.$$

FIG 13 —Sarcina ventriculi
From the contents of a diseased stomach a. Individual cell, b. the same divided in one direction only, c. the same divided in three directions, d packet colony of cells

The *Sarcina maxima*, frequently met with in malt mashes, may be cited as an example of this form of growth A comprehensive classification and accurate characterisation of all known species of the genus *Sarcina* has been given by Th Gruber (I.).

Subdivision in more than one direction, and therefore the formation of sheet-like or packet-shaped aggregations of daughter-cells, has hitherto been observed solely among iso-diametric and a few thread bacteria. The first group (of coccus form) is constituted by *Sarcina* The behaviour, in this connection, of the second group (*e.g Crenothrix*) will be subsequently discussed.

§ 45.—Form of the Daughter-Cells.

Cocci, when observed immediately after their production by subdivision, exhibit a more or less angular outline, since the surface of separation between the mother- and daughter-cell is flat, appearing to the eye as a straight line. This shape, however, very soon undergoes alteration, the cell-membrane being caused to bulge outward by the pressure of the plasma, whereby the plane surfaces are rounded and the cell assumes the customary appearance of the non-facetted coccus. By the same cause the initially plane circular outline of the smaller side of a cylindrical bacillus, newly formed by fission, becomes dome-shaped.

§ 46.—Division of the Nucleus.

As is known, the division of the nucleus can be effected, in the higher plants as well as in animals, in two ways. The simpler of these, generally known as **segmentation**, or as **direct or amitotic division of the nucleus**, occurs, in the higher plants, only in such cells as have ceased to subdivide, and whose multiplication has therefore ceased. The nucleus elongates, becomes constricted at an intermediate point, and finally falls apart in two halves. More complicated, however, is the other process known as **indirect or mitotic division of the nucleus.** In this case the final separation of the nucleus into two portions is preceded by a far-reaching transformation of the substance of the nucleus of the mother-cell, an operation entitled **karyokinesis**; and it is in this manner alone that the subdivision of the nucleus occurs in the segmentation cells of higher plants and animals. Fundamental researches into this process have been made by FLEMMING (I.), and in the narrower domain of the *Thallophytes* the investigations of SCHMITZ (I.) merit attention.

In so far as the *Schizomycetes* are concerned, BÜTSCHLI (I. and II.) made various observations, from which Zacharias concluded that the division of the central body of fission bacteria is amitotic or direct. WAHRLICH (I.), FRENZEL (I.), and SJÖBRING (I.) studied the matter more closely, and, according to them, the chromatin granules of the central body (§ 35) are first dissipated, and then the latter stretches and subdivides. Concurrently, the new septum interposes itself between the two moieties of the cell and then splits up into two lamellæ, thus effecting the separation of the two cells.

As will be remarked, the *Schizomycetes* assume an exceptional position as regards the behaviour of their nucleus during cellular subdivision, the operation being in this case alone direct, whereas in all other plants karyokinesis occurs

§ 47.—The Rate of Reproduction

is, naturally, influenced by external circumstances, especially by the method of nutrition and the temperature. It also varies under the same external conditions in the different species. The time required for the formation of one bacterial cell from another (a new generation) is known as the **period of generation.** This period was determined by BREFELD (I.) and by PRAZMOWSKI (I.) as twenty minutes at $35°$; thirty minutes at $30°$, forty-five minutes at $25°$, ninety minutes at $18\frac{3}{4}°$, and four to five hours at $12\ 5°$ C, for the hay bacillus (*Bacillus subtilis*). Similarly rapid is Koch's *Vibrio choleræ asiaticæ*, the period of generation for which under favourable conditions is only twenty minutes, as ascertained by BUCHNER, LONGARD, and RIEDLIN (I.) If the number of cells present in a bacterium culture at the commencement of an experiment

... at a given time, t, has increased to b, the ... period of generation is

$$\frac{t \log 2}{\log b - \log a}$$

... tion that the period of generation is half an ... ... If we take a single bacillus measuring ... with a weight of 0.00000001571 mgrm., it ... ...resaid assumption, at such a rate that in two ... ... to 281 billions, and will occupy a volume ... ... cub. ins.). Within a further three days the ... ...ufficient to completely fill the beds of all the ... ... of the progeny would be expressible only ... ... an inordinate development does not occur is ... ...ot of external influences, and especially to the ... ... species themselves. A no less powerful and ... ... the transformation products excreted as a ... ...reproducing cells, which finally arrest further ... ... nutriment is still available.

CHAPTER V.

THE PERMANENT (REPRODUCTIVE) FORMS OR SPORES.

§ 48.—The Formation of the Endospores.

THE cell forms, hitherto considered, produced by fission, and generally designated **vegetative** forms of growth, have only relatively low powers of resisting the multifarious dangers to which bacteria are exposed in Nature. The fact that nevertheless, these tender organisms hold their ground is due to their faculty for producing special forms, which, on account of their physiological function are known as **permanent forms** (spores). These may be of two kinds, viz., **endogenous** spores, and arthrospores. Any account relative to the continuation of the species will therefore have first to deal with the formation of the endospores

When a bacterial cell commences to develop such a new form, it condenses its cell contents into a smaller space, and then surrounds them with a tough smooth, colourless membrane (probably composed of two layers) The form thus produced is enclosed on every side by the membrane of the mother-cell (Fig. 14), in which it is developed, and is therefore called an **endogenous spore** or **endospore.** The greater density of its contents is evidenced by their greater refractive properties, which, were they confined to the spores, would enable these to be detected with certainty by the optical method alone. This is, however, not the case, large, highly-lustrous drops of a fatty nature, and which cannot, without other means, be accurately identified, occurring frequently in the cell plasma of the fission fungi In such cases germination tests—dealt with in the following chapter—must be resorted to for the purpose of differentiation.
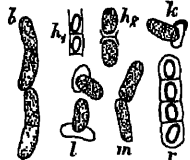
FIG 14.—Bacillus megatherium

Spore formation

r chain of four cells with ripe, tough-walled endospores (*After De Bary*) Magn 600.

With regard to the transformations undergone by the individual parts of the contents of the mother-cell precedent to spore formation, uncertainty still prevails According to the observa tions of P Ernst (§ 35), the chromatin granules of the central substance appear to play an important part in these changes, on which account this worker entitled them "*sporogenic granules*"

Soon after spore formation has terminated, the membrane of the mother-cell is dissipated, swelling up and dissolving in the surrounding liquid, and thus leaving the spore free. This is the ordinary course, but in many instances deviations occur, one of them being in the case of the *Spirillum endoparagogicum* discussed rather more fully below. In this case the membrane of the mother cell encloses the spore long after the latter is mature, and is still present when the spore germinates. This is described in the next chapter.

§ 49.—Alterations in the Form of the Mother-Cell.

In many instances the mother-cell undergoes alterations of form during the process of spore formation, and swelling occurs.

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When this happens at one of the polar terminations of a rod-shaped cell, the latter then assumes the form of a nail or drumstick. Bacteria exhibiting this peculiarity are styled nail-bacteria or helo-bacteria by Billroth, or *Urocephalum* by Trécul, and in medico-bacteriological literature they are also frequently called pin-head bacteria. The earliest known example of this kind was the *Vibrio rugula* (Fig. 15), frequently encountered in pools, and another is the
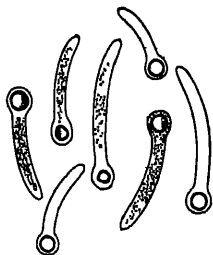
FIG 15.—Vibro rugula.

Seven rods, each with a ter-
minal spore (*After Praz-
mowski*) Magn. 1020
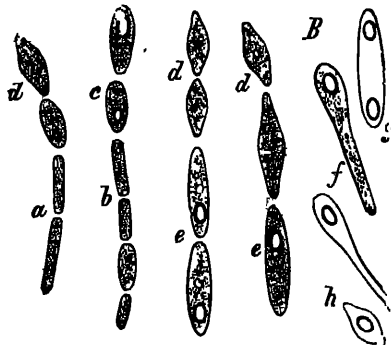
FIG 16.—Clostridium butyricum.

Spore formation

a, b purely vegetative cells, d commencement of spore
formation, c-e progress, f-h completion, a-f contain
granulose stained blue by iodine, h. devoid of this car-
bohydrate, unstained by iodine, g cell with two spores
(*After Prazmowski*) Magn 1020

"drumstick bacillus," found in human fæces by BIENSTOCK (I) The author has often found morphologically similar fission fungi in the skin developing on the surface of boiled infusions of hay, and as a matter of general interest an example from the pathogenic bacteria may be cited, viz, *Bacillus tetani*, by which tetanus is produced

Not inferior in number are the species wherein the sporogenic rod ordinarily swells up in the middle and gives rise to a spindle-shaped outline resembling that of a lemon or cop of yarn. TRÉCUL (I.), who in 1865, in the course of his studies in butyric fermentation, first became acquainted with this bacterial form, gave it the name of Clostridium, a term adopted as a generic name by Prazmowski. The two species described by this worker, viz., *Clostridium butyricum* (Fig. 16) and *Cl. Polymyxa*, were supplemented by LIBORIUS (I.) with *Clostridium fœtidum*, a fission fungus, which is isolated from old cheese, and produces a repellent odour in artificial nutrient media. A fourth species is the *Bacillus alvei*, discovered by CHESHIRE and CHEYNE (I.), which causes the so-called "foul brood" in bees. The *Bacillus inflatus* (Fig. 17), discovered by ALFRED KOCH (I) in 1888, also belongs hereto. Beyerinck investigated the conditions under

FIG 17.—Bacillus inflatus

Spore formation

a, b, e cells of Clostridium form, each
containing one elongated cylindrical
endospore, c, d, f, g cells with two
spores of unequal size (*After A
Koch*) Magn. 2100.

which the Clostridium form is assumed by a number of species, having close affinities with *Clostridium butyricum*, which have been grouped under the genus *Granulobacter*.

§ 50.—The Number of Spores

produced in a single mother-cell exceeds unity in but few species. The fi
communication on this subject was made by Prazmowski, who found that
exceptional cases *Clostridium butyricum* developed two spores in a cell. A rep
sentation of this is given in Fig 16. ED KERN (I.) observed in Caucasi
kephir granules a bacillus to which, on account of its faculty of producing t
spores, the name of *Dispora caucasica* has been given. This bacillus produce:
spore at each of its two poles, without any alteration of size or shape bei
undergone by the latter The contrary report, met with in many books, vi
that this microbe during spore formation swells up in such a manner that
assumes the form of a dumb-bell, is a pure invention. The doubt raised
MAOÉ (I.) in 1889, and shared by many others, against the sporous nature
this form, is also groundless, since a perusal of Kern's treatise shows that tl
inquirer confirmed by observation the germination of the doubtful spores in
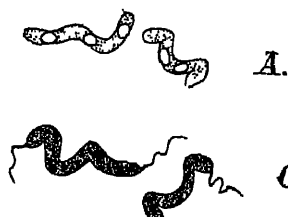
FIG 18 —Sphillum endoparagogicum

C, vegetative cells , A, two cells, one with two
and the other with three endospores   (*After
Sorokin*)

FIG 19 —Bacillus tumescens

Chain of seven cells, six of which have (
veloped one spore apiece, whilst the seven
and central cell has remained barren. I
plasma is granular. (*After A Koch*) Mag
1100

new rods A third species in which this unusual fruitfulness has been observe
is the above-named *Bacillus inflatus*, in which, however—as is shown by Fig 1
—the situation of the spores is not polar, but central. E KRAMER (I) repoi
that the *Bacillus saprogenes vini III*, isolated by him from turned wine, swel
up at first at one of its poles and develops an endogenous spore therein, anothe
spore being then formed in the handle of the drum-stick form thus produced
so that two spores are developed in the same cell.

The formation of *more than two spores in a single cell* has hitherto bee
noticed in but one species of fission fungus, the *Spirillum endoparagogicun*
This was repeatedly observed by SOROKIN (I) in a small pool of rain-wate
collected in the cavity of an old black poplar tree. A representation of thi
microbe is given in Fig. 18 In A is seen a cell containing *two*, and anothe
with *three* endospores, and Sorokin found as many as *six* in a cell The attemp
to obtain artificial cultures of this organism was as little successful as in the cas
of so many other spirilla, there being (it may be mentioned *en passant*) up to th
present only a few known species wherein attempts of this kind have succeede(
The first of these species is that which was isolated as a pure culture by ESMARCI
(I.) from putrescent fluid, and which formed rose-red colonies (*Spirillur
rubrum*); the second is the *Spirillum desulfuricans*, discovered and thoroughl
investigated by BEYERINCK (II), which readily reduces sulphates to sulphides
A third is the *Spirillum luteum*—developing a citron-yellow colouring matter—
obtained by H JUMELLE (I) from a bog; and the fourth is the *Spirillur
marinum*, described by H. L. RUSSELL (II) as a frequent inhabitant of the mu(

and water of the Bay of Naples . The sporula, as Mühlschlegel (I.) h
are extremely susceptible to variations in the temperature and nutrient :

With these exceptions, only one spore is produced in the bacter...
which event the spore formation does not result in an increase in the r
the cells. It is scarcely necessary to remark that it is not every cell that
a spore, it being a matter of frequent observation that individual (
bacterial chain are sterile, leaving their neighbours on either hand to ca
maintenance of the species. An attempt is made to represent this st
things in Fig. 19, which is a drawing of *Bacillus tumescens* made from nat
A. Koch (I.).

§ 51.—Form and Size of the Spores.

These characteristics vary of course in different species, the spores of *Bacillu*
subtilis, for example, being ellipsoidal and measuring 1–2 μ in length by about
0.6 μ in breadth, whilst the similarly shaped endospores of *Clostridium butyricum*
are 2–2 5 μ long and 1 0 μ broad The general shape is oval, but there are
noteworthy exceptions to this rule One of these is exhibited by the *Bacillus*
inflatus, which has already been frequently referred to. As can be seen from
Fig. 17, the spores of this microbe have the form of an elongated cylinder, and
are often curved in the shape of a bean. With a breadth of about 0.7 μ the
largest of them attain a length of 3.8 μ, in which respect this species is as yet
unrivalled. We may here mention that in A Koch's work, as also in Eisenberg's
treatise, already alluded to, a number of spore measurements are given. The
duration of spore formation has been determined by Prazmowski for *Clostridium*
butyricum as 10 to 18 hours at 30°–35° C.

§ 52.—The Conditions Influencing Spore Formation

have been frequently investigated, but no generally satisfactory elucidation has
yet been obtained H. Buchner (II.), on the basis of his studies, sought for
the explanation in the *exhaustion of the supply of nutriment*, but this is
contested by Osborne (I). Turro (I.), on the other hand, sees the cause in the
accumulation of noxious transformation products, against which the vegetative
form seeks protection and the maintenance of the species by developing the
hardier reproductive spores.

A. Koch established the fact that *Bacillus inflatus* in hanging-drop cultures
forms spores when a 1 to 2 per cent solution of meat-extract is employed as
nutrient medium, but that they are not formed if grape-sugar be added thereto.
Clostridium butyricum forms spores only in the absence of oxygen, whilst the
morphologically similar *Cl Polymyxa*, on the other hand, produces them only in
presence of this gas. Kotljar (I) found, in a microbe named *Bacillus pseud-*
anthracus, that spore formation was influenced favourably by violet light but
unfavourably by red light.

Past experience has shown that the formation of endogenous spores is
confined to the rod-shaped species (bacilli). This observation has been utilised
in the classification of bacteria, as will be seen in § 69. The reports to the
contrary found in the literature of the subject lack the force of proof, since they
ignore the fact that the sporous nature of the growths seen to originate in the
cocci has been demonstrated by germination tests.

§ 53.—Resisting Power of the Endospores.

The forms in question are endowed with the character of reproductive cells,
since they are able to withstand those adverse conditions which would inevitably

I   D

be fatal to the vegetative forms. As in the following sections—especially that dealing with sterilisation—occasion will often arise for a closer investigation of this faculty, so important for the maintenance of the individual species, an exhaustive and tedious list of individual cases need not be given here; it will therefore suffice if we cite one example, viz, *Bacillus subtilis* According to the researches of BREFELD (I.), which were confirmed by M. GRUBER (I.), a continuous exposure of twenty minutes to the action of boiling water suffices to destroy the sporeless rods of this microbe, whereas to kill the spores requires three hours' boiling at 100° C., or a quarter of an hour's exposure at 105° C, or, finally, the action of a temperature of 110° C. during five minutes The assertion made by Koch, that the continuous action of steam at 100° C for fifteen minutes will destroy the spores of any of the bacteria, was subsequently negatived by his pupil E. VON ESMARCH (II.) According to SWAN (I.), spores of *Bacillus megatherium*, dried on a cover-glass, retained their vitality and germinating power for more than three years.

Use may be made of these powers of resistance for separating the sporiferous from associated non-sporiferous bacteria. By skilful handling, e g. by the aid of sufficiently high temperatures, the weaker species in a mixture of bacteria can be killed off, leaving only the spore-producing individuals. A process based on this mode of procedure, and known as the **boiling method**, was introduced into bacteriology by ROBERTS (I.) for obtaining pure cultures of the hay bacillus, and the same method was employed by Prazmowski for preparing cultures of *Clostridium butyricum*.

The seat of this high power of resistance has already formed the object of numerous researches. One school looks for it in a peculiar modification of the spore plasma—for instance, in the presumably low water-content thereof, as suggested by LEWITH (I.). Others, again, attribute to the spore membrane an exceptionally low heat-conducting power, and a very slight degree of permeability by noxious substances. This latter opinion seems the more probable one, considered from a physical point of view, and is further supported by

§ 54.—The Behaviour of the Endospores towards Dyes.

As already observed in a previous chapter, the dead plasma of the bacterial cell absorbs colouring matters greedily and copiously The staining of the endospores is, however, more difficult, and consequently they have to be exposed to the dye a much longer time before they will absorb any of it However, the colour thus taken up is retained by them more firmly than by the vegetative forms.

This property has been utilised in microscopy to obtain a differential staining of the spore-bearing bacterial cells, for which purpose the latter are treated with a suitable (e g red) colour solution until the spores are thoroughly impregnated therewith, the preparation being then steeped in a decolorising liquid (generally slightly acidified alcohol), wherein it is left until the vegetative cells are deprived of the colour. These latter are thereafter stained anew by a short immersion in a second colour (e g blue) solution, a two-colour preparation (double-staining) being thus obtained, the spores in this case being red and the rods blue. More detailed directions for double-staining will be found in Hueppe's handbook, Eisenberg's treatise, and in Bernheim's "Taschenbuch" (Pocket-book). Fig 20 gives a black and white reproduction of a cover-glass preparation of spore-bearing *Bacillus subtilis* stained only once, so that the spores are unchanged, and appear colourless (white)

The aforesaid behaviour of bacterial endospores towards colouring matters is characteristic of all. From this fact it is not infrequently, though erroneously, assumed by medical bacteriologists that any formation, in the interior of the

cell, that behaves similarly towards dyes is to be considered as an endospore, whereas it is not yet proved that spores alone exhibit this power. A general report concerning the spore formation in any bacillus must therefore be received with due reserve when it rests merely on the result of staining experiments. The sole decisive proof of the sporous nature of such bodies is afforded by their germinating power alone, a subject discussed in the next chapter. When this property has been observed, the staining flask is no longer needed, its use in such case being confined to the preparation of a coloured slide, which, in itself, is now valueless as a criterion.

FIG 20 — Bacillus subtilis Cover - glass preparation from an eight-days-old gelatin culture grown at room temperature Stained with Vesuvine The spores, not having absorbed the dye, show up as white specks against the (dark-stained) vegetative cells (After Baumgarten) Magn 950

The property of offering considerable resistance to decolorising agents, possessed by the endospores, is also shared by the vegetative forms of a few species of bacteria, among which are the tubercle bacilli and the leprosy bacilli. This unusual behaviour greatly facilitates their detection by microscopical examination alone, and is of particular utility in this respect in the examination of milk and of the sputa of consumptive patients.

The differential staining of tubercle bacilli, also experimentally applied to non-pathogenic bacteria by many bacteriologists, will be found dealt with in each of the above-named books.

§ 55.—Arthrospores.

As already remarked, the capacity for forming endogenous spores is not universal among the fission fungi. The question then arises as to the means whereby those species not endowed with this faculty protect themselves against adverse external influences.

In many cases the resistance of such cells, and consequently the maintenance of the species, is secured by the development of a protective wall of cells. This is most frequently met with in zooglœa-masses of bacteria.

In other cases actual spore formation occurs. This, of course, takes place not within the bacterial cell, since that would imply endospore formation, but by a thickening of the membrane of the individual cell in question, which thereby plays the part of a reproductive cell. This procedure is known as arthrospore formation, since the spore detaches itself from the chain of moribund cells, encysts, and becomes dormant until conditions are once more favourable for its germination, when the cell increases in length and subdivides in the same manner as the vegetative form.

The thickening of the cell membrane of the incipient arthrospore proceeds, in many instances, to such an extent as to form spiny excrescences on the exterior surface. This was observed by HANSGIRG (I) in two species of bacteria, viz , *Mycacanthococcus cellaris* and *Mycotetraedron cellare*, found by him on the walls of a cellar at the Castle of Pleissen, at Leipzig. The arthrospores of the latter species, which are tetrahedral in form, exhibit at each of the four angles a spiny thickening of the membrane 2 μ in length.

The name arthrospore will be understood when it is remembered that this kind of spore is met with particularly in the thread bacteria, from which it becomes, as it were, actually dismembered. Examples of this are exhibited by the *Crenothrix* observed by Cohn, *Cladothrix* by Zopf, *Leptothrix* by Miller, and *Streptothrix Fuerteri* by GASPERINI (I.) The discovery of these organs in species of cocci, &c.— such as the urea bacterium found by JAKSCH (I.), in *B. vermicosum* by ZOPF (II.), in *Bacterium Zopfii* by KURTH (I.), and so on—was only a secondary matter.

CHAPTER VI.

THE GERMINATION OF THE ENDOSPORE.

§ 56.—First Type.

WHEN the spore is exposed to favourable conditions as regards nutrition, it abandons its dormant state and begins to germinate by commencing to absorb liquid from the surrounding medium It then becomes distended, its high refractive power gradually diminishing in the same proportion Further development can then proceed in three ways.

The first type of spore germination was accurately observed by H. BUCHNER (III.) in *Bacillus anthracis*, the producer of anthrax, and afterwards discovered

FIG 21
Bacillus anthracis
Germination of spores
s the ripe spore before germination begins, 1, 2, 3 three successive stages of germination, 3 the fully developed rod (*After De Bary*) Magn about 600-700.

FIG 22 —Clostridium butyricum.
Spore formation
a ripe spore
b ditto expanding in nutrient solution
c final dimensions attained, and separation of exosporium from endosporium visible
d, e young rod escaping from polar extremity of spore capsule.
(*After Prazmowski*) Magn 1020

in other kinds, as, for instance, by PRAZMOWSKI (II.) in a fission fungus named by him "mistbakterie" (dung bacterium), and in a second species isolated from fermenting urine The progress of germination in this type is very simple (Fig 21) The spore, gradually acquiring the normal dimensions and functions of the vegetative form, soon divides and reproduces by fission According to several observations made by Brefeld, the external layer of the spore-membrane ("exosporium") separates during this germination process and swells up This harmonises well with the remark made in a previous paragraph, that the spore capsule probably consists of two layers, distinguished as **exosporium** and **endo sporium**.

§ 57.—Second Type of Spore Germination.

Microscopically the initial stage of the process is identical with that described in the foregoing paragraph, the refraction of the spore diminishes and an increase in size occurs Then, however, the contents of the spore are elaborated into a new rod, which is surrounded by a thin membrane, and which, by its further growth, bursts the spore capsule

This rupture is effected at the point of least resistance, the position of which—and consequently the mode of escape of the germ—varies in different species.

In *Clostridium butyricum*, *Cl. Polymyxa*, and a few others, the spore capsule opens at one of the poles, so that the direction taken by the young rod in its escape is in a line with its length, as shown in Fig 22, *d*. The expulsion is

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effected by the spore membrane, the tension of which is gradually increased to such an extent, through the expansion of the spore contents, that it finally squeezes out the mature spore. The capsule then shrinks at once to its original size, and gradually disappears from view by swelling up and dissolving in the surrounding liquid If the spore has originated from any species of motile bacteria, the liberated rod begins to rove directly one of its extremities is free.

It not unfrequently happens that the empty spore capsule is not completely detached from the germ, but rests as a well-defined cap on the rearward pole for some time, until finally it disappears In a few species the persistence of this membrane is very considerable, whilst the force of contraction is small and insufficient to expel the matured rod An example of this is afforded by the *Bacillus sessilis*, discovered by L KLEIN (I and II.), which—as its distinctive name implies—remains embedded in the spore membrane This captivity,
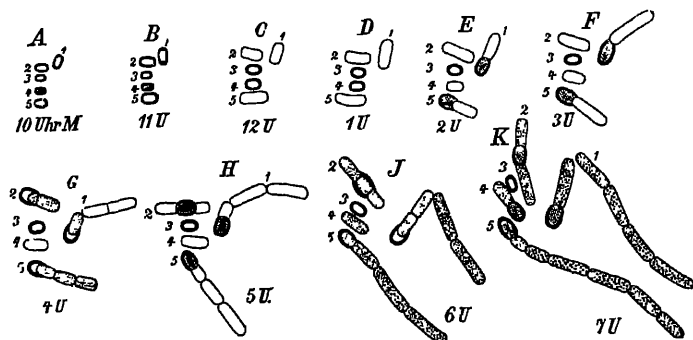
Note —10 to 12 Uhr M (U) = 10 A M to 12 noon , 1 to 7 U = 1 to 7 P M

FIG 23 —Germination of spores of Bacillus sessilis

Progressive observation of the germination of five endospores (1–5) under the microscope at 30°–35° C Hanging-drop culture in meat-extract solution The time of the observation (from 10 A M to 7 P M) is given under the illustrations of the separate stages of development (A–K) of the germination Spore 3 had not germinated even at 7 P M (*After L Klein*) Magn about 1000

nevertheless, in nowise retards its nutrition and reproduction, since by fission it forms new rods, which it protrudes through the polar openings at both extremities in the muff-shaped capsule, the said rods quickly forming spores in turn An illustration is given in Fig 23

§ 58.—Third Type.

In *Bacillus subtilis* (Fig. 24), *Bacillus megatherium* (Fig. 25), and a few other species, the spore membrane does not burst at the poles, but along a line coinciding with the equator of the spore This line, however, extends only part of the way, not right round the spore, so that the two halves of the membrane still remain attached together at one point The rod then makes its exit by bending somewhat at the centre, and, by turning one of its extremities, pushes it out of the capsule, one half of which often remains on the other pole for some time like a cap, the other half hanging down empty Sometimes the germ cannot liberate even one end from the capsule, both poles remaining wedged between the two halves of the membrane (these acting like a pair of tongs), and the central portion alone projecting. This position, however, does not prevent reproduction,

but gives rise to horse-shoe chains (Fig. 26), which only separate into their individual members when the spore membrane has become swollen and flaccid.
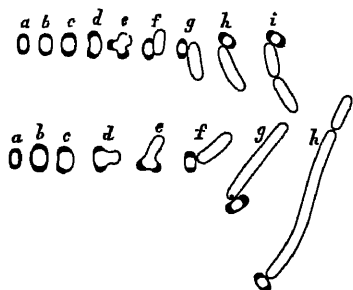
FIG 24 —Bacillus subtilis

Spore germination

a. ripe spore, b placed in a nutrient solution, the refraction disappears, c enlargement begins, d the equatorial fissure is formed and the young germ begins to escape, e in the upper row the central portion of the germ is just protruding, in the lower row one pole is already freed, f the young rod is at liberty, g it grows to its normal size, h reproduces by subdivision Extra long cells are seen at g and h in the lower row (After Prazmowski) Magn 1020
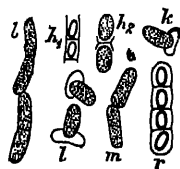
FIG 25 —Bacillus megatherium

Spore germination

h_1 two dried ripe spores enclosed by the walls of the mother-cell
h_2 the same spores after forty-five minutes' immersion in a nutrient solution
l, l the spore contents have invested themselves with a new membrane and are escaping from the old capsule
m two full-grown rods (After De Bary) Magn 600
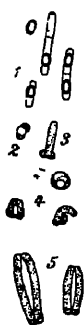
FIG 26 —Bacillus subtilis.

Impeded germination.

1 Cells with ripe spores causing the mother-cell walls to bulge.
2 Commencement of spore germination, capsule fissured equatorially
3 Ordinary unimpeded escape of the germ.
4 Exit somewhat impeded, one pole being eventually liberated
5 Both poles of each germ remain fixed, germ divides into two cells (After De Bary) Magn 600
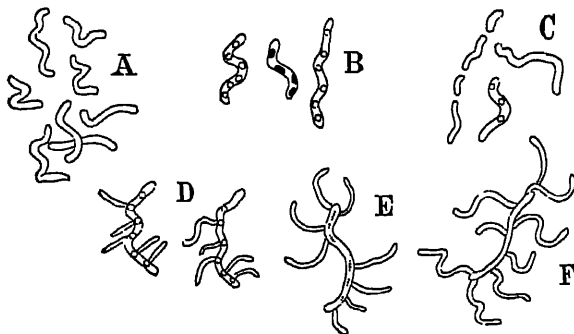
FIG 27 —Spirillum endoparagogicum

Spore germination.

A, purely vegetative cells in brisk motion.
B, three spirilla with four to six spores, those in the central cell being ripe
D, mother-cell with germinating spores, from which proceed
E, branched forms, subsequently dismembered into single cells
C, moribund spirilla, one with three spores (After Sorokin) Magn 1375

The time occupied by *Bacillus subtilis* in germinating is, according to Prazmowski, generally 3–4½ hours at 30°–35° C., but frequently much longer.

A unique procedure is manifested in the germination of the spores of *Spirillum endoparagogicum*, the membrane of the mother-cell remaining unimpaired (as already mentioned) after the spores are formed, so that the germs proceeding from the spores have to penetrate the membrane of the mother-cell in order to attain their freedom. Not infrequently they remain attached by the one end, thus giving rise to a branched form, as shown in Fig 27.

§ 59.—Importance of this Process in the Classification of Bacteria.

Since, according to observations made thereon, the course of spore germination differs in the various species, it may be utilised, in the characterisation of species, as an invariable and therefore reliable indication. One example, serving for Bacteriology in general, may here be cited. H. BUCHNER (II.) ascertained that by a suitably modified method of culture it was possible to deprive the anthrax bacillus of its virulence and render it harmless. Sundry other experiments (subsequently found to be defective and deceptive) with the hay bacillus (*B subtilis*) induced him to assert that these two species were *identical*, the hay bacillus being an anthrax bacillus that had lost its virulence, and *vice versâ* Now it has already been shown that the germination of the endospores of *B anthracis* follows a different course to that occurring in *B subtilis*, consequently, if the former were by Buchner's treatment not only rendered harmless, but also actually converted into *B subtilis*, then the course of germination must also have become correspondingly changed. Starting with this assumption, PRAZMOWSKI (III.) subjected a weakened anthrax bacillus (presumably transformed into *B subtilis*) to examination with regard to the nature of the formation and germination of its spores. He found that both operations pursued exactly the same course as in the virulent (unweakened) anthrax bacillus, thereby disproving the assumption of the identity of the two species.

The first observation of the production of developing reproductive cells within the bacterial cell was made by PERTY (I.) in 1852, who designated the rods he found to possess this property *Sporonema gracile* (Fig 28) His communication, however, met with no recognition and fell into oblivion. About fifteen years later PASTEUR (VI.), unaware of Perty's observation, re-discovered the

FIG 28

Spore formation according to Perty

b–g Sporonema gracile , *b*. with one terminal spore , *c* with two spores , in *d* the spore has escaped from the mother-cell , *e–g* shows the gradual development of the spore until ripe In *a* is shown the (formerly) so-called *Metallacter*, a chain of short threads which when viewed under a low power is apt to be mistaken for a long rod, hence its name.

same fact in the course of his researches on the causes of lethargy in silkworms (*gattine*) The fission fungi which he found in large number in the alimentary canal of the diseased (lethargic) worms, and which he experimentally ascertained to be the cause of this generally fatal epidemic, frequently exhibited internal lustrous enclosures, the formation and function of which he explained to be reproduction by endogenous germs ("*réproduction par noyaux intérieurs*"), without, however, observing them more closely in order to ascertain the accuracy of this hypothesis.

Only one proof was needed to set this beyond doubt, namely, the demonstration that these forms have the faculty of germinating and of developing into new individuals. FERD. COHEN (VII.) first succeeded in doing this, in

1876, with a bacillus isolated by him from an infusion of hay. This fact defi-
nitely proved that the bacteria belong to the vegetable kingdom, since spore
formation exclusively characterises the sub-kingdom *Thallophyta*, and is unknown
in animals. A more accurate account of this important process was given two
years later by OSKAR BREFELD (I) for *Bacillus subtilis*, two years subsequently
by A. PRAZMOWSKI (I) for *Clostridium butyricum*, in 1882 by HANS BUCHNER
(II) for *B. anthracis*, then by DE BARY (I) for his *B. megatherium*, and by
others.

SECTION II.

GENERAL BIOLOGY AND CLASSIFICATION OF BACTERIA.

CHAPTER VII.

THE BACTERIA UNDER THE INFLUENCE OF
PHYSICAL AGENCIES.

§ 60.—Influence of Electricity.

THE effects of this agency were first recorded by SCHIEL (I.) in 1875, the earliest exhaustive researches being carried out by COHN and MENDELSOHN (I) in 1879, succeeded in following years by the labours of APOSTOLI and LAQUERRIÈRE (I.), PROCHOWNIK and SPATH (I), and DUCLAUX (III)   The same method of experimenting was followed by all these observers, and consisted in passing an electric current through the culture. Cohn found that, to produce an appreciable weakening effect by this means, a battery of at least two cells was required, the current from which, when passed for 12-24 hours through a nutrient solution inoculated with bacteria, was unable to kill the germs, but nevertheless rendered the medium unsuitable for further culture. This result, as explained by Cohn, was due to the action of the current in forming decomposition products inimical to fungi   Bearing this in mind, such a method of experiment is therefore unsuitable for affording a clear insight into the influence of the current itself   These labours are, nevertheless, worthy of mention, since, having been further pursued with a practical aim, they have led to the elaboration of a process for the purification of sewage water (as developed and tested by WEBSTER (I.) in particular). The water to be purified is led through a trough into which dip large iron plates, acting as electrodes for a powerful current generated by a dynamo machine and passed through the liquid. FERMI (I) tested the process from a bacteriological point of view, and ascertained that—under the conditions of the experiment—a current of o 5 to 1 o ampère reduced the number of germs to between $\frac{1}{50}$th and $\frac{1}{100}$th of the initial quantity

To ascertain the effect of the electric current, unaffected by secondary chemical influences, BURCI and FRASCANI (I) proceeded by drying the bacteria (i e a small portion of inoculated nutrient solution) on a pad of glass wool at 37° C , and then dipping the pad into mercury through which a constant galvanic current was being passed   In this case the bacteria were killed, but the method of experimenting is not free from objection, since the dried constituents of the medium were present along with the bacteria, and might retain moisture and form decomposition products noxious to the latter

These injurious secondary influences can only be perfectly excluded when the electric current is prevented from coming into contact with the nutrient medium, a condition first attained in the experimental method selected by SPILKER (I) and GOTTSTEIN (I.). The glass flask containing the bacterial culture was enveloped by a coil of the line wire, and an induction current then passed

Micrococcus prodigiosus—washed by sedimentation in pure water or in water qualified by nutrient gelatin—was killed when the liquid (250 c.c. in volume) was exposed for twenty-four hours to the influence of a current of 2.5 ampères and 1.25 volts   Other species of bacteria offered greater resistance. as, for example, those occurring in milk, which are gifted with the power of forming endospores capable of retaining their vitality under very adverse conditions. For this reason the above-named observers never succeeded in thoroughly freeing milk from living germs by electrical treatment, although the number of the germs could be reduced thereby.

D'ARSONVAL and CHARRIN (I) studied the influence of the electric current on the blue pigment of the *Bacillus pyocyaneus* found in the pus discharged by wounds.   They placed a culture of this organism in the cavity of a solenoid traversed by a current of 10,000 volts; an exposure of twenty minutes sufficed to destroy the chromogenic power of the bacilli almost completely.   A similar decrease of virulence was observed by S KRÜGER (I) in the case of a few pathogenic bacteria; and, finally, reference may be made to a research of this nature performed by H FRIEDENTHAL (I).

At present, owing to the high cost entailed, the utilisation of the anti-bacterial powers of electricity in the food-stuff industries is out of the question. Use has, however, been made of these powers in the fermentation industries, although the primary object of the process is not the destruction of germs, but the chemical changes effected by the electric current.   Alcoholic beverages (wine, cognac) are artificially matured, and a slight esterification, and consequent mellowing of flavour, produced by allowing the liquors to flow slowly through an electrical field.   A more detailed consideration of this process is, however, beyond the scope of the present work.   A review of the methods proposed for this purpose and the experiments made therewith is given by A. SCHROHE (I.)

D'Arsonval and Dubois have made a few observations on the influence which magnetism (so closely allied to electricity) has on bacteria, but unfortunately these have not been followed up any further.

§ 61.—Influence of Temperature.

The ordinary conceptions with regard to the favourable or prejudicial influence of certain temperatures on organic life cannot be applied, without modification, to bacteria, and this is particularly the case with respect to the effects of cold

J. FORSTER (I.) was the first to find (in 1887) that there are species of bacteria which at a temperature of $0°$ C are not only alive, but actually reproductive. This report is based on a luminous bacterium isolated by him from the surface of a phosphorescent salt-water fish.   B. FISCHER (I) next discovered fourteen other species, some in sea-water, others in the soil, all of which were very reproductive at $0°$ C   Returning to the subject, J FORSTER (II) then examined more narrowly the natural habitat of similar bacteria, and found that

Commercial milk contained up to 1000 per 1 c c
Drain water contained up to 2000 per 1 c c
Garden soil contained up to 140,000 per 1 gram
Street mud an innumerable quantity per 1 gram

MIQUEL (I)., by keeping a sample of sea-water at $0°$ C, found that an initial number of 150 germs per 1 c c. increased to 520 in twenty-four hours and to 1750 in four days   These facts indicate that glacier water, hail, and snow may also contain bacteria. Quantitative researches on this point have been carried out by L. SCHMELCK (I.), O. BUJWID (I), W. FOUTIN (I.), TH JANOWSKI (I.), and especially P MIQUEL (I).

The resistance of bacteria to low temperatures extends considerably below zero Centigrade, FRISCH (I.) having shown that some species will bear cooling down to $-110°$ C. for a short time without injury. R PICTET and E. YUNG (I.) found that bacteria (species unknown) could be kept at $-70°$ C for 108 hours and at $-130°$ C. for twenty hours without succumbing; certain (unnamed) species even withstanding the effects of a short exposure to $-213°$ C. in solidified oxygen These facts are not merely of general biological interest, but also, at the same time, important as regards the question of the suitable treatment of stored food-stuffs. This will be discussed in a subsequent paragraph.

Antithetical to these cold-loving bacteria is the *Bacillus thermophilus*, discovered by MIQUEL (II.), which thrives and reproduces with great activity at $70°$ C, a temperature which instantly kills animal cells, coagulates egg albumin and blood serum, and produces painful burns on the skin. When kept at $50°$ C this aerobic bacillus occurs as short rods, about 1μ in thickness, which become longer as the temperature rises, threads beginning to form at $60°$ C., and constituting at $70°$ C. the sole occupants of the field The lowest limit of temperature at which development of this organism can be observed is about $42°$ C , above $72°$ C. the vegetative forms die off This non-ciliated fission fungus is but seldom met with in atmospheric dust, but is very frequent in sewage, and therefore also in sewage-contaminated waters It is likewise present in the alimentary canal of human beings and mammals. This locality seems to possess a highly suitable temperature for the growth of this saprophyte, although accurate knowledge on the subject is still lacking When the temperature rises above $50°$ the medium undergoes putrefaction as a result of the activity of the bacillus.

Between *Bacillus thermophilus* and the aforesaid cold-loving species there are numerous species forming intermediate links in the chain. In the case of Forster's microbe, already mentioned, the highest limit of supportable temperature is $35°$ C , and it cannot retain its vitality when exposed, even for a few hours, to a temperature of $35°$ to $37°$ C. GLOBIG (I) isolated from garden soil twenty-eight species of bacteria, each of which still developed luxuriantly at $60°$ C , whilst the minority were able to grow at even higher temperatures. In connection with their occurrence in nature the question of the limits of temperature—range of climate—within which they can grow is of interest. In this respect great differences were observed, one of them growing as well at $15°$ as at $68°$ C , whilst most of the others required a temperature of over $50°$ C , and one exhibited signs of development only when the temperature exceeded $60°$ C. It must therefore be concluded that, under natural conditions, the reproduction of these organisms proceeds only in the height of summer, when the soil is sufficiently heated by prolonged sunshine These warmth-loving bacteria are not found in the ground exclusively. LYDIA RABINOWITSCH (I.) isolated from the excrement of various animals, as well as from manures, milk, &c , eight widely distributed species, for which the highest limit of temperature at which growth was possible was found to be $75°$ and the minimum about $39°$ C. These organisms are therefore able to reproduce freely in the alimentary canal of warm-blooded animals and human beings. Warmth-loving fission fungi are also not infrequently encountered in sea-water. One example of this is afforded by a phosphorescent bacterium found in the West Indies and described in chapter xv. under the name of *Bacterium phosphorescens* This inhabitant of the tropics thrives best at $20°$–$30°$ C., and ceases growing at $15°$ C Living bacteria have also been found in boiling springs, e g that discovered by Cortes and Garrigon in the basin of a mineral spring, the temperature of which was $64°$ C J KARLINSKY (I.) in 1895 discovered in the hot sulphur springs at Ilidze, near Sarajevo, in Bosnia, two species of *Schizomycetes* which he named *Bacterium*

Ludwigs and *Bacillus Ihdzensis capsulatus*, the former developing only when the temperature rose above 50° C., and the second producing endospores able to withstand four hours' exposure in water at 100° C without succumbing

DIEUDONNÉ (I.) drew attention to the fact that, owing to the possession by bacteria of a certain power of adaptation to climatic conditions, no *hard and fast* lines can be drawn respecting the limits of temperature within which growth is possible; but that by carefully controlling the stages of transition it is possible to somewhat extend these limits

In ciliated bacteria spontaneous motion ceases when the temperature of the environment approaches the lower or higher limit, and they fall into a state of **torpidity** through cold or heat, from which they recover as soon as the temperature once more becomes favourable

Reference to the morphological influence of temperature has already been made above (as also in § 29), and will be exhaustively described and illustrated, with a particularly fine example, in a subsequent paragraph. The transforming and modifying power of warmth also extends to other properties of bacteria, for example, to the **virulence** of pathogenic bacteria, *i e.* their capacity for engendering disease. In the present work, however, not more than a single one (on account of its general interest) can be referred to, viz , Pasteur's process of **preventive inoculation for anthrax.** If *Bacillus anthracis* be cultivated in meat-broth for twenty-four days at 42°–43° C , a virus (*premier vaccin*) is obtained the virulence of which is so attenuated that sheep (the animal most subject to anthrax) inoculated therewith experience only a mild form of the complaint If then inoculated with a second culture prepared by exposure to the attenuating influence of a temperature of 42°–43° C. for only twelve days (*second vaccin*), the animals no longer sicken, even if inoculated by unattenuated *B anthracis*, and are therefore immune against inoculative anthrax.

§ 62.—Influence of Light.

The old empirical hygiean maxim concerning the disease-banishing power of the sun's rays—which is well expressed by the Italian proverb, " Where the sun does not enter the doctor does "—finds a full explanation in the bacteriological discovery that the overwhelming majority of the fission fungi thrive much better in darkness than in the light, and are, in fact, under certain circumstances, killed by direct sunshine. This question of the influence of light on bacteria has already formed the subject of innumerable researches, most of which, however, are of purely medical and hygienic interest, on which account their consideration here must be restricted to a mere recapitulation of the main points involved. A summary review of the literature of the subject up to 1889 will be found in a work by J RAUM (I.), which in this particular is to some extent supplemented by the more recent publications of TH JANOWSKI (II.) and TH GEISLER (I.)

Most of our knowledge of the question was obtained from the earliest investigations therein, published in 1877 and 1878 by DOWNES and BLUNT (I and II), who found that the growth of the bacteria is restricted by the influence of diffuse white daylight and is completely stopped by sunshine. The blue and violet rays proved the most injurious, the red and orange rays being weaker in their action   The authors explained the injurious effect of light as an indirect one, in that it strengthens the decomposing power of oxygen, the result being the decomposition and destruction of the bacterial plasma. JAMIESON (I), in 1882, gave another explanation of the phenomenon by attributing the injury observed to the increase of temperature affected in the cells by the sun's rays. The fallacy of this hypothesis—which had been rejected by DOWNES (I)—was demonstrated in 1885 by D'JCLAUX (IV.), who was also the first to employ pure

cultures—viz , of *Tyrothrix scaber*—in the study of this question. He proved, at the same time, that the duration of exposure to sunlight necessary to kill the microbe is dependent on the composition of the nutrient medium employed for the culture, cells cultivated in bouillon proving less capable of resistance than those of the same species grown in milk.

Several other explanations have been given regarding the particular and more intimate reactions that occur in a bacterial culture exposed to the rays of the sun. Some observers adhered to the opinions expressed by Downes, and attempted to show that, by exposure to sunshine, decomposition products are formed in the medium and act fatally on the bacterial cell Support for this view is found in the observation made by G. ROUX (I), that the destruction of the germ goes on much more rapidly when there is a concurrent admittance of air , and an indication pointing in the same direction is afforded by the fact, determined by RICHARDSON (I), that hydrogen peroxide—a substance highly poisonous to bacteria—is formed when sterilised urine is exposed to sunlight On the contrary, other observers—WARD (I) in particular—have shown that the presence of such oxidising agents is not essential, but rather that sunshine alone suffices to destroy the vitality of even the strongest bacterial spores Probably in nature both agencies co-operate in producing the same results.

The last-named investigator also examined more closely the degree of influence exerted by the individual colours of the spectrum, and found that, in the case of red to green, this action is almost *nil*, increasing thence to its maximum at the violet end of the blue, and then falling away again in the violet and ultra-violet rays According to the researches of SANTORINI and GEISLER (I), a similar, though less powerful, injurious action is exerted by the electric light , and F MINOK (I) has performed several experiments on the effect of the Rontgen rays on bacteria.

The anti-bacterial influence of sunlight is of the highest importance, especially in regard to the self-purification of rivers. As is well known, the amount of organic matter and the number of bacteria in river-water diminish in proportion as the water increases its distance from the point of contamination This property, on account of its hygienic and technical importance, has already formed the subject of investigation HANS BUCHNER (IV and V) in 1892 pointed out that all previous explanations of this occurrence had omitted one factor, viz., the influence of light He showed that a natural water to which about 100,000 cells of *Bacterium coli commune*—an organism constantly and abundantly present in fæces—had been added per 1 c c , contained, after one hour's sunlight, no living germs To bring this action into specially prominent notice, he poured peptonised meat-juice-agar-agar, inoculated with a copious supply of typhus bacilli, into Petri basins, on the under side of which were affixed the letters **TYPHUS** cut out of black paper. The basins were then exposed to the sun's rays for one to one and a half hours, or to diffused daylight for five hours, and afterwards left in a dark room for twenty-four hours. On the paper letters being then removed, their form was found to be marked out by the thickly clustered whitish colonies composed of the bacteria that had been protected from the fatal effects of sunlight by the paper cover, and had consequently remained alive, whilst the residual uncovered portion of the medium was destitute of any such colonies. Fig 29 is a reproduction of the photograph taken by Buchner from one of the plates. The same result was obtained by illuminating the care fully-closed culture basin under water. Experiments made in the clear waters of Lake Starnberg showed that the anti-bacterial influence of the sun's rays extends to a depth of some two metres (about eighty inches) below the surface of the water Therefore, to the already known factors in the self-purification of rivers—viz. sedimentation, oxidising influence of air, consumption of filth by

algæ, &c.—all of which are more concerned with alterations of chemical composition—must be added the influence of sunlight in diminishing the number of bacteria. A critical review of the most important labours and researches performed in respect of the self-purification of rivers is given by E. DUCLAUX (V)

All the pathogenic *Schizomycetes* seem to succumb under the influence of sunlight. This has been shown by Arloing and Ward in respect of *Bacillus anthracis*, Gaillard for *B. typhi abdominalis*, Pansini for *Vibrio choleræ asiaticæ* and a
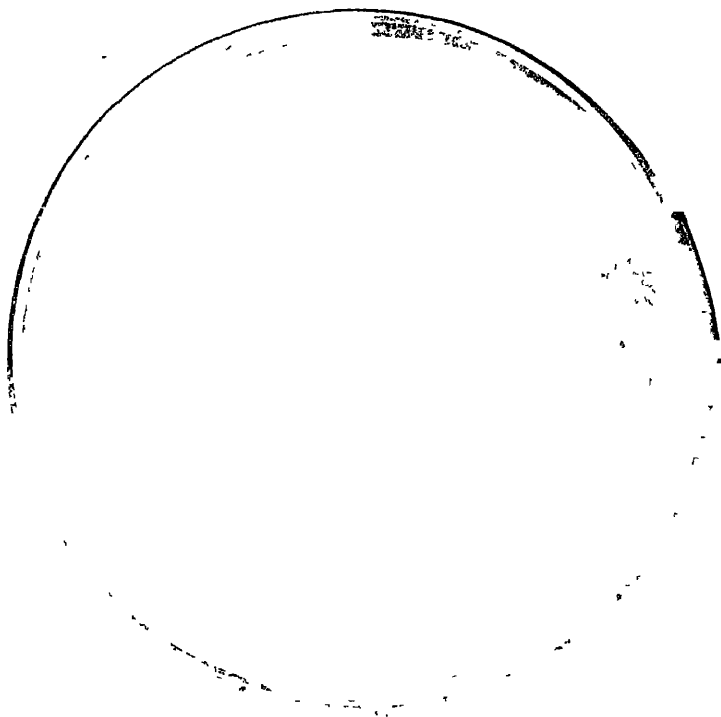
FIG 29 —Thickly-sown plate culture of typhus bacilli on agar-agar Covered with paper letters and exposed to the sun's rays for 1½ hours, then kept twenty-four hours in the dark, whereupon development of thickly congregated whitish colonies was found only at the parts covered by the letters (*After H Buchner*) Nat size

fungus giving rise to white pus in wounds (*Staphylococcus pyogenes albus*), Chmihewski for the organism which induces the formation of yellow pus (*St pyogenes aureus*), and the bacillus of erysipelas (*Streptococcus erysipelatis*), Rob. Koch for *Bacillus tuberculosis*, Charrin for the organism producing swine-erysipelas; and others Most of the non-pathogenic fission fungi also succumb to the influence of light. GALEOTTI (I) arranged a number of chromogenic species in the following descending series, the first member of which resists the action of diffused daylight the longest *Bacillus ruber, Micrococcus prodigiosus, Sarcina rosea, Bacillus violaceus, B pyocyaneus, B lactis erythrogenes* According to the researches of GROTENFELT (I.), the last-named fission fungus does not produce red colouring matter at all when strongly illuminated. R. DUBOIS (I.) ascertained that the luminous bacterium, *Photobacterium sarcophilum*, found on

spontaneously phosphorescent flesh, temporarily loses its light-producing power on prolonged exposure in a light room.

Great differences in susceptibility to sunshine are also exhibited in the *Schizomycetes*. At the extreme end of the series stand the purple bacteria, examined more closely by Engelmann, which always seek out the more highly illuminated positions. One of the species was named by ENGELMANN (I) *Bacterium photometricum*, on account of its variable susceptibility to the colours of the spectrum and degrees of brightness. These organisms, which will be fully noticed in a subsequent chapter, also display the phenomenon known as **movement of alarm** If a microscopic preparation containing one of them in large numbers be illuminated in such a manner that the light rays can fall only on one sharply defined portion, then all the roving bacteria collect within this space and bustle about briskly therein. If now one of them in its onward career passes beyond the circle of illumination into the dark portion, it stops instantly, and then returns by the same road into the illuminated field. This is the phenomenen of the **movement of alarm** Consequently each sharply defined illuminated portion of the field acts as a trap for the bacteria, from which they cannot escape until the illumination has been altered. If a definite form be given to this trap, such, for instance, as the shape of a W, and the closely congregated cells be fixed and stained in this position, then a so-called **bacterial photogram**—*i.e.* a coloured picture of the trap, composed of the organisms themselves—is obtained

§ 63.—Influence of Mechanical Shock.

The first to inquire whether the vitality of lower organisms can be influenced by agitation was A HORVATH (I.) in 1878. He made his observations with bacteria because he assumed that, on account of their small size, the possibility of mechanical injury (rupture) due to agitation would, in the case of these organisms, be reduced to a minimum. On gently agitating bacterial cultures (in Cohn's nutrient solution) he was unable to detect the manifestation of any retarding influence on the growth of the organism The results were, however, different when the sample was made to undergo, by means of a shaking machine, about a hundred movements—in a direct line and of an amplitude of about 10 inches (25 cm)—per minute. This treatment for a period of twenty-four consecutive hours diminished the reproduction of the bacteria in question ; and when continued for forty-eight hours the agitation proved fatal On the basis of his researches Horvath formulated the opinion that " for the development of the living organism, or the physiological reproduction of the elements constituting the organism, a certain degree of repose is necessary," meaning thereby that rest mainly favours, whereas movement injures, reproduction This generalisation was opposed by NÄGELI (II) and E. Oh Hansen, the former of whom drew attention more particularly to the reproduction of algæ living beneath large waterfalls and exposed to much more violent agitation than was effected by Horvath's shaking apparatus

In 1879 E Oh HANSEN (I) instituted experiments in order to test Horvath's assertions Working with beer yeast (*i e* not bacteria), he ascertained that this organism developed better when the liquid (beer wort) was set in motion by stirrers The probability of this favourable influence of movement being due to aeration is, according to Hansen, inadmissible, this latter effect having been but slight.

A year later the question was taken up by J REINKE (I) An objection raised by NÄGELI (II) led him to try the effects of movements more nearly approximating in amplitude to molecular movements than were those produced in Horvath's experiments. To this end he made use of **sound waves**, the end of a metal rod, caused to emit sound by friction, being immersed in a glass filled

with Cohn's nutrient solution containing bacteria, and thereby transmitting the wave-motion to the liquid   The experiments showed that a considerable restriction, but not cessation, of growth occurred.   From this Reinke concluded that "if it be assumed that the molecules of living protoplasm are endowed with specific vibratory movements, the idea appears feasible that when those specific molecular vibrations are crossed by other molecular motions of external origin, the vital functions of the protoplasm will be weakened"

The labours subsequently made public by L Tumas, O Roser, H Buchner, H. Cramer, H Miquel, H Leone, A. Gartner, B. Schmidt, and others, did not produce anything having a material bearing on this question.   A treatise by H. Russell (I.), who worked with *Monilia candida*, *Saccharomyces mycoderma*, and *Oidium albicans*, and found that the form and dimensions of the cells are but little altered by agitation, and that the percentage of germs in agitated samples is almost double that in samples left at rest for the purpose of comparison, is, however, worthy of mention

The results appear to contradict one another.   It should, however, be remembered that the experimenters who obtained favourable results with agitation subjected their cultures to comparatively gentle movements, whereas the motion set up by Horvath was violent and prolonged   The conditions of his experiment were first repeated by S Meltzer (I) in 1891, who worked chiefly with *Bacillus megatherium*   He made numerous experiments, but we will only draw attention to those that gave results in advance of those previously obtained.   A New York mineral water works placed at Meltzer's disposal their agitator, with which apparatus he was enabled to subject the test samples to 180 reversed movements— of an amplitude of 15½ inches (40 cm)—per minute.   The flasks employed were only one-third full.   Meltzer found that the number of germs (ascertained by the plate method) in the agitated example in no instance amounted to as much as one-tenth of those in the unshaken check samples, and was, in fact, almost invariably smaller than at the commencement of the experiment   The restriction of reproduction thus indicated increased with the duration of the treatment, so that by this means the liquid could be completely freed from germs   The effect was even more powerful when sterilised glass beads were added before commencing the operation, the complete annihilation of the germs being accomplished under these conditions by ten hours' agitation   In addition to *B megatherium*, Meltzer also included a micrococcus (presumably *M radiatus*, Flugge) and a short motile bacillus (*albus ?*) in the scope of his investigations   A difference in the degree of resistance to this kind of inhibition is inherent in these organisms, since it was found possible to successively eliminate each form from a mixture of the three species.   *B megatherium*, as the most susceptible, disappeared first, and was followed, in order, by *Micrococcus radiatus* and *Bacillus albus*   The cells were, as a result of the shaking, split up, not into visible débris, but into an indistinguishable fine powder, a circumstance showing that the destruction of the vitality of the cell was not the result of a coarse mechanical disruption, but was due to a much more refined process, as was, in fact, shown by the further researches made by the same observer   He left several flasks containing cultures of *B. megatherium* or *B. subtilis* in solutions of common salt, to stand for several days in the engine-house of a large New York brewery, wherein, in consequence of the uninterrupted working of the engine, an incessant vibration was produced throughout the room   After four days all the germs in the several flasks were dead, whilst energetic reproduction had proceeded in the check samples placed in a quiet spot. Consequently, not only violent shocks, but also minute vibrations, exhibit the power of retarding the growth of bacteria, and even killing the organism

Motion may, however, also exert a favourable influence, and especially when it is comparatively weak, reproduction being thereby accelerated, as has been more

particularly demonstrated in the case of *B ruber*. Meltzer therefore arrived at the following conclusions Slight concussion favours the vitality of micro-organisms and has a stimulative effect, the rate of reproduction being highest when the optimum of vibration is obtained ; but from this point onwards the restrictive effects of concussion become manifest. The constants of optimum and maximum effect have different values for different organisms That degree of concussion which is injurious for one species may be favourable to a second, and without any appreciable effect on a third. This explains the contradictory reports made by the pioneers in this field, each of whom experimented on different organisms.

The influence of **gravity** on the direction of growth, which comes into play in the higher plants, and the effects of which are known in Vegetable Physiology as **geotropism**, has also been observed in the *Schizomycetes*. BOYCE and EVANS (I) found that vertically disposed puncture-cultures of *Bacterium Zopfii* in nutrient gelatin arranged themselves in the form of a feather, and in such a manner that the individual rays grew in a slanting *upward* direction. When the tubes containing the cultures were placed radially on a rapidly-revolving horizontal glass disc, the vegetation then developing assumed an appearance corresponding to that already described, the individual rays, which extended from the axis of the puncture, formed acute angles therewith, the apertures of which were reflected towards the centre of the disc This species therefore exhibits **negative geotropism.** BEYERINCK (III)—erroneously, as the author conceives—has denied this fact

The lower fungi generally, and bacteria in particular, remain, within wide limits, unaffected by high gaseous pressure Thus, SCHAFFER and FREUDENREICH (I.) and others have inoculated samples of milk with different bacteria (those of anthrax and typhus among them), and then exposed them for seven days to carbon dioxide at a pressure of fifty atmospheres, without being able to cause any appreciable injury to the organisms. Similar behaviour was also observed with oxygen under a pressure of twenty-one atmospheres, prolonged for a week. There is, therefore, no reason for hoping that liquids which are injuriously affected by heat can be sterilised in the cold by the aid of gas (CO_2, O, air) under high pressure. For exhaustive experiments on the influence of high gaseous pressure on living creatures generally, and the pathogenic *Schizomycetes* in particular, we are indebted to Paul Bert.

I   E

CHAPTER VIII.

BACTERIA IN THEIR RELATION TO ONE ANOTHER.

§ 64.—Symbiosis, Metabiosis, Antagonism.

It is only in exceptional cases that a sample of a natural liquid contains but a single species of micro-organism when in a state of fermentation. Nearly always we have to deal with a mixture of several species, the separation of which one from another, and the reproduction of the isolated individuals, is termed **pure cultivation.** A liquid or solid nutrient medium inhabited by a single species is called a **pure culture,** the methods of preparing which will be considered in the next section

When two or more species are *simultaneously* engaged in the consumption of a given nutrient medium, their association is termed **Symbiosis.** A couple of examples will serve to make this clear, one of them being the **Kephir granules,** which will be described in a later chapter. These granules chiefly contain two classes of organisms, lactic acid bacteria and yeasts; and when introduced into milk the fission fungi generate acidity, whilst the yeasts decompose a portion of the milk-sugar and produce alcohol and carbon dioxide. In this way an acid, foaming liquor known as " kephir " is obtained. A second, cognate example is afforded by the ginger-beer yeast, investigated by WARD (II.), and used in England for making ginger-beer. This is another instance of symbiosis, viz , the association of *Saccharomyces pyriformis* with a fission fungus, *Bacterium vermiforme,* the latter of which—as is described in chapter xxv —induces lactic fermentation in (spiced) cane-sugar solutions

The mutual relation of two or more species contained in the same culture may, however, be such that the one species, by the exercise of its vital functions, renders the nutrient medium suitable for the growth of the second species. This preparatory function of the one species may consist either in the absorption and elimination of certain constituents of the medium which retard the development of the other species, or in the excretion of certain products otherwise lacking in the medium, and either indispensable or highly favourable to the other organism. This kind of dependence was styled by GARRÉ (I.) **Metabiosis,** an excellent example of which is afforded by the decomposition set up in natural wine-must If this be allowed to stand in an open vessel as soon as it comes from the press a decomposition characterised as alcoholic fermentation rapidly sets in. The skin of the grape is the habitat of an abundant flora of fungi, which are introduced into the must in the operation of pressing. Of these (exceptional instances apart), the organism exciting alcoholic fermentation is the first to develop because the constitution of the must favours it the most, the result being that the sugar therein contained is split up, and carbon dioxide and alcohol are produced. When this decomposition is effected, the character of the liquid has become changed, and now a new species, exciting acetic fermentation, comes into play. This organism was already present in the must, but could not make head way against the predominant yeast, because, in the first place, the alcohol without which it feeds but indifferently, was lacking. Secondly, even had this substance been present, it could not have been utilised, because of the atmospher

of carbon dioxide, immediately above the liquid, preventing the free access to the latter of the copious supply of oxygen without which the oxidation of the alcohol cannot proceed Now, however, that both substances are present, the liquid commences to undergo a second alteration, and turns sour, the acetic acid bacteria being now on the surface , and this condition endures so long as there is any alcohol left. When this is exhausted, a third group of organisms comes to the front, thread fungi establish themselves in the strongly acid liquid and consume the acetic acid, carbon dioxide and water being found. This accomplished, the once again altered nutrient medium is attacked by putrefactive bacteria, which have been carried into the vessel along with the dust in the atmosphere, but can only develop now that the alcohol and acid, which are poisonous to them, are wanting The liquid is seized upon by these *Schizomycetes*, and, with their activity, the series of metabiotic phenomena which the wine-must presents to our notice closes.

The mutual influence of two or more species may be of such a nature that it is impossible for them to live together, the presence of the one species retarding the development of the other This set of conditions is termed **antagonism**, a number of examples of which will be given in subsequent sections.

§ 65.—Mixed Cultures.

When a nutrient medium is inoculated with two or more species of symbiotic organisms, we obtain a **mixed culture.** Such a culture may, under certain circumstances, yield fermentation products that cannot be obtained from any of the component species cultivated singly, but owe their origin partly to the coalescence of the normal products of the individual species, and partly to the reciprocal stimulative action exerted by the associated organisms. A few highly instructive examples of this are given below.

The first of these—which was discovered by NENCKI (I.)—is afforded by the bacillus of symptomatic anthrax (*Rauschbrand*) and *Micrococcus acidi paralactici.* Fuller information concerning the individual behaviour of these two *Schizomycetes* will be found in subsequent paragraphs, which we will here anticipate in respect of the fact now coming under consideration, viz., that the first-named bacillus yields, in nutrient solutions containing cane-sugar, the following fermentation products: hydrogen, carbon dioxide, normal butyric acid, and inactive lactic acid. On the other hand, *Micrococcus acidi paralactici* forms, almost exclusively, optically active paralactic acid, and that, too, in a quantity almost identical with the theoretical yield from the sugar eliminated If, now, both these organisms be cultivated together in the nutrient solution aforesaid, fermentation proceeds much more rapidly, and the final products consist not only of the already mentioned substances (yielded by the organisms singly), but also of a large amount of normal butyl-alcohol. This substance, therefore, owes its production in this case to the co-operation of two species of bacteria, neither of which singly is capable of such power

Interesting as this fact, that *new* fermentation products can be formed by the association of organisms, may be, the following one, which was first established by BURRI and STUTZER (I.), is so in a still greater degree. In this case two organisms are concerned, neither of which is capable singly of liberating nitrogen from nitrates , but, when acting conjointly, they decompose the same nutrient medium with violent disengagements of gas. The one organism is the *Bacterium coli commune,* already mentioned, and very abundant in human fæces and that of domestic animals, whilst the second microbe was named by the above-named naturalists *Bacillus denitrificans I.* A bouillon containing three grams of sodium nitrate per litre, inoculated with both these organisms and

then maintained at 32° C , began to disengage gas in a short time, the **nitric acid** in the nitrate being **reduced to nitrogen** so completely that, even at the end of forty-eight hours, the extremely delicate test with di-phenylamine-sulphuric acid gave only negative results.

Apart from the purely scientific interest excited by these facts, new vistas are also opened up in a practical sense. Up to the present, investigators have contented themselves with the examination of the transformation products resulting from the pure cultivation of single species of bacteria. In future researches, however, the question whether or not a species can be spurred on to more extended activity by the collaboration of a second, so as to give rise to the development of powers which without such stimulant, would remain unobserved and unutilised, cannot be neglected.

This claim is not restricted to the domain of schizomycetic fermentation, but applies also to the ferments of the *Eumycetes* class. The mode of action exhibited by mixed cultures of different species of yeasts is of great importance in brewery and distillery practice. In this connection we are already in possession of several studies by E. Ch Hansen and others, the results of which will be considered in a subsequent section.

CHAPTER IX.

CLASSIFICATION OF THE BACTERIA.

§ 66.—First Attempt by O. F. Muller.

It has already been mentioned in the Introduction (§ 2) that Leeuwenhoek observed bacteria as far back as the end of the seventeenth century. For a long time, however, nothing more was done than merely to admire the appearance presented by these organisms under the microscope; and since many of them were observed to exhibit brisk movements, they were considered as animals and denominated *animalcula*.

The first to study these organisms from a scientific standpoint, and to arrange and systematise the multitude of forms, some of which were already known, while others were discovered and described by himself, was the Danish investigator Otto Friedrich Muller of Copenhagen. In his important work "Animalcula infusoria fluviatilia et marina," published in 1786, all the small animals unsuitable for inclusion in Linnæus's sixth class, *Vermes*, were classed by him under the name of *Infusoria* (infusion animalculæ), and he divided these into two main groups: those provided with external organs and those devoid of same. He also originated the generic names *Vibrio*, *Monas*, and *Proteus*, still in use.

The next worker to whom we are indebted for important conclusions respecting the character and species of bacteria is Christian G Ehrenberg In his work "Die Infusionstierchen als vollkommene Organismen" (The Infusoria as Perfect Organisms), published in 1838, the generic names *Bacterium*, *Spirochæte*, and *Spirillum* first occur. He also classed all these organisms with the animal kingdom, by reason of their (frequently very active) spontaneous motion.

It was left to the Breslau botanist FERDINAND COHN (V.) to ascertain, in 1853, that the organisms we now know as bacteria are of a vegetable nature This he established by proving the lack of animal organisation, and also from the fact that these creatures increase by subdivision after the manner of the algæ, from which they differ, as he says, merely in one characteristic the absence of chlorophyll. Four years later NÄGELI (V) bestowed on these organisms the name of *Schizomycetes*, which they still retain

§ 67.—Cohn's Classification.

The first point was to bring the confusion of forms into order. What characteristic should be taken as a guide thereto? Were there several at disposal on which one could rely? These questions COHN (I.) may well have asked himself when, in 1872, he felt himself impelled to attempt a classification of the bacteria, and finally thought his object attained by the following system :

I. *Sphærobacteria*, globule bacteria
    Genus 1 *Micrococcus*

II *Microbacteria*, short rod bacteria.
    Genus 2 · *Bacterium.*

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III. *Desmobacteria*, thread (long rod) bacteria.
    Genus 3 *Bacillus*.
    Genus 4 . *Vibrio*.

IV. *Spirobacteria*, spiral bacteria.
    Genus 5 *Spirillum*
    Genus 6 *Spirochæte*

The sarcina organisms have no place in this system, because Cohn did not consider them as belonging to the fission fungi. As may be seen, the basis of classification employed was the form of the cells, *i e.* their form of growth. However, since methods of pure culture were then undiscovered, the diagnosis of the individual species was as yet impracticable, and the question whether the form of the cells in each species is definite and unchangeable was, in particular, still unsolved. The answer to this question is, nevertheless, of vital importance to the Cohn system, and, if negative, causes it to break down (as was subsequently the case)   The weakness of the system was recognised by Cohn himself, and he particularly stated that his classification was only a provisional one. A number of over-zealous disciples, however, overlooked this reservation, and, by degrees, expounded the system as meaning that each separate species has a single well-defined and invariable cell form, the one species appearing only as short rods, the second only as cocci, and so on   This constitutes the theory of constant form, also known as **Monomorphism.**

§ 68.—Billroth's Coccobacteria Septica.

The exaggeration resulting from the misapprehension of Cohn's attempt at classification soon brought about a corresponding reaction. In proportion as assiduous microscopic research revealed the certainty that bacteria do undergo changes of form, so the hasty assumption of monomorphism of species had to be given up   In 1852, PERTY (I.) had already observed a short-rod bacterium, which, on account of its faculty of changing into the thread form, he named *Metallacter*. Twenty-one years later LANKESTER (I) studied a species of red-coloured bacterium, named by him *Bacterium rubescens,* and observed that, under varied conditions of cultivation, its cells underwent different modifications of form—an observation which led him to deny that specific constancy of form existed. He would thereby have anticipated subsequent decisions had the basis on which he relied proved free from objection. This was, however, unfortunately not the case, and, indeed, such a condition was at that time unattainable owing to the lack of irreproachable and reliable methods of cultivation, without which, and the resulting pure cultures, the problem in question cannot be solved. A culture intended for modification experiments may, when examined under the microscope, present a perfectly uniform appearance, and nevertheless contain a few unnoticed individuals of another species, which by their rapid increase when transferred to a medium favourable for their development may lead to the erroneous supposition that a second and modified form of growth has been produced   By another re-inoculation a third species may be brought into prominence, and so forth.

A very instructive example of the possibility of similar self-deception is afforded by LISTER's (I.) striking experiment. He allowed ordinary milk to become sour spontaneously, and then introduced a drop of the liquid into boiled milk, beet-extract, and into urine; from thence into Pasteur's nutrient solution; thence into urine again; and finally back again into milk. Finding, then, that from identical sowings differently shaped cells made their appearance in the various media, he concluded that he had to do with so many changes of form of

one and the same organism, which, on account of its origin, he named *Bacterium lactis*.

It must not be understood that similar errors were confined to the island of Britain; on the contrary, they attained their culmination on the Continent in the assumptions of HALLIER (I) concerning the metamorphosis of one fungus into another. It need, therefore, be small matter for surprise that the Austrian surgeon TH. BILLROTH (I), in a comprehensive work published in 1874, not only attributed all infectious diseases to the agency of a *single* species of bacterium, susceptible of multiform modifications, but also considered all known bacteria generally as vegetation forms of this one species, viz., *Coccobacteria septica* This observer was supported by the botanist NÄGELI (VI), in so far that the latter declared that no necessity existed for the division of the bacteria even into only two specifically different forms. This opinion he still maintained in 1882, notwithstanding the appearance in the interim of a work by Cohn containing a number of fresh data calculated to complete and support the theory of difference of species in bacteria. This treatise has already been mentioned in § 24, because its author upheld the relationship of the fission fungi to fission algæ and advocated their collection into one group, *Schizophytes*. As at present, however, we are not concerned with the relationship of the *Schizomycetes* to other organisms, but with the separation of the former into genera, we must confine ourselves to remarking that the new classification in the said treatise rested too exclusively on morphological characters to be of practical value.

§ 69.—De Bary and Hueppe's Classification.

Gradually an accumulation of facts arose which afforded a basis whereon a new system of grouping the fission fungi was attempted. Differentiation based on cell form only was still considered justifiable up to 1878, but could no longer be maintained in the face of incontrovertible observations made, in the course of the following years, with absolutely pure cultures of various species of bacteria, and all leading to the same conclusion, that mutability, *i e* modification of form, unquestionably does occur in the fission fungi. This knowledge is the result of various researches, amongst which may be mentioned in 1879, that of E. CH. HANSEN (II) on *Bacterium aceti* and *B Pasteurianum*, in 1882 those of W ZOPF (III) on *Bacterium merismopedioides* (Fig 30), and by H. BUCHNER (VI.)

FIG 30 —Bacterium merismopedioides
Found in the mud of the river Panke (Berlin)

1 A thread form breaking up into 2 long rods, 3. short rods, 4. cocci; 5. a chain formed of rods of different lengths (*After Zopf*) Magn 700

on *Bacillus subtilis;* in 1883 that of KURTH (I) on *Bacterium Zopfii* (Fig 31), afterwards also examined by H SCHEDTLER (I), in 1885 that of G HAUSER (I) on a few species of putrefactive bacteria of the genus *Proteus*, and others   The adherents of Koch at first unconditionally opposed the theory of the pleomorphism of bacteria; but, not being able to sustain this view in the face of the facts

brought to light, they then asserted pleomorphism to be peculiar to the non-pathogenic bacteria   Even this restricted assumption has, however, given way, since undoubted pleomorphism was proved in 1882 by ARCHANGELSKI (I) and ROLOFF (I) for *Bacillus anthracis*, and by Friedlander for *Pneumobacillus* (§ 33) ; in 1883 by TH EHLERS (I) for the *Rauschbrand bacillus* (of symptomatic anthrax), in 1889 by E. METSCHNIKOFF (II.) for his newly discovered pathogenic *Spirobacillus Cienkowskii* (of *Daphnia magna*), and in 1892 by F. FISCHEL (I.) for *Bacillus tuberculosis*   It may be remarked *en passant* that Metschnikoff prefaced the report of his discoveries with a short review (well worthy of perusal) of the development of the pleomorphism theory.

At the end of the "seventies" Cohn had established beyond doubt the ability of certain fission fungi to produce endospores, and thereby obtained reliable
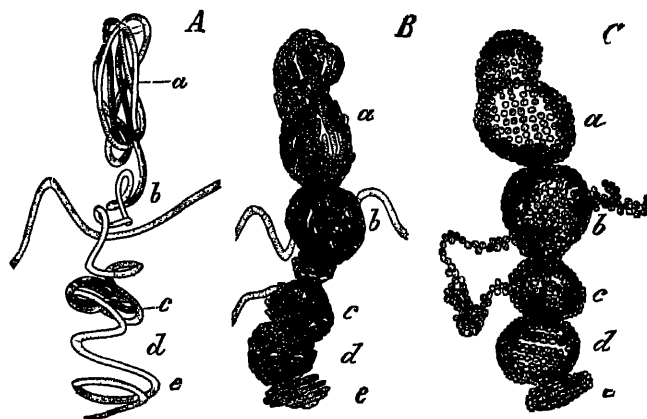
FIG 31 —Bacterium Zopfii, Kurth

Gradual changes in the same thread observed under the microscope

A, thread without apparent articulation , B, breaking up into rods which finally form cocci in C , *a—e* are corresponding cells   (*After Kurth*) Magn 740

means of differentiation.   Very soon after, De Bary showed that several of the species which do not form endospores protect themselves from injurious influences in another way, viz , by the formation of arthrospores   Hence a classification was devised in 1883 by VAN TIEGHEM (II), which was further developed by DE BARY (I.) and HUEPPE (II), in 1886, and in which two main groups were recognised, viz , the endospore- and arthrospore-forming bacteria. The second group also comprises all the species in which the formation of repro-ductive cells has not yet been observed   Fuller details of this system can be seen in Hueppe's treatise, but the system need not be further developed here, as it has not yet been generally accepted in scientific circles

For fuller information regarding Van Tieghem's system, as well as for parti-culars relative to the systems proposed by P. Miquel and by Woodhead in 1891, which may be properly designated as "diagnostic tables," reference may be made to WARD'S (III) readily accessible and comprehensive treatise   The new system published by W. MIGULA (II.) in 1896 may also be simply referred to.

In this connection there remains only one remark to be made, and this concerns the term **Bacillus**   This word has been hitherto employed by us to designate only a well-defined form-phase of cell, viz , the cylindrical bacterial cell, the length of which is at least double the breadth   Hueppe's system, however, applies the generic name *Bacillus* only to such rods as have been proved capable

of developing **endospores** This definition has not yet been accepted by the majority of bacteriologists; hence it happens that newly discovered rod-shaped species of fission fungi are still occasionally assigned to the genus *Bacillus*, although the describer may have no knowledge whatever as to their capability of forming endospores. The author has not considered it within his province to change this nomenclature, and therefore this fact must be borne in mind in perusing the present work. It should also be remembered that in the following paragraphs the generic name *Bacillus*—Hueppe's definition notwithstanding— means nothing more than that the species of bacterium so entitled *exhibits, preferentially and under normal conditions, the bacillus form of growth*

A comprehensive collection of the relative dimensions and forms characteristic of growth in various nutrient media, &c., of about three hundred species of fission fungi was prepared by EISENBERG (I), and may be advantageously employed as an aid to determining whether any species under examination is identical with any known species A descriptive table of eighty-seven of the bacteria of most frequent occurrence in drinking and utilisable water is given by ADAMETZ (I) Reference may also be made here to the very valuable book of TIEMANN and GÄRTNER (I) in connection with the bacteriological analysis of water. FRANKLAND and WARD (I.) give a comprehensive account of the literature published up to the year 1882 on the bacteria occurring in natural and mineral waters, and a comparative investigation into the distribution of a number (twenty-eight) of well-known bacterial species in various well-waters has been made by W. MIGULA (III.).

§ 70.—Pathogenic, Chromogenic, and Zymogenic Bacteria.

The attempts hitherto made to obtain a method of classification of bacteria have always been restricted to the morphology of the organisms themselves. It will now be well to remember that the attention of Applied Mycology is preferentially directed to the influence exerted by the fungi on their nutrient media. The interest aroused by these organisms has always, from the outset, had its practical side Bearing this in mind, it will be readily conceivable that, long before the establishment of Cohn's first classification, there had appeared in the literature of the subject a division of bacterial species into three main groups . pathogenic, chromogenic, and zymogenic bacteria

It is quite unnecessary to remark that this grouping is just as faulty as the division of the *Schizomycetes* into cocci, bacilli, and thread bacteria Nevertheless it was exceedingly convenient, as it was based on some well-marked primary characteristics. If the fission fungus in question excited any form of disease in men or animals, it was referred to the **pathogenic** group, if it possessed the faculty of producing colours, it was relegated to the category of **chromogenic** bacteria , and if it exhibited a capacity for effecting those chemical changes which were comprised in the term "fermentation" (§ I), it was considered as **zymogenic**. A strict adherence to this method of partition is impracticable, because there are some bacteria which, on account of their range of activity, would have to be placed in two, or even all three of these classes. A large number of examples could be adduced in support of this assertion; it will be sufficient to cite merely a single one, viz., *Staphylococcus pyogenes aureus*, the cause of osteomyelitis (bone caries), and therefore pathogenic. However, since it also, as its name implies, produces a golden-yellow colouring matter, it is also chromogenic , and, finally, from its power of setting up lactic fermentation in suitable nutrient media, it is therefore also zymogenic.

From this example it will be evident that the domains of Pathological and Technical Mycology cannot be rigidly kept separate On the contrary, their

further coalescence will undoubtedly result—and that soon, we hope—in proportion as fermentation physiologists acquire a greater insight into the chemical changes effected by bacteria, and pathologists determine the precise action the bacteria exert on the organs of animals and plants.   A fine, but unfortunately still very isolated, example of the successful combination of these two fields of research is afforded by the labours of L. NENCKI (I) on the bacterium which is the cause both of "blown" cheeses and of inflammation of the udder in the cow.

The distinction between chromogenic and zymogenic bacteria can also be further maintained, not because there is any essential reason for it, but because there are certain species of *Schizomycetes* which are interesting to the technicist solely because they produce colouring matters.

So far as the zymogenic bacteria, in the narrower sense of the term, are concerned, *i.e.* those either cultivated, or dreaded, on account of the chemical changes they produce, there is the same need for a well-established consistent classification as in the two groups just noticed   The changes effected by them are expressed in terms having reference to the predominant fermentation products , hence it is we speak of the bacteria of lactic fermentation, acetic fermentation, and so on   This purely practical method of classification will be adopted in the description about to be given.   Before passing thereto it will, however, be necessary to consider the methods practised in the examination of these organisms, this knowledge being essential for the study of the organisms themselves.   This will form the subject of the two following chapters

PRINCIPLES OF STERILISATION AND PURE CULTIVATION

CHAPTER X.

METHODS OF STERILISATION.

§ 71.—Sterilising.

To sterilise an object, *e.g* a nutrient solution, piece of apparatus, &c , means to treat it in such a manner that it no longer contains any living germs, and is therefore sterile.

The reader must not expect to find in the present work a detailed description of even the most important of the methods of working adopted for this purpose. Those who have an opportunity of studying the methods of Technical Mycology in a laboratory devoted to Fermentation Physiology will learn all they need much more speedily and intelligibly from oral instruction than from a printed book. On the other hand, those who have access to the latter only will attain their object by the exertion of a little diligence in consulting the books referred to later on, and especially

Hueppe, Ferdinand · *Die Methoden der Bakterien-Forschung*, 5th edition, 1891, Wiesbaden (O. W. Kreidel).

Lindner, Paul *Mikroskopische Betriebskontrolle in den Gdrungsgewerben*, 1895, Berlin (P. Parey)

In the first-named compendium the reader will find a better description than the present author could give of all the methods used in general Microbiology. The second, very useful, work treats, with great experience, a narrower field, wherein it will afford reliable guidance and help to the student on all matters relating to fermentation technology. In the newest edition (1895) of the work on water-analysis by TIEMANN-GARTNER (I), already referred to (and which should be in every efficient chemical laboratory), the reader will also find descriptions of the most important manipulations and methods employed in sterilisation, pure cultivation, re-inoculation, &c. In selecting apparatus for installing a new laboratory for Fermentation Physiology work, the beginner should seek the advice of an expert, and should compare the illustrated catalogues of such firms as make the supply of these appliances a speciality, *e.g* O Desaga, of Heidelberg , Erhardt and Metzger, of Darmstadt, &c

It is not our purpose now to give a detailed initiation into the work of a fermentation physiologist's laboratory, but rather to describe, in bold outline, only so much as is necessary to facilitate the object of the present work, viz., the study of the character and modes of action of the organisms of fermentation.

§ 72.—Freeing the Air from Germs.

There are two chief methods by which liquid substances and gases can be sterilised, viz , either by killing the germs present therein, or by removing them

by passing the liquid or gas through a suitable filter. The sterilisation of air on a large scale is effected exclusively by the latter method, the prototype of which was constituted by the tubes, plugged with cotton-wool, first employed by Schröder and Dusch. The air is therefore passed through a cotton-wool filter, as it is termed, such a one being used, for example, to purify the air admitted to the sterilised wort in an apparatus for the pure cultivation of yeast. It will not be out of place to lay stress on the fact that such a filter will only work efficiently provided it be thoroughly dry, otherwise the *Eumycetes* spores entangled therein will germinate and develop into long-thread cells, which will penetrate right through the filter and quickly form new spores, so that the air at the end of the filter nearest the wort is not only not freed from germs, but is probably richer therein than before. Attention to the air filters must, consequently, not be neglected. E. CH. HANSEN (III.) has reported on experiments made by Poulsen concerning the time during which such filters continue, under normal conditions of practical working, to pass the air in a germ-free state.

The cotton-wool plugs with which, since the time of Schröder and Dusch, it is customary to close test-tubes, bottles, and flasks in which cultures of organisms or stores of nutrient media are kept, are simply small cotton-wool filters. They are especially brought into action when currents of air pass into the vessels as a consequence of the partial vacua formed within them by a lowering of temperature, the germs in which are retained by the plugs. The efficiency of the filter depends on its being kept dry. Its reliability is not, however, permanent, since, though the fission fungi are always retained, this is not the case with the spores of mould fungi, which are so abundantly met with in the air. These latter are very troublesome, as they often produce much mischief even when the mycologist has taken the greatest care. If the room in which the cultures are kept be free from moisture, then the cultures dry up very rapidly, which, in order to preserve their vitality, necessitates their being frequently re-inoculated into fresh media—a tedious and unpleasant task. On the other hand, if the surrounding air be too moist, then it not infrequently happens that the spores of the mould fungi on the surface of the cotton-wool stopper germinate, and the resulting cell threads penetrate to the other end of the plug, and there form spores, which, falling into the culture, contaminate and spoil it.

Various remedies have been proposed to overcome this evil, one of them being a previously sterilised indiarubber cap, which is drawn over the mouth of the vessel (test-tube, &c.) after the outer end of the stopper has been burnt away. This latter operation must always be performed when one begins a re-inoculation, since the germs resting on the surface of the cotton plug are thereby annihilated, and consequently prevented from falling into the culture when opened. Instead of the rubber cap, one can be made out of a double layer of filter-paper tied on with a string; many cultures specially requiring air are covered with a cap of this kind only, the cotton-wool plug being dispensed with.

It is not essential that the working layer of the filter should consist of cotton-wool, various other stuffs being employed for special purposes. Thus, for example, Pasteur, in carrying out his researches (referred to in § 7) on the organised bodies present in the atmosphere, passed the air through gun-cotton. This was then immersed in a mixture of ether and alcohol, which dissolved out the nitro-cellulose and left the entrapped organisms behind, so that they could be more closely examined as to their size, form, and structure. This was the first microbiological analysis of air. Of the numerous methods since proposed for the estimation of the number of germs in the air, that given by FRANKLAND and PETRI (I.), which is a successful modification of the Pasteur prototype, is the most suitable for the purposes of the technical mycologist. These observers deprive a measured quantity of air of its germs by passage through a filter

charged with sterilised glass powder or sterilised fine sand, the contents of the filter being then intimately mixed with a gelatinised nutrient medium, and the whole poured into flat glass basins The separate germs then develop into multi-cellular families (colonies) When counted their number—referred to unit volume—gives the germ content of the air. The difficulty in the way of study-ing the cultures, caused by the presence of the powdered glass and sand, can be overcome by substituting a soluble filtering medium, such as coarsely powdered crystals of sodium sulphate of about o 5 mm in diameter. This is specially recommended by MIQUEL (III.), to whom (be it remarked *en passant*) we owe the most comprehensive experiments on the percentage of germs in the air Regular reports of his researches appear in the Year-Book (published annually since 1879) of the observatory established, under his direction, for studies of this kind, in the southernmost district of Paris. Readers are hereby referred to this "Annuaire de l'Observatoire de Montsouris." The percentage of germs in the atmosphere of breweries was more particularly investigated by E Ch HANSEN (II); and PETRI (I) has summarised all the methods of examination proposed up to 1887.

The method, originally performed by Th. Schwann, of purifying air by exposure to a red heat is at present used by fermentation physiologists in one instance only, viz, when working with the so-called Pasteur flasks. When liquid is poured out of the lateral tube—whether for the purpose of taking a sample or for inoculating a similar flask with the contents—the air coming in in its place is purified by holding the aperture or the first bend of the swan-neck tube in the flame, *i.e.* heating it to redness.

§ 73.—The Filtration of Drinking Water.

The methods of sterilising liquids are various, but are not all equally suitable for any given case. For example, the employment of poisonous substances is precluded when the liquid to be sterilised is intended for human consumption, and the use of heat—which next suggests itself—is frequently inapplicable on account of the expense entailed. Such, for instance, is the case with the drinking water of towns deriving their supply from a river Under these circumstances a so-called sand-filter is employed, the true filtering layer of which is not the strata of sand and gravel, but the mud which is gradually deposited thereon. A fuller consideration of this subject, which belongs to the domain of Practical Hygiene, may be passed over the more readily since it has been treated in Tiemann-Gartner's work already alluded to. This may be referred to, as also a very practical investigation performed by A. REINSCH (I.), bacteriological adviser to the Altona Waterworks.

The filtrate obtained from such filters intended for use on a large scale is, when the service is carefully regulated under bacteriological control, found to be .very low in germs, though not perfectly free therefrom. If it be desired to .attain such perfection—which is necessary in times of epidemic—other filters, of greater powers of retention and correspondingly diminished delivery, must be resorted to, and employed solely for the water intended for human consumption. The prototype of these is the apparatus invented by TIEGEL (I) in 1871, and subsequently (1884) improved, especially by Chamberland. In the form devised by the last-named, the effective constituent of the bacterium filter consists of a candle-shaped hollow cylinder of hard-burnt, porous, unglazed porcelain ("biscuit"), with an effluent aperture at one end, which, before use, is sterilised by dry heat. This candle (*bougie*) is enclosed in a somewhat wider metallic cylinder, the liquid to be freed from germs (the suspected potable water) being forced into the intervening space, and, finding its way through the porous wall

of the candle, collects in the interior of the latter and escapes through the aforesaid aperture at the bottom   Fine *kieselguhr* (diatomaceous earth) is employed by NORDTMEYER (I) and Berkefeld for making the candle, and GARROS (I.) uses asbestos of fine fibre.   The reliable working of this filter is, however, not illimitable, and that for two reasons ∙ first of all, the pores become gradually obstructed by the fine, slimy deposits separated from the liquid, which necessitates the cleansing of the filter from time to time; secondly, the bacteria grow by degrees through the pores of the filter, a circumstance first observed by Bourquelot and Galippe.   In some cases the pores of the filtering candle are too large; consequently, a germ-free filtrate is unobtainable   For testing the efficiency of the filter the photo-bacteria can, according to BEYERINCK (IV.), be employed with advantage.   A comparison of the capacity and efficiency of the Chamberland and Berkefeld systems was drawn up by (*inter alia*) DACHNJEWSKI (I.), and the columns of the *Centralblatt fur Bakteriologie* contain numerous articles respecting the advantages and defects of the aforesaid apparatus   Mention should be made of the filter constructed by Breyer, which, according to an investigation made by WICHMANN (I.), acts satisfactorily.   PLAGGE (I) instituted exhaustive experiments in respect of the efficiency of all the known water-filters designed for use on the small scale.

§ 74.—The Bacterium Filter in the Service of Enzymology.

In many instances the filter affords the sole reliable means of sterilising a given liquid ; as, for example, when a species of bacterium is to be tested with regard to its capacity of producing enzymes.   For this purpose it is necessary to free the culture, containing any such chemically active substance, from germs, since otherwise it would be impossible to determine whether the chemical reaction obtained by means of the sample is effected by the enzyme itself, or primarily by the vital energy of the bacterium   The sterilisation admittedly necessary in such case cannot be effected by heat, since this agency would at the same time destroy the readily decomposable enzyme   There remains, therefore, but *one* way open to us, viz., removing the germs by filtration ; and of the above-named apparatus (filters), therefore, there is likewise only one that is reliable and suitable for use for the purpose in view, namely, that of Chamberland. This is, however, unfortunately expensive, and consequently not accessible in every laboratory.   For this reason the pattern described by A. KOCH (II.), which is both efficient and cheap, forms a welcome substitute.

Whichever of the two appliances be employed, it must never be forgotten that, in its passage through the filter, the bacterial culture under examination is not only deprived of germs, but may also, under certain circumstances, part with some of its chemical constituents, so that the equation, Filtrate = bacterial culture − bacteria, does not always hold good.   The filtering cylinder, especially when used for the first time, retains varying amounts of the individual constituents of the liquid passing through it, a fact that was first recorded by FLÜGGE and SIROTININ (I) in 1888, and more closely examined by ARLOING (I) in 1892   We will, in this place, merely refer to the oxidising influence of the air, observed more particularly by Miquel in the separation of urase from cultures of uric-bacteria.   It is therefore advisable to perform such filtering operations in an atmosphere of pure hydrogen.

§ 75.—The Beer Filters

used in the brewery must also be briefly considered here.   The object of these appliances is to render the beer bright, i e. perfectly clear and transparent, when

drawn from the storage cask and sent out to the purchaser. Under normal conditions this clarification is effected in sufficient degree in the storage cask, and recourse should therefore be had to the filter only in such cases where, by reason of defective treatment or other unfavourable circumstances, a turbid lager beer is to be made similar to a beer of standard quality. Such was the practice in Bavaria until a few years ago, but since the great breweries in that country began to cater for the export trade, they have had to conform to the tastes of their foreign customers, who judge the quality of beer by the eye, and would, without having tasted it, set it down as inferior if it were not perfectly bright. Therefore, in order to render it acceptable to this large and continually increasing *clientèle*, the beer has to be passed through the filter The South German connoisseurs in beer, who judge their beverage by the flavour, raised objections, and with reason, since filtration causes—apart from the exception aforesaid—an uncalled-for depreciation of quality. This applies primarily to the chemical composition, the filter removing from the beer sundry mucoid substances, extremely minute in quantity and of as yet undetermined composition, but which, nevertheless, contribute to the fineness of the flavour, so that an experienced palate can distinguish with certainty between a filtered and unfiltered beer. This defect, regretted though it be by connoisseurs, is, however, the lesser evil when compared with the dangers, from a biological point of view, that are obviated by filtration.

Two main types of beer filters are in general use The one constructed by Enzinger consists chiefly of a number of chambers, the walls of which are composed of perforated plates lined with thick filter-paper, specially prepared for the purpose, and through which the beer is forced by compressed air acting on the storage cask. The second type of filter, recommended for brewery work by Stockheim, contains as its acting ingredient purified (and therefore tasteless) cellulose of a felty nature. No objection can be raised against the use of such appliances in exceptional cases, since by this means a clear filtrate is obtainable when all other methods of clarification have failed to remedy turbidity. This decision must, however, be amended when it is a question of beer already in good condition, this latter often suffering, under such treatment, a considerable alteration (in certain circumstances) with regard to its flora, apart from the depreciation of flavour already alluded to. The filter removes the yeast cells, but allows the (much smaller) bacteria to slip through, so that the latter appear in almost their original numbers in the filtrate, where, moreover, they have free play, owing to their previous competitors, the yeast cells, having been got rid of. This unfavourable modification in the relative condition of the two classes of organisms becomes especially objectionable when a filtering material that has already been in use before is employed, without having been sufficiently purified in the interim. In this manner the filtrate can be actually enriched with bacteria, as the author ascertained by experiments with the Enzinger filter in 1894.

Respecting the wine filter in continually extending use in cellar management, a full report can be perused in the handbook issued by BABO and MACH (I).

§ 76.—Destroying Germs by Dry Heat.

Strictly speaking, the term "germ-free" should be applied only to such objects as have actually been devoid of germs from the beginning or have been brought into this condition by filtration In the language of bacteriological practice, however, it is also applied to objects wherein all the germs have been destroyed and are only present in a defunct condition. Hence it would be more correct to say that the object in question is "free from living germs," but this distinction, being practically unimportant, is not generally drawn.

For the destruction of germs a number of methods are available, and may l classified into two principal groups the one **physical** and the other **chemica** The former may be subdivided into germ-killing by **warmth, electricity, ligh mechanical concussion**, or, finally, by **gas under high pressure**. We will confii ourselves to the first of these five methods, the employment of the remainii four being, for the purposes of the mycologist in general and of the fermentatic physiologist in particular, either too costly or too cumbersome. In so far, hov ever, as their influence is of general biological interest, we have already reviewi them in the preceding section.

On the other hand, **sterilisation by heat** is the method always resorted t unless found undesirable on other grounds. Before giving it more detailed co sideration, we must first ascertain which group of organisms exhibits the greate tenacity of life and is able to longest withstand influences adverse thereto. Tł group alone has to be borne in mind in testing the efficacy and general applic bility of a method of sterilisation, since if the same is capable of destroying tł organisms exhibiting the greatest power of resistance, it will certainly, and mu more quickly, deprive all the remaining weaker ones of life. On the other han when the contrary is not proved, it must always be assumed that the object be sterilised is infested with organisms of the highest resisting power.

These hardy organisms we are already acquainted with, namely, the bacteri **endospores**, which in this respect have no equal, and can therefore be made serve as test objects for determining the reliability of any germ-destroying pi cess coming under examination It has been already stated, in § 53, that gre differences exist in the resisting powers of the spores of the various species bacteria, but of course we have only to take the *strongest* into consideratic According to the investigations hitherto made, these are among the non-patł genic varieties, those species commonly known as the **hay—and potato—bacill** and among the pathogenic bacteria, the **anthrax bacilli**. Bearing this in mir ROBERT KOCH (I.), the eminent medical bacteriologist, employed as reagent i testing the efficacy of various disinfectants spores of anthrax bacilli, which, i greater convenience in application, he allowed to dry on silk threads

The articles of metal or glass to be sterilised are placed in a case, formed the plan of the drying-ovens used in chemical laboratories, wherein they s heated to 150° C for an hour During this time no diminution of temperatu is permissible, because if such a fall occurs, the labour will have been bestowed vain. KOCH and WOLFFHÜGEL (I) have shown that there are bacterial spo· that are killed only after an exposure to air at 140° C. for three hours. Ho ever, by an exposure to 150° for one hour we may be sure that all the ger present have been killed ; and air-filters fitted with cotton-wool (freed from f are also sterilised by the same treatment, the cotton-wool assuming thereb; yellowish to brownish coloration. Both the apertures of such a filter must hε been previously closed with plugs of cotton-wool, which must not be remov until the filter is about to be used. It is necessary that glass articles should *dry* before they are introduced into the hot-air sterilising apparatus, since oth wise they will crack.

Small metal instruments, such as forceps and inoculating needles, as well the glass stoppers used for closing Pasteur flasks, can be conveniently purif in the flame of a Bunsen burner or spirit-lamp.

§ 77.—Destroying Germs by Moist Heat.

The opinion expressed in a former chapter, that the seat of the high pow of resistance enjoyed by bacterial spores is to be sought in their membrane supported by their behaviour towards the influence of warmth, in so far—as

been ascertained by numerous experiments—that, under otherwise identical conditions, moist heat, e g in the form of steam, exerts a more violent action and kills them much sooner than dry heat at the *same* temperature. .This behaviour is explained by the unusually low heat-conducting power of the unaltered spore membrane. By the influence of moisture, however, the structure of this protective envelope is loosened and its permeability to heat rays increased

Although the use of moist heat may thus appear preferable to the method described in the preceding paragraph, it is nevertheless inapplicable in many special instances. For example, air-filters must be sterilised by dry heat alone, but when liquids have to be freed from living germs by the aid of heat, then moist heat must be decided upon. This can now be employed in one of two ways either by boiling the liquid over a naked flame, or by exposing it to the influence of water vapour heated to a sufficiently high temperature.

That every liquid can be sterilised by simple boiling at 100° O. was shown by HUEPPE (III) in 1882, the time of exposure necessary in order to secure the desired result with certainty being, however, very long. In this connection we may recall the experience of Brefeld, mentioned in § 53, according to which the killing of the spores of the species of hay bacillus examined by him necessitated their exposure for full three hours in boiling water. However, the nutrient solutions destined for the cultivation of organisms, and requiring to be sterilised anterior to use, must not be treated in this manner, since they would be concentrated too much by such prolonged boiling Such solutions are generally sterilised by exposure to low-pressure steam, for which purpose the so-called " steam steriliser," proposed by Gaffky, R Koch, and Loffler, and resembling in arrangement an ordinary potato-steamer, is employed. It consists principally of a high cylindrical tin pot, covered over with asbestos board or felt, and fitted with two bottoms, the upper one, which is perforated, serving as the support for the vessels to be sterilised by exposure to the steam evolved by the boiling water below This process is known as sterilising by **direct steam**, it obviates the inconvenience arising from the evaporation of the nutrient media, and also prevents local overheating. The samples are surrounded on all sides by steam, which drives away the protecting envelope of air and raises the temperature uniformly throughout to that of the boiling water. This is, of course, dependent on the prevailing atmospheric pressure, and generally ranges between 96° and 100° O A reduction of the time of exposure is not to be thought of, since here, as before, we have to do with a temperature of only about 100°, this must be particularly emphasised, since the Koch school at one time fell into error on this point, by promulgating the maxim that " the spores of bacilli cannot withstand the temperature of boiling water for more than a few minutes." We have already recalled a fact controverting this, and will now cite a second example, given by GLOBIG (II.), viz., that the endospores of a species of bacterium, discovered by this observer on potatoes, originating therefore in cultivated soil, and named the "potato bacillus," resisted the influence of a current of steam at 100° O. for as much as six hours This is the most powerfully resistant of all organisms hitherto observed

Numerous modifications have been made in Koch's steriliser, in accordance with the special purposes for which it is intended. Thus, for example, the water-chamber has been separated from the steam-chamber, and the steam introduced into the latter from above This pattern is specially preferred in the case of the large apparatus employed for disinfecting invalids' linen, hospital bedding, and the like Readers desirous of obtaining full information on this point are referred to a treatise by DUNCKER (I), who subjected a

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number of steam-disinfectors to a careful examination. The simple and inexpensive form described above is sufficient for the purposes of the fermentation physiologist.

In the fermentation industries the method of destroying germs by steam is highly prized on account of its convenience and efficacy. In breweries, for instance, all the piping is steamed out, as also the wort cooler, and so on. The Enzinger filter, however, cannot be treated in this way, owing to the softening action of moist heat on the filter paper.

The duration of exposure requisite for the destruction of germs by moist heat can be considerably shortened by employing supersaturated high-pressure steam. If, for example, the steam be used at a temperature of 120° C. (corresponding to an extra-pressure of one atmosphere), an exposure of twenty minutes suffices for sterilising liquids up to 50 c c. in volume with certainty. For larger quantities a corresponding additional exposure at 120° C. (five to ten minutes) is given. The use of supersaturated high-pressure steam is attended with much smaller outlay, but requires a strongly built autoclave. Laboratories already possessing such an apparatus—which is required, for example, in the determination of starch in cereals, &c.—can also employ it to advantage for sterilising. In many instances, too, a method of this kind is advisable, not only on account of the saving in fuel, but also by reason of the fact that the chemical composition and nutritive quality of the liquid to be sterilised are less impaired by fifteen minutes' exposure to 120° than by three hours' exposure in the Koch apparatus.

On the other hand, there are liquids which are so readily decomposed that neither of the above methods of treatment can be thought of An example of these is afforded by the medium so frequently employed in mycological laboratories under the name of nutrient gelatin, a solution of bouillon, wort, &c, containing 8–10 per cent. of gelatin. This mixture, which sets at the ordinary temperature of a room to the consistency of soft glue, and liquefies at about 25° C., would lose its property of setting if exposed to such degrees of heat, and would thereby become useless. In such cases another method of killing the germs must be employed—namely, that first proposed by Tyndall, and known as—

§ 78.—Intermittent Sterilisation.

The powerful methods hitherto described have been considered necessary, fo the sole reason that the sample to be sterilised had to be regarded as presumably containing highly resistant bacterial spores. In the absence of such forms, th object in view is attainable by much milder means, and the liquids, &c., to b sterilised can be converted into this more favourable condition by causing th spores (possibly present therein) to germinate. It then becomes a much easie task to deal with the resulting vegetative forms, since these latter perish a temperatures *below* 100° C, and therefore so much the more certainly in current of steam For this reason then the sample to be sterilised—which, ε before, is supposed to contain the most highly resistant types of bacterial spore in addition to the comparatively feeble vegetative forms—is exposed at first to temperature of 100° C. in the Koch steriliser for a short time The duratic of his first treatment depends on the volume of liquid in the individual sample For flasks containing a charge of 10–15 c c each, fifteen minutes will suffice larger quantities warm through more slowly, and must be left in the steamer f; a correspondingly longer time, In every case the liquid should remain at temperature of 100° C for about fifteen minutes. By this treatment only tl vegetative forms and weaker spores are killed, and the next step is to ensu that the still living spores germinate, which is generally effected by simp

leaving the samples to stand at room temperature. At the end of twenty-four hours the first treatment in the steamer is repeated, whereby the vegetative forms that have in the meantime developed from the spores are killed. It being, however, possible that, owing to the known irregularity of germination, some of the spores have not developed, the samples are again left at rest for a day, and thereafter steamed a third time to kill the residual cells proceeding from these tardy spores. The medium, liquid, &c., will in this manner be entirely freed from living germs without having been subjected to injury from over-prolonged or excessive heating. This method is known as the intermittent process of sterilisation, and is the only one in use for the preparation of nutrient meat-juice gelatin. The success of this method of killing germs depends on *the whole* of the spores being caused to germinate. Now we know, from statements already made, that there are certain species of bacteria which will only develop under high temperatures, and for whose germination the temperature of the air of a laboratory is therefore insufficiently high. On this account it will be evident that, under certain circumstances, the samples will have to be left to stand at high temperatures. Since, however, these temperatures will, on the other hand, retard the development of the spores of such species as thrive only at low temperatures, it is therefore impossible to neglect either consideration. The samples must, consequently, be kept for a certain time at room temperature, and for another interval at higher temperatures. In no case, however, must this be relied on without further examination, but it must be laid down as a *fundamental rule of conduct*, that any nutrient medium apparently rendered sterile by fractional sterilisation, may only be considered as actually sterile, and used as such, when it is found that after a short storage, following the above treatment, no spontaneous development has taken place. This regulation, urgently necessitated by reason of the insecurity of the sterilising process in question, must not be neglected. Nevertheless, if the work is cleanly done, it will seldom be found necessary to reject samples on account of insufficient sterilisation, since the highly resistant spores, now in question, are generally absent in the majority of the substances employed in the preparation of artificial nutrient solutions, and only creep in when the manipulations are performed without due care. Cultivated soil is rich in such organisms, so that if such soil is, by any means, introduced into these media, an unsuccessful result may readily ensue, as was, for instance, observed by L HEIM (II). The occurrence of such spores in meat-extract is no rarity, and the remarks just made should therefore be recalled when such material is employed.

It may happen that a nutrient medium, which cannot be exposed to a temperature of 100° C. without decomposing, will have to be sterilised. An instance of this is afforded by the solution employed in the study of uric fermentation, which, in addition to the nutrient substances, contains also an admixture of urea. This body, as is well known, is gradually converted at 100° C. (in aqueous solutions) into ammonium carbonate. In preparing a medium containing this amide the directions of LEUBE (I.) should be followed, the solution of the other nutrient substances (*e.g.* a bouillon) being first treated by itself in the steamer, and the urea sterilised separately by heating it in the dry state at 106° C. for half an hour. By this treatment it is maintained unaltered, and may, when cooled, be added to the cold sterile bouillon.

Under particularly favourable circumstances, exposure to a temperature much below 100° C. can bring about either the complete mortification of the germs present in a solution, or else render them so debilitated that their further development is prevented, so that the liquid will remain for a long time (months and years) without alteration. This result is attainable when the influence of

warmth is seconded by suitable antiseptics, substances which we must first consider before noticing the combined process of sterilisation to which we are gradually leading up.

§ 79.—Mineral Antiseptics.

The substances exerting a toxic action on micro-organisms are still often divided into two groups those serving to annihilate the pathogenic bacteria being termed disinfectants , whilst the substances capable of retarding fermentation and putrefaction are denominated antiseptics There are, however, no good grounds for this distinction, since, as we know, there are bacteria capable of originating both disease and fermentation

Very exhaustive researches on the efficacy of the various antiseptics are available.   Those of R KOCH (I) were undertaken in the interest of medical hygiene.   As in the case of other agencies inimical to bacteria, so is it in the case of toxic substances · the destruction of the life of vegetative forms of growth is relatively the easiest to effect , stronger means being necessary to prevent the germination of the endospores, and the most powerful influences of all to kill these latter.

The strongest antiseptic is **corrosive sublimate**, or mercuric chloride, $HgCl_2$; but, unfortunately, this substance cannot, for hygienic reasons, be employed in the fermentation industry.   In the laboratory, however, the fermentation physiologist always keeps a stock of this reagent for disinfecting (inter alia) the bell glasses used for storing fresh plate cultures   A sufficient quantity is also put in vessels containing cultures that are no longer needed, but which should not be placed in the hands of the cleaner until they have been killed.   Again, in the laboratory of the chemist in large works a solution of sublimate should always be kept, along with materials for bandages, as being the first remedy to apply when the workmen are injured or wounded.   In washing wounds with this solution, one should always be mindful of the fact that the first treatment has a preponderating influence in the restoration of health.   The strength of solution employed, both in the laboratory and for this Samaritan service, is one gram of $HgCl_2$ per litre of distilled water   Calcareous well-water must not be used, and the author would recommend any chemist who cannot afford to purchase distilled water to prepare his stock of sublimate solution in rainy weather, using pure rain water for that purpose.   Like most of the salts of mercury, sublimate forms insoluble compounds with albuminoids (e g. in the blood), and has then no longer any effect on bacteria.   This reaction is prevented by adding 5 grams of sodium chloride per litre of solution, since this salt forms with the mercuric chloride a double salt soluble in water.   According to the researches of R. Koch, the spores of Bacillus anthracis perish in an hour when immersed in this solution. For the prevention of their germination the presence of 1 part of sublimate in 300,000 parts of water suffices.

The earliest disinfectant employed was **sulphurous acid**, the use of which for **sulphuring** wine casks has been handed down from remote ages   In this process, so-called sulphur threads are ignited and placed in the cask, being prevented from falling by the bung.   These sulphur threads are strips of linen about the breadth of the finger, steeped in melted sulphur   The germicide properties of gaseous sulphurous acid (sulphur dioxide) were examined by G WOLFFHÜGEL (I.); and G LINOSSIER (I.) endeavoured to express in figures the relation between the percentage content of a solution of this dioxide and the length of exposure necessary to kill various germs.   His experiments were not conducted with bacteria, but with Eumycetes , they are, nevertheless, given in the following Table

Species.	Fatal dose of SO_2 in c c per litic for an exposure lasting—			
	15 min.	6 hours	24 hours	5 days
Beer yeast	200	100	20	—
Wine yeast	100	20	20	10
Mycoderma vini	200	100	100	40
Aspergillus niger	50	20	10	—

With regard to the deadening of wine-must by sulphurous acid, referred to in § 11, mention may be made of the discovery of this observer that 25 c.c of SO_2 per litre sufficed not only to hinder the inception of fermentation in wine-must, but also to bring it to a standstill when already in progress. The presence of a small quantity—by itself inert—of another mineral acid was found to increase the power of the sulphurous acid in a remarkable degree. The subsequent fate of this latter in sulphured wine varies a small portion combines with the aldehydes, a little (often merely a trace) of which is always present, to form aldehyde-sulphurous acid, a compound of agreeable odour, but the bulk is converted into sulphuric acid, and is then found as potassium sulphate. Several experiments in this connection have been conducted by E CHUARD and M. JACCARD (I.). Apart from the cases already mentioned, this antiseptic is not used in a gaseous form in fermentation industries, since it attacks the metal fittings, irritates the workmen's lungs, &c. It is, however, employed in combination with lime, as calcium bisulphite, $CaO.2SO_2.H_2O = Ca(HSO_3)_2$, with which the fermenting tuns, &c, in the brewery are purified. On the basis of his experiments on this point with beer yeasts and film yeasts, H. WILL (I) recommends an aqueous solution of this salt containing 10 grams of SO_2 per litre. As the commercial salt contains 70–75 grams of SO_2 per litre, one part by weight of this liquid must therefore be diluted with six parts of water.

The suitability of Pictet's solution (*liquide Pictet*)—a mixture of CO_2 and SO_2 (1 1)—for disinfecting purposes has been reported upon by DE RECHTER and LEGROS (I.).

As a rule, the germicidal power of carbonic acid (carbon dioxide) is over-estimated by non-professional people The researches of CARL FRAENKEL (I), confirmed by C STEINMETZ (I), have shown that this acid has no power at all on certain bacteria, these latter thriving even in an atmosphere of the pure gas Other species are less able to stand it, and the remaining kinds, though retarded in their development, are killed by it only with great difficulty The most important literature on the subject has been arranged by P. FRANKLAND and WARD (I). The above-mentioned fact suffices of itself to destroy the hope that carbonated mineral waters are necessarily devoid of germs (as was assumed by Leone some years back), the researches of P. SIEDLER (I.) having shown that this is not the case. The influence of this acid on the vital activity of yeast and the progress of alcoholic fermentation will be dealt with in the second volume.

Chlorine, also, is not employed in the gaseous state, but as *chloride of lime* (calcium hypochlorite) This substance was recommended by H. WILL (II.) for the disinfection of the sacks—made wholly or in part of wool—used for filtering off the "cooler sludge" in the brewery. As these bags are rendered unusable by hot-water washing, their purification has to be effected by a cold process. That cold washing does not produce the desired effect was proved by Will, who found the sacks to be strongly infected with bacteria and wild yeasts, especially

around the stitches, a circumstance sufficient to account for the bad repute in which wort- and beer-droppings are held. Disinfection experiments have, however, shown that these germs can be killed by exposure to the action—assisted by careful brushing—of a chloride of lime solution containing 1 per cent. of active chlorine. As good commercial chloride of lime yields 30–35 per cent. of chlorine, the solution may be prepared for use by mixing 3–3½ kilos. (6 6–7 7 lbs) of the chloride with 1 hectolitre (22 gallons) of water—i e. about 5 oz. per gallon—stirring the mixture up frequently, and, after settling, pouring off the clear liquid from the (useless) sediment. According to R Koch, 0.2 per cent chlorine water will kill the spores of *B anthracis* within an hour. Exhaustive experiments—conducted chiefly from a medico-hygienic point of view—on the anti-bacterial properties of chlorine and bromine have been carried out by BERNHARD FISCHER and B PROSKAUER (I.).

Among the inorganic acids, **hydrofluoric acid** and its alkali salts have proved to be particularly poisonous to bacteria In the last few years this substance has, by the labours of Effront, been utilised in distilleries, on this head more detailed reports will be given in a later section

Boric acid, either *per se* or in the form of borax, is occasionally—in despite of prohibitory regulations—used for preserving food-stuffs (*e g.* milk). A permissible and useful application of this substance may be made in the preparation of starch paste by employing an aqueous solution of borax as a substitute for water. Paste prepared in this way can be recommended, for instance, for affixing the labels on wine bottles kept in store, the occurrence of the uncleanly formation of mould, otherwise intervening, being thereby prevented.

The effect of ozone and **hydrogen peroxide** on bacteria is due to a common cause, viz., the decomposing power of the oxygen liberated. According to the determinations made by H. SONNTAG (I.), ozone has only a weak germicidal power, but other experimenters, *e.g* OBERDÖRFFER (I.) and WYSSOKOWITSCH (I.), obtained somewhat more favourable results. According to the researches of OHLMÜLLER (I.), this gas acts more powerfully when it is passed, along with oxygen, through the culture. When the volume of the liquid amounted to 500 c c. an ozone-content of 90 m grms. of O_3 per 100 c.c of the gas was requisite in order to kill the germs of the spores of anthrax bacillus present. According to the researches of CHRISTMAS (I.), the germicide power of ozone sinks to *nil* when its amount falls below 0 05 per cent by volume; so that no effect can be anticipated from the much lower proportion (1–10 m grms. per 100 litres) of ozone present in the atmosphere. With regard to the purification of river water —intended for drinking purposes—by the aid of ozone, prepared artificially on a large scale, an exhaustive report has been drawn up by E. VAN ERMENGEM (I.).

Owing to the great expense entailed, the utilisation of the anti-bacterial power of **hydrogen peroxide** in the service of the fermentation industry is as yet impracticable. The invention of a less expensive method of production would, however, ensure it an extensive sphere of operation, since this bacterium poison offers the advantage that during its action it is resolved into water and oxygen. When the latter has killed the organism, nothing is left of the antiseptic but harmless water. Great advantage might be derived from this property in connection with the manufacture of **conserves**; but hitherto its value does not seem to have been sufficiently appreciated A few experiments have, however, been made with it in connection with the freeing of drinking water from germs. In partial improvement on the results reported by Van Tromp, it has been proved by ALTEHOEFER (I.) and P. SCHILOW (I) that an addition of 1 part *per mil* of H_2O_2 to drinking water will, within twenty-four hours, be fatal to the common (innocuous) water bacteria, the microbes usually present in conduit waters, and the organisms which produce cholera and typhus. No alteration in

flavour results from this application, and an injurious influence on health is the less likely, since the peroxide is quickly decomposed. A reduction of the dose below 1 *per mil* would naturally interfere with the efficiency of the reaction, a circumstance which explains the unfavourable results obtained by other experimenters, reported by A. SCHROHE (I.) A proposal, worthy of being followed up, has been made by A. GOTTSTEIN (II). A sample of water containing 1000 bacteria per 1 c.c. was found to evolve bubbles of gas at its upper edges fifteen minutes after the addition of H_2O_2, the gas being oxygen liberated from the peroxide by the activity of the microbes. Since the extent of this evolution of gas fluctuates in accordance with the number of living bacteria present, this behaviour might perhaps be utilised in arranging a simple method for controlling the efficiency of water filters at frequent intervals. No appliances beyond a stock of hydrogen peroxide and sterilised test-glasses would be required. Of course, this crude method neither could nor should be used to replace the examination of the efficiency of the filter by bacteriological tests, but is intended for the sole purpose of enabling the engineer in charge to convince himself, every quarter of an hour (or at other selected intervals), that the filtrate has fewer bacteria than the unfiltered water According to the critical researches of HUGO LASER (I.), the Gottstein method is not sufficiently reliable.

Milk of lime is, when fresh, a fairly good disinfectant, but loses its disinfecting property as soon as the calcium hydroxide becomes converted into carbonate, the latter being innocuous towards many organisms, and even favourable to others (especially the acid-forming microbes) In the absence of other disinfectants this liquid may be successfully used. According to the researches of E. PFUHL (I.), it is sufficient to add two volumes thereof, and leave them to react for an hour, to ensure the death of the typhus bacilli and cholera bacteria in liquid fæcal matter L STEUBER (I) has made several experiments as to the influence of milk of lime on yeast-cells, and on its suitability for disinfecting brickwork in the brewery

§ 80.—Organic Antiseptics.

The antiseptic most appreciated—next to sublimate—in surgery, viz, carbolic acid (Phenol, C_6H_5OH), which is used as a 4 per cent solution for washing wounds, is never employed for industrial purposes. Nevertheless, it merits brief mention here because the discoverer of its antiseptic action, viz, J. LEMAIRE (I and II), established the interesting fact that this constituent of coal-tar, whilst capable of restricting the development of organised ferments, leaves the efficiency of the enzymes unimpaired, a differential behaviour which afforded support to Pasteur in his campaign against the Liebig theory of fermentation. The toxic action of phenol on the individual species of the bacteria varies, a circumstance which is utilised in the bacteriological analysis of water. In order to determine if the water under examination for impurities contains *Bacterium coli commune*, a small quantity is, in accordance with Péré's suggestion, placed in bouillon containing one part of carbolic acid *per mil*. This will retard the development of most of the water bacteria, but not that of *B coli commune*, which will therefore increase in the culture, and can then be more readily detected by supplementary means (plate cultures). Crude carbolic acid is soluble with difficulty in pure water, but readily so in sulphuric acid, combining therewith to form sulpho-acids, an aqueous solution of which, under the name of aseptol, is employed in surgery. According to the researches of R Koch, the strength of aqueous carbolic acid solution requisite to prevent the germination of the spores of *B. anthracis* is 1 part in 850 In a 5 per cent solution the death of these spores is caused only after more than forty days.

The three succeeding higher homologues of phenol, viz., the **cresols**, $C_6H_4.OH\,CH_3$, are also used in surgery. The so-called **kreolin** or **creolin** is a mixture of soap with a tar-oil, containing a small quantity of phenols (cresol, &c) and a large amount of hydrocarbons   As the last are insoluble in water, a milky emulsion is produced by pouring creolin into that liquid   **Lysol** and **sapocarbol** are mixtures of soap and tar-oils containing more phenols and a smaller proportion of hydrocarbons than the substance last described, both these mixtures will dissolve in water without producing turbidity   The solubility of the cresols in water is slight : about 1 part per 100 aq , but can be increased considerably (as ascertained by Hueppe) by the presence of other substances Thus, when sodium cresotate is used, **solveol** is obtained.   An alkaline aqueous solution of sodium-cresol will absorb a very large quantity of cresol, thereby forming **solutol**   By adding to a 50–60 per cent. crude carbolic acid about 20 per cent. of its weight of mineral oil, a mixture known as **saprol** is obtained, which is lighter than water and floats when applied to fæcal matter   The suitability of this preparation for the continuous disinfection and deodorisation of the contents of cesspools and closets was tested by SCHEURLEN (I and II) Mention of the foregoing seven antiseptics is only made here for the purpose of stating their composition as a matter of interest to the technical chemist   They are, however, unimportant so far as fermentation industries are concerned.   A derivative of orthocresol, viz., **salicylic acid**, $C_6H_4.OH\,COOH$, is still occasionally used, e g. for the preservation of jams, to arrest the formation of mould on wine, &c. The time when H. Kolbe (who held the first patent for the manufacture of this substance on a large scale) strongly recommended its employment has long gone by.

On the other hand, another derivative of cresol, viz , potassium orthodinitrocresol, $C_6H_2(NO_2)_2\,CH_3.OK$, finds extensive employment, its explosibility being entirely done away with by the use of a small addition of glycerin, soap, &c The red pasty mass thus obtained is put on the market, as a patented preparation, by the Bayer Farbenfabrik under the name of **Antinonnin**, this name being given to it on account of its having been first used on a large scale in practice in 1892, for the destruction of the " nonnen " (*Monacha*) larvæ infesting the forests of Bavaria and Wurttemberg.   This paste dissolves in water in proportions up to 5 per cent , forming a clear solution, dark yellow in colour and of a soapy smell, possessing no corrosive action and attacking neither metals nor fabrics, but penetrating deeply into wood and other porous substances, and remaining fixed therein without volatilising or imparting any odour to the material.   Reports on the applicability of this antiseptic are unanimously in its favour   TH. STETTNER (I.), for example, has drawn up an exhaustive account of its usefulness in preserving wood employed for building purposes, and it forms a reliable means for the annihilation of the dreaded dry rot in timber (respecting which, it may be casually remarked, a comprehensive monograph has been written by R HARTIG (I.))   To prevent the spreading of this fungus, all the woodwork (and especially that forming the floor joists) is treated, by dipping or brushing the ends to be imbedded in brickwork, with a ½ per cent. (1 200) solution of antinonnin.   Dipping is also recommended for preserving railway sleepers and wood blocks for paving.   The latter are at present steeped in creosotic tar, and render the streets malodorous in hot weather by the vapours they evolve.   Antinonnin will equally counteract putrescence without inconveniencing the olfactory organs.   Telegraph posts, fencing, hop-poles, and vine-props are treated by setting the butt ends in a 0.5 to 1 per cent. aqueous solution of antinonnin for a day, whereby they will acquire great powers of resistance against rotting.   The packing for spaces between ceilings, for which purpose building waste is generally employed, and which is so often the breeding-ground of pathogenic germs (particularly tetanus bacillus), should be

impregnated with this disinfectant. Antinonnin is also a very suitable material to employ when it is a question of keeping the brickwork of a building dry and arresting corrosion, the cause of which latter phenomenon is probably bacterial. The evil may be remedied by brushing the walls with a 1 per cent. solution of antinonnin   If it be desired to prevent the inception of such corrosion—as will be specially the case when a wall is to be decorated with fresco paintings—then the mortar applied directly to the wall should be mixed with about 5 per cent. of antinonnin.   The walls of hospital wards, &c., may be cheaply and reliably disinfected by brushing them over with a saturated (5 per cent.) solution of this agent.   Full information concerning its successful employment in the brewery has been given by AUBRY (1), who recommends its use for purifying all utensils not brought into direct contact with the beer   The walls of the fermenting and storage cellars, which are frequently damp and form the habitat of mucinous and malodorous fungi prejudicial to the beer, may be dried and freed from mould by brushing them over with antinonnin solution.

Ethyl alcohol, in an undiluted condition, behaves as a fairly powerful poison towards bacteria, and, according to R. Koch, will hinder the germination of the spores of *Bacillus anthracis*, even when diluted with twelve times its own volume of water.   The use of this compound—of 90-96 per cent. strength—is strongly recommended to the fermentation physiologist, since it possesses the advantage over sublimate of rapidly attacking the spores of those mould-fungi that coat themselves with an excretion of fatty matter, owing to which they are able to resist the influence of aqueous antiseptics for a long time   It is advisable, before performing inoculations in Pasteur flasks, to wash the flasks all over with alcohol, more particularly the part of the lateral tube covered by the caoutchouc tubing, and the mouth closed by the glass stopper.   The surface of the table on which the inoculation is effected should also be cleaned with alcohol of about 50 per cent. strength

The disinfection of the hands is, as shown in particular by FÜRBRINGER (I.), a very tedious labour when it has to be absolutely efficient.   This, however, is necessary only in the case of surgeons, and the fermentation physiologist may rest contented with simply washing them with soap and water, and finally with alcohol, before undertaking a delicate inoculation   The latter precaution should in no wise be omitted before handling the ends of the caoutchouc tubing of Pasteur flasks   The susceptibility of the different species of bacteria to alcohol is various, a few of them being able to resist it very well when dilute; and some even utilise it as a source of energy, e g the acetic acid bacteria, which still thrive freely in presence of 10 per cent by volume of this alcohol.

Ethyl ether is also a very powerful antiseptic, and is recommended by R. WOLLNY (I.) for use in sterilising by the cold process   For this purpose the ether is added in the proportion of 10 per cent. to the liquid, and then, after the germs have been killed, removed by the air-pump.   The advantage of this method over that of heat is that it has no effect on the albuminoids coagulable by the temperature of boiling water.

Formaldehyde, also known as formol (formalin), will in the near future enjoy extended employment as a powerful disinfectant   Many objects, such as clothing dyed with delicate colours, furs, &c., must not be disinfected with liquid antiseptics or by steam, gaseous germicides alone being suitable   Among these there is but little range of choice, chlorine and sulphur dioxide not only destroy the germs, but also the materials to which the latter adhere; and the only other resource at our disposal is in formaldehyde   The antiseptic properties of this substance were indicated by O Löw (I.) and by BUCHNER and SEGALL (I), and have since been thoroughly investigated by TRILLAT (I).   Meat-broth containing one-twelfth part of formaldehyde *per mil* was found to be perfectly free from

germs at the end of several weeks. Aronson (I.) found that typhus bacilli, *Staphylococcus pyogenes aureus*, and *B anthracis* could not develop in bouillon containing one-twentieth part *per mil* of this aldehyde   According to the researches of J. Stahl (I.) and of E van Ermengem and Sugg (I), the spores of *B. anthracis* and those (very tenacious of life) from garden soil were killed by an exposure of one hour to the influence of a *1 per mil* solution of formaldehyde, and a solution containing 1 part in 750 proved fatal to the germs in a quarter of an hour   This disinfectant is therefore on a par with the strongest mineral (bacterium) poison, corrosive sublimate, as regards efficiency, and surpasses it in point of general applicability. Moreover, unlike the mercury salt, formaldehyde is but slightly dangerous to man and the higher animals. The air may be impregnated with sufficient of the vapour for the purpose of disinfection without causing any greater inconvenience than coughing, which, however, soon disappears, since one quickly gets acclimatised to this reagent. Formaldehyde is generally met with in commerce as a 40 per cent solution known as **formalin**. Trillat (II) gives a few methods for testing its strength and disinfecting value.—A few pads of cotton-wool or kieselguhr, &c , are moistened with the liquid formalin and transferred to a box or other receptacle, wherein the articles to be disinfected (clothing) are suspended , or the same are laid between linen cloths moistened with the liquid. By this means K B Lehmann (I.) thoroughly disinfected a complete suit of men's clothing, even when infested with anthrax bacilli, by the aid of 30 grams (a fraction over 1 oz) of formalin in twenty-four hours. For the preparation of formaldehyde on a small scale R Cambier and A Brochet (I) recommend a burner, and B. Tollens (I) a lamp, both fed with methyl alcohol. In the latter apparatus a dome or cap of platinum gauze (2 cm. high and 1 cm. wide) is placed over the slightly projecting lighted wick, and as soon as the gauze is red hot the flame is extinguished, whereupon the formation of formaldehyde goes on uninterruptedly   It should not be forgotten that—as pointed out by A Brochet (I)—this incomplete combustion of methyl alcohol also produces some 3 to 5 per cent of carbon monoxide. An apparatus constructed by Krell, and resembling the Barthel soldering-lamp, has been described by A Dieudonné (II), by means of which a constant current of formaldehyde vapour can be produced from methyl alcohol and blown into crannies and corners that require disinfecting   The different degree of susceptibility exhibited by the various bacteria towards this poison has been utilised by E. Schild (I. and II) for the **differentiation of typhus bacilli** from the very similar *Bacterium coli commune*, which, in the bacteriological analysis of water, is both very important and difficult. The latter species develops freely in a bouillon containing 1 part of formaldehyde in 7000, whereas the former will not do so. Therefore, if a species of fission fungus isolated from the sample of water, and suspected to be typhus bacillus, produces turbidity in such a medium, this behaviour shows that it is *not* the bacillus which causes typhus. The applicability of this method—which gives a *negative* characterisation—has been confirmed by Rud. Abel (I). The researches above noticed deal only with the action of formaldehyde on bacteria, but for the fermentation industry it is also important to know how the higher fungi, and especially the alcohol yeasts, behave towards this disinfectant   In this connection it has been established by W. Windisch (I.) that yeast cells show much less susceptibility , consequently this aldehyde is not a suitable means for killing them. Fortunately, however, they are readily affected by the influence of hot water vapour, chloride of lime, &c., so that there is no lack of available remedies.

The antiseptic power of iodoform, CI_3H, was studied by Behring (I.), with the result that this compound was found not to injure (kill) bacteria, except in the rare cases when iodine was liberated. In all other instances (which thus

constitute the rule), its favourable action in the healing of wounds is based exclusively on the counteraction of the poison produced by the pus-forming bacteria, without, however, the appearance of the latter being prevented. The use of chloroform for disinfection is only, as a rule, resorted to when it is desired to sterilise milk for use as a culture medium, in which case it is necessary to dispense with strong heat   This will be discussed in a subsequent chapter.

The **organic acids** have a fatal effect, even in small quantities, especially on putrefactive bacteria. Frequent and regular use is made of this property in technical processes of sterilisation, as also in distillery work ("souring the mash"), as will be frequently noticed in the course of the present work   On the other hand, fairly high degrees of concentration are required for killing such bacteria as are themselves active producers of acid. Benzoic acid, though prohibited by law, is occasionally employed for increasing the keeping properties of milk   This acid—even in very small quantities—has a very restrictive influence on alcoholic fermentation, and it is to this influence that the difficulty of exciting fermentation in the juice of the whortleberry (*Vaccinium Vitis Idæa*) is to be ascribed, considerable quantities of this acid being present therein, MACH and PORTELE (I) having found o 64 to o 86 grm per litre.

§ 81.—The Combined Method of Sterilisation.

The influence exerted on micro-organisms by the substances already considered is subject to the same fundamental law as has been established for physical force, viz , that **the effect produced varies with the intensity of the causative influence.** A solution containing so large a proportion of antiseptic that it is capable of killing a given microbe, will, when sufficiently diluted, have a merely restrictive influence on development, without, however, proving fatal. Proceeding farther in the same direction, a condition of dilution will be attained which will exert a favourable effect, stimulating the vital activity of the organism ; and finally, if the degree of dilution be extended beyond this point, no effect will be observable. This fact was expressed by HUGO SCHULZ (I) in the following phrase · "Each impulse exerts on each cell an action whose effect on the activity of the cell is in inverse proportion to the intensity of the impulse "   A series of researches, which confirm this law, have been made on microbe poisons, but it will be sufficient to simply mention two examples, viz , that of CH RICHET (I.), treating of the bacteria of lactic fermentation, and that of BIERNACKI (I), which deals with alcoholic fermentation

This law forms the basis of the **theory of toxic action** originated by O. Löw (II) in a book the perusal of which is commended to the reader, and more especially for the complete critical digest it contains of the literature, relating to the action of poisons, published anterior to 1893   According to Low, the ultimate cause of toxicity is to be sought in the **lability** of the albuminoid matter of the cell protoplasm. The activity of the latter consists in a continuous chemical change of the atomic groups composing the molecule, the briskness of which alteration is increased by slight stimuli. Larger quantities of the irritant (poison) exert such a strong preponderating influence on the change, that the lability of the plasmic albuminoid is arrested, and the life of the cell is consequently destroyed. Probably, then, toxic action may be the means of throwing light upon the obscure problem of the chemical dynamics of the cell; just as, in many other branches of natural philosophy, the study of disturbing influences has afforded the deepest insight into the normal course of phenomena

A knowledge of the nature of toxic action—the progress of which depends more or less upon chemists obtaining a clear idea as to the constituents of the albuminoids—is of the greatest importance, both to the study of organic life in

general, and to that of the pathogenic and fermentative microbes in particular. It is also important, as we shall soon see, for the technique of sterilisation.

The destruction of germs by heat in certain nutrient solutions and food-stuffs is often a very difficult task, because it necessitates temperatures that damage the sample both as regards nutritive value and palatability. Success may, however, be attained by combining the influence of heat with that of poison, although the isolated action of either is incapable of killing the germs. This is the leading idea on which is based the process of **mixed** or **combined** sterilisation, wherein the death of the micro-organism is caused by the simultaneous application of two factors, one of which (the poison) is without influence on the chemical composition of the sample, whilst the other (heat) is too low to set up any injurious decomposition.

At first sight it may seem that the presence of poison restricts the application of the process to such cases as the sterilisation, pure and simple, of a liquid, and precludes its use when such liquid is intended for the cultivation of micro-organisms or for human consumption. On more mature deliberation, however, a contrary conviction will be formed

Many of the substances named in the preceding paragraph are in themselves innocuous to the health of man, provided the quantity present is not too large; this is particularly the case with alcohol and the organic acids, and it is precisely these acids that are generally employed for the preservation of numerous food-stuffs. A fuller account of this subject will be given in a future chapter, so we will simply refer to it here and pass on to the consideration of the second question · Is the combined method also suitable for sterilising nutrient media intended for mycological work?

Let us recall the observation that has been frequently made in previous paragraphs with reference to the behaviour of micro-organisms under the influence of physical and chemical forces. Just as a certain degree of heat is fatal to one species, simply retards the development of a second, is favourable to a third, and insufficient to allow the cells of a fourth species to grow at all—so given amounts of poison may be fatal to one species of organism, inert towards a second, and even stimulating to a third   In other words, the constants of influence of a given poison vary with different organisms.

We are indebted to TH SCHWANN (II.) for the first observations on the variations in behaviour thus exhibited, but to PASTEUR (I) for the first practical application thereof. Attention has already been directed to the susceptibility of the putrefactive bacteria to the influence of acids, a property of which Pasteur availed himself to protect his cultures of ferments (in the restricted sense of the term) against injury on the part of such interlopers   For example, in order to study acetic fermentation, he first acidified the artificial medium with acetic acid   By means of a skilful combination of various anti-bacterial forces, properly adapted to each particular case, a given nutrient medium can be freed from germs without diminishing its suitability for the culture in view   One factor of this combined method of sterilisation is usually heat.   Many examples of this will be given in the course of subsequent paragraphs, so that we will now simply refer to that afforded by the **boiling of beer-wort**

At the moment when the still unhopped wort runs from the mash-tun into the copper, it contains innumerable bacteria, chiefly derived from the malt. Not only do these survive the mashing process uninjured, but their increase is such that o 07-0.12 per cent of lactic acid is produced. The acidity of the wort is somewhat further increased by the addition of the hops placed in the copper before boiling is commenced. But, as a consequence of the conjoint influence of the boiling temperature ($100.5°-103°$ C.), the lactic acid and the hops, the germs in the wort are—as found by G. H. MORRIS (I.)—at the end of fifteen minutes' boiling, partly killed and partly so far weakened that they are

incapable of further development, the wort is therefore practically sterile. Sometimes—but, as E. Ch Hansen (III.) has shown, not always—the complete destruction of all the germs (absolute sterility) is attained in this way. However, the residual living germs in the wort do not develop therein, though they will do so if transferred to a more favourable medium—e.g meat-broth. In this case we have to do with relative sterility. The rapidity of the effect is chiefly attributable to the influence of the hops, which, in turn, owe their germicidal powers to the possession of certain resinous bodies, generally known under the collective name of hop-resins. The chemical properties and biological effects of these bodies have been investigated by M. Hayduck (I), who found three different resins in hops, all of which are soluble in alcohol, ether, and chloroform. One of these, viz., the brittle, tasteless γ-resin, insoluble in petroleum spirit, does not interest us in the present instance, the germicidal properties of the hop not being due to its influence, but to that of the two (extremely bitter) soft resins, the α-resin and β-resin. These two act powerfully on the lactic acid- and butyric acid bacteria, but are innocuous towards acetic acid bacteria, sarcina, and higher fungi (especially yeast). The latter organisms are, however, subject to the influence of the boiling temperature, so that the wort is delivered in a sterile condition to the cooler, where it is infected anew. The attempts of all discerning brewing technicists to abolish the cooler and to effect the rapid cooling of the wort (as well as its aeration by the injection of germ-free air) in closed vessels fitted with refrigerating appliances, are thus easily accounted for. This method of procedure, which, from the fermentation physiologist's point of view, is the only correct one, is, however, beset with a difficulty as regards the separation of the sedimentary matter. Therefore the hot wort from the copper is generally allowed to stand until the sediment has subsided, the still hot goods being then carefully drawn off and conveyed to suitable cooling and aerating apparatus. For a description of the latter, reference must be made to Handbooks on Brewing, three of which are recommended · that of Thausing (I) studies the wants of the practical brewer, whilst Moritz and Morris's (I.) work is intended for the brewing chemist familiar with chemistry and microbiology, to whom it presents a large amount of lucid information These two books being supplementary one to the other, the student will do well to leave neither unread Finally, the third work, O J Lintner's (I) "Handbuch der landwirtschaftlichen Gewerbe," is adapted for imparting instruction in High Schools.

The sterilisation of wort in Pasteur flasks—the medium most frequently employed in the fermentation physiologist's laboratory—will be briefly described as an addendum to the preceding remarks. In order to produce a clear liquid, poor in precipitated albuminoids, &c , the Pasteur flask is half filled with wort (not from the hop copper, but from the cooler), which will now contain numerous germs, several hundreds to thousands per c.c. The flask is then placed on a heated sandbath and the steam evolved is allowed to escape for ten minutes—counting from the moment boiling begins—through the short caoutchouc tube on the lateral tube of the flask, whereupon the former is closed by a glass stopper previously purified in the flame. Then, for a further ten minutes, the steam is allowed to escape through the swan-neck, and the flask is left to cool, being for that purpose placed on a hollowed cork or a ring of millboard one inch in height. When the liquid has again sunk to the temperature of the room, the moisture condensed in the swanneck is driven off by means of the gas-flame, and the neck is closed by a small plug of asbestos, which subsequently serves as a germ filter. Any organisms capable of passing through this are deposited in the first bend of the tube, which is then freed therefrom, by heating it to redness in the flame, before proceeding to inoculation. Concerning the sterilisation of the large copper apparatus for pure yeast culture, detailed instructions have been given by E. Qh. Hansen (III)

CHAPTER XI.

METHODS OF PURE CULTURE.

§ 82.—Nutrient Solutions.

In § 15 of the Introduction it was stated that Liebig's theory regarded the disintegration of the albuminoids as the true active agency in fermentation. Pasteur (VII), the active opponent of this theory, interested himself in the preparation of artificial media which, though free from albuminoids, began to ferment when inoculated with a minute quantity of fermentative organisms (e g. a trace of yeast). The oldest of these, generally known as Pasteur's fluid, consists of—

	Grams
Water	100 0
Ammonium tartrate	1 0
Cane-sugar	10 0
Yeast-ash (corresponding to one gim. yeast)	0 075

and was intended preferably for the cultivation of the higher fungi (yeast in particular). Its suitability for bacterial cultures was examined by Cohn, who found that for this purpose the sugar could be dispensed with   On the basis of researches into the requirements of yeast as regards mineral matters, Adolf Mayer (I) proposed to employ, in place of the yeast-ash, which is soluble only with difficulty, an artificially prepared solution of the salts of which this ash is known from experience to consist   Utilising this report, Cohn (I) prepared a nutrient solution which he named "normal bacterial liquid," and which was composed of—

	Grams
Water	100 0
Potassium acid phosphate (KH_2PO_4) . .	0 5
Tribasic calcium phosphate ($Ca_3(PO_4)_2$)	0 05
Crystallised magnesium sulphate	0 5
Ammonium tartrate	1 0

Nägeli (IV.), relying on the results of his researches (indicated in chapter ii) on bodies suitable for the nutrition of the lower fungi, prepared three "normal liquids for fission fungi," one of them having the subjoined constitution :

	Grams
Water	100 0
Di-potassium phosphate (K_2HPO_4). . . .	0 1
Ciystallised magnesium sulphate . . .	0 02
Calcium chloride	0 01
Ammonium tartrate	1 00

The nutrient solutions hitherto described play a great part in earlier mycological literature, on which account their constitution is now given, though at present they are but seldom used

On the other hand, a second nutrient solution given by Pasteur, viz , yeast-water, is still frequently used. To prepare this solution, about 100 grms. of thick brewer's barm (or 75 grms. of pressed yeast) are placed in a tin can with

one litre of water over the fire, and boiled for a quarter of an hour, and are then passed through a folded filter. If the liquid passing through is turbid it is returned to the filter, and in this way a clear, pale yellow filtrate is obtained, which is made up to one litre by the addition of distilled water, and is then sterilised (either in bulk or in portions) by exposure to 100° C in a steamer on three consecutive days, or by a single operation of twenty minutes at 120° C. under pressure. By a preliminary addition of 5 to 10 per cent. of sugar a very useful nutrient medium for yeast is obtained   When acidified with acetic acid and qualified with alcohol, yeast-water rendered good service in Pasteur's studies in acetic fermentation.

For the cultivation of beer-yeasts the most suitable medium is hopped **beer-wort**, sterilised in the Pasteur flask as already described   The hop-resin in this liquid exerts a toxic action on many organisms, and among them the lactic acid bacteria, which play an important part in distillery work ; so that hopped wort must not be employed to cultivate these organisms in the laboratory, unhopped wort being advisable for this and sundry similar purposes.   Unhopped wort is an advantageous medium for numerous fermentative organisms, and therefore requires special care in sterilising

Wine-must serves for the artificial multiplication of wine-yeasts and fruit-yeasts, and a concentrated form of it is kept in stock in the laboratory.   On this point fuller particulars will be found in chapter xx.

Saprogenic and most pathogenic bacteria thrive particularly well in meat-juice.   This is used in the form of so-called **bouillon**, and, following the lines indicated by the researches of PETRI and MAASSEN (I), is prepared as follows Half a kilogram (1.1 lb) of finely minced beef, *free from fat*, is placed in a tin pan or earthen crock along with one litre (1¾ pints) of well-water, and, after standing for an hour at the ordinary temperature, is heated to about 60° C. during three hours, with frequent stirrings   At the conclusion of this period of extraction the mixture is boiled for half an hour and then filtered through a folded filter   The pale yellow effluent liquid is made up to one litre when cold, and exhibits an amphoteric reaction.   Its primary salts of orthophosphoric acid (*e.g* KH_2PO_4) redden blue litmus paper, whilst on the other hand the secondary phosphates (*e g*. K_2HPO_4) also present behave in the contrary manner.   In presence of phenolphthalein, however, only the tertiary phosphate (K_3PO_4) acts as a base, and consequently meat extract behaves as an acid towards both *blue* litmus and phenolphthalein.   As a general rule, 10 c c. of this broth require an addition of 1 8 c c of deci-normal alkali to prevent the colour change from taking place with blue litmus, and an addition of 3 c.c to enable it just to redden phenolphthalein   This acid reaction of meat-broth being a hindrance to the development of many bacteria, it is on that account rendered *very slightly* alkaline, the resulting liquid containing a smaller or larger percentage of alkali according to the indicator used, and which should be selected in accordance with the requirements exacted of the medium in each case   After neutralisation, 1 per cent of dry peptone and a ½ per cent. of common salt are added to the liquid, which is then boiled again for a quarter of an hour (but not longer), and filtered hot; the resulting liquid, generally known as **nutrient bouillon**, is filled into small bottles (*e.g* 5–10 c.c) and sterilised by either a thrice-repeated treatment in the steamer or once under pressure

When, under particular circumstances, suitable meat cannot be obtained, meat extract is used instead   Hueppe's formula for making **meat-extract bouillon** is   30 grms. dry peptone, 5 grms grape-sugar, and 5 grms meat extract, dissolved in 1 litre of water, and boiled, filtered, and neutralised as previously described   The sterilisation of the (once more boiled and filtered) bouillon must be performed with scrupulous care, the meat extract being rich

in bacterial spores which are very tenacious of life. If this or the previously described bouillon refuses to filter clear, the white of an egg, previously beaten to a froth, is added, and the whole warmed up, boiled, and filtered, whereupon the liquid will run through bright.

The power of thriving in a solution of salts devoid of albuminoid matters was first observed by DUJARDIN (I.) in 1841, in the case of a fission fungus allied to *Bacterium termo*, and was afterwards decisively proved, as regards the zymogenic fungi, by Pasteur. In 1893 USCHINSKY (I.) demonstrated that the majority of pathogenic bacteria (of typhus, cholera, diphtheria, tetanus, swine-erysipelas, &c.) could also be cultivated in a liquid containing ammonium lactate and sodium asparaginate as its sole supplies of nitrogenous nutriment. Cultures in such media are specially suitable for the study of the poisonous substances (**toxins**) excreted by these originators of disease, the separation of the former being easy on account of the absence of albuminoids. The fact that these toxins (which are probably allied to the albumoses and peptones) can also be elaborated in non-albuminous media proves that they are not derivatives of albumen, but are the result of synthetical processes occasioned by the vital activity of the organisms.

This matter has been investigated by FERMI and SCHWEINITZ (I.), PROSKAUER and BECK (I.), O FRAENKEL (II), and others. Since bacteria rapidly increase in such a solution, they are therefore also endowed with the faculty of effecting the synthesis of albumen. Comparative researches instituted by E. CRAMER (II) with cholera vibrio showed, however, that the percentage content of albumen (calculated to dry substance) in the cells cultivated in Uschinsky's solution is lower than in the case of cultures grown in media containing albumen.

In the sixth and seventh decades of the present (nineteenth) century the preparation of a medium suitable as a **universal nutrient medium** for all possible bacteria formed the object of the repeated exertions of many bacteriologists. Such an attempt is now regarded as hopeless on account of the knowledge which has been gained of the very opposite conditions governing the vitality of the several species.

§ 83.—The Dilution Method and Fractional Cultivation.

It has already been remarked in chapter viii. that it is quite the exception for a natural bacterial growth to consist of merely a single species, but that, as a rule, we have to deal with a mixture of several. To separate these from one another, and to further multiply each species by itself, so as to obtain therefrom a **pure culture**, forms the aim of the **methods of pure cultivation**

We start with the assumption that we have to deal with a number of different bacteria inhabiting a liquid, inasmuch as there is a second condition possible, *i e* when the organisms are distributed within a solid body (such as cheese, butter, soil, &c.) In the latter case a finely divided suffusion of the sample must be made with sterilised water and treated in the same manner as liquid bacterial samples

Very often the mycologist is set the task of determining the **germ content**, *i.e.* ascertaining how many individual cells are contained per unit of space in a sample. This contingency is often met with in fermentation experiments with yeasts, in order that, from the result of the counting, the extent of the cell multiplication occurring during the fermentation may be ascertained. For such purposes a so-called **counting chamber**, such as supplied, *e g.*, by Carl Zeiss of Jena, is used. The arrangement of this appliance is shown in Fig. 32, in plan at A and in vertical section at B. On a thick glass slide there is mounted a cover-glass (*a*) with a circular hollow, within which is cemented a second glass

disc (*o*) o.1 mm thinner. On the upper side of this latter are etched two systems (crossing each other at right angles) of twenty-one parallel lines at regular intervals of o o5 mm., and therefore enclosing compartments each of which has an area of o oo25 sq. mm.

If now a sufficiently large droplet of the sample to be counted be laid on the centre of *c* and covered with a cover-glass (*b*) about o 5 mm. thick, then various portions of the liquid, which has a uniform thickness of o.1 mm., can be examined under the microscope for the number of germs present therein.

A number (ten to fifty) of the square divisions are counted and the mean of the resulting figures is taken. This being denoted as M, the germ content of the liquid will then be $\frac{M}{o\ oo o25}$ = 4000 M per cubic millimetre.

In order to arrest the movement of motile forms or prevent the multiplication of rapid-growing cells (*e.g.* yeast), a portion of the sample, well shaken up, is previously mixed with an equal volume of 10 per cent. sulphuric acid, which
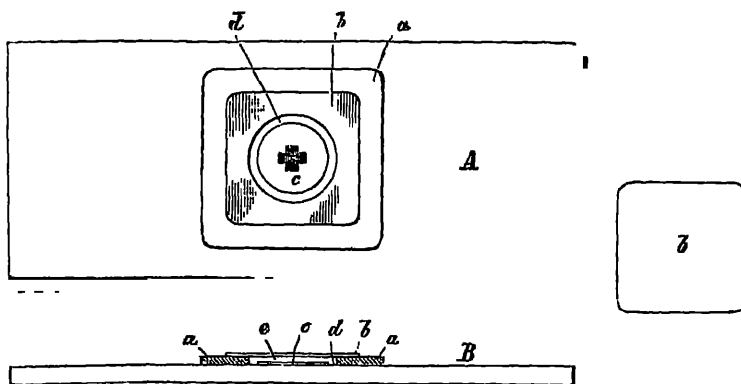
FIG. 32.—Counting Chamber   Nat size   Description in text

will kill the organisms. This dilution must be taken into account in calculating the germ content from the numbers found in the counting, so that M must be multipled by 8000 instead of 4000

Assuming the germ content to have been ascertained in the way described above in the bacterial mixture, the different species in which are being isolated, then, the number of cells present per unit of space being known, a portion of the sample—but not that diluted with acid—must be thinned down with sterilised water to such an extent that only one cell is present in two to five drops. One drop of this diluted liquid is then placed in each of a series of flasks containing a sterile nutrient medium, which flasks are subsequently kept at a suitable temperature, whereupon some of them will, after a while, exhibit signs of development, these will constitute the wished-for pure cultures. They are, however, not unconditionally reliable, since it not infrequently afterwards becomes evident that, in despite of calculation, some of the flasks contained more than one germ. By this method, generally known as the **dilution method**, Lister in 1878 prepared a pure culture of *Bacterium lactis*, which was (chronologically) the first bacterial pure culture, and FRITZ (VII.) also employed the same method in his studies on fermentation by fission fungi   The first six species of the *Saccharomycetes* studied by E Oh. Hansen, and which stand out so prominently in the literature of fermentation physiology, were also isolated by

I   G

the aid of an improved form of the dilution method, further mention of which will be made in the second volume.

The so-called fractional method of culture, as it was afterwards styled by Klebs, was employed in particular in Pasteur's experiments on fermentation. It consists in taking from a sample of fermenting liquid that has attained its maximum of development a small aliquot portion and transferring this to a new, sterile medium. By recalling the remarks made in the paragraphs of Section II. dealing with symbiosis, it will be understood that, at the period of highest fermentation in a natural liquid—and therefore one rich in different species—that species which is the cause of the fermentation in question will preponderate. Therefore, if merely a single droplet thereof be placed in a medium analogous in composition to the original habitat, this species will be favourably situated from the outset, and will increase at a relatively quicker rate than its associates By repeating this transference (" re-inoculation ") several times over, cultures will finally be obtained wherein impurities, i e. extraneous species, can only be detected by more searching methods of separation, such as are described in the next paragraph.

These subjugated species will, however, come to the front again if the (apparently pure) bacterial culture be inoculated in a *different* medium forming a favourable environment for their development. Mention of this has already been made in a previous section, when referring to the older evolutionary labours of Lister, Lankester, Hallier, Billroth, and others. We are now in possession of another more convenient method for the purposes of pure cultivation, which will be described in the succeeding paragraph, and consequently a criticism of the dilution method can be omitted.

At present, only a couple of words will be devoted, as supplementary to the remarks already made, to the examination of brewery water for the presence of dangerous organisms. For the brewer those water bacteria alone are important that develop in wort and beer and are capable of producing injurious changes therein. Consequently, sterilised samples of both these liquids are employed in the biological analysis of brewing water The method employed was first proposed by E Oh. Hansen (IV.), according to whom fifty small Freudenreich flasks are used, twenty-five of them being charged with 15 c.c of sterilised wort apiece and the remainder with a similar quantity of sterilised beer. In each of the first fifteen flasks in both series is placed one drop (0.04–0 05 c c), and in each of the remaining flasks 0 25 c.c., of the water to be tested These fifty flasks are then kept at a temperature of $24°$–$25°$ C. for fourteen days, and are examined to ascertain how many become turbid or throw up a skin, i e. exhibit signs of the development of organisms. The ratio of the number of flasks with turbid contents to the total number is referred to 1 c.c of water, and a standard for determining the destructive capacity of the sample in question is thus obtained. Assuming that, for instance, three out of the fifteen wort-flasks (inoculated with 0 04 c c.) exhibit turbidity, then three growths have proceeded from 15×0.04 c.c., or five from 1 c c of the water Or, on the other hand, suppose that of the beer-flasks only one has become turbid, and that this is one inoculated with 0 25 c.c. In this case, then, there is but one growth per $10 \times 0 25 = 2 5$ c c.

H. Wichmann (II) attempted to add, as a co-factor influencing the conclusion arrived at, the length of time required for the turbidity to develop; of this fuller particulars will be found in the reference just given. Hansen showed that a large number of species of water-bacteria are incapable of developing in the two solutions last named, and this is particularly the case with beer, the flasks charged therewith seldom becoming turbid after inoculation with water. J. Oh. Holm (I) has, for several consecutive years, regularly examined the well-

water and main-water of the breweries at Alt-Carlsberg, near Copenhagen, by this method, and found that the spores of mould-fungi are comparatively the most frequent, cells of bacteria capable of thriving in wort and beer being less general, and yeast cells very rare. If the water be used merely for malting and mashing purposes, its germs are unimportant, being, as we have already seen, unable to withstand boiling in the hop-copper There is, however, one unavoidable opportunity afforded for the contact of the beer with the water in its unchanged condition, and that is in the washing out of the storage casks and of the trade casks in which the beer is sent out to customers In Germany these casks, being lined with pitch, will not stand cleaning with hot water or steam, and are cleaned with cold water, a small quantity of which is always left behind in the casks; so that, if this water be rich in organisms injurious to beer, serious inconveniences may arise. Dr. Will has reported to the author an instance coming under his knowledge where the beer from a brewery was constantly so turbid that no customers would take it. After prolonged investigation the cause was eventually discovered in the well-water, used for swilling out the casks, which was found to be rich in the organisms producing turbidity in beer Subsequent examination showed that the well was connected with the drains by means of fissures in the soil.

§ 84.—Liquefiable Solid Media.

If a species is represented in a bacterial mixture by a few individuals only, its isolation by the dilution method requires an inconveniently large number of culture vessels In order to overcome this difficulty (with others that need not now be touched upon), Robert Koch, utilising a method practised by Schroeter, devised a new method of separation, generally termed plate-culture. The essential part of the method consists in the addition of a gelatinising substance to the nutrient solution, whereby the latter acquires the property of becoming liquid at a moderate warmth but is solid at room-temperature The medium thus liquefied is inoculated with a little of the bacterial mixture to be separated, and, after being well shaken up, is poured, whilst still fluid, on to sterilised glass plates, on which it sets as a thin film. In this film (under favourable conditions) each one of the cells inoculated therein is held fast and isolated from the others, and can subsequently multiply, undisturbed, into an aggregation of similar cells known as a colony.

Gelatin is the substance most frequently used for this purpose, and nutrient media containing it are called by the generic name of nutrient gelatin, a distinction being drawn between wort gelatin, meat-juice gelatin, must gelatin, &c., according to the kind of nutrient solution used. The amount of gelatin added is about 9 or 10 per cent., and this produces a medium that is liquid above 30° C and solid below 24° C., so that inoculation can be conveniently performed at 35° C., a temperature exerting no injurious influence on organisms Bouillon gelatin, often called peptonised bouillon gelatin, is prepared by making up the meat extract—prepared as already described—to its former volume, i.e 1 litre, with distilled water, after boiling, filtering, and mixing it in a glass flask with 1 per cent. of peptone, 0.5 per cent. of NaCl, and 10 per cent. of gelatin. The flask is carefully warmed in the water-bath or steamer until the gelatin liquefies, and the liquid is then neutralised in the manner prescribed for bouillon. It is next boiled in the steamer for half an hour and filtered hot through a moist folded filter, to remove the precipitated albuminoid matters and those thrown down in neutralising. Samples that clarify badly are improved by egg-albumen, since the filtrate has to be perfectly clear and transparent. The liquid is filled whilst warm and fluid into vessels for use (e g test-tubes holding 5-8 c c.), and

sterilised by intermittent heat, being left for twenty to thirty minutes in the steamer on three consecutive days, as explained in the preceding chapter In bacteriological treatises frequent mention is made of "nutrient gelatin" pure and simple without any qualifying term , in such cases the peptonised bouillon gelatin referred to above is always meant. The tubes spoken of in the colloquial language of the bacteriological laboratory as "gelatin tubes" are ordinary test-tubes containing 5 to 10 c c of nutrient gelatin. The preparation of **wort gelatin** is very simple, the unhopped or hopped wort (according to the purpose it is intended for) being mixed with 10 per cent. of gelatin, melted, boiled for half an hour in the steamer, filtered hot, filled into vessels, and sterilised by the intermittent process **Must gelatin** requires a little care in preparation, the high acidity (— 0.7 to 1 0 per cent of tartaric acid) of the must having to be previously almost exactly neutralised by caustic potash, since otherwise the setting power of the gelatin is impaired After the acid has been neutralised, 10 per cent of gelatin is added, liquefied, cooled down to between 30° and 40° C., and mixed with the white of an egg beaten up to a froth ; then boiled for half an hour in the steamer, filtered off, filled into the recipients and sterilised as pre-scribed. During storage, numerous crystalline concretions (up to the size of millet seed) of potassium tartrate separate out in the solid medium, and by presenting the appearance of colonies, give rise to the supposition that the medium has been imperfectly sterilised Attention is therefore now called to this phenomenon.

As already stated, nutrient gelatin liquefies above 30° C., and therefore also at the usual temperature prevalent in the incubator, viz., 38°–39° C , on which account it is unsuitable for use in the separation of such organisms as require higher temperatures for their development. In such cases a medium tempered not with gelatin, but with agar-agar, is used This substance, obtained from Eastern Asia, and fully described in a treatise by N K Schultz (I), is a dried vegetable jelly prepared from various marine algæ and put on the market in the form of thin strips or as a powder Its manipulation being more conveniently effected in the latter condition, the use of agar-agar powder is recommended as preferable. In French literature this gelatin is generally known as "*gélose.*" For use, not more than 2 parts of agar-agar per 100 of nutrient solution should be taken. It dissolves very slowly and with great difficulty. For the prepara-tion of **peptonised bouillon agar-agar** Hans Buchner recommends the following process The meat-broth (bouillon), prepared in the usual manner, is qualified with peptone, NaCl, and 1 or 2 per cent. of agar-agar, and boiled under pressure at about 105° C., neutralised after cooling down to 100° C. ; then boiled up again, filtered hot, and filled into vessels for use. It is sterilised by exposure to 120° C., under pressure, for a quarter of an hour. The agar-agar media do not readily adhere to the glass walls of the vessels, a circumstance which in many operations may be very troublesome, but may be obviated if the adherent properties be increased by adding to the agar-agar employed (1 to 2 per cent) the same amount of gelatin or gum For the study of the lactic acid bacteria of the distillery, which thrive best at about 48° to 50° C , a 1 per cent. unhopped wort agar-agar medium containing 2 per cent. of gelatin is used. Moreover, these agar-agar media do not lose their power of solidification if stored for a long time at 100° to 120° C They are liquefied only at temperatures exceeding 40° C , and since this last-named temperature is for many organisms the highest supportable maximum, the agar-agar is used in the following way when designed for the separation of a bacterial mixture The recipient tubes are immersed in boiling water to induce liquefaction of the contents, which are then cooled down to 40° C. (at which temperature they are still just fluid), inoculated quickly, shaken up and mixed thoroughly, and poured out on to the aforesaid glass plates, which rest on a support warmed to 40° C

For pure cultures at temperatures above 50° C agar-agar cannot be used, since it then begins to soften For such (rare) cases, Miquel, when experimenting with *Bacillus thermophilus*, replaced agar-agar in the nutrient solution by 2.5–3.0 per cent. of **Caragheen moss (Irish moss)**, from *Chondrus crispus* —For special purposes suitable indicators are also added to the nutrient media. For example, if it is desired to separate merely the acid-forming species from a bacterial mixture, then a little litmus is added to the medium before sterilising , the colonies of acid-forming bacteria in the subsequent plate culture will then become surrounded by a red halo standing out conspicuously against the blue background. For the same purpose BEYERINCK (V) recommended an addition of fine levigated chalk, which forms an opaque **chalk nutrient medium**, becoming, however, clear at the parts of the plate culture occupied by acid-forming bacteria, in consequence of their solvent action on the calcium carbonate.

Certain organisms, such, for example, as the nitrifying bacteria, do not thrive in the solidified nutrient media hitherto described. Therefore, in order to prepare cultures of the same by the aid of the plate method, recourse is had to the medium prepared from precipitated silica, proposed by W KÜHNE (I). Silica precipitated from water-glass (alkali silicate) and carefully purified will, when used as a 3 4 per cent aqueous solution, set within an hour to a firm mass if mixed with 0.25 per cent. of NaCl. The salt is added to a sterilised solution which also contains the other requisite nutrient substances. In this solution is distributed a small portion of the bacterial sample to be separated, the cells of which will, when the medium has set, be fixed and develop into colonies Further particulars concerning the preparation of this **silica medium** will be found in the above-mentioned treatise, as also in one by Winogradsky which will be referred to later

The numerous nutrient media employed in practical mycology are more fully described in the handbooks of Hueppe, Eisenberg, Tiemann-Gartner, and Bernheim, but only one need be briefly noticed, viz., the potatoes employed for the so-called **potato cultures** The potatoes—the better sorts used (in Germany) for salad-making—after being carefully cleaned externally, are steeped for an hour in a 1 *per mil* solution of sublimate, then swilled with water and sterilised in a wire basket by two hours' exposure in a current of steam. When this is effected, and they are so far re-cooled as to be fit for handling (with disinfected fingers), they are cut into halves by a sterilised knife and placed under a sterilised bell-glass. When cold, inoculating streaks are drawn on the cut surfaces, and subsequently develop into **potato cultures**.

§ 85.—Koch's Plate Cultures

are, as previously indicated, prepared by pouring out the liquefied and inoculated medium (*e.g.* 5–8 c c. in a test-tube) on to colourless glass plates, rectangular in form, about one-twelfth of an inch thick, 5 to 6 inches long, and $3\frac{1}{2}$ to 4 inches broad, previously sterilised in batches in a copper or iron box, from which they are taken as required. The plates are laid on a plate-pouring apparatus arranged horizontally —as described and shown in the above-named handbooks— and the distribution of the stratum of gelatin or agar-agar is assisted by the aid of the rim of the test-tube To sterilise the latter, it should be held for a short time in the Bunsen flame and allowed to re-cool sufficiently before proceeding to pour. When the gelatin layer is set the plate is transferred to a sterile damp chamber, which is placed in the thermostat and maintained at the constant temperature required.

These plates are rather inconvenient to handle, since, in following up the development of the growing colonies the plate must be frequently taken out of the chamber During each observation the mould spores in the air are liable to fall

upon the medium, where they rapidly develop into such masses of branched threads that the bacterial colonies are smothered, thus rendering all the care bestowed upon the preparation of no avail. In order to prevent this, the flat plates are replaced by double shallow glass dishes, in which the cultures can be examined under low powers without being exposed to the air. These dishes were first introduced into bacteriology by Salomonsen, but are generally known in Germany as **Petri dishes**, this latter worker having been the first to test them. Their use for this purpose can be recommended.—Instead of pouring out the inoculated gelatin, the closed tube can be held almost horizontally under the stream from the water-tap and slowly turned round on its axis, whereby the contents are distributed uniformly over the walls, and will set as a thin stratum wherein the germs then develop into colonies. These cultures are generally called **Esmarch tubes or roll cultures**, and were first proposed by W. Hesse.

Plate cultivation affords useful assistance, not only for the separation of a bacterial mixture into its several species, but also for the determination of the number of cells present therein, a few gelatin tubes being charged with various quantities of the sample and poured on to plates. This method is of particular importance in the **quantitative bacteriological analysis of water**, for which reference should be made to Tiemann-Gartner's handbook. The counting of the colonies grown on the plates is effected by the aid of special counting apparatus, that of Wolffhugel being used for the Koch plates. For counting the colonies on gelatin plates in Petri dishes the author, in 1893, constructed a cheap counting-plate, obtainable from F. Mollenkopf, of Stuttgart (10 Thor Strasse). The number of germs thus found is always smaller than the living cells actually present in the inoculating mixture, since only such as have developed into colonies are enumerated, whereas a number of germs in the original have failed to develop under the conditions prevailing, owing to the medium being unsuitable for some, and the temperature of the incubator, though favourable to the majority, being too hot or too cold for a minority. The medium relatively most suitable for the purpose of ascertaining the number of germs is, in most cases, gelatinised meat-juice, and this is therefore the one most frequently used. Considerable influence on the number of developing germs is exerted by the degree of **alkalinity** of the medium, a fact first conclusively demonstrated by A. REINSCH (II) and confirmed by MAX DAHMEN (I.). If it is a question not of ascertaining, as nearly as possible, the *total* germ content of a sample, but only how many of the cells are capable of development in a given medium, then the latter is arranged in a solidified condition as a plate culture. For example, wort gelatine is generally used—unless the contrary be expressly stated—when determining the number of germs in brewery water. It is important to know for certain whether the colonies in a plate culture are each developed from a single cell, since it is only in such cases that a pure culture can be obtained on re-inoculation. This aim is attempted by thin sowing and thoroughly shaking the liquefied medium, in order to separate the cells from each other. Nevertheless, there is always some uncertainty, which we must endeavour to remove by discarding the first series of plates and by preparing a second series wherein any impurities may become manifest, then, if the colonies are found to stand this test, the reinoculations therefrom may be considered as pure cultures. This can be ensured from the outset if the growth of the colonies, *i.e* from the single cells, be followed by the aid of the microscope from the beginning. This test is, however, feasible only with large cells (*Eumycetes* spores, yeast cells, &c), and will be enlarged upon in a subsequent chapter in connection with the pure culture of yeast. On the other hand, it is, as a rule, impracticable for bacteria, since their examination necessitates the use of such a high (short focus) objective that the latter has to be brought so near the plate as to impinge on the gelatin stratum.

Pure cultivation with solidified media is almost exclusively applicable to fungi alone, its application to other groups having been successful in the case of a few only of the lowest unicellular algæ. In this manner BEYERINCK (VI.) prepared pure cultures of the last-named organisms (frequently found in the microscopic examination of river-water), viz, *Chlorella vulgaris, Scenedesmus acutus, Chlorosphæra limicola, Chlorococcum (Cystococcus) humicola, Stichococcus major*, and a second species of Chlorella. WILHELM KRÜGER (I.) prepared by the plate method pure cultures of two algæ, which he named *Chlorella protothecoides* and *Chlorothecium saccharophilum*, from the sap of the lesser maple. Quite recently BEYERINCK (VII.), A ŒLLI (I), FR SCHARDINGER (I.) and others, have also successfully attempted the cultivation of *Amœba* in the same manner

- The solid nutrient media in question are, except those containing chalk, transparent, so that the plates prepared therefrom can be laid on the stage of the microscope and their colonies examined under a low power by transmitted light. The differences thereby observable form valuable indications for the identification of the individual species. A number of them, *Bacillus subtilis* for example, excrete a peptonising enzyme, in consequence of which the gelatin is liquefied as far as the solvent enzyme proceeding from the colonies is able to diffuse, and thus a liquefactive colony is obtained. The converse was supplied by such organisms as produce no enzyme capable of dissolving gelatin, and which therefore do not liquefy the medium, but grow as solid colonies. The development of these latter may proceed in various ways, the colonies of *Bacterium aceti*, for instance, gradually assuming a stellar form, whilst those of the lactic acid bacteria have a circular outline *Bacillus ramosus*—a fission fungus frequently occurring in soil and in natural waters, and generally known as wurzel (root) bacillus—which M. WARD (IV) subjected to exhaustive morphological and physiological examination, grows on agar-agar to colonies built up of entangled, branched, and plaited threads resembling the roots of a tree.—A comprehensive description of these characteristics, as presented by the separate species of bacteria, will be found in Eisenberg's work.

If a platinum wire, previously heated to redness and then dipped in a bacterial culture, be thrust into a solid medium contained in a test-tube, the cells so implanted in the passage formed by the wire will develop to a so-called puncture culture, the appearance of which also affords valuable indications for the recognition of individual species. Organisms requiring air grow only on the surface, whereas those shunning the air will develop only in the deepest part of the channel, and those dissolving the gelatin will form a liquefied funnel. This latter indication is one developed in a highly characteristic manner by the cholera bacillus, and is, therefore, made use of in the bacteriological analysis of water. If a test-tube containing about 8 or 10 c c. of liquefied nutrient gelatin or agar-agar be held at a sharp angle, the contents will set in the form of a wedge, and if the plane surface be stroked over with a small quantity of a culture, then a so-called streak culture will be developed. This also in many cases assumes a characteristic form that should be taken into consideration in the identification of a species. The potato cultures already referred to are nothing more than streak cultures on the cut surfaces of steamed potatoes. We are indebted to C FRAENKEL and R PFEIFFER (I) for an excellent atlas of photographs of the colonies, streak cultures, cover-glass preparations, &c, of a number of (mostly pathogenic) bacteria. An atlas of coloured plates of these objects has been issued by K B. LEHMANN and R NEUMANN (I.)

It may be valuable, for purposes of instruction, to preserve in the cultures the appearance they present at the time of their most vigorous growth According to G HAUSER (II.), the preservation of cultures on gelatin or agar-agar can be most conveniently ensured by means of formaldehyde Cultures in

test-tubes can be treated by moistening the cotton plug with formalin and the covering it with a rubber cap to prevent desiccation. Plates and cultures in Petri dishes can be kept for some time by covering them with filter-paper moistened with the same antiseptic, which kills the cultures without destroying their form. It also penetrates the medium, hardens the gelatin, and makes 1 unsuitable for the further development of organisms, so that the preparation thus treated are exceedingly durable. Fuller information on the preservation of pure cultures of fermentative organisms, and practical hints concerning the arrangement of mycological museums, which are very useful both for teaching and for reference purposes, will be found in the treatises prepared by J. Soyk (I.), F Král (I.), H. Plaut (I.), E. Czaplewski (I.), and E. Krückmann (I.)

The necessity will not infrequently arise for reliable (living) pure culture of authoritatively named species of bacteria, &c., either for use as a starting point for study or for comparing, and, as far as possible, identifying some newly-discovered species Král's Bacteriological Laboratory (11 Kleiner Ring Prague I.) can be recommended as a source from whence to obtain them. The institution supplies living pure cultures, streak-cultures on oblique solidified agar-agar, in test-tubes at the moderate price of one to two marks (= shillings per tube.

Koch's plates can also be used with advantage when it is a question o ascertaining which nutrient media are suitable for a given microbe. For the purpose Beyerinck (VIII.) has devised a method which he calls **Auxanography** A 10 per cent. gelatin or 2 per cent agar-agar in distilled water is prepared both of which substances in the *pure* state form very bad media, whether fo bacteria or higher fungi Plate cultures of the micro-organism whose nutritiv requirements form the object of the investigation are then prepared These, 1 left to themselves, will not exhibit any appreciable degree of development, s the surface of the plates is stippled with a few drops of aqueous solutions of th substances whose nutrient properties are to be tested. These drops are absorbe by the gelatin or agar-agar, and form circular fields of diffusion around th spots in question. The thickly sown cells of the species under examination wi then develop into strong colonies on those spots only where the requisite nutrien materials are encountered, so that the organisms inscribe, as it were, with thei bodies, the answer to the question propounded as to the suitability of th nutrient substances at hand. Such a plate of colonies grown in this manner 1 called by Beyerinck an **Auxanogram.** This method may also be employed fo testing the toxic action of various substances on given organisms. Beyerinc (IX) also employed this process as a basis for the qualitative and quantitativ method of microbiological analysis proposed by him in the reference just given

SECTION IV.

CHROMOGENIC, PHOTOGENIC, AND THERMOGENIC BACTERIA.

CHAPTER XII.

CHROMOPAROUS BACTERIA, PRODUCING RED AND YELLOW COLOURING MATTERS

§ 86.—Coloured and Colouring Bacteria.

IN classifying the chromogenic (colour-producing) bacteria, the situation, as well as the nature, of the colour has an importance that cannot be disregarded. An examination for this first-named characteristic in individual species quickly leads to the differentiation of the chromogenic bacteria into coloured bacteria on the one hand and colouring bacteria on the other, the cells in the latter being themselves quite colourless, but excreting a coloured transformation product these species have been designated **chromoparous** by Beyerinck

In the coloured bacteria, on the contrary, the colouring matter remains within the cells This group may be divided into two sub-groups, the one comprising those coloured bacteria the colouring matter of which performs an important physiological function, as in the case of the purple bacteria treated of in the following chapter; such bacteria are termed **chromophorous**. On the other hand, the second sub-group includes those coloured bacteria in which the colouring matter has no such function, and must be regarded as a purely passive metabolic product, which is, nevertheless, not excreted (as in the chromoparous species), but remains *within* the cell without manifesting any apparent activity These bacteria are termed **parachromophorous**.

The chemical properties of the bacterial colouring matters and their importance for distinguishing one species from another were discussed by PAUl SCHNEIDER (I.) after exhaustive experiments with thirty different species. His results in this connection may be thus summarised (1) The bacterial colouring matters can to some extent be differentiated by their behaviour towards solvents (2) A given species, grown under identical conditions, always produces the same colouring matter. (3) Two species, differing as regards form and conditions of growth, may in certain cases produce the same colouring matter (4) Most of the species apparently producing the same colouring matter, and also analogous in other respects, can be differentiated by the reactions of their colouring matter.

§ 87.—Micrococcus Prodigiosus.

This being the oldest known chromogenic bacterium, will be dealt with first Many a victim of the proceedings taken against witchcraft during bygon centuries must have been consigned to the stake on the charge of having fabri cated the blood-red spots that were occasionally found developed on the Host

and which filled the credulous mind of the masses with horror ; and even in 18
the entire province of Padua was set in a commotion by the frequent appearan
of such spots and drops on various articles of food. This red, slimy coati
was examined by SETTE (I.), who recognised it as endowed with vitality a
named it *Zoogalactina imetrofa* A small quantity applied to still unalter
food-stuffs, &c., sufficed to produce red spots on these latter. This phenomen
was first more closely investigated in 1848, when it was of frequent occurrer
in Berlin. Ch. Ehrenberg studied the spots and drops, and found them to cons
of minute oval cells 0.5 to 1.0 μ in length ; and bearing in mind their form a
observed powers of locomotion, he classified this wonderful organism as a n
species of his genus *Monas*, and called it *Monas prodigiosa*, a designation sub
quently changed by Cohn to *Micrococcus prodigiosus*. As this microbe (mos
appearing as approximately spherical cells) will, under certain conditions of envir
ment, assume an elongated form, it is also frequently named both *Bacteria
prodigiosum* and *Bacillus prodigiosus*, as was done by Flügge in his handbo
These names, therefore, indicate one and the same species of fission fungus, a
are also synonymous with the older names *Palmella prodigiosa* and *Bacteridv
prodigiosum*.

This fission fungus excretes a peptonising enzyme, and consequently lique
the gelatin medium. A temperature of $25°$ C. is the most favourable one
its **growth**, and it thrives most luxuriantly on boiled potatoes, the formation
trimethylamine becoming at the same time apparent. Starch paste, boiled r
boiled egg-albumen, boiled carrots, boiled meat, milk, and many other food-stu
form suitable media for this microbe, which, however, will not develop on r
potatoes, raw meat, or uncooked steeped rice. It is therefore evidently a t
saprophyte, occurring only in defunct or destroyed and converted nutrient mec
When cultivated in thinly fluid solutions it exhibits--as was established
SCHOTTELIUS (I.)—brisk powers of locomotion.

The red **colouring matter**, which is produced in presence of air only,
according to the researches of this observer, at first diffusely distributed throu
the young cells. It is then excreted, and collects into various-sized granu
which lie between the cells and so impart a red coloration to the culture   ʻ
tone of the colour changes with the age of the culture, beginning as a pale r
and passing through a bright scarlet stage into dark brown-red   Accordin
the researches of J. SCHROETER (I.) and SCHEURLEN (III.), the colouring ma
is insoluble in water, but readily soluble in alcohol, xylene, chloroform, car
bisulphide, and, to a slighter extent, also in ether and in fats (*e.g.* olive
The alcoholic solution exhibits in the spectroscope three absorption bands
beyond D, the second just before E, and the third before F. The element
formula was determined by A. B. GRIFFITHS (I) as $C_{38}H_{60}NO_{6}$, though
analytical results obtained by Scheurlen are not in conformity therewith.
opinion expressed by O. ERDMANN (I) that the colouring matter generatec
M. prodigiosus is identical with fuchsine has been contradicted by C
HELM (I.) and BORDONI-UFFREDUZZI (I.).

This fission fungus forms a very suitable object for the study of mutabil
E. WASSERZUG (I.) traced the changes of form which this species underw
in consequence of alterations of the conditions of nutrition By repes
cultivation on faintly acid media—0 3 to 0 4 grm. of tartaric acid per litr
cultures are obtained the cells of which are no longer globular or oval,
exhibit the form of actively motile long rods and threads ; the modificat
being the more pronounced as the number of inoculations is increased As s
however, as an inoculation is made from such acid liquids into an alka
medium, the typical short cells reappear. This reversion also occurs when
cells remain for some time in the original acid medium, after the reaction

become alkaline from the transformation products (trimethylamine, &c) excreted by the microbe.

In addition to the form of the cells, the development of colouring matter is, as Schottelius has found, also dependent on the nutritive conditions; since, if a *prodigiosus* culture, grown at $10°-25°$ C and already red in colour, be inoculated on sterile potatoes (steamed and cut in halves), and the temperature kept at $38°-39°$ C., the inoculating streaks develop into *colourless* streak cultures. From these again a red culture can be once more obtained by suitably modifying the conditions of the culture, *i.e.* reverting to a lower temperature

§ 88.—Lipochromes.

With the organism mentioned in the last paragraph are classified a number of other species also producing red colouring matter. One of these, *Bacillus erythrosporus*, first discovered by ED EIDAM (I.) in putrefying egg-albumen, is of particular interest. This is a slender motile bacillus, which does not liquefy gelatin As is indicated by its second name, the seat of the dirty red colouring matter is not in the vegetative form of growth, but in the endospores. The so-called (motile) "*Kiel bacillus*," found by Breuning in the Bay of Kiel, occurs generally as long rods (0 8 μ broad, by 2.5–5 μ long), which liquefy gelatin. There is great similarity in the red colouring matter produced by this bacillus and *Micrococcus prodigiosus*, but the former microbe is distinguished by its greater susceptibility to direct sunlight, which, according to the researches of E. LAURENT (I.) permanently destroys its chromogenic power. A similar effect is produced by the presence of carbohydrates in the medium, the *Kiel bacillus*, in such event, elaborating no colouring matter. The following chromoparous red species will only be briefly alluded to *Bacillus ruber*, discovered by Frank and described by COHN (II), *Bacillus indicus*, discovered in the contents of the stomach of an East Indian ape, the *Bacillus granulatus* of Babes, *Bacillus corallinus*, isolated by O SLATER (I) from atmospheric dust, and the *Bacillus rubellus*, discovered by OKADA (I.), which forms endospores and thereby assumes the clostridium form

Greater interest attaches to several red and yellow species studied by ZOPF (IV.), and especially as regards their colouring matters, which were named by him lipochromes or fat-colouring matters. These are excreted from the cells and collect between them to form dendritic crystalline aggregations, which are luminous in the darkened field of the polariscope. The lipochromes known at present are red and yellow, the former being styled **liporhodine** and the latter **lipoxanthine** The reagent for these is concentrated sulphuric acid, whereby they are converted into deep blue acicular crystals of **lipocyanine**, which remain isolated when derived from **lipoxanthine**, but arrange themselves in characteristic groups when produced from **liporhodine**. Illustrations of these will be found in OVERBECK's (I.) work on this subject. These colouring matters can be extracted from the cultures by means of ethyl alcohol, in which they are just as soluble as in methyl alcohol, chloroform, carbon bisulphide, and benzene On evaporating the solvent, a fatty mass, furnishing the acrolein reaction, remains behind This being saponified and salted out with a hot solution of sodium chloride, the liquid underlying the soapy layer will contain the colouring matter, which can then be extracted by shaking up with petroleum spirit and examined spectroscopically. Of these species the following were more closely examined by ZOPF (V.) *Micrococcus rhodochrous*, isolated from the contents of a goose's stomach, is about 0 9 μ in diameter, and will grow on nutrient gelatin, potato discs, &c , to form deep red masses. The absorption spectrum of the liporhodine extracted therefrom shows an absorption band in F. The *Micrococcus erythromyxa*, obtained from

the town-water of Halle, has a diameter of 1.0–1 2 μ, and its colonies on the nutrient media referred to resemble in appearance those of the first-named species. In addition to liporhodine, however, it produces a yellow colouring matter, soluble in water, but of unknown nature.

Antithetical to these two red-producing species are *Bacterium egregium*, obtained from atmospheric dust, then *Bacterium chrysogloia*, and finally the *Staphylococcus pyogenes aureus*, already several times referred to in the previous chapter. These three develop into yellow cultures producing lipoxanthine, the absorption spectrum of which consists of two bands, one near F and the other between F and G

§ 89.—Red Coloration in Milk

may be due to various causes, one of them being an admixture of blood derived from a broken blood-vessel in the udder. In this case the coloration of the liquid is not uniform, but is due to scattered patches of red, flocculent blood coagulum. If, on the other hand, the milk is found to be uniformly coloured red or reddish throughout the entire mass as soon as it is drawn from the udder then other causes are in operation. If the colour undergoes no change on standing, it is attributable to the fodder having contained a large quantity of madder (*Rubia tinctorum*) or of *Galium verum* Should, however, the red-drawn milk precipitate a red sediment on standing, the colour of the liquid concurrently decreasing, then the coloration is due to a transudation of hæmatin. This case is analogous to that of " red water," and is a consequence of the consumption of highly stimulating fodder.

When, however, a normal milk *gradually becomes red after standing*, the micro-organisms are at work (*Micrococcus prodigiosus* being frequently the agent and the colouring matter excreted by the organism is absorbed by the f globules of the milk This microbe has already been more minutely characterise in the preceding paragraph.

A second milk-reddening organism is the *Bacillus lactis erythrogenes*, d covered by Hueppe and more closely examined by GROTENFELT (I) The no motile rods of this organism are o 3–0.5 μ in diameter, with, usually, a length 1–1 5 μ, but attaining to 4.3 μ in bouillon This fission fungus (which liquefi gelatin) grows on solid media to yellow colonies, which excrete around the periphery a red colouring matter. When inoculated into sterile milk it produces a gradual coagulation attended with a sickly-sweet odour, without affecting the reaction of the liquid to any appreciable extent. The serum gradually clarifying from the deposited casein absorbs the resulting deep red colouring matter which develops most copiously in the dark and ceases to form if the culture exposed to light or the medium has an acid reaction. Its absorption spectrum exhibits two strong bands between the lines D and E and a third in the bl A species of fission fungus allied to *B. lactis erythrogenes* has been isolated from red milk by A. BAGINSKY (I).

Of the sarcina group two species are known to be endowed with the facu of reddening milk One of these was discovered by K. MENGE (II.), *Sarcina rosea* Menge , and the second was isolated from red milk by L. ADAM (II), and was identified with the *Sarcina rosea* Schroeter, very frequently with in the air Apart from the red coloration, Menge's sarcina produ no noteworthy changes in milk, but Schroeter's sarcina, on the other hand, precipitates the casein which is subsequently gradually re-dissolved Con quently a milk-culture (growing dark brown in colour) kept at 25° C for to five weeks no longer exhibits any deposit beyond a sediment consistin sarcina packets.

Red coloration in cheese may arise from various causes, those of a pu

chemical nature being merely referred to here for the purpose of differentiation According to the researches of H. BURSTERT and F. J. HERZ (I.), a number of rhodanate (thiocyanate) compounds develop in ripening curd, and when the ripe cheese is cut the iron compounds therein oxidise and combine with the thiocyanates, the resulting ferric thiocyanate being in sufficient quantity to impart a red or reddish tinge to the cut surface   A sample of cheese thus reddened is decolorised by immersion in a solution of oxalic acid, so that this reagent should be employed in doubtful cases.   If, on the other hand, micro-organisms are at work, the result is different   This disagreeable phenomenon is more rarely encountered in hard than in soft cheeses   Red spots gradually form on the surface and spread by degrees, but do not penetrate more than a few millimetres into the substance of the cheese   The spots may be composed of red *Eumycetes* or of pigment bacteria.   ADAMETZ (III) isolated two species of micrococcus from red cheese, both of which develop a red colouring matter in milk and cheese ; but more frequently *Eumycetes, i e* higher fungi, are the cause of this phenomenon in cheese   These will be discussed in a subsequent section

The cause of the reddening of dried codfish (stock-fish) was investigated by LE DANTEC (I).   About one-third of this article put on the market is said to be affected by this evil, and is thereby rendered unsaleable, not only by reason of its salmon colour, but also because of a popular belief that reddened stock-fish is poisonous   The annual loss thus entailed is estimated at ten millions of francs (£400,000). Le Dantec isolated three organisms from such spoiled fish ; the first being a sporogenic red-producing bacillus, morphologically similar to tetanus bacillus, and liquefying gelatin , secondly, a coccus of 3–5 μ diameter, developing on gelatin to solid red colonies, but not producing a red colouring matter in the fish unless accompanied by a second species of coccus, which, by itself, is incapable of developing colouring matter.   In order to render the reddened fish fit for sale again, it is brushed in cold water and re-dried   In America borax is added to the salt used in curing the fish, in order to prevent the development of the evil ; and an addition of from 10 to 15 per cent of sodium bisulphite or potassium nitrate (saltpetre) is also said to be efficacious.

§ 90.—Bacteria Producing Yellow Colouring Matters.

These were first studied by C J. FUCHS (I.) in 1841, the starting-point of his researches being the so-called yellow milk, i e milk that on standing develops a pale- to orange- yellow coloration.

The cause of this appearance was traced to a microbe named *Vibrio synxanthus* by Ch. Ehrenberg, and afterwards known also as *Vibrio xanthogenus* and *Bacterium synxanthum*.   The same phenomenon was also investigated by J. SCHROETER (I) in 1870 , according to whom it occurs in boiled milk only, inoculations from a slightly yellowed milk into normal unboiled milk being unsuccessful   The activity of lactic acid bacteria was presumably the cause of this prevention   On inoculation with this microbe, boiled milk coagulated after twenty-four hours, and the yellow coloration made its appearance after the lapse of a second period of equal duration.   Thereafter the precipitated coagulum gradually disappeared and became re-dissolved, so that in six days the milk had become converted into a citron-yellow, watery, strongly alkaline liquid containing merely a few particles of casein in suspension.   The pigment is insoluble in alcohol or ether, but soluble in water ; it is unchanged by alkalies, but acids combine with it to form a colourless compound   The absorption spectrum is devoid of characteristic bands, and merely exhibits a darkening of the rays on either side of the yellow. Schroeter proposed the name of *Bacterium xanthinum* for the microbe investigated by him, which designation was converted in later

text-books to *Bacillus synxanthus*. It is desirable that the study of the organisms of yellow milk should be taken up anew, since Schroeter's investigations were not performed upon pure cultures in the present acceptance of the term.

With the species hitherto described (*Bacterium egregium*, *B. chrysogloia*, *Staphylococcus pyogenes aureus*, *B. synxanthum*) there can be associated a number of others, equally characterised by the faculty of producing yellow colouring matters. Of these, mention will here be made merely of the *Micrococcus ochroleucus*, discovered by O. PROVE (I) in human urine in a state of incipient decomposition. When kept in the dark this microbe develops into colourless cultures, but if exposed to diffuse daylight or the sun's rays it elaborates a sulphur-yellow colouring matter. This may, therefore, be regarded as a probable means of protection.

Many species of bacteria producing orange-yellow or orange-red colouring matters are met with in air, water, and soil. Since they are of no particular importance, it will be sufficient to merely mention a couple of them, viz., the *Micrococcus aurantiacus* (= *Bacteridium aurantiacum*), described by Cohn and Schroeter, and the *Bacillus aurantiacus*, discovered by Frankland

The group of globular *Schizomycetes* to which the generic name of *Sarcina* has been applied is rich in species producing red or yellow colouring matters

A few examples of the former having been already given in previous paragraphs, it will now be sufficient to consider merely those developing into yellow colonies. The first observation of any sarcina species whatsoever was made in connection with a species of this group. JOHN GOODSIR (I) in 1842 discovered, in the vomit of a patient suffering from a diseased stomach, colonies of microbes resembling bales of merchandise in form, to which he applied the name *Sarcina ventriculi* (Fig. 33) His opinion that the microbe was a vegetable organism led to a controversy only terminated in 1847 by VIRCHOW (I.), who agreed with his English colleague. *Sarcina ventriculi* produces, however, but a faint yellow colouring matter. The cells of the *Sarcina flava*, described by De Bary, which measure 1–2 μ in diameter, produce a yellow colouring matter, and this species is distinguished from the *Sarcina lutea*, discovered by Schroeter, by its power of liquefying gelatin. Paul Lindner prepared pure cultures of *Sarcina aurantiaca* from Berlin "Weissbier" (white beer), the colouring matter of which organism is, according to the researches of H VON SCHRÖTTER (I), allied to lipoxanthine. No sarcina producing blue, violet, or green colouring matters are as yet known

FIG 33—Sarcina ventriculi
From the contents of a diseased stomach *a–d.* various stages of development.
(*After Zopf*)

CHAPTER XIII.

PURPLE BACTERIA AND THEIR BEHAVIOUR TOWARDS LIGHT.

§ 91.—Their Morphology.

WHEN in September 1826 the "Versammlung Deutscher Naturforscher und Aerzte" (Association of German Naturalists and Physicians), founded by Lorenz Oken in 1822, was held in Jena, Ch. Ehrenberg was among those present   In the course of a stroll to the neighbouring village of Ziegenhain he observed in a pool in the brook below the church a number of large red patches, about a hand's-breadth across.   These he found to be composed of enormous swarms of a unicellular cylindrical organism provided with a single cilium and measuring 10–15 μ long by 5 μ broad, which he named *Monas Okenii*.   Later on, PERTY (I.) arranged this species, along with other similar ones, into a new genus, *Chroma-*

FIG 34
Chromatium Okenii
Magn 600   (*After Cohn*)

FIG 35
Monas Warmingii
Magn 600   (*After Cohn*)

tium, and the Monas in question then received the name *Chromatium Okenii*, which it still retains.   This organism is shown in Fig 34

The main reason for considering this organism here, separately from the other red bacteria previously noticed, is on account of its physiological action rather than its morphological character, which action places this bacterium (and many other similar species) in quite a distinct category from all other pigment bacteria, and, in fact, all other bacteria whatsoever.   Even in 1875 COHN (II) expressed a doubt as to whether the ciliated *Chromatium Okenii* and allied forms could be classed as bacteria at all, since it was at that time assumed that the latter organisms were not endowed with special organs of locomotion.   However, as explained in chapter iii , the improvements made in the methods of optical and micro-chemical examination have led to this opinion being modified.

The chief characteristic of the *Chromatia* and their congeners is their behaviour towards light , but before considering this more closely we will throw a glance over the multitude of organisms now in question, with some of which we have already had an opportunity of becoming acquainted   Thus we know from § 68 that Lankester studied the ciliated red monads and classified them all as special forms of growth of a single species ("*Bacterium rubescens*").   This unproved pleomorphism we declined to recognise at the time, but must now revert

to it in order to append the remark that, though we are not indebted to the last
named worker for any revelations regarding the morphology of these organisms
we have to thank him for an exhaustive study of their colouring matter, which
he termed bacterio-purpurin   A form similar to *Chromatium Okenii* was dis
covered by E WARMING (I) on the coast of Seeland and afterwards examined
by Cohn, and by him entitled *Monas Warmingii* (Fig. 35), so that Perty's pro-

position with respect to the generic name *Chromatium* seems not to have
been accepted by the Breslau bacteriologist   Whilst the species hitherto
mentioned differ among themselves in point of size, but not appreciably in
form, and all more or less correspond with the plump, short rods shown in
the drawing, a second group of similar species exhibits the **spirillum** form of
growth   One example of this is afforded by *Spirillum rubrum* (Fig. VI
Plate I), a second being the *Spirillum volutans*, shown in Fig. 36, and a third
the *Ophidomonas* (Fig. 37), described by Ehrenberg. A third sub-group finally
comprises organisms of elongated spindle form, and therefore resembling a
whetstone in outline, e g. *Rhabdomonas rosea* (Fig. 38)—4–5 μ wide and
20–30 μ long—first observed and described by Cohn. These organisms are not
infrequently to be found in ponds and lakes; sometimes being so abundant
as to *colour the water red*. A series of observations respecting such occurrences
was made by CHARLES MORREN (I).
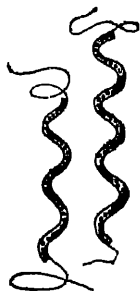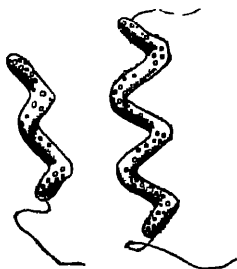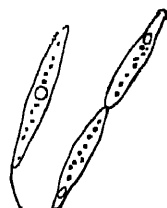
§ 92.—Influence of the Individual Colours of the Spectrum.

The necessity for a separate consideration of these red species, grouped
together by ENGELMANN (V.) as *purple bacteria*, is soon apparent when an
attempt is made to study their physiology. The bacteria described in the
previous chapter, of which *Micrococcus prodigiosus* may serve as an example
also produce red colouring matter , but, in their case, the latter is a mere iner
waste product, appearing under certain conditions, or absent under others
without the growth of the cell being thereby seriously affected   Contrary to
this, the purple bacteria do not excrete any colouring matter into the environ
ment, but the pigment occurs exclusively within the cells (Fig 39) in the par
of the contents which immediately adjoins the cell-wall, and which is described
in chapter ii as the parietal layer   The bacterio-purpurin is not always distri
buted throughout the whole of this layer, but is frequently restricted to isolated
spots therein, and, in exceptional instances, is altogether absent. It is no
present in any definite shape, such as granules, bands, or plates, like the chloro
phyll of the higher plants, but occurs diffused in the plasma.

It has already been stated in chapter iii. that the purple bacteria exhibit great avidity for light, and therefore always strive towards the sunlight. Closer observation shows this behaviour to be intimately connected with the presence of bacterio-purpurin. It was a happy thought on the part of Engelmann to investigate the nature and extent of the influence exerted on the vitality of the purple bacteria by the several colours of the spectrum.

If a preparation rich in such organisms be placed in a drop of water, and a micro-spectrum of a few millimetres in length be projected thereon, a rapid movement towards certain parts of the spectrum will be observed under the microscope, the organisms collecting and resting there in micro-scopically visible bands. By quickly killing the cells they will remain in position and constitute a permanent preparation, or, as Engelmann appro-priately named it, **bacteriospectrogram**. This, when submitted to examination, is found to correspond, with the absorption spectrum of bacterio-purpurin, showing a sharply defined band in the ultra-red (wave length $\lambda = 0\ 9$ to $0\ 8\ \mu$), a second, less power-ful, small band in the orange ($\lambda = 0\ 61$ to $0\ 58\ \mu$), and, finally, a pale washed-out band in the green ($\lambda = 0.55$ to $0.52\ \mu$). Engelmann then determined by an accurate quantitative photometric examina-

FIG 39 —Chromatium Okenii

Optical longitudinal section

The cell-wall (the thickness of which is somewhat exaggerated in the figure) immediately adjoins the outer layer of protoplasm, which carries the colouring matter—shown as fine dots   Large sul-phur granules are present in the interior of the cell   Magn about 1600   (After F. Forster)

tion, with the aid of the bolometric method, that a remarkable ratio prevails between the intensity of the physiological action and the extent of the absorp-tion, i.e the attractive force of a given colour of the spectrum is greater in proportion as the latter is retained by the colouring matter   From this is deducible the further conclusion, that the purple bacteria have great need, not merely of light in general, but of certain components thereof in particular, and especially those corresponding to the lines A_0, D, E, of the spectrum

§ 93.—Assimilation and Oxygen Elimination.

This behaviour of the purple bacteria, unique in the bacterial kingdom, reminds one so much of the connection between light and the activity of chloro-phyll in the higher plants, that not only does the question naturally arise as to the nature of the reactions occurring under the influence of the selected rays, but also the idea that here likewise there is assimilation accompanied by the elimination of oxygen. This is, in fact, the case, the purple bacteria excreting oxygen in the presence of light.

ENGELMANN (VI.) proved this in a variety of ways. One of his experiments, which demonstrates it in a very elegant manner, is based upon that already given in § 41.   Quiescent forms, resembling a zoogloea, of one or other of these species are employed, a portion about 2 sq mm in size being placed in a drop of water   To this are added a number of aerobic organisms (e.g. *Spirillum tenue, Sp undula*, various infusoria, &c), capable of reacting on even small quantities of oxygen, and the cover-glass is surrounded with vaseline to prevent the admission of air. The oxygen dissolved in the water is very quickly exhausted, and the organisms come to a standstill   If, now, the preparation be illuminated, it will be seen directly that the wanderers scattered about rise and

I

hasten to the red skin where the oxygen they need is produced. On the other hand, when replaced in the dark, they disperse again in all directions. That oxygen is the attraction is demonstrated by the circumstance that the phenomenon just described does not occur when the experiment is performed on a preparation the cover-glass of which is not made air-tight, and to which air can consequently penetrate by diffusion.

This peculiarity gives the purple bacteria a unique position amongst the *Schizomycetes* as a connecting link between them and the green plants. From its capacity of converting the actual energy of light into potential chemical energy, and of changing vibrations of light into force, bacterio-purpurin deserves the title of a true chromophyll, since it plays in the purple bacteria the same *rôle* as chlorophyll does in green plants. These two substances are antithetical, accomplishing similar tasks by different methods of working. On more closely examining the individual spectral colours for their power of eliminating oxygen, this latter faculty is found to be proportional to the absorptive capacity of bacterio-purpurin for the colour in question. The maximum effect is produced in the case of the aforesaid ultra-red rays ($\lambda = 0 \ 8$–$0.9 \ \mu$), whilst, on the other hand, the rays between the lines B and C are inert. With chlorophyll the converse is the case, this being quite inactive in the ultra-red rays, and exerting its greatest effect in the red rays (between B and C)

The aforesaid ultra-red rays ($\lambda = 0.8$ to $0 \ 9 \ \mu$) are well known under the name of "invisible heat rays," being inappreciable to the eye as light The discovery that they are the rays that not only enable the purple bacteria to exhibit activity, but also spur the latter on to their highest degree of efficiency, allows the wider conclusion to be drawn that the elimination of oxygen through the activity of the vegetable cell *is not dependent on the co-operation* of visible *light rays*, but may also proceed *in the dark.*

So far for the facts, but looking beyond them, it may be asked if the faculty of absorption in the dark is inseparably connected with the presence of bacterio-purpurin, or if there are also colourless bacteria similarly endowed The answer to this query will be found in chapter xxxvi., which treats of nitro-bacteria. This is a matter of such great interest as to deserve special consideration; at present, therefore, we will merely review the facts hitherto discovered in the case of the purple bacteria, their general importance being so great that we shall certainly not regret having bestowed attention on these organisms, notwithstanding that they are devoid of technical application. The scientific harvest they are capable of yielding is, however, still far from being exhausted. It will be remembered that it is in the outer layer of the inner substance of the cell of these red organisms that this very influential bacterio-purpurin has its abode, and that the central substance is surrounded by this layer. The study of this from a morphological standpoint by Bütschli led to the conclusions respecting the structure of bacterial plasma already recorded in an earlier section. The investigation of the physiology of this central substance has yielded a second series of weighty results, which will be given in chapter xxxv.

CHAPTER XIV.

CHROMOPAROUS BACTERIA, PRODUCING BLUE, GREEN, AND VIOLET COLOURING MATTERS.

§ 94.—Blue Coloration of Milk

is a phenomenon known from time immemorial. The opinions as to its cause are widely divergent, but the earliest of them are at present only of historical interest. They may be found in MARTINY's (I.) useful handbook, which (it may be parenthetically observed) affords a rich supply of information respecting the literature published on milk, butter, and cheese up to the year 1871

The first to arrive at the opinion that the blue coloration of milk might proceed from some external infection penetrating into the liquid, was STEINHOF (I.) who, however, made no attempt to prove by experiment the correctness of his hypothesis. This was only effected three years later, viz., in 1841, by O. J. FUCHS (I), who inferred from the results obtained during numerous inoculation experiments and much microsopic research that the blue coloration of milk is induced by the development of a pigment microbe, which he first named *Vibrio cyanogenus* and then *Bacterium syncyaneum*.

Unfortunately, however, for the study of this question, Liebig just at this time promulgated his theory of fermentation, and fetterad philosophers in his dogmatic shackles. This explains why HAUBNER (I) lost sight of the object for which Fuchs had striven, and, by endeavouring in 1852 to adapt the result of his numerous and careful experiments on this point to preconceived opinion, came to the conclusion that the blue coloration of milk is not caused by vibrions, but by a lifeless chemical ferment contained in the decomposing casein If, then, this work is mentioned now, it is not for the purpose of controverting its untenable conclusions, but because it contains an instructive description of the development of the milk disease in question. It runs verbally as follows

"Under ordinary domestic conditions blue milk occurs only in the warm season, and persists from early summer to autumn. In small households where the milk is not kept in a separate chamber, but in warmer apartments (living rooms), the evil may be prolonged through the winter A case of this kind is recorded by Steinhof as lasting for twelve years, without interruption, in the house of a farmer. The blue coloration appears from twenty-four to seventy-two hours after the milk is drawn from the cow, the process being accelerated by warm, close weather, and retarded by cold. At a temperature of $15°-20°$ R ($18\frac{1}{2}°-25°$ C.), it may ensue in twenty hours—the shortest limit of time observed by H —whilst, at only a few degrees above zero, it may linger on to the seventh day. The coloration always begins to form at the surface, never in the depths of the liquid, and generally appears at first as isolated patches or dots, immovable, and increasing in circumference and depth, so that there are various stages, ranging from single superficial patches to almost complete impregnation of the mass with blue colour."

F. NEELSEN (I.), in 1880, took up the work commenced by Fuchs. Convenient and reliable methods of pure cultivation were, however, lacking at that time, and, in fact, the cultures prepared by Neelsen, when subsequently examined analytically

at the laboratory of the Berlin State Board of Health, were found to consist of a mixture of four species of bacteria, only one of which proved capable of developing blue coloration in milk. This one was named by HUEPPE (IV.) *Bacillus lactis cyanogenus*, and consists of actively motile, spore-producing rods, o. 3-o 5 μ broad, 1-4 μ long, such as are shown photographically in Fig. III. of Plate I They are non-liquefactive towards gelatin, and are extremely sensitive to higher degrees of acidity in the medium, a circumstance explaining the facts (noted by Haubner) that sour milk does not turn blue, and that blue patches produced in sweet milk cease to spread when the latter turns sour.

This fission fungus is highly aerobic, and consequently requires oxygen as an essential factor for its development. In order that this need may be supplied the organism always grows on the surface of the liquid, so that the colour is produced in that situation solely, and only becomes disseminated through the bulk of the milk by diffusion.

This microbe grows not only on milk (and equally well on human milk as on that of the cow, ewe, goat, mare, ass, and dog), but also on many other media. On several of these (*e g.* almond milk, boiled rice, boiled potatoes, vegetable casein, Cohn's nutrient solution qualified with ammonium lactate) it also develops colouring matter which, on the other hand, is not formed in cultures on animal albumen (egg-albumen, blood serum), gum, and a few other media In artificial inoculations the period of incubation, *i.e.*, the time elapsing between the inoculation and the visible appearance of the blue coloration, is found to be about twenty hours, but, as Haubner ascertained, depends on the prevailing temperature. Milk with a tendency to this disease transmits the property to the butter prepared from it.

The colouring matter is not stored up within the cell, but is merely produced there and excreted into the surrounding medium; *Bacillus lactis cyanogenus* is therefore chromoparous. The constitution of the colouring matter has not yet been determined with accuracy, but O. ERDMANN (I.) was led by his comparative experiments to consider it as one of the aniline group, viz., triphenylrosaniline. Neelsen's endeavours to prepare it in a pure condition were frustrated by reason of its instability. Dilute solutions of acetic acid, hydrochloric acid, phosphoric acid, sulphuric acid or nitric acid produce no noticeable alteration. Ammonia gives rise to a violet tint, whilst under the influence of the hydroxide or the carbonate of potassium or sodium, conversion into a beautiful rose red occurs, the original colour being restored by acidification. Frequently the low degree of acidity of the milk, under which condition the bacillus is still able to develop, is insufficient to enable the colouring matter to assume a deep blue tint. The colour of the surface of the liquid is merely greyish-blue, and only becomes a pure full blue when the lactic acid bacteria come into action and raise the acidity to a sufficient degree. The colour shade in individual instances may exhibit an intermediate tint between a delicate light blue and the deepest indigo. According to Neelsen, the absorption spectrum of this colouring matter, which consists of the strong lines E and F and of a broad band in the yellow, is almost identical with that of triphenylrosaniline. From the researches of C. GESSAR (I) and K THUMM (I), it appears that *Bacillus cyanogenus* also produces, in addition to the blue colouring matter, a yellow fluorescent substance. L HEI (III.) and P. BEHR (I.) have given an account of a variety of this bacillus that had lost its faculty of producing colouring matter in nutrient gelatin nutrient agar-agar, and skim milk W. ZANGEMEISTER (I) found on milk that turned blue spontaneously a fission fungus (*B cyaneo-fluorescens*) allied to *Bacillus cyanogenus*, further particulars of the properties of which will be found in the treatise referred to.

Bacillus cyanogenus is not injurious to health. The poisonous properti

attributed to blue milk by early observers—Steinhof and Mosler—have been controverted by Haubner, therefore, if illness has actually ensued on the consumption of such milk, the fission fungus in question was not to blame. Harmlessness apart, blue milk is not a merchantable article, and its sale should be prohibited, since its appearance is, to put it mildly, unappetising.

Since the causes of this blue coloration in milk have become known, its occurrence has, as a rule, been very limited, and when it is observed, many ways of combating it are employed, chief among them being scrupulous cleanliness in all appliances and utensils with which the milk comes into contact. The dairy or milk-room is then thoroughly sulphured several times, and, finally, a little salt or Glauber salt (sodium sulphate) is added to the cows' dietary. This last named remedy must, however, be employed with discretion, since, under certain circumstances, it may prejudicially affect the health of the animals and the composition of the milk, as was ascertained by E. HESS, F. SCHAFFER, and M. LANG (I). The milkers' hands and the cows' udders should be carefully washed before milking. The sudden appearance of the evil, and its frequent disappearance after a change of fodder, permit the conclusion that the bacillus occurs not infrequently on certain vegetables, from which it finds its way into the dung of the animal and thence into the milk.

As has already been stated, the blue coloration of milk by bacterial agency is effected gradually when the liquid is left to stand, and first makes its appearance on the surface. If, on the other hand, the milk is of a bluish or blue colour when freshly drawn, this results from the cow having partaken freely of the flowering rush (*Butomus umbellatus*), which contains a blue colouring matter (indigotin ?) that is taken up by the gastric juices, conveyed in an unaltered condition into the arterial circulation, and thus finds its way into the milk.

§ 95.—Blue Coloration in Cheese.

This is an evil to which the Dutch dairy industry is particularly liable. Since it very often makes its appearance only after the ripening stage is over—that is to say, at a time when the article has already passed into the hands of the salesman—it is the cause not only of monetary loss to the cheese-maker, but also of unwelcome complaints on the part of the purchaser. The phenomenon manifests itself in various forms. Either the whole bulk of the cheese is of a bluish cast, or exhibits blue patches internally, or, finally, is interspersed with blue spots (*Dutch*, "stipjes") from 1 to 2 mm ($\frac{1}{16}$ to $\frac{1}{12}$ inch) in diameter, the latter form (blue grain. *Dutch*, "blauwstippigkeit") being the most common.

The causes of blue cheese are twofold, one of them being chemical, and due to iron sulphide, as was demonstrated by M SCHMOEGER (I.) and TH KLARVERWEIDEN (I). Normal cheese contains but a very small quantity of iron, Cheddar, for instance, having o oo9 per cent., Gouda, o o11 per cent, and so on. If, however, a larger quantity of iron obtains admittance to the milk or freshly precipitated curd, it will then gradually, during the ripening process, enter into combination with the sulphuretted hydrogen separated from the albumen by bacterial action, and will form iron sulphide, which, as is well known, exhibits a blue shade when in a dilute condition. When the iron has been admitted in the soluble form, then cheeses coloured a fairly uniform blue or bluish shade throughout will result. If, on the other hand, the metal be present in coarser particles, e g in the form of rust, then a patchy-blue or blue-grained cheese will be obtained. This explains the more frequent occurrence of the phenomenon since the introduction of the centrifugal machine into the dairy industry, the numerous rivets of the iron cylinder of this machine being, as demonstrated by Schmoeger, so many sources of contamination of the milk by rust. Careful

tinning of the interior of the cylinder is therefore advisable, and should be renewed in good time.   One observation made by Klarverweiden still remains to be mentioned, viz., that the frequency of the occurrence of blue cheeses in Holland is coincident in point of time (August and September) with the highest percentages of the iron bacterium, *Crenothrix Kuhniana*, in natural waters.

Blue cheese may, however, also result from the activity of micro-organisms. H. DE VRIES (I.), in 1887, asserted that a microscopical examination showed that the blue dots in question ought to be considered as colonies of a blue pigment bacterium; but he made no attempt to prepare cultures thereof.   The subject of his investigations was Edam cheese, a kind very frequently affected by blue grain.   BEYERINCK (X.) identified, as the cause of this disease, a fission fungus which he named *Bacillus cyaneo-fuscus*.   However, before proceeding to the consideration of the properties and functions of this organism, we must devote a little attention to the microscopy of cheese.

The structure of sound cheese, as exemplified by the appearance of thin sections immersed in water under the microscope, is made up of the following elements.   The ground mass or matrix consists of amorphous casein enclosing small drops of fat and bubbles of gas, which are, however, for the most part, not spherical, but irregular in outline, owing to the pressure of the enveloping curd. Along with these two enclosures—readily detectable by their optical and micro-chemical behaviour—a closer examination will reveal crystalline spheroids of a substance allied to or identical with tyrosine; also yeast cells, and, finally, a large number of bacteria.   The crystalline spheroids are oval or kidney-shaped, and about as large as big yeast cells (some 10 μ long by 8 μ broad).   Each of these crystalline aggregations exhibits (like starch granules) a central nuclear spot, around which the crystal needles are arranged radially, and constitute in their totality a crystalline spheroid.   A thin section cut from a blue patch or speck of a blue cheese reveals, in addition to these normal constituents, sundry granules of colouring matter, mostly bluish-black, but frequently brownish in colour, and forming the actual pigmentary substance   The crystalline spheroids are also highly coloured.   Bacteria will be found more plentifully assembled in the centre of the blue patch or dot than in any other position.   The (usually globular) colour granules, the diameter of which varies from 1 to 5 μ, are an excretory product of the *Bacillus cyaneo-fuscus*, which can be isolated from the blue patches

The dimensions of this motile microbe vary according to the conditions of nutrition.   On nutrient gelatin the cells 'grow to a length of 1.0–1.5 μ by 0 2–0 3 μ in breadth; whereas in a solution of 0 5 per cent. of peptone in ditch-water they only develop to 0 3–0 6 μ by 0 15 μ.   If we follow up the alterations gradually occurring in a culture of this fission fungus in the latter medium at 10° C , we find that after four or five days the liquid, which has hitherto been pale yellow, assumes a more and more decided greenish cast.   A sample taken from the bacterial skin covering the surface discloses two constituents a number of colourless rods connected to form bands, and, secondly, lying between them, the blue pigment granules, almost globular in form and 1.5–3 5 μ in diameter The colour of the liquid then successively changes by degrees (beginning at the surface) into brownish-grey, brown-black, and finally into persistent yellow, the conversion being effected by the oxidising influence of the air, which slowly decomposes the blue colouring matter and forms brown intermediate products, all finally oxidised to a colourless compound   The micro-chemical analysis of these pigment granules shows them to consist of a framework of albuminous matter on which the pigment crystals rest, but the constitution of the colouring matter itself has not been established, although Beyerinck, on experimental grounds, considers it to be allied to indigo.   It resists the reducing action of the bacteria in cheese, especially those productive of lactic acid.

Bacillus cyaneo-fuscus is very susceptible both to desiccation—on which account it is not found in atmospheric dust—and high temperatures, as also to acids This explains why the microbe cannot be cultivated from the blue granules in old ripe cheese, since it is no longer alive therein, but has succumbed under the influence of the lactic acid (of which Edam cheese, for example, contains between 1 3 and 1.8 per cent) produced from lactose by the other bacteria in the cheese during the ripening process. Considered from this standpoint, the good result obtained by so-called **ropy whey** (a culture of long thread and lactic acid bacteria, more particularly described in chapter xxix) as a preventive of blue grain becomes obvious. Since, as already stated, oxidising agents destroy the colour, it has been proposed by De Vries to decolorise blue cheese by exposing it for some time to the action of oxygen in a closed vessel

This *Bacillus cyaneo-fuscus* (not infrequently met with in natural waters and soils) is the sole species that as yet has been positively identified as capable of producing blue grain in cheese. It is uncertain whether this faculty is also inherent in other organisms, though Hollman, who, in his " Handboek voor den Kaasmaker " ("Cheesemaker's Handbook"), deals thoroughly with this disease, ascribes the responsibility for its appearance to *Bacillus cyanogenus*—an assumption which, however, according to the inoculation experiments conducted in this connection by Adametz and Beyerinck, is untenable.

For the sake of completeness it should be recorded that, according to Beyerinck's report, *Bacillus cyaneo-fuscus* has been found to make itself unpleasantly apparent in a glue factory by giving rise to black glue, which, by reason not only of its undesirable colour (due to the pigment developed by the microbe), but also of its diminished setting power, was thus seriously depreciated in value and merchantable quality. The source of infection was discovered in a dirty pipe previously used for the conveyance of ditch water, and afterwards for delivering the finished glue into the setting pans. When this pipe was cleansed the evil disappeared entirely

§ 96.—The Fermentation of Indigo.

As is well known, the indigo so highly prized for dyeing, and constituting such an important article of the world's commerce, is obtained from certain species of the Leguminous genus *Indigofera*. The province of Bengal alone produces from *Indigofera tinctoria* over twelve millions of pounds of indigo per annum, the value being about £2,000,000 sterling. Some five hundred thousand workers make a livelihood in that province by the cultivation and treatment of this plant. The colouring matter does not exist ready formed in the plant, but is developed therefrom by the fermentation of a glucoside constituent known as **indican**. The plants are cut down shortly before flowering-time, and are left immersed in a five to eight-fold quantity of water, to undergo a continuous fermentation for eight to fifteen hours at an atmospheric temperature of $25°–35°$ C., the liquid gradually becoming yellow in colour, with an alkaline reaction, and throwing up a blue-violet scum. The most important of the fractional processes constituting this fermentation is the splitting up of the indican into sugar (**indiglucin**) and **indigo white**, which remains in solution in the alkaline liquor. The liquor is then drawn off into another vessel and beaten with rods, whereby numerous and fresh points of attack are presented to the air and the **indigo white** ($C_{16}H_{12}N_2O_2$) is oxidised by atmospheric oxygen into **indigo blue** or **indigotin** ($C_{16}H_{10}N_2O_2$), which is precipitated as an insoluble body and deposited at the bottom of the vessel The sediment is then boiled in a pan, strained through cloths, dried, and brought into commerce in the form of irregular fragments, cubes, balls, &c.

Several noteworthy observations on the organisms taking part in this

fermentation have been recorded by ALVAREZ (I.). The fermentation may be performed on a small scale by comminuting fresh leaves of the indigo plant and leaving them to stand in water, whereupon fermentation, attended with evolution of heat, will ensue in twelve to twenty-four hours, the surface of the liquid becoming covered with a thin blue skin, which, when broken, subsides to the bottom and is succeeded by a new one. Microscopical examination shows that this skin consists of bacteria surrounded by fine blue acicular crystals. A sterilised extract of the leaves, when inoculated with a little of this skin, exhibits normal indigo fermentation, whereas without inoculation it remains unaltered even when air is freely admitted. Indigo fermentation is therefore due to the activity of a fission fungus, the *Bacillus indigogenus*, which is associated in the said skin with other species of bacteria that need not be taken into account here. This bacillus is of variable dimensions, but generally 3 μ long and 1.5 μ broad, and is always surrounded by a gelatinous envelope. Its microscopic appearance is almost the same as that of Friedlander's pneumonia microbe (see Fig. 7), and being motile, it is thereby able to collect at the surface of the liquid, where the desired supply of oxygen is found. It also produces a disengagement of gas, to which the formation of a froth or head on the liquid is attributable. When introduced into the blood of the guinea-pig, *Bacillus indigogenus* proved pathogenic, and therefore belongs to the group of organisms endowed with both zymogenic and pathogenic properties. A second observation made by Alvarez is also worthy of note, viz., that the microbe of pneumonia can set up indigo fermentation, whereas on the other hand many other pathogenic bacteria proved incapable of so doing.

Apparently unacquainted with these results obtained by Alvarez, O. J. VAN LOOKEREN (I. and II.) expressed the opinion that the decomposition of indican is not produced by micro-organisms, but by an enzyme present in the living protoplasm of the leaf-cells. The reasons whereon this hypothesis is based do not, however, carry conviction, since attempts made to isolate the alleged enzyme proved unsuccessful. Apart from this, however, the treatise can be read not without profit, and it also affords several welcome supplementary additions to the bibliographical references collected by G. v. GEORGIEWICZ (l.) in his monograph on indigo.

Alvarez's researches ought to be regarded as a thankworthy preliminary work incentive to further study of the subject, but they do not afford a closer insight into the progression of the fermentation process in question. It is in this case not merely a matter of determining the nature of the ferment or ferments, but rather of the solution of a whole group of problems of both scientific interest and technical importance. Commercial indigo, as is well known, contains, in addition to indigotin, a number of other organic constituents, such, for instance, as the **indigo red** or **indirubin**, isomeric with the blue and soluble in alcohol, also **indigo brown**, obtained by treating the indigo with alkalies, and finally, **indigo gluten**, soluble in dilute acids. The proportion of these subsidiary constituents influencing the shade of the colour is variable in different samples of indigo. An investigation of the conditions affecting their production is a necessary preliminary to the establishment of the most suitable method of fermentation, capable of yielding, on the one hand, the maximum quantity of indigo (in Lookeren's experiments 0.2 per cent of the weight of the plant), and on the other, producing at will any desired variety of the pigment. A very promising field of profitable enterprise is thereby opened up to mycologists residing in the districts where the indigo plant is cultivated. Moreover, it is incumbent on fermentation physiologists in the centres of consumption to study the fermentation of the indigo dyeing-baths—the woad-vat, the so-called potash bath, and, finally, the urine bath—the preparation and employment of which are

carried on according to old-fashioned rules and without any knowledge of the internal reactions occurring therein. One thing is certain : these reductions are not purely chemical operations, but are true fermentations, as already emphasised by A. Fitz (IV.) in 1878. Frequently they proceed in an undesired direction; two such maladies of the indigo bath being destruction of the colour, and blackening. However, as regards these, mycological investigations are still lacking.

§ 97.—Varieties of Bacillus Pyocyaneus.

The number of bacterial species capable of producing blue colouring matters is by no means exhausted by those mentioned in the three preceding paragraphs A fourth, which has been repeatedly referred to in earlier chapters, is the *Bacillus pyocyaneus*, described by O. Gessard (II). This organism, conveyed in atmospheric dust, comes in contact with the pus exuded from wounds, and developing therein, produces, as its name implies a coloration ranging from blue to verdigris green. This microbe, which really belongs to pathological and not to technical mycology, is very mutable—a property which must also be briefly dealt with here In addition to the blue pigment designated **pyocyanine**—which may be separated from the cultures by shaking them up with chloroform, and which was first prepared in the pure crystalline state by Fordos (I) —the parasite in question, when cultivated in bouillon, produces a **green** fluorescent colouring matter By skilfully modifying the conditions of nutrition Gessard (III and IV.) obtained three varieties, morphologically indistinguishable, one of them producing only pyocyanine, the other only the fluorescent pigment. and the third no colouring matter at all. Whilst these ·three varieties can be re-transformed into the typical species, such change cannot be effected with a fourth variety, cultivated by Charrin and Phisalix (I), which had permanently lost its chromogenic faculty. Unaware of this variability, P. Ernst (III.) proposed to distinguish between two species—*Bacillus pyocyaneus* α and β, a course which Gessard considers incorrect In contradiction to the results obtained by the latter worker, and confirmed by Hans Buchner and Rohrer (I.), K. Thumm (I.) asserted that *B pyocyaneus* produces only a single pigment, the blue Undoubtedly this investigator, as a result of the system of cultivation preferred by him, unwittingly deprived the bacillus of its property of elaborating the green fluorescent colouring matter.

During the past two decades there has been isolated from water, air, and soil a number of blue-producing species of **Schizomycetes**, which, however, are of no special importance, and can therefore merely be mentioned here, though fully described in Eisenberg's work. They are *Bacillus janthinus*, obtained by Zopf from the river Panke, Berlin, *Bacillus berolinensis indicus*, by H Claessen (I) from the Spree; *Bacillus lividus*, by Plagge from Berlin town-water, *Bacillus cœruleus*, by Allen J. Smith (I.) from the water of the Schuylkill river It is probable that these species are chromoparous, as was established with certainty in the case of a fission fungus obtained by Voges from natural water in Holstein (and also named *Bacillus cœruleus*), which excretes the blue colouring-matter into the surrounding medium, where it collects into small granules

At the present time a fairly large number of species producing **violet** pigments are known , they are found in the same places as the above-named, and are equally of little practical importance. A few may be cited as examples, the oldest species known being the *Bacteridium violaceum*, observed by Schroeter, and named *Micrococcus violaceus* by Cohn This organism develops on solid media (gelatin, potatoes, &c) to solid-growing violet-blue colonies In contrast to this is *Bacillus violaceus*, which liquefies gelatin and produces a deep violet colour

Bacillus membranaceus amethystinus was discovered by Jolles in Spalato well-water, and produces a dark violet pigment exhibiting a metallic lustre.

§ 98.—Green Bacteria   .

were observed by Schroeter, and COHN (I) named one such species (producing a
sap-green colour) *Micrococcus chlorinus*.   VAN TIEGHEM (III.) introduced into the
literature of the subject two new green non-motile species under the names of
Bacterium viride and *Bacillus virens*.   The *Bacterium chlorinum*, discovered by
ENGELMANN (VII.), is more highly interesting.   It is endowed with powers of
locomotion, and when present in microscopic preparations where there is a lack
of oxygen it strives to reach such spots as are illuminated by white, yellow,
or red light.   The three last-named species are not chromoparous, but chromo-
phorous bacteria.   W. SYMMERS (I) described a *Bacillus viridans*.   Reference may
be made in this place to a treatise on the green bacteria by P. A. DANGEARD (I.).

Green fluorescent transformation products are excreted by very many species
of bacteria, a number of which are described in Eisenberg's work.   At present
mention of three will suffice, viz , the gelatin-liquefying *Bacillus fluorescens lique-
faciens*, and the *Bacillus fluorescens non-liquefaciens*, which grows on the same
medium into solid colonies.   Both organisms occur in natural waters   The
author in 1891 discovered a fission fungus of very frequent occurrence in Munich
butter, and named it *Bacillus butyri fluorescens*.

O. GESSARD (V) recorded a beautiful observation in connection with the
formation of fluorescent pigment by *Bacillus pyocyaneus*.   This substance is—in
the case of nutrient solutions prepared artificially from mineral salts—produced
only when phosphates are present in quantity equivalent to at least 0 25 grm. of
potassium phosphate per litre   When this supply is reduced, the organism
develops, but produces no fluorescence   This behaviour is also exhibited by
other fluorescent bacteria, so that these can therefore be regarded as very delicate
tests for potassium phosphate.

K. THUMM (I) comparatively examined a number of bacterial species
yielding fluorescent cultures, viz., *Bacillus fluorescens tenuis*, *B fl putidus*, *B fl.
albus*, *B. erythrosporus*, *B viridans*, *B pyocyaneus*, *Bacterium syncyaneum*
(*Bacillus syncyaneus*)   These species produce, in alkaline gelatin, a fluorescence
initially sky-blue, but afterwards moss green, caused by an excreted yellow
pigment, which is formed only when the medium contains magnesium sulphate
and potassium phosphate.

The green coloration of cheese is, as first determined by CARLO BESANA (I),
due to the presence of copper, this evil being especially manifested by Lodisan
cheese, the Parmesan cheese produced in Lombardy—*formaggio di grana
lombardo*.   The initially yellow cut surface of this cheese becomes green by
exposure to the air.   For the successful preparation of this cheese a certain
fairly high degree of acidity in the milk—equal, according to J. RAVÀ (I.), to
0.22 per cent of lactic acid—is essential, and therefore it is the custom in
Lombardy to leave the milk to acidify in untinned copper vessels, whereby it
takes up a considerable quantity of copper.   In fact, the progress of the souring
is determined by the gradual disappearance of the metallic lustre from the
previously polished surface of the vessels.   G. MARIANI (I), who examined
twenty-five samples of these cheeses for their content of copper, found the
minimum to be 54 mgrms of Cu per kilogrm. of cheese, and the maximum 215
mgrms., the average per kilo of Lodisan cheese being 100-110 mgrms. of
copper.   That this metal alone is actually responsible for the green coloration of
cheese is evidenced not only by comparative laboratory experiments with tinned
and untinned milk vessels, but also by the fact that the Parmesan cheeses made
south of the river Po (especially in Reggio), and brought to Parma for sale,
undergo no alteration in their yellow colour when cut.   In that region, however,
the milk is left to acidify in wooden tubs.

CHAPTER XV.

PHOTOGENIC BACTERIA.

§ 99.—The Genus Photobacterium.

THE first occasion of witnessing the phenomenon known as marine phosphorescence will never be forgotten by the beholder The boat cleaving the gleaming waves inscribes its track in glittering lines, and every movement of the water causes pronounced phosphorescence. A rain of sparkling drops of light trickles from the poised oar, each apparently becoming the centre of fresh evolutions of light. This phenomenon, the sight of which is calculated to captivate the senses of the coast-dweller, and lead him to forget all trouble for a time, has a counterpart which filled the mind of earlier races of mankind with terror and lent great support to credulity, viz , the phosphorescence of meat, fungi, and decaying wood in forests. It is only in our own day that an insight into the cause of this wonder has been gained, the microscope, in this case also, being the instrument used to open the door to knowledge. To devote a few words to this phenomenon would be in any event justifiable, on account of its general scientific interest; and moreover, the matter cannot be avoided in the present work, since some of the facts established in this connection also deserve a brief consideration, both from a chemical and a technical standpoint.

The first attempt made to investigate this long-known phenomenon in a scientific manner was that of G. FABRIZIO (I) in 1592. The treatises subsequently published thereon (up to 1887) are to be found included in a historical and critical review of the subject written by F LUDWIG (I.) This has been supplemented (up to 1891) by a work issued by O. KATZ (I.) which may be referred to in this place.

We are indebted to Pfluger for the first microscopical examination of this phenomenon. He examined, in 1875, the white mucus covering the surface of fish exhibiting a silvery phosphorescence, and found it composed of globules, frequently united to form chains. When these forms were mixed with water and the mixture passed through a doubled layer of dense filter-paper, the latter became phosphorescent, whilst the filtrate ceased to exhibit this property, thus proving the phosphorescence to be due to the minute organisms themselves These, which were recognised as bacteria, were in 1878 named *Micrococcus phosphoreus* by Cohn. Whereas Pfluger's studies were concerned with the carcases of phosphorescent salt-water fish, the first microscopical examination of the phosphorescent flesh of cattle slaughtered for food was made by Nuesch, who also found a fission fungus, which he named *Bacterium lucens*, to be the cause. Ludwig, in 1882, showed that a transference of the mucus of phosphorescent sea-fish on to sound animal flesh rendered the latter phosphorescent in turn Unaware at the time that the organism had already received two names, he bestowed on it the title of *Micrococcus Pflugeri*. This was the first phosphorescent bacteria obtained (in 1885) as an undoubtedly pure culture. Three years later B. FISCHER (II.) proved the existence of other species, three of which he himself described, one of them (from the West Indian seas) being named *Bacillus phosphorescens*, and the other two (found on German shores)

he called respectively *Bacterium phosphorescens* and "native phosphorescent bacillus "

In 1890 BEYERINCK (XI) proposed the generic name of *Photobacterium* for the entire group of phosphorescent bacteria, and more closely investigated the six undermentioned species:

1. *Photobacterium Pflugeri.*
2. *Ph phosphorescens*
3. *Ph. balticum* = Fischer's " native phosphorescent bacillus."
4. *Ph Fischeri* = *Bact phosphorescens*, F.
5. *Ph. indicum* = *Bacillus phosphorescens*, F
6. *Ph luminosum.*

With these six (motile) species Katz in his above-mentioned treatise associated a second half-dozen species collected on the coast of Australia, and EIJKMANN (I) added a thirteenth (*Photobacterium javanense*), repeatedly found by him on phosphorescent sea-fish in the market at Batavia

§ 100.—The Food Requirements of Phosphorescent Bacteria

formed the subject of comprehensive investigations by Beyerinck, a few of whose multifarious results obtained therefrom may be given here.

A remarkable difference exists between the first four and the last two of the six species named in the foregoing list. The former require at least two organic nutrient materials, the one (supplying nitrogen) being a substance resembling peptone, and the second a compound supplying the carbon · peptones alone, or amides and the like, by themselves producing neither growth nor phosphorescence. *Ph indicum* and *Ph luminosum* behave differently, peptone (or any other albuminoid substance) being of itself sufficient as an organic food-stuff therefor

A slight addition of sugar to the medium increases the luminosity, but a higher percentage arrests both growth and phosphorescence, a circumstance due not to any injurious effect of the carbohydrate itself, but to the acids produced therefrom by the vital activity of the organism, the luminous bacteria thriving solely on neutral or faintly alkaline media Concerning the extent of the injurious content of sugar, and also the varying influence exerted by the different saccharides, Beyerinck arrived at noteworthy conclusions in respect of his six species. Thus, for example, maltose is taken up by *Ph phosphorescens*, but is discarded by *Ph Pflugeri*, *Ph. Fischeri* is very susceptible to cane sugar, a content of o 5 per cent sufficing to retard growth and suppress phosphorescence, whereas, on the other hand, *Ph. balticum* will stand 5 o per cent without injury A similar relation in respect of glucose obtains between *Ph. luminosum* and *Ph indicum*, the luminosity of the former ceasing when the medium contains 1 per cent of this sugar, whilst the latter produces light even in presence of 4 per cent.

The six species of photobacteria examined by Beyerinck are halophil, i e., absolutely require sodium chloride, of which the medium must contain at least 3 5 per cent. Consequently it follows that none of these species is able to thrive on the flesh of land animals slaughtered for human food The luminosity appearing on this latter substance is caused by other species, among them being the above-named *Micrococcus Pflugeri* discovered by Ludwig The presence of oxygen is essential to the production of phosphorescence, but is not requisite for mere growth. For preparing pure cultures a nutrient gelatin made from fish bouillon is employed, and, for cultivation on a large scale, boiled salt-water fish forms an advantageous medium.

§ 101.—The Luminous Bacteria as Tests for Enzymes.

The different behaviour of *Ph phosphorescens* and *Ph. Pflugeri* towards maltose can be utilised when it is desired to ascertain whether this sugar is produced in any diastatic process. For this purpose plate cultures of the two organisms are prepared, a mixture of sea-water with 8 per cent of gelatin, 1 per cent. of peptone, and ¼ per cent. of boiled potato-starch being used. On these cultures are placed small drops of a solution of the substance whose saccharifying properties are to be tested Then, if this elaborates glucose or levulose from the starch, the (thickly sown) plates will shortly become luminous at the points affected, whereas if maltose alone is formed, the culture of *Ph Pflugeri* will remain dark In point of delicacy this reaction has but one compeer, viz , the Bunsen flame reaction, whilst in respect of the duration of the phenomenon it is unequalled. The method may also be used with advantage in the solution of the question whether any given microbe has the power of elaborating an enzyme, and if so, of what nature. Thus, for instance, if *Saccharomyces Kefyr*, a higher fungus occurring in Kephir granules, is to be tested on this point, a plate culture thickly sown with *Ph phosphorescens* is prepared in a medium composed of sea-water, gelatin, and peptone. On several of these non-luminous plates are laid a couple of drops of an aqueous solution of-lactose, on others cane-sugar, and on a third series raffinose, none of which sugars are taken up by the photo-bacterium, and consequently the plates remain dark. The drops are quickly absorbed by the gelatin and formed patches named by Beyerinck **diffusion-fields**, in the vicinity of which inoculating streaks of *Saccharomyces Kefyr* are then drawn, and, on developing, finally enter the field of diffusion After a short time the colonies of *Ph phosphorescens* gradually begin to become luminous at the points where the diffusion-fields are in contact with the *Saccharomycetes* cultures, this luminosity occurring in all three series, and thus proving that *Saccharomyces Kefyr* produces an enzyme (known as **lactase**) which penetrates into the diffusion-fields of the lactose, saccharose, and raffinose and inverts these di- and tri-saccharides to assimilable hexoses, which then cause the photo-bacterium to become luminous

§ 102.—The Phosphorescents.

The question whether the light proceeds from within the organisms, or whether they are in themselves dark, but excrete luminous metabolic products into the surrounding medium, has been much disputed. The latter opinion was upheld, notably by Br. RADZISCEWSKI (l.), according to whose exhaustive researches the aldehydes and aldehyde-ammonia derivatives are, in general, endowed with the faculty of becoming luminous in alkaline solution, and are thereby gradually oxidised by atmospheric oxygen The quantities coming into play per unit ot time are comparatively small , a solution of 1 8 grms of lophin in 25 c c. of caustic potash remaining luminous for over three weeks. If, then, the fact be remembered that the photobacteria are luminous only in alkaline nutrient media, and in presence of compounds which are partly already aldehydes (especially the sugars) and partly exhibit a similar constitution (e g glycerin and asparagin) , and if it be also remembered that the luminosity only occurs in presence of oxygen, and that in the cultures acids, i e. oxidation products, are formed from the said luminous materials, then it will be readily understood why Radziscewski sought the source of this beautiful phenomenon, not in the bacterial cell, but in aldehydic metabolic products, **phosphorescents**, which are oxidised, with evolution of light, outside the organism The same opinion is

held in the main by Quatrefages, Owsjannikow, Ludwig, and Dubois, the observer last mentioned designating the assumed phosphorescents luciferin. The most important effect of the photobacteria, viz., marine phosphorescence, stands, however in the way of the general applicability of this interpretation, the external conditions under which this phenomenon is produced being still unknown. It is consequently uncertain whether the food-stuffs (aldehydes and ammonia bases) necessary for the production of the phosphorescents are at such times exceptionally present in sufficient quantity in the waves.

The spectrum of the bluish-green light emitted by *Micrococcus Pflugeri* is, according to Ludwig, a continuous one, and extends from the line *b* (green) into the violet. Equally continuous and more extensive—occupying the entire breadth from D to G—are the spectra of two European species discovered by B. Fischer, and a luminous bacterium described by J Forster (I). Here the blue and violet rays predominate, and, consequently, these organisms can be photographed by their own light, a result first successfully attained by Forster in conjunction with Van Haren Noman One year later B. Fischer also demonstrated that the light from streak cultures of these microbes is strong enough to illuminate and photograph other adjacent objects, such, for example, as a watch.

Marine phosphorescence can be caused not only by photobacteria, but also by a variety of low forms of animal life. When these latter come into play, then the illumination of the water only becomes well developed provided the latter is in motion and the necessary supply of oxygen is thereby copiously supplied to the photogenic animals. If, on the other hand, photobacteria are in question, the entire surface of the water glistens uniformly and continuously with a soft lustre. Ludwig was the first to successfully produce marine phosphorescence artificially and on a small scale, and the experiment was then repeated on a larger scale by B Fischer in the Berlin Aquarium. The demonstration well repays the slight trouble involved. Cultures of photobacteria are prepared on salt-water fish, on which they grow and form a mucinous coating, which, on being stripped off in salt water and dispersed through it, immediately produces marine phosphorescence capable of prolonged duration.

A beautiful observation made by A Giard (I. and II.) must be recorded here, viz , his cultivation—from the luminous *Talitrus*—of a bacterium which is both pathogenic and luminous, inhabiting the abdominal cavity of the aforesaid aquatic animal, fully permeating all its organs, and, finally, causing its death During the prevalence of this malady the victim shines with a green light, visible nearly a dozen yards off, and persisting for a few hours after the death of the animal This fact, established as it has been by successful inoculation experiments, induces the supposition that the luminosity of other small marine animals (infusoria, polyps, and medusæ) may also be due to photobacterial infection.

CHAPTER XVI.

THERMOGENIC BACTERIA.

§ 103.—Spontaneous Combustion.

ᴑ state of our knowledge on the thermic side of the process of fermentation ᴉ𝒔 not extend beyond a few crude, isolated determinations, so that nearly 𝗿ything in this department has still to be accomplished, even the primary ᵴtion—whether there exist fermentative organisms with purely exothermic 𝟷 others with purely endothermic cell-activity—being as yet unsolved   One ᴆg only has been established for certain, viz, that many microbes under 𝐭ain conditions generate heat and give it off to the environment   Hence the ᴍical changes then occurring are exothermic processes.

An example of this is afforded by the organisms effecting the so-called ᴨtaneous heating of hay and cotton.   For sundry researches hereon we are ₑbted to F Cohn (VlII), from which it appears that fission fungi, allied to hay bacillus already seveial times referred to, are here concerned.   That the ᴛing is actually the result of microbial activity was proved by Haepke, who ₑrtained that sterilised cotton-waste, under otherwise identical conditions, 𝘺 became heated when moistened with washing-water from fresh unsterilised ᵼte.   The heating only occurs in presence of oxygen, and comes to a standstill ₑn this substance is lacking, the action being the result of brisk oxidising ᵼvity (respiration) on the part of the bacteria in question.   The fluffy greasy ᵼte material formed during the cleaning and spinning of raw cotton, consisting ᴄotton fibres, seed capsules, &c., spontaneously rises in temperature up to

O., according to the observations of Haepke, and becomes gradually conᴛed into a humous mass with evolution of the vapours of trimethylamine. 𝒔 observer attributes the fermentation occurring in this case to various ᴄies of micrococcus.

The heating of vegetable matter to high temperatures should not, however, ₐys be ascribed to the action of fission fungi.   For example, the temperature in ly managed heaps of germinating (malting) barley may rise to 60° C. and over, cause of which, accoiding to the researches of Cohn (IX and X.), is a mould, , the *Asp·rgillus fumigatus,* nearly allied to the common mould fungus ᵼt the diastase in the germinating barley is thereby greatly injured is certain. In many instances the spontaneous heating of the aforesaid vegetable ᵼters may develop into **spontaneous combustion,** whereby barns and spinning ks have often been set on fire.   Nothing definite is known of the precise ᴉlitions concerned in this phenomenon.   In the first place, a knowledge of the ᴛing temperature of the various substances under consideration is necessary, as this temperature is probably higher than the maximum heat supportable iving organisms, the actual ignition in such cases cannot be directly attributed ʰeir vital activity.   Hence, the probable explanation of spontaneous ignition ᵼat certain micro-organisms, by their oxidising action, convert the vegetable ᵼs into a humous porous mass, which is then (like finely divided iron, &c.) ᵼble of occluding oxygen, whereby ignition is induced.   More detailed researches this phenomenon are still required

§ 104.—The Spontaneous Heating of Hops

is well known to every brewer from wide personal experience For a more intimate knowledge of the subject we are indebted to J BEHRENS (I), who has not only discovered a cause for the phenomenon, but has also traced a connection between the latter and the well-known presence of trimethylamine in hops, first shown by GRIESSMAYER (I). Behrens found, in hops that had become warm, a fission fungus, in a condition of almost pure culture, which he named *Bacillus lupuliperda*, and explained to be nearly allied to the *Bacillus fluorescens putidus* described by FLÜGGE (I.).   The cells of this newly-discovered motile microbe are about 0.7 μ in breath, and vary in length, according to the conditions of cultivation, from 0.7 to 2 5 μ. It liquefies gelatin. Peptone alone is insufficient for its support, a second substance from which it can derive carbon being required, consequently it belongs to the group of peptone-carbon bacteria established by Beyerinck   It thrives freely in hop extract and quickly renders sugar-free media alkaline by excreting copious quantities of ammonia bases, especially trimethylamine   In presence of sugar the reaction of the medium at first becomes acid, butyric acid being formed, although in Behrens' experiments only to the extent of 0.1 per cent. at most.   This microbe, which gives rise to spontaneous heating in hops, and causes them to give off an odour of trimethylamine, was found by Behrens in all the samples of hops examined by him   It appears to be chiefly domiciled in the soil, and passes thence to the hop cones, which, being fairly hygroscopic, attract moisture when bagged, and thus enable the bacillus to develop, the hops becoming " warm " and commencing to decompose, whereby they are reduced in value. Timely prevention may be ensured by removing the bagging in which the hops are packed, and thus admitting cool and dry air into the interior of the contents, in consequence of which the activity of the microbe is lessened.   Closer studies both on the spontaneous heating of hops, and especially into the transformation products of *Bacillus lupuliperda* and their relation to the production of rancidity in hops, constitute a productive field for future research

The above remarks on the spontaneous heating of stored vegetable substances may now be supplemented by a few observations concerning

§ 105.—The Fermentation of Tobacco,

which cannot well be included in subsequent chapters. The tobacco leaves, when gathered, are allowed to become somewhat withered, and are then arranged in moderate-sized heaps, where they undergo a so-called "sweating." The rise of temperature occurring during this process is, as determined by Muller-Thurgau, a consequence of the activity of the leaf-cells, which transpire their store of carbohydrates and convert their albuminoid matters into amides, the heat thereby liberated effecting the gradual drying of the leaves. The water vapour evolved condenses into the matting employed to cover the heaps, which are then said to " sweat." The alteration of the nitrogenous bodies in the leaves can also be effected by " shed drying " On this point more will be said in a later chapter dealing with *Botrytis cinerea* in the second volume.

As soon as the tobacco leaves have finished sweating and become "shed ripe," they are made to undergo fermentation, for which purpose they are tied in bundles and arranged in great heaps, containing as much as fifty tons of tobacco.   Hereupon active decomposition quickly ensues and the temperature rises. NESSLER (I) found this to be as much as 54° C even on the second day, but, as a rule, the heaps are not allowed to become warmer than 50° C., further

increase being prevented by turning the heap, so that the outer layers of the first heap become the central portion of the new one. This fermentation is due to the action of bacteria, and was studied thoroughly by E. SUCHSLAND (I. and II.), who, however, furnished but scanty reports thereon. A patent was granted to him in connection with the use of pure cultures of bacteria for the purpose of favourably influencing the fermentation of tobacco. He prepared pure cultures of bacteria from fine West Indian tobacco and transferred them to inferior German tobacco in course of fermentation. By this means the flavour of the latter was so greatly improved that it was no longer recognisable as such even by connoisseurs and experienced smokers of native tobacco.

The chemical changes hereby produced have been investigated by J. BEHRENS (II), according to whom the loss of matter amounts to 4 to 5 per cent., and consists principally of soluble carbohydrates and fixed organic acids, the former disappearing almost entirely. Carbonic acid is evolved as the result of these changes, while FESCA and IMAI (I.) ascertained that nitrates are no longer present in the fermented mass. The amount of nicotine is also reduced, only 70 per cent. of that originally present being afterwards found (in one experiment) by Behrens

The flora of the fermenting tobacco heap does not consist solely of bacteria. For example, JOH. BEHRENS (III.) frequently met with the *Aspergillus fumigatus* already mentioned, and Dávalos and Behrens also very often detected *Monilia candida*, remarks on which will be given in a subsequent chapter.

A few researches have been made into the fermentation of snuff, the most important being those carried out by TH. SCHLÖSING (I. and II.) relative to the chemical changes involved With respect to the part played by micro-organisms he expressed himself as follows "The fermentation begins at the ordinary temperature under the predominant influence of micro-organisms, but above a certain (still to be determined) limit, which is over 40° C. and below 70° C., and is probably about 50° C., the changes become purely chemical reactions in which the living organisms have no longer any share." When giving utterance to this opinion Schlösing was unacquainted with the newer researches in connection with the heat-loving organisms which thrive at 70° C. It is therefore desirable that his experiments should be repeated and extended, with this fact borne in mind.

The tobacco subjected to this fermentation is usually moistened with a liquid containing sugar, syrup, honey, and the like, in addition to various aromatics, and not infrequently alcohol as well In many cases this "sauce" also has added to it wine yeasts, particularly for the grades known as St. Omer, St. Vincent, and Paris tobaccos. These additions (found by experience to be partly essential and partly useful) probably furnish the material for, on the one hand, a weak alcoholic fermentation, and, on the other, for the formation of esters. More detailed knowledge is, however, lacking with regard to this primary fermentation, and to the subsequent after-fermentation of the tobacco, either packed tightly into casks or rolled up in "pigtail" form and wrapped in linen cloths. The snuff yielded by the latter method is finer than that obtained by the quicker cask-fermentation process. More definite researches on this subject are highly desirable.

§ 106.—The Preparation of Burnt Hay

must also be briefly described in this place There are two chief methods, differing from one another, for the preservation of green fodder, viz , either by acidifying it, allowing it to ferment, and so producing "sour " fodder or ensilage —for which see also chapter xxvi ; or it is dried, and then forms hay. The

I   I

removal of the water, of which the plants now under consideration contain som
85 per cent , can be effected in two different ways.   Either the necessary hea
is applied from outside, *i.e.* they are exposed to the sun's rays, thus producir
air-dried hay, or else the same result is obtained by spontaneous heating, ar
therefore by the action of the thermogenic micro-organisms dwelling on th
plants   Here again there are two possible methods of procedure, by followir
one of which burnt hay is obtained.   On this subject we are indebted
BÖHMER (I) for a critical research.   According to him, the mown grass is pile
up into heaps about 10 to 13 feet high and 13 to 16 feet in diameter, the ma:
being trodden down as tightly as possible in order to prevent the admissic
of air—which might favour the development of mould—into the interior.   I
these heaps spontaneous heating goes on and becomes apparent, often withi
twelve hours, but generally in twenty-four to thirty hours.   The operation
watched, and as soon as the temperature inside the heap reaches 70° C —whic
is mostly the case in forty-eight to sixty hours—the heaps are opened and th
contents spread out thinly, a single turning being then sufficient to complete th
curing of the hay.   By this fermentation, about which nothing definite is know
from the physiological point of view, not only is the desired degree of drynes
attained, but the hay also becomes friable and acquires an aromatic odour

As mentioned above, the heaps must be opened when the temperature ha
risen to 70° C., since, if this be neglected, the spontaneous heating will quickl
become spontaneous ignition   If, as is possible, it is rainy when the hay
ready for spreading out, such preliminary labour is futile.   To this circun
stance is due the fact that burnt hay is seldom prepared, as also that,
districts where the weather is too uncertain to allow of ordinary haymakin
being conveniently practised, another method of drying has been developed,
which the necessary heat is likewise generated by a process of fermentation, froi
which a product known as " brown hay " is obtained.   This will be dealt with i
the Section on Lactic Fermentation.

SECTION V.

THE HEAT-RESISTING BACTERIA

THEIR PLACE IN NATURE AND THEIR IMPORTANCE IN THE FERMENTATION AND FOOD-STUFF INDUSTRIES.

CHAPTER XVII.

BACILLUS SUBTILIS AND ITS CONGENERS.

§ 107.—Roberts' Heat Method.

THE unfavourable conditions to which the bacteria inhabiting the soil are therein exposed result in the accumulation of such species as are capable of developing reproductive forms endowed with great vitality   These are carried from the surface soil on to plants, and in this way hay becomes infested with
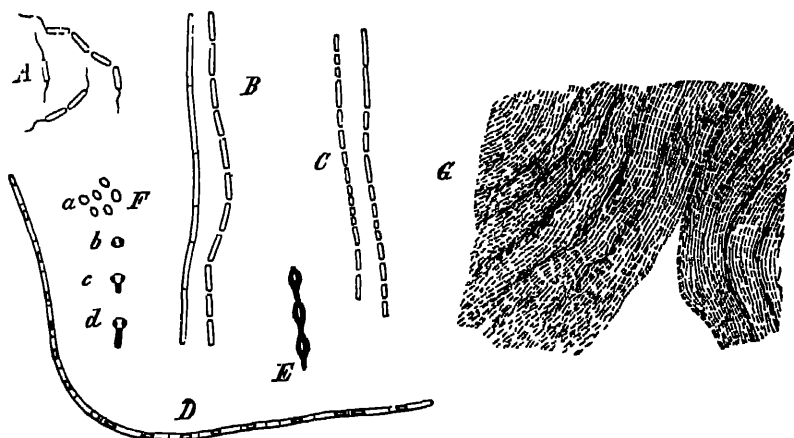
FIG 40—Bacillus subtilis

G, a fragment of the skin formed on hay infusion, magn 200 times   Consists of tightly packed filamentary groups of cells   B and C show the individual parts of these threads at an early stage   D, a thread, each separate part of which contains an oval endospore   E, the mother-cell membrane swells up and the spores are liberated.   F, progress of spore-germination   B–F, magn 600. (*After Brefeld and Zopf*)

the spores of highly resistant *Schizomycetes*, which can withstand the temperature of boiling water for several hours.   Early observers, being unacquainted with this property, noticed with astonishment that a development of bacteria occurred spontaneously in vegetable infusions (especially infusions of hay) that had previously been exposed to boiling heat for an hour   The cells were almost

exclusively in the form of short rods, and were named *Vibrio subtilis* by Ehrenberg and *Bacillus subtilis* by Cohn. To obtain them with certainty the following process, known as the heat method, which was devised by ROBERTS (I.), and already referred to in § 53, is employed Dry hay is chopped up fine, suffused with a little water, and then left to stand four hours, at about 36° C, the usually somewhat acid liquid being afterwards poured off (*without filtering*), exactly neutralised, and diluted until it shows a density of 1 004. This liquid is then boiled gently for an hour over the sand-bath in a flask plugged with cotton-wool. Since the number of heat-resisting spores present on the hay is frequently but small, sufficient hay and water are taken at the outset to yield at least half a litre after an hour's boiling, which quantity will be sure to contain some living germs. The flask is removed from the sand-bath, left to cool down to the temperature of the hand, and then placed in the incubator, the temperature of which is regulated to about 36° C during the ensuing twenty-four hours. At the end of this time there will have appeared on the surface of the infusion a thin skin, which subsequently thickens and develops to a typical zoogloea. A little of this, examined under the microscope, will present the appearance shown at G in Fig 40, viz., a number of closely adjacent rows of short rods.

§ 108.—Morphology of Bacillus subtilis.

That, by the aid of Roberts' method, pure cultures (in the present acceptance of the term) cannot be obtained, need hardly be insisted upon, all that is produced being a culture of heat-resisting bacteria ; hence the "hay bacillus" prepared in this way by different observers will vary Really pure cultures may, however, be obtained therefrom by modern methods of pure cultivation The organism examined and styled *Bacillus subtilis* by Brefeld is not identical with that of the same name described by Prazmowski, though allied thereto, and indeed so closely that the physiologically important phenomena of spore-germination are alike in both kinds. This circumstance has already been fully noticed in § 58, and the reader is therefore referred thereto A few supplemental morphological facts will now be added, the opportunity being also favourable for remarking that a reliable culture of *B. subtilis* can be prepared by mixing crushed malt and rye in a flask with about four times their volume of water, inserting a plug of cotton-wool, boiling up the mixture, and then leaving it to stand at 35°-40° C. A thick, wrinkled skin will rapidly develop on the surface of the liquid, and the contents of the flask acquire a characteristic sickly-sweet odour. At a somewhat earlier stage, before the surface is entirely covered with . , the liquid (which on this account becomes tu id) swarms with numerous actively motile rods. merly, with the defective instruments at comm , only a single cilium could be discerned on each the terminal poles (see A, Fig. 40), but subseque researches established the fact that *Bacillus subtili*, like many other species, is richly endowed with cilia, as may be seen from Fig. 41, which is reproduced 'rom a photograph. The de-velopment of the motu rods into the multicellular

FIG 41 —Bacillus subtilis.
Cilia staining
Magn about 1500 (*After A Fischer*)

chains constituting the skin must be regarded as a transition to the quiescent stage. The formation of this wrinkled cover first becomes noticeable when the nutrient medium is in an advanced state of decomposition. In most of the cells composing the chain there will then be found a firm, brilliant endospore (D, Fig 40) producing an uncommonly beautiful and remarkable appearance.

The walls of the mother-cell swell up, the chain is dissolved, and the endospores thereby liberated. Some facts indicative of their power to resist adverse influences have been given in a previous section (§ 53). The spore membrane is capable of swelling up, so that when the spores are placed in water each of them soon appears to be surrounded by a dull halo—the swollen external layer of the membrane.

§ 109.—Influence of the Mode of Nutrition on the Form of Growth.

This factor was exhaustively described, for the bacillus in question, by HANS BUCHNER (IV.). A few of the forms of growth observed are shown in Fig. 42.

By employing a faintly alkaline 5 per cent. solution of meat extract, rods (as at 1a) are obtained, o 5 μ broad and 6–10 μ long If a neutral solution of 5 per cent. of sugar and o.1 per cent. of meat extract be taken, then the forms shown in 2a appear, viz., short rods o 8 μ broad and only 4–6 μ long. Finally, in an infusion prepared from hay in which woody stems predominate, the cells (3a) have a length of 12 μ and a breadth of 1.0 μ. Under all the above conditions reproduction goes on with vigour, the fission being very rapid. The new partition walls formed during the operation are at first so thin and so faintly refractive as to escape the eye in the case of unstained preparations If, however, a solution of iodine be added, then the apparently uniform long cells are seen to be divided into short cells in the manner diagrammatically sketched at b and c in the figure. All these shapes belong to the cycle of
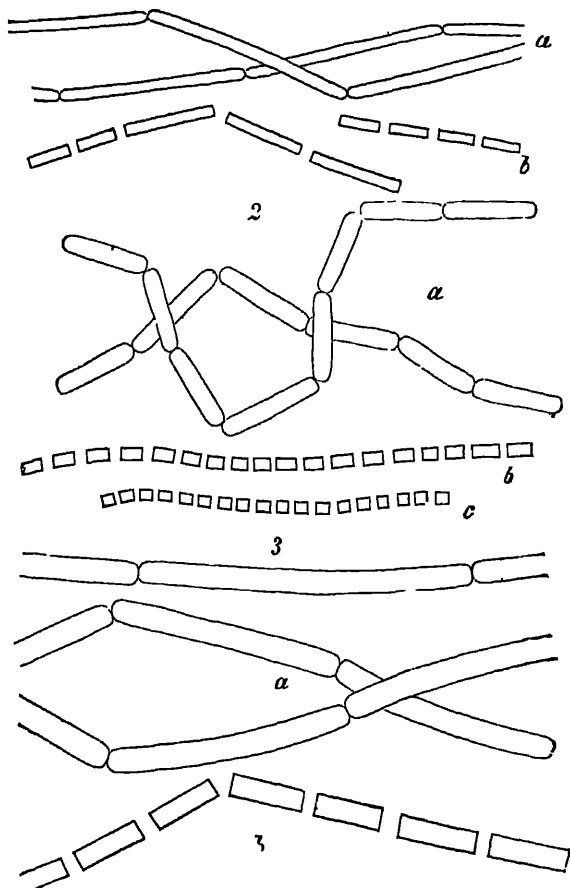
FIG 42 —Bacillus subtilis under various conditions of cultivation For explanation see text Magn about 4000 (After H Buchner)

normal forms of growth, of strong vitality, and capable of reproduction. When, however, the composition of the nutrient solution is, from the first, unfavourable (e.g a solution of o 1 per cent of asparagin or albumen, and 10 per cent of sugar), or becomes so subsequently in consequence of the excretion of injurious waste

products on the part of the cells, then there result involution forms, that have lost their reproductive faculty and must be regarded as diseased and moribund modifications. A couple of these are shown in Fig. 6 Similar forms are also produced in nutrient media containing a larger percentage of acids than usual, the hay bacillus being very susceptible to these reagents. The locomotive powers, as well as the form of the cells, are influenced by the method of nutrition. Thus, for example, the cells grown in a 1 per cent solution of asparagin at 25° C are devoid of cilia.

Bacillus subtilis liquefies nutrient gelatin Streak cultures on agar-agar develop into a wrinkled white pellicle This microbe must be classified among the extremely aerobic organisms, *i e* those essentially requiring the presence of oxygen for their development. Care must therefore be taken that air has admittance to the cultures. In reference to this matter, a valuable observation was made by LIBORIUS (I.). If air be excluded, the reproduction of the cells ceases, but the formation of a peptonising enzyme is not interrupted so long as the medium contains sugar. As already remaiked, this microbe is extremely sensitive to acids, even the small quantity present in normal beer-wort and beer —and which, expressed as lactic acid, amounts to only 0 09–0 12 per cent. in the former case, and up to 0 2 per cent. in the latter—sufficing to suppress the development of the bacillus in question, so that the brewing industry is exposed to no danger from this quaiter

The decompositions effected by this microbe were first studied by G. VANDE-VELDE (I.) in 1884, who was, however, unable to make use of pure cultures. On the other hand, such cultures were used in the researches carried out by ADRIAN J. BROWN (II.), in 1895, which were specially directed to the decompositions sustained by the various sugars under the influence of this fission fungus. It oxidises dextrose to an (unspecified) acid, which is thereafter entirely consumed, and a levo-rotatory volatile dissociation product, of unknown nature, but exerting an exceptionally high reducing power on copper solutions (such as those prepared by Fehling and others). The decomposition of the total sugar supplied is effected completely when the acid, by repeated neutralisations, is kept down below 0 04 per cent Saccharose undergoes a preliminary inversion, and is then oxidised.

§ 110.—The Potato Bacilli.

Before the great tenacity of life possessed by many of the bacterial spores inhabiting the soil was recognised, it frequently happened that potatoes, which had been presumably thoroughly sterilised, became (when employed for streak cultures), infested with a spontaneously developed, wrinkled zoogloea of rod-shaped *Schizomycetes*, which, starting from the potato skin, rapidly extended over the cut surface. These species, observed by different workers, are, with reference to their habitat, named the "potato bacillus." This is naturally a very comprehensive appellation, which has to be more narrowly defined in each separate case. These uninvited guests have their origin in the soil, sufficiently large quantities of which remain in the depressions (known as "eyes") in the potato; and the germs adherent thereto will withstand any heating that is not pushed too far. Of the species belonging to this group a considerable number is already known, and a few of them will be referred to later on, *e g.* in the chapter treating of "blown" cheeses. A few others must, however, be briefly dealt with in this place, namely, the three species of most general occurrence These possess in common the property of growing on solid media *e g.* potato cuttings, to form a pellicle, the surface of which becomes more and more wrinkled and convoluted, and recalls the appearance presented by the mesentery

The most common of all is the *Bacillus mesentericus vulgatus*, discovered by

Flügge, a plump, actively motile rod, shown in Fig. 43. The colonies on potatoes are dirty white, can be drawn out into threads, and develop equally well in the incubator and at room temperature. This bacillus excretes several enzymes, one being peptonising and producing liquefaction of the medium; another diastatic; and a third resembling rennet, whereby the casein of milk is at first precipitated, but is subsequently redissolved by the peptonising enzyme. It forms endospores, the final stage of germination of which is shown at *b* in Fig. 43.

Bacillus mesentericus fuscus was first described by Flügge (I.). The cultures on agar-agar and on potatoes are initially yellow, but as their age increases deepen progressively into brown. It also produces a peptonising enzyme, which liquefies nutrient gelatin. The production of spores is in this species less copious than in the foregoing and following kinds.

FIG. 43.—Potato bacillus

a two motile rods; *b* a newly germinated rod, just leaving the spore capsule, and unprovided with cilia. Magn 1000. (Drawn from a photograph by *Neuhauss*.)

The vegetative cells of the *Bacillus mesentericus ruber*, discovered by Globig (II.), are short rods, rather more slender in form than those of *B. m. vulgatus*. Like the other two species, the vegetative cells are motile and produce a peptonising enzyme. The colour of the streak cultures on potatoes is at first reddish-yellow, but subsequently becomes rose-red. The tenacity of life exhibited by the endospores of this species was minutely examined by Globig. A 1 per cent. solution of sublimate kills them after $1\frac{1}{2}$ hours' exposure; but they resist the action of 5 per cent. carbolic acid for more than a fortnight. For their destruction by a current of steam at 100° C. an exposure of $5\frac{1}{2}$ to 6 hours is necessary, and they will bear without injury an immersion of three-quarters of an hour in high-pressure steam at 109°–113° C. On the other hand, they perish in twenty-five minutes in steam at 113°–116° C., in ten minutes at 122°–123° C., in two minutes at 127° C., and immediately in steam at 130° C.

The great difficulty of sterilising articles contaminated with traces of soil is due to the great powers of resistance possessed by the spores they contain of the species just described, as also of a large number of their congeners, which are the hardiest of all organisms. The preservation of numerous food-stuffs, milk in particular, is thereby rendered more expensive, as will be sufficiently demonstrated in the two next following chapters. At present, attention will be drawn to a phenomenon which could not well be referred to there, viz., a disease in bread (due to the potato bacilli), which becomes manifest by the crumb of the loaf gradually changing into a sticky mass, capable of being drawn out into long threads, and having a repulsive sour sweet smell. Emil Laurent (II.) was the first to examine this disease closely, and he attributed it to a fission fungus, which presumably also plays a part in the normal fermentation of dough (dealt with in the second volume), and has therefore received the name of *Bacillus panificans*. This is undoubtedly a species of the group of potato bacilli. A second case of this disease was investigated by Kratschmer and Niemilowicz (I.) by the aid of the plate culture method, whereby *B. m. vulgatus* was recognised as the exciting agent. According to the researches of Aimé Girard, reported by Balland and Masson (I.), the temperature prevailing in the interior of loaves of bread during the baking process ranges between 100° and 102° C. The duration of exposure to this temperature being insufficient to kill the spores of potato bacilli, those originally present in the flour will still be found alive in the finished loaf. The sites occupied by these organisms, which germinate and reproduce immediately, will then become the headquarters of active masses of microbes, as already described. Batches of bread containing

much bran are especially liable to this disease, since it is on this part of the grain, and not on the enclosed flour, that the bacteria reside, and, as a matter of fact, it is commissariat (military) bread and Graham bread that are most frequently affected, as was pointed out both by Loeffler and UFFELMANN (I.)   The latter observer found the *Bacillus mesentericus vulgatus* to be associated in this disease with a second allied species, viz, the *Bacillus liodermos*, discovered in cows' milk by LOEFFLER (III.), which owes its other name of "gum bacillus" to the thick gummy appearance of its zoogloea-like cultures on cut potatoes.   That the bread disease in question does not make its appearance so frequently as might be expected from the (often very large) number of potato bacilli found in the flour is due to the strongly acid reaction of the dough, which facilitates the extinction of the germs.

§ 111.—Bacillus Fitzianus.

If a cold-prepared infusion of hay be left to stand at room temperature, there quickly forms on the surface of the liquid a skin composed of various organisms, including the bacillus named above, the chemical activity of which was first examined by A. FITZ (III.).   If a little of this skin be transferred to a sterilised solution of 2 per cent. of meat extract and 5 per cent of glycerin that has received an addition of some 10 per cent of calcium carbonate, then the said microbe develops and acts on the glycerin ($C_3H_8O_3$) in such a manner that ethyl alcohol and volatile acids are the chief products formed   Fitz obtained, in two experiments, a yield of alcohol amounting to 25.7 and 25 8 per cent. respectively.

FIG 44 —Bacillus Fitzianus.

a, b, f, g Cocci gradually changing into short rods, then (*c, e*) into long rods, *d* The same with an endospore   Magn. 4000. (*After H Buchner*)

The fermentation is very brisk, and attains its maximum within twenty-four hours.

At the time Fitz made his experiments, no method of preparing pure cultures had as yet been devised.   It is therefore of interest to record that his reports were tested by H. BUCHNER (VI) by the aid of the dilution method. The results were confirmatory, not only of the fermentative activity, but also of the pleomorphism of this glycerin-ethyl bacterium, which was later named by Zopf *Bacillus Fitzianus*   As shown in Fig 44, this fission fungus occurs both as cocci and as short and long rods, and is able to produce endospores   Though of no practical importance, it is mentioned here chiefly in order to show that the production of ethyl alcohol during fermentation can be effected, not only by the higher fungi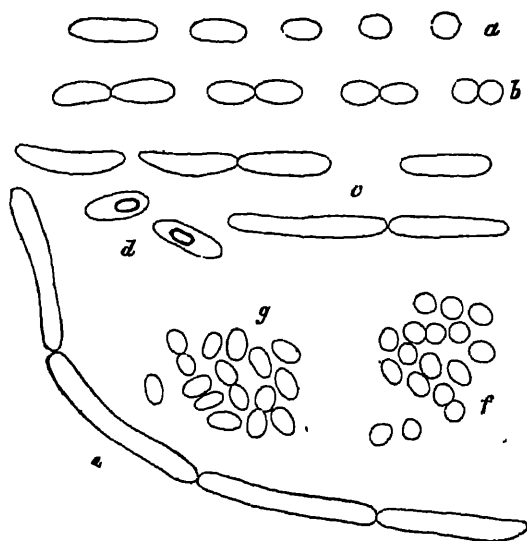 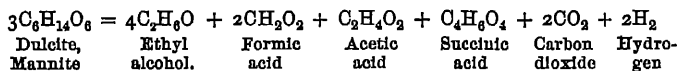(yeast in particular), but also by *Schizomycetes*. This fact is overlooked by many chemists when they speak of "alcoholic fermentation fungi," meaning thereby yeast alone.

Allied to the last-named bacillus as regards fermentative activity is the

Bacillus ethaceticus, cultivated from sheep-dung by P FRANKLAND, J. FOX (I), and MACGREGOR (I). This is a motile rod about 0 8–1.0 μ broad and 1.5–5 1 μ long, which, however, seems to lack the faculty of producing spores. It ferments glycerin, mannite, and arabinose in such a manner that the chief products are, in addition to small quantities of formic acid and succinic acid, ethyl alcohol and acetic acid, the ratio found being, in the first instance 2.11 · 1; in the case of mannite, 1 63 . 1; and in the last, 1 : 1.96. In a subsequent communication FRANKLAND and FREW (I) demonstrated that glyceric acid, $CH_2 OH—CH.OH—COOH$, is decomposed in the same way, the molecular ratio of the ethyl alcohol to the acetic acid being about 1 to 4

Whereas the last-named bacillus leaves dulcite unattacked, this hexavalent isomer of mannite is fermented by *Bacillus ethacetosuccinicus.* This microbe was discovered by P. FRANKLAND and W. FREW (II) in a solution of ammonio-ferric citrate, which, originally intended for photographic purposes, was found to have spontaneously fermented with vigour These observers give the following equation as a deduction from their experiments

$$3C_6H_{14}O_6 = 4C_2H_6O + 2CH_2O_2 + C_2H_4O_2 + C_4H_6O_4 + 2CO_2 + 2H_2$$

Dulcite, Mannite	Ethyl alcohol.	Formic acid	Acetic acid	Succinic acid	Carbon dioxide	Hydro- gen

Many other bacteria also produce ethyl alcohol, but only one more will be noticed, and that a pathogenic organism, viz , Friedlander's *Bacillus pneumoniæ.* According to the researches of F. BRIEGER (I.), and of P. FRANKLAND, STANLEY, and FREW (I.), this bacillus, when grown in nutrient solutions containing sugar (saccharose, glucose, mannite), produces ethyl alcohol and acetic acid, together with other fermentation products in smaller amount. These four examples may suffice to support the assertion made above, that ethyl alcohol is producible by bacterial activity. No practical application of this is made in the arts, the higher fungi known as "yeasts" being exclusively used , consequently this property of many *Schizomycetes* will not be referred to again. We will now supplement the account given of the hay- and potato-bacilli by a few remarks on the

§ 112.—Bacterial Content of the Soil.

To determine this quantitatively the procedure followed is to finely divide a weighed quantity of soil in sterilised water and then prepare cultures from the washings in the usual manner. P. MIQUEL (IV.), to whom we are indebted for the first determinations made in this connection, fixed the unit of weight to be taken as one gram, and it is to this unit that all the subjoined data refer.

It would, from the first, be expected that layers of the same soil at different depths would exhibit differences, both quantitatively and qualitatively, with regard to their bacterial inhabitants The property of soils (especially clays) of combining with the fertilising materials supplied in manures, prevents these substances from penetrating in any quantity to great depths, and from this circumstance alone one would expect the number of bacteria in the subsoil, at considerable depths below the surface, to be but small The filtering action of the upper layers of the soil also conduces to the same end, these layers fixing not only the fertilising substances, but also (and that in a purely mechanical way) the bacteria applied to the soil in manures. Thus it happens that subsoil water is perfectly free from, or at least very poor in, bacteria, a fact established by PASTEUR and JOUBERT (I.), and of which O. FRAENKEL (III) has given instructive examples. (A remarkable exception to this rule was reported by S ROHN and H. WICHMANN (I)) Finally, the influence of aeration should not be forgotten This, in the lower and comparatively undisturbed layers, is almost

nil, and the qualitative nature of the inhabitants of the soil is greatly influenced by this factor, the upper layers being relatively the richest in aërobic bacteria.

The greatest percentage of organisms should not be expected in the uppermost layer, since this is exposed to a too rapid alternation of excessive moisture and dryness, heat and cold, as well as to the anti-bacterial influence of the sun's rays. For these reasons the largest content of germs is always found at a depth of 10 to 20 inches below the surface, a fact first shown by ROBERT KOCH (I.). As the depth increases beyond this, the finds become smaller and smaller, and approximate to *nil* at about 60 to 80 inches.

It is manifest that the germ content of a soil is also dependent on the presence of nutrient substances, and that a soil rich in humus will be much more thickly infested than a poor sandy soil. Thus BEUMER (I.) found in dune sand only some 1000 germs per gram, which is very few, and an almost identical result was attained by A MAGGIORA (I) in the examination of a sample of sandy soil from a hill near Turin. On the other hand, he found in tilled agricultural soil some 11 millions of germs per gram, and in the same weight of a sample of soil taken from a street in Turin no less than 78 millions of bacteria.

That the degree to which a soil is warmed, as also its condition as regards moisture and meteorological factors, all influence its bacterial population needs no further argument. It follows naturally that the percentage of germs is higher in summer than in winter, and that it falls in dry, but increases in wet weather.

Any reader desirous of more closely studying the bacterial content of the soil, especially from a hygienic point of view, will find a good introduction thereto in Fodor's work, "Hygiene des Bodens" (Hygiene of the Soil), forming the 4th part of the useful "Handbuch der Hygiene" issued by Weyl (Jena, 1894 *et seq.*).

In addition to bacteria, the soil harbours a large number of higher fungi, comprising not only numerous innocuous mould fungi, but also the spores of phytopathogenic *Eumycetes* These are of no immediate importance to agricultural chemistry, and therefore do not need any further consideration here. E. Ch. Hansen demonstrated that the wine yeasts winter in the soil, but on this point reference must be made to the chapter devoted to *Saccharomyces apiculatus* in the second volume.

CHAPTER XVIII.

BUTYRIC ACID FERMENTATION AND ALLIED DECOMPOSITION PROCESSES.

§ 113.—Anaërobiosis.

THE first mention of the fact that butyric acid, discovered by Chevreul in 1814, can also be produced by fermentation, was made by R. MARCHAND (I.) in 1840, in connection with his researches on the composition of the milk of the South American cow-tree (*Galactodendron americanum*). In the following year Noellner described, under the name of **pseudo-acetic acid**, a substance which he had found to result from the spontaneous decomposition (fermentation) of calcium tartrate, and which was then recognised by Berzelius as a mixture of butyric and acetic acids. Two years later TH. PELOUZE and A. GÉLIS (I) observed that the lactic fermentations instituted by them did not progress satisfactorily, butyric acid and considerable quantities of hydrogen being produced In following up this observation they formulated the recipe, still current in many text-books on chemistry, that to start butyric acid fermentation a solution containing about 10 per cent. of sugar should be mixed with chalk and a little cheese, and left to stand at 25°–30° C.

These observers did not endeavour to follow the progress of this fermentation more minutely, as their attention was entirely devoted to the new fermentation product ; the only remark they make is that the butyric fermentation is not set up at once in their method, but is preceded by a lactic fermentation "without its being possible to influence the progress thereof." It was naturally far from the thoughts of Liebig's former fellow-worker at Giessen to attribute the decomposition to the activity of living organisms

The discovery of the true state of the case was made in 1861 by PASTEUR (VIII.), who showed that two successive processes are here involved · first, the conversion of sugar into lactic acid or calcium lactate, and afterwards the transformation of the lactate into butyrate. He demonstrated that each of these changes is due to a special ferment, of which the second is the only one we have to deal with now The microbe (2 μ broad and 2–15 μ long) causing butyric fermentation, and which we now recognise as a bacillus, was regarded by Pasteur, not as a plant, but as an animal, one of the infusoria, because it was observed to possess powers of locomotion. Nevertheless he laid but little stress on this distinction, the point being one of minor importance in comparison with the property he recognised in this "*vibrion butyrique*," viz , the **faculty of existing without air.** This observation formed one of the main supports on which this gifted philosopher founded his theory of fermentation, mentioned in § 16, and with regard to which a few additional remarks will now be made

At present we have first to consider the said peculiarity by itself, irrespective of the resulting decomposition effected in the nutrient medium. Creatures requiring oxygen for the continuance of their existence are termed **aërobiontes**, whilst the term **anaërobiontes**, or, shortly, **anaërobes**, is applied to creatures capable of development in the absence of this gas A distinction is drawn between two sub-groups, viz., **strictly anaërobic** organisms, *i.e.* such as can live *only* in an

environment devoid of oxygen, and for which this gas is therefore a poison, and facultatively anaerobic organisms, *i e.* those to which oxygen is neither injurious nor essential, and which can consequently thrive either in presence or absence of air. Thus, for example, the acetic acid bacteria are strictly aerobic, certain lactic acid bacteria facultatively anaerobic, and the majority of the butyric acid bacteria strictly anaerobic

At the present time numerous species of anaerobic fission fungi are known. PASTEUR (IX.) himself, in 1863, associated with the "*vibrion butyrique*" a second anaerobic species, viz, the microbe which sets up fermentation in calcium tartrate. This process of decomposition, already observed by Noellner, but which requires more careful investigation, often occurs spontaneously in tartaric acid works, destroying the tartaric acid and occasioning great loss. The different species of butyric acid bacteria will be thoroughly discussed later on. At present, by reason of their general interest, the known pathogenic anaerobic species, three in number, will be considered. The first of these, as regards priority of discovery, is the "*vibrion septique*," found by PASTEUR, JOUBERT, and CHAMBERLAND (I), and subsequently examined more closely by R. Koch and Gaffky, and now generally known to bacteriologists as *Bacillus œdematis maligni.* According to an opinion expressed by Pasteur, this bacillus is identical with the one effecting the fermentation of calcium tartrate The decompositions set up by the bacillus of malignant œdema in nutrient media containing carbohydrates were studied by R. KERRY and S FRAENKEL (I). Grape-sugar yielded ethyl alcohol, ethylidene lactic acid, and butyric acid. From calcium lactate were produced butyric acid, a little formic acid, and propyl alcohol. Milk-sugar and also cane-sugar were gradually fermented to ethyl alcohol, formic acid, butyric acid, and ethylidene lactic acid. Starch was also attacked, and yielded the three last-named acids With *Bacillus œdematis maligni* have been associated two other pathogenic anaerobic species of fission fungi, namely, the bacillus of symptomatic anthrax, by Feser and Bollinger in 1876–1878, and that which causes tetanus —*Bacillus tetani*—by Nicolaier in 1885. The latter is of somewhat frequent occurrence in arable soil, and was also discovered by S A. SEVERIN (I) in horse-dung. The bacillus of symptomatic anthrax, according to the researches of M. NENCKI (I.), when cultivated in media containing grape-sugar, produces chiefly normal butyric acid, along with acetic acid and optically inactive lactic acid, accompanied by the evolution of CO_2 and H_2. The remarkable symbiosis of this bacillus with *Micrococcus acidi paralactici* has already been briefly mentioned in § 65.

FIG. 45 — Gruber's tube for anaerobic cultures Ready for use Somewhat reduced in size. (*After Gruber*)

§ 114.—Methods of Cultivating Anaerobic Bacteria.

Pasteur covered the nutrient liquid with a layer of oil in order to prevent access of air. This method of covering up the medium with a protective stratum has been variously modified in order to render it applicable to solid media as

well. Thus R. Koch, in 1884, proposed to cover the gelatin plates with a film of mica, which, however, according to the experience of P. LIBORIUS (I), is not always sufficient in the case of strictly anaerobic bacteria. On the other hand, a second method (also emanating from the German State Board of Health) has proved highly suitable, viz., that of cultures in deep layers, as prescribed by W. Hesse in 1885. For these a puncture culture is made in test-tubes containing nutrient gelatin or agar-agar, the medium being covered, after successful inoculation, with a sterile stratum of the same liquefied substance

Another method, which has also been variously modified, is that first employed by Pasteur in connection with his studies of the "*vibrion septique*," which consists in exhausting the air (oxygen) from the vessel containing the nutrient medium inoculated with an anaerobic organism. The modification made by MAX GRUBER (II.) is convenient and reliable, and is in general use, especially in the laboratories of Fermentation Physiologists. Strong test-tubes (Fig. 45), about 7 inches long, with a much constricted portion in the upper third of their length, are used. These are filled with about 10 c c. of nutrient medium, then closed by a cotton plug, and after inoculation are immersed in water at about 30°–35° C., and then connected with an air-pump. The air is all driven out by the water vapour given off under the diminished pressure, whereupon the narrow part of the tube is closed by fusion and the upper portion removed By the use of nutrient gelatin this method also facilitates the cultivation of colonies, so that the individual anaerobic species in a bacterial mixture can be isolated For this purpose the still warm liquid contents of the tubes are converted into Esmarch roll-cultures, as shown in Fig 46

In place of removing the air from the culture vessel by mechanical means—pumping or driving it out by vapour—recourse may be had to oxygen-absorbing chemicals. For this purpose a solution of pyrogallic acid $[v-C_6H_3(OH)_3]$ in caustic potash, a mixture that takes up oxygen with avidity, and which, as is well known, has long been in use in gas analysis, is employed It was introduced into physiological work by Nencki in 1880, as a test for the presence of anaerobic organisms, but it was not until the publication of H. BUCHNER'S (VII.)

FIG 46

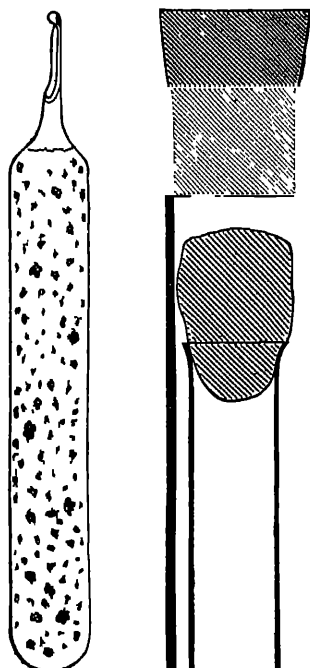Gruber's anaerobic tube exhausted of air

Upper portion removed by fusing Contents arranged as an Esmarch culture wherein the germs have developed to colonies Somewhat reduced in size (*After Gruber*)

FIG 47

Buchner's anaerobic tube

The pyrogallol solution is at p, the wire support resting therein and carrying the test-tube with the inoculated nutrient medium (n) Somewhat reduced (*After Buchner*)

method in 1888 that it came into general use in Bacteriology. The test-tube containing the inoculated nutrient medium—as a roll culture if desired—is placed in a large test-tube (Fig 47) about $1\frac{1}{4}$ inches wide and 10 inches long, at the bottom of which has just previously been inserted 1 gram of dry commercial pyrogallic acid and 10 c c. of deci - normal caustic potash. The smaller tube rests on a small wire support, in order to prevent it from dipping into the liquid. The "Buchner pyrogallol tube" is closed by a well-fitting previously-moistened rubber stopper, and may then be placed in the incubator. When kept at $37°$ C the absorption of oxygen is complete in twenty-four hours, or in two days at $20°$ C. To treat plate cultures by this method, the culture is placed over a basin containing a sufficient quantity of the said solution and resting on a flat plate of ground glass, the whole being covered with a well-sitting bell-glass, the edge of which has been rubbed over with vaseline. Another method for the culture of anaerobic organisms consists in expelling the air from the culture vessel by another gas, e g. carbon dioxide, hydrogen, coal-gas, or nitrogen. Carbon dioxide is frequently recommended by the French school, and particularly by Pasteur, but its employment is not without objections, since it is not an inert gas, but is absorbed by the medium, which it then renders acid, and hence has the power of restricting growth. Moreover, according to the researches of P. FRANKLAND (I.), it acts as a fatal poison on many bacteria. Although experience shows that hydrogen gas is not inert, still it may be accepted as the best to use for anaerobic cultures. Ordinary illuminating gas was recommended for this purpose by R. WURTY and A. FOUREUR (I.), but, according to the researches of TH KLADAKIS (I), it must be rejected, since he found it acting as a poison on many bacteria. Nitrogen may be regarded as perfectly innocuous, and would long ago have been employed for anaerobic cultures were it not that the method of preparation is too cumbrous and costly, at least for the physiologist. Many methods have been proposed for the expulsion of oxygen by one of the above gases, but only two will now be briefly mentioned here That of C. FRAENKEL (IV.) is concerned with the treatment of test tube cultures, ordinary wide test-tubes with two-holed stoppers being employed. One of the glass tubes inserted therein reaches almost to the bottom of the test-tube, whilst the second is cut off close below the stopper. When filled with nutrient gelatin, agar-agar (or bouillon, wort, &c), the tubes are sterilised in the steamer in the usual way, and inoculated, a current of hydrogen being introduced through the longer tube and passed through. When all the air is expelled the small tubes are hermetically

FIG 48

Fraenkel's anaërobic tube.

Contents arranged as an Esmarch culture round the walls, and developed colonies appearing as black spots. Somewhat reduced (After Fraenkel)

sealed by fusion (Fig 48) and the stopper smeared with warm paraffin. Esmarch roll cultures can then be prepared   If plate cultures (e g in Petri dishes) are to, be exposed to an inert gas, they are (according to P. LIBORIUS (I.)) placed under a bell (of copper, &c) which can be tightly fixed by screw clamps against a caoutchouc plate. The gas (when hydrogen is used) enters through a tube fixed in the crown of the bell, and leaves by way of another tube situated below.

It is impossible to refrain from mentioning that there is another method capable of taking rank with those described, and fulfilling all the conditions usually prevailing during anaerobic growth in Nature, viz, the simultaneous presence of strongly aerobic organisms. It is certain that anaerobic organisms can often be detected in liquids to which air has unrestricted access, and such associations of aerobic and anaerobic organisms are not difficult to bring about by artificial means, this having been successfully attempted by R PENZO (I), BEYERINCK (II), and others. The application of this method is, however, somewhat limited, since, of course, only mixed cultures can be produced by its aid.

It was remarked by Pasteur that the growth of anaerobic organisms could be promoted by an addition of sugar to the nutrient medium   Now, an alkaline solution of grape-sugar is well known to have a strongly reducing action; hence these two facts induced KITASATO and WEYL (I) to ascertain whether other reducing bodies were equally efficient; and they strongly recommended the addition of o 3–o 5 per cent. of sodium formate, or of o.1 per cent. of sodium indigo-sulphate   A solid nutrient medium, qualified and stained blue by the last-named substance, is decolorised as far as the growth of the reducing organism extends. The use of indigo-sulphuric acid as a test for reducing action was first practised in 1858 by M TRAUBE (I.) in his researches on ferments, and A. SPINA (I.) was the first to employ the sodium salt as a reagent for the same purpose.

§ 115.—Clostridium Butyricum (Prazmowski) and Bacillus Butyricus (Hueppe).

Pasteur's discovery that organic life is possible without free oxygen, and that certain organisms can obtain the energy they need by so breaking down organic compounds as to liberate heat, is one of the highest importance for physiology generally. The amount of heat so evolved is naturally much less than it would be if the compounds in question were directly converted into carbon dioxide. Pasteur, however, went somewhat too far in founding on this newly-discovered fact a theory of fermentation which culminated in the assertion that "Fermentation is a universal phenomenon, and consists of life without air, life without free oxygen," because, if this definition be accepted, we should be able to speak of but few phenomena as fermentation, and, in particular, it would be necessary to discontinue the application of the term to those decomposition processes that from time immemorial have been, and are even now, principally borne in mind in speaking of "fermentation," viz, the alcoholic fermentation excited by yeast, an operation which proceeds both with and without free oxygen.

To return to the "vibrion butyrique." In the years 1877 to 1880, A. PRAZMOWSKI (I.) published a careful morphological investigation of a butyric acid bacterium, presumably identical with the vibrion butyrique—though this cannot be stated with certainty   Elevating the designation Clostridium (first used by Trécul, and then only to indicate a form of growth) to a generic term, Prazmowski named two new species of bacteria Clostridium butyricum and Clostridium Polymyxa. The latter completely coincides with the former both in morphology and life history, but differs from the strictly anaerobic Cl. butyricum

both by its inability to exist in the absence of oxygen, and also by its incapacity to incite fermentation (in the restricted sense of the term). Fig. 49 reproduces the vegetative forms of growth depicted by Prazmowski as those of his butyric acid bacterium. They are mostly plump rods, some 1 μ broad The generation period was determined by Prazmowski as about thirty to thirty-five minutes at 35° C., and forty-five to fifty minutes at 30° C. Under certain conditions, and especially whilst young, the rods store up in their plasma a substance which resembles starch (amylum) in being stained blue by iodine This phenomenon had already been noticed by Trécul, who gave it expression in the generic name of *Amylobacter*, which he applied to these *Schizomycetes*. During the formation of spores the rods swell up, as related and shown in § 49 The power of withstanding heat possessed by the endospores of *Cl. butyricum* was examined by Prazmowski. They are able to remain in boiling water for five minutes without injury, but if the treatment be prolonged to twice that duration, then only the
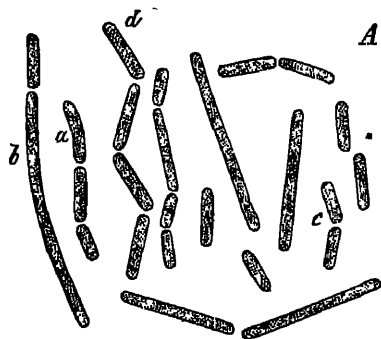
FIG 49 —Clostridium butyricum

Vegetative forms of growth, short rods of different lengths, partly straight (c, d), partly curved (a, b) Magn 1020 (After Prazmowski)
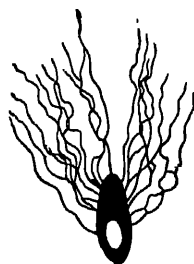
FIG 50

Clostridium butyricum With endospore

Stained cilia. Magn 2000 (After A Fischer)

hardiest spores of all are left alive, and even these succumb if the boiling be extended to fifteen minutes The progress of the germination of the endospores has already been described in § 57 (q.v.). The brisk locomotion of the rods is produced by a large number of cilia, shown in Fig 50. Prazmowski did not have pure cultures, in the present acceptance of the term, at disposal for his researches, but was obliged to confine himself to an approximately pure culture prepared by means of Roberts' boiling method (§ 107). The same remark applies to a valuable treatise by A. FITZ (VII.), wherein the fermentative activity of a fission fungus named *Bacillus butylicus* is reported upon.

After E. Ch. Hansen, in 1878–79, had established, in connection with acetic fermentation, the new and important fact that this decomposition process is effected by at least two different species of bacteria, F HUEPPE (IV) in 1884 found the same to be the case with butyric fermentation, and discovered a *Bacillus butyricus* which exerted its decomposing activity in presence of air. This fact was confirmed by MAX GRUBER (II), working with a reliable method of pure culture in 1887, and it was at the same time demonstrated that the *Clostridium butyricum* of Prazmowski consists of a number of closely allied, but nevertheless distinct, species Nearly related to this is a ferment isolated by P. LIBORIUS (I.) from old cheese, and introduced into literature under the name of *Clostridium foetidum*. This organism liberates very foul-smelling gases, in

addition to producing butyric acid, and forms one of the many connecting links between the butyric acid bacteria (in the restricted sense of the term) and the so-called potato bacilli. No sharply defined limit can be drawn between these two groups. In the same category must also be included one of the two species of bacteria which were isolated in 1894 by W KEDROWSKI (I) from a butyric fermentation produced by a method approximating to that of Pelouze. In still closer relation to the potato bacilli are a few anaerobic species isolated by O. FLÜGGE (II) from boiled milk, as also the *Bacillus hodermos*, frequently observed by LOEFFLER (III) in imperfectly sterilised milk.

§ 116.—The Genus Granulobacter.

As the reader will be aware, organic chemistry distinguishes between two kinds of butyric acid, only one of which, viz , the propyl carboxylic acid, having the subjoined formula—

$$CH_3 - CH_2 - CH_2 - COOH,$$
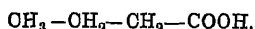
is, in the present state of science, known to result from butyric acid fermentation, on which account it is also called **fermentation butyric acid.** On the other hand, the isomeric acid, which, in accordance with its constitution—

$$\underset{CH_3}{\overset{CH_3}{>}} C \underset{COOH}{\overset{H}{<}} = \underset{CH_3}{\overset{CH_3}{|}} CH\ COOH$$
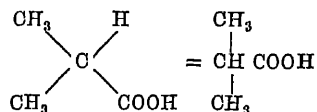
is also styled dimethylacetic acid or isopropyl formic acid, has not hitherto been obtained by the aid of fermentation. However, not only the first-named acid, but also the corresponding alcohol, viz., normal butyl alcohol,

$$CH_3 - CH_2 - CH_2 - CH_2OH,$$
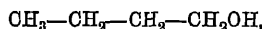
can be produced by the activity of fission fungi ; so that we may also speak of a group of the **bacteria of butylic fermentation.** In this connection we are indebted to M. W. BEYERINCK (XII.) for some thoroughgoing researches, which have not only brought new facts to light, but also led to a more definite characterisation and limitation of a number of species of butyric acid bacteria. This observer has given to the bacteria of butylic fermentation the common generic name of *Granulobacter*, since they all possess the faculty of storing up **granulose** in the interior of their cells, owing to which they are stained blue by iodine. The characteristics of this genus are given by Beyerinck as follows "Strictly or temporarily anaerobic fermentative bacteria, which in a condition of complete anaerobiosis become partly or entirely filled with granulose, and then assume the clostridium form In presence of traces of oxygen, short motile rods are quickly produced, which are stained yellow by iodine. Endospores make their appearance in the clostridia. They are able to remain uninjured for a few seconds or minutes at a temperature of 95°-100° C. Among the products of the fermentations set up by individual species of this genus are . carbon dioxide always and hydrogen generally, but methane is never found "

Granulobacter butylicum is the species producing butyl alcohol. It is presumably identical with Gruber's *Bacillus amylobacter I.*, and is frequently met with in the flour of cereals. It is anaerobic, and produces from maltose normal butyl alcohol, hydrogen, and carbon dioxide, but no butyric acid ; diastase is formed concurrently, but not glucase. A spontaneous butyl-alcoholic fermentation can be set up by gradually adding to 100° c.c of boiling water as much coarsely ground, unsifted, fresh barley-meal (from *Hordeum distichum nudum*)

I   K

as will produce a thick gruel, and then cooling down to about 35° C. so quickly that the final portion of barley-meal will have been exposed to 100° C. for a few seconds only The mixture is kept at a temperature of 35°–37° C. At the expiration of twelve hours bubbles of gas will be perceptible, and the presence of butyl alcohol will be manifest, by its odour, after a further twenty-four hours. If the aforesaid temperature be strictly maintained, almost pure *Granulobacter butylicum* will develop in the liquid, and a pure culture can be obtained therefrom, unhopped malt-wort gelatin forming a suitable medium, and one of the methods described in § 114 being employed. In this medium the fission fungus in question will develop into milk-white, visco-mucinous non-liquefactive colonies. The fermentations induced therewith (*s.g.* in unhopped malt-wort of not more than 10° Sacch), and which must be carried out in the absence of air, progress in two stages so long as any free oxygen remains dissolved in the liquid, development will proceed but slowly, only carbon dioxide and hydrogen (no butyl alcohol) being produced. When the liquid is finally purified, then not only can the appearance of the alcohol be observed, but also an unusually vigorous increase of the cells, which will be found to be so full of granulose that a drop of the liquid will become stained quite blue-black by iodine. The endospores, which soon make their appearance, attain, with a breadth of 1 μ, a length which may be as much as 2 μ This species is very sensitive to butyric acid.

A second species is *Granulobacter saccharobutyricum*, the true butyric acid bacterium, generally so called, and presumably identical with the *Bacillus butylicus* examined by Fitz. It is more widely distributed and of more frequent occurrence than the last-named species, with which it is associated on cereal grains and in the green malt, groats, and flour prepared therefrom. It is this species, also, which occurs, and gives rise to damage, in badly prepared distillery yeast-mash. Glucose and (but with greater difficulty) maltose are decomposed by this species, butyl alcohol, carbon dioxide, and hydrogen in variable proportions being produced, in addition to butyric acid. From a morphological point of view, it is closely allied to the first-named species, but the spores are somewhat smaller ; also, like the other, it does not liquefy gelatin Probably identical with, or at least very closely related to *G. saccharobutyricum* is an anaerobic ferment (*Bacillus butyricus*), isolated by S. Botkin (I.) from Berlin and Breslau milk, and also frequently noticed by FLÜGGE (II.) in market milk.

Granulobacter lactobutyricum is probably identical with the organism causing butyric acid fermentation in calcium lactate, described by Pasteur. When cultivated **in the absence of air**, it grows in the form of plump, short clostridia, which stain violet-blue (not pure blue) with iodine and convert calcium lactate into butyrate, hydrogen and carbon dioxide being liberated. The endospores are smaller and shorter than those of the first-named species. When kept in presence of air, this organism converts calcium lactate into large spheroidal crystals of the carbonate, and in this case takes the form of slender short rods, resembling those of *Bacillus subtilis* and staining yellow with iodine From the fact, recorded by Beyerinck, that this species in a state of pure culture dies out after several re-inoculations, whether air be admitted or excluded, it may be presumed that the organism needs for its prosperous development the symbiotic association of another, still undetermined, species of fission fungus.

Granulobacter Polymyxa is frequently found on cereal grains, and is presumably identical with Prazmowski's *Clostridium Polymyxa*. This species develops most satisfactorily in an unrestricted supply of air, and then assumes the form of motile rods. When the aeration is deficient, spore-bearing clostridia appear, and a weak fermentative action is noticeable, a small quantity (traces) of butyl alcohol, together with carbon dioxide, being formed, but neither hydrogen nor butyric acid —The *Leptothrix buccalis*, very frequently found in dental mucus,

and whose thread chains are generally stained blue by iodine, is also classified b Beyerinck along with the Granulobacteria.

§ 117.—The Equation of Butyric Fermentation

is set out in a very simple form in most text-books on chemistry, that for th decomposition of the hexoses (glucose, &c) being given as follows—

$$C_6H_{12}O_6 = 2H_2 + 2CO_2 + C_4H_8O_2,$$
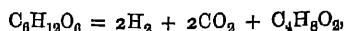
or with lactic acid (or its lime salt) as the raw material—

$$2C_3H_6O_3 = 2H_2 + 2CO_2 + C_4H_8O_2$$

In the preceding paragraphs we have, however, made the acquaintance of a very large number of bacterial species with divergent methods of action, so that we must at once admit that a general equation for butyric acid fermentation is not to be thought of All that can be hoped for is the discovery of more accurately defined equations for each of the various species and their characterisation, as e.g., the equation for the fermentation set up by *Granulobacter saccharobutyricum* and so on. Nevertheless, even this limitation is not sufficiently strict, as will be evident from what follows

L. PERDRIX (I) examined the fermentative capacity of an anaerobic spore bearing butyric acid bacterium (closely allied to Botkin's *Bacillus butyricus*) which he isolated from the water in the Paris mains and named *Bacille amylozyme* by reason of its property of bringing starch into solution (sacchari fication). When grown in a meat-broth containing glucose and calcium car bonate, with exclusion of air, this fission fungus produces acetic acid and butyric acid, in addition to hydrogen and carbon dioxide. The mutual ratio of these four fermentation products changes with the increasing age of the culture. In the first three days it can be approximately expressed by the equation

$$56C_6H_{12}O_6 + 42H_2O = 156H_2 + 114CO_2 + 39C_2H_4O_2 + 36C_4H_8O_2,$$
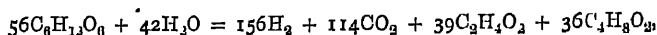
but later on by the equation

$$46C_6H_{12}O_6 + 18H_2O = 112H_2 + 94CO_2 + 15C_2H_4O_2 + 38C_4H_8O_2.$$
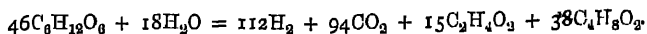
Finally, the transformation becomes simplified, acetic acid being no longer produced, and the sugar then splitting up very nearly as follows .

$$C_6H_{12}O_6 = 2H_2 + 2CO_2 + C_4H_8O_2$$
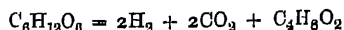
Similar ratios were established for the fermentation of saccharose and lactose, which is not preceded by inversion Starch is, as already mentioned, saccharified by the *Bacille amylozyme*, and is then fermented, amyl alcohol and ethyl alcohol being formed.

Along with these *Schizomycetes* must be ranked the *Bacillus suaveolens,* described by SOLAVO and GOSIO (I), which converts starch into dextrin and glucose, and ferments these with excretion of alcohol, aldehyde, formic acid, acetic acid and butyric acid, which then partly unite to form sweet-smelling esters. Butyric acid bacteria that produce aromatic substances as well are important for the ripening of cheese, being essential for the development of the characteristic odours of the various kinds of cheese. However, in this matter our knowledge is still only in a rudimentary state. E. VON FREUDENREICH (I.) separated from milk a *Clostridium fœtidum lactis*, which develops, in this medium, an odour resembling that of Limburg cheese, and the same observation was made by H WEIGMANN (I) The *Bacillus saccharobutyricus*, isolated from

so-called " Quargelkase" (small country cheese, a sour soft variety) by V. von KLECKI (I.), and examined by him for its fermentative power, also belongs hereto.

The influence of the age of the organisms used as " seed " and the reaction of the nutrient medium on the progress of butylic fermentation was shown by L. GRIMBERT (I.) in the case of the anaerobic *Bacillus orthobutylicus*. This was isolated as a pure culture from a fermenting aqueous liquid containing calcium tartrate and leguminous seeds, the said salt being, however, as little affected by the bacillus as is calcium lactate. On the other hand, saccharose, lactose, maltose, invert sugar, glucose, and the like, form favourite nutrient substances, normal butyric acid, acetic acid, normal butyl alcohol, and a little iso-butyl alcohol, together with hydrogen and carbon dioxide, being the products of fermentation. The ratio of these products is found to depend on the reaction of the medium, the yield of butyl alcohol increasing and that of butyric acid diminishing with the increased acidity thereof, whilst the amount of acetic acid remains unaffected. In harmony with this determination is the further fact that, as the age—and concurrently the acid content—of the fermenting liquid increases, the amount of butyl alcohol produced per unit of time becomes larger. So far as the **age of the " seed "** (*i.e.* the germs and organisms transferred in the process of inoculation) is concerned, it is found that, as regards the production of butyl alcohol, the fermentative power of young cultures is greater than those of more mature age.—Sundry experiments in the technical preparation of butyric acid by fermentation were made by L. LEDERER (I.), but these leave much to be desired from a bacteriological point of view.

The faculty of producing starch-dissolving enzymes is widespread among the bacteria, and is in no wise restricted to the above-named species. Our knowledge of these **amylases** or **diastases** (in the general sense) is, however, still in its infancy. In this connection we may refer to a treatise by BEYERINCK (XIII.) on glucase, the enzyme of maltose. After J. WORTMANN (I) had already, in 1882, made a few investigations thereon, but only in bacterial mixtures, CL. FERMI (II) approached the matter more closely in 1890. According to his observations (made exclusively with pure cultures), diastatic enzymes are excreted by the following species · *Bacillus subtilis, B megatherium, B. anthracis, B. tetragenus, B. ramosus, B. Fitzianus, Vibrio cholerœ asiaticœ*, and others ; this faculty being, on the other hand, lacking in *Bacillus pyocyaneus, Micrococcus prodigiosus*, &c. According to the researches of A. VILLIERS (I.), there occurs among the fission products of the action on starch paste of a fission fungus belonging (presumably) to the group of Granulobacteria, a small quantity (o 3 per cent. of a new carbohydrate, known as **cellulosin**, which has the formula $C_{12}H_{20}O_{10} + 3H_2O$.—The starch-dissolving action of bacteria also probably comes into play in the preparation of the alcoholic beverage known in Central America as chicha. V. MARCANO (I) states that this liquor is prepared by steeping maize for four to six hours in water, then boiling for a short time, and afterwards leaving the mixture to settle, whereupon a brisk fermentation quickly ensues. More accurate information respecting the fission fungus concerned is still lacking.

§ 118.—The Fermentation of Cellulose.

To dissolve and get rid of what has ceased to live is (according to an appropriate remark made by Pasteur) the task of the fungi in general, and of the fission fungi in particular. Without their activity the circulation of the elements of which the organic world is constructed would quickly come to a standstill, and the surface of the earth become in a few years thickly covered with the dead bodies of animals and plants. Respecting the constituents of the

latter a few words will now be devoted to the fate of the Cellulose, of which the greater part of the cell walls of plants is composed.   Here the question arises as to how the carbon in this substance is set free again, and the gradual, and finally complete, accumulation of this element in a useless form prevented   In this case once more assistance is afforded by bacteria, which split up the cellulose and remove it out of the way.

E MITSCHERLICH (I) was the first, in 1850, to comment on the natural decomposition of cellulose, by expressing his opinion that it was attributable to the fermentative activity of vibrios.   The probability of his view was increased by Popoff's (I) discovery in 1875, that this decomposition process can be moderated or completely arrested by the addition of substances poisonous to bacteria   A closer investigation of the organisms in question was undertaken two years later by VAN TIEGHEM (IV.), who gave them the name of *Bacillus amylobacter*, and (V), from microscopical examination only, declared them identical with Pasteur's *vibrion butyrique*

It would be useless at the present time to argue on this assumption, since both observers worked with what were probably complex mixtures of several species, certainly not with pure cultures   On the other hand, Van Tieghem's further demonstration that petrified cells of (morphologically) similar fission fungi are also to be found in the fossil coniferæ of the Carboniferous period is worthy of mention.

A H. C VAN SENUS (I), in 1890, endeavoured to obtain a pure culture of the organism giving rise to cellulose fermentation,   According to him, a symbiosis of two species is here in question, the one of them—which he named *Bacillus amylobacter*—occurring in the form of rods 0.8–1 μ broad and 2–10 μ in length, which, under special conditions, are stained blue by iodine.   When air is admitted they form endospores, which then germinate only when air is excluded. The second of these symbiotic species is of much smaller dimensions, and is by itself, like the *B. amylobacter*, incapable of fermenting cellulose.   For this purpose the conjoint effect of both species is necessary, an enzyme being then excreted which dissolves the cellulose   Van Senus isolated this enzyme, and demonstrated its solvent power on cellulose by applying the alkaline solution (containing chloroform in order to suppress bacterial growth) to the cell walls of slices of beans.

V. OMELIANSKY (I.), in 1895, obtained very different results   He inoculated a mineral nutrient solution (containing potassium phosphate, magnesium sulphate, ammonium sulphate, and chalk), in which strips of Swedish filter-paper were held in suspension, with a small quantity of mud from the Neva, and then kept the whole at 30°–35° C, air being excluded.   Fermentation rapidly set in, the strips of paper gradually becoming thinner, and finally disappearing altogether. By repeated transferences of small portions of the fermenting liquid to fresh sterile media the ferment was purified, and finally brought into a state of pure culture by anaerobic cultivation on discs of boiled potato   Omeliansky describes the organism as an unusually slender bacillus, measuring only 0.2 to 0.3 μ in breadth for a length of 6–7 μ, and forming terminal globular endospores 1 μ in diameter, whereby the pole at which the spore occurs is swollen up.

As in many other directions, here also, in the case of the fermentation of cellulose, the extension of our knowledge is dependent on the elucidation, still to be made by chemists, of the composition of the substances subjected to investigation for the products they yield on fermentation.   At the present time the essential requirement that the ferment shall be used in a state of pure culture is almost fulfilled, but so far as the purity of the cellulose to be decomposed is concerned matters are by no means on a satisfactory footing.   ERNST SCHULZE (I), in 1895, in a treatise setting forth the present state of our know-

ledge on the subject (and one well worthy of perusal) shows how very divergent are the substances which now bear the name of "cellulose" He separated a number of these, and collected them into the group of **hemicelluloses**, distinguished by their solubility in hot dilute mineral acids, whereby they are converted into glucose, whilst the remaining celluloses do not undergo this change. Considering how divergent these substances are, it is not surprising that different investigators do not always obtain concordant results as regards the products of cellulose fermentation This decomposition occurs on a large scale in the mud of marshes where there is no lack of decaying plants, and where, moreover, the other conditions are favourable It has long-been known to chemists that, in such water, a somewhat copious discharge of gas bubbles rises out of the ground, which discharge consists for the most part of methane (CH_4), i e. the gas to which the name of **marsh-gas** has been given, from the places where it is found in Nature. Carbon dioxide is also liberated at the same time. The proportional quantity of the two products was reported in several analyses communicated by POPOFF (I) in 1875, and more accurate researches on the same point were published by HOPPE-SEYLER (I) in 1886 The latter kept, with exclusion of air, either mud from marshes and rivers, or else clean paper inoculated with a little mud and then distributed in water, and demonstrated that in the mixture of gases evolved therefrom methane predominated greatly at the outset, but thereafter gradually diminished to the proportion 1 : 1, so that Hoppe-Seyler, being unable to discover any other decomposition products, expressed the opinion that the cellulose is hydrolised by the action of the bacteria—which from microscopic examination he asserted to be the same as Van Tieghem's *Bacillus amylobacter*—and is then split up into equal volumes of methane and carbon dioxide, according to the equations—

$$C_6H_{10}O_5 + H_2O = C_6H_{12}O_6$$
$$C_6H_{12}O_6 = 3CO_2 + 3CH_4$$
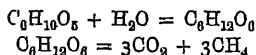
He found the ratio different when sulphates (gypsum, &c.) or ferric salts were present in the water or mud. In such case the nascent methane, by its reducing action on these salts, converts them into carbonates, sulphuretted hydrogen being liberated, according to the equation—

$$CaSO + CH_4 = CaCO_3 + H_2S + H_2O$$
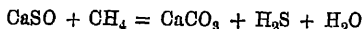
The sulphuretted hydrogen is, then, under natural conditions, acted upon by the sulphur bacteria which are always present in such waters. This will be dealt with in chapter xxxv.

The importance of cellulose fermentation in the physiology of nutrition (especially of cattle) must also be briefly adverted to The opinion long held by Emil Wolff, that the vegetable fibres consumed with the food pass out of the alimentary canal unaltered, was contradicted, in the case of ruminants, as far back as 1854, by Haubner, who showed that even in the case of sawdust and paper pulp mixed with the fodder, only a portion (less than half) was expelled again in the excrement, a fact confirmed by the exhaustive researches of Henneberg and Stohmann. The difference between the amounts of cellulose (crude fibre) taken in and rejected was highest in the case of ruminants (up to 75 per cent), being only 50 per cent. at most in horses, and still less in the human subject and in swine In carnivora (dogs), on the other hand, no such difference could be detected. The quantities of cellulose thus disappearing in digestion were, it was thought, **digested**, and were regarded as approximately equivalent in nutritive value to the soluble carbohydrates A number of animal physiologists maintained that the cellulose was dissolved by an intestinal enzyme, which, however, was sought for in vain. The earliest reliable determinations

on this point were made by H. TAPPEINER (I) in 1884, who showed the fate of all cellulose taken into the body and not evacuated in the fæces to be, not digestion and absorption into the arterial circulation, but fermentation into marsh-gas. The operation, however, does not proceed exactly according to the above equation, but yields, in addition to methane, volatile fatty acids (chiefly acetic acid, then butyric acid, &c) Bearing this in mind, this decomposition process cannot be regarded as totally without value for the animal body, although, naturally, the coefficient of digestibility, hitherto usually assumed, necessarily suffered considerable depreciation Moreover, this process is indirectly favourable, in so far that, by the solution of the cell-walls, the cell contents of the vegetable nutriment are laid bare, and thus rendered more readily accessible to the digestible fluids. Although by this discovery the chief source of the intestinal gases so copiously evacuated by herbivorous animals is made manifest, still it should not be assumed that the methane therein is exclusively derived from the fermentation of cellulose, since Ruge and Planer proved that, even in cases of a purely flesh diet, methane is to be found in the intestinal gases (of man and the dog).

Cellulose fermentation also plays a part in the preparation of **brown hay, sweet ensilage**, and **sour fodder**, considered in chapter xxvii, where it forms one of the causes of the great loss of matter inherent in these processes, in connection wherewith reports have been made by Weiske, O. Kellner, M. Maercker, and others. This process also goes on—as shown in 1884 by P. DEHÉRAIN (I.) and U GAYON (I.)—in **manures** If, favoured by special circumstances, it proceeds more rapidly than the decomposition of all the other (and especially the nitrogenous) constituents, then an irregularly fermented product—deficient in the fibres necessary to impart porosity to the mass—and known as **fatty manure**, is the result

The evolution of marsh-gas and hydrogen by the agency of bacteria also occurs, not infrequently, in other situations; for instance, in the **diffusers** in sugar-works, and very often so strongly that the amount of gas suffices to produce powerful **explosions** when the diffusers are incautiously approached with a naked light. The teaching of experience, that frozen beet is particularly liable to such a form of decomposition, is probably explicable by the circumstance that such beet cannot be entirely freed from adherent particles of soil, and the ferments present therein. It is also conceivable that, by the agency of frost, the pectins in the beet are transformed into a more readily decomposable condition. On this point a few observations have been made by Millot and Maquenne, and, more recently, by P DEHÉRAIN (II), more accurate investigations thereon are, however, still lacking.

§ 119.—The So-called "Retting" of Flax and Hemp.

The present forms the most fitting occasion for making a few explanatory remarks on this matter As is well known, it is the bast fibre of these plants that is referred to when hemp or flax is spoken of in the textile industry In order to lay these fibres bare and obtain them in a pure state, it is necessary to dissolve the intermediate intercellular substance (the so-called **central lamellæ**) —which consists, not of **Pectose**, as stated by J. Kolb, but (according to the researches of Mangin) of **calcium pectate** This can be effected by chemical means, by a (patented) process which Baur successfully introduced into Silesia on a large scale in 1882, and which consists mainly in treating the plants with very dilute sulphuric acid, and then neutralising the adherent acid by a weak alkali bath. The solution of the cementing calcium pectate can, however, be brought about by a fermentation, known as "retting," that has been practised

from remote ages without any special knowledge of the more delicate proᵢ
involved   According as the moisture necessary for this fermentation (or fᴄ
development of the fission fungus effecting the same) is imparted to the rɪ
stalks by means of dew, sprinkling them with water, or by immersion
process is known as dew-retting, water-retting, or, finally, mixed retting.

With reference to the bacterial species taking an active part in this pr
VAN TIEGHEM (VI.) in 1879 expressed the opinion that they should be ass
to his *Bacillus amylobacter*, which he, as already mentioned, also regarded ε
cause of cellulose fermentation. The inaccuracy of this view is evident,
if the retting (Fr *rouissage*, Ger. *rosten*) of the flax were mainly a procᵢ
cellulose fermentation, there would not be much of the fibre (which chiefly
sists of cellulose) left. It was V. Fribes who—working under the direᵢ
of S WINOGRADSKY (I.)—in 1895 clearly proved the true state of the casᵢ
made known the active agent of this pectin fermentation. This organisɪ
fairly large-celled species, occurring in the form of rods, which, when y
have a length of 10–15 μ with a breadth of 0 8 μ, but subsequently bᵢ
broader)1 μ), and swell up to a thickness of 2 μ at the one end (tadpole sl
where a long endospore (1 2 × 1.8 μ) is developed. This (anaerobic) bacillᴜ
not grow on gelatin, but when supplied with nitrogenous food in the fo
peptone will ferment glucose, saccharose, lactose, and starch, leaving these,
ever, untouched when the peptone is replaced by ammonia salts. On the
hand, even in this latter case, any pectin bodies that may be present, *i.e.*]
and pectic acid, are fermented, and that, too, even more readily than the ᵢ
hydrates already mentioned The organism, however, has no action on cel
and gum-arabic. When clean portions of plants, previously washed, first
acidified, and then with faintly alkaline, water (free from bacteria), are ex
to the action of pure cultures of this bacillus, they quickly lose the gɪ
portion of their pectin content, the loss of weight they suffer being a
exclusively due to this cause. As reported by E. PFUHL (II.), a paten
been obtained in the United States by Allison and Pennington for the m
tested by them, whereby the retting of flax can be effected in a few days i
class of water by the addition of salts promoting the growth of the d
ferment Perhaps an inoculation by water from a locality—such as the
Lys, a tributary of the Scheldt—where flax-retting is extensively carried
also to be made.

§ 120.—The Rancidity of Fats, particularly Butter,

will be briefly dealt with here parenthetically, as there is no other appropriate
for it. The characteristic indication of this well-known phenomenon is an in
in the percentage of free acids (chiefly the volatile acids, butyric, caproic
This may be attributed to four different causes · (1) The activity of lactɪ
bacteria converting the residual lactose in the butter into lactic acid, which
is then transformed into butyric acid by *Granulobacter lactobutyricum* ; (
decomposition of albuminoids (casein) by bacteria capable of forming b
acid therefrom , (3 and 4) the dissociation of the fats into glycerin anᵢ
fatty acids, either by bacterial agency on the one hand, or by the action oɪ
and air on the other. The preponderance of one or other of these ᵢ
depends on the attendant circumstances.

E DUCLAUX VI) rendered valuable service in demonstrating the inf
of air on the fatty matter of butter and cheese, and by showing tha
influence is twofold, viz., it first, by saponification, breaks the matter u
glycerin and fatty acids, and then converts the latter (especially oleic acid
oxy compounds. In the dark, and in presence of a copious supply of air, s

fication predominates, the butter then smelling strongly of butyric acid ; but if, on the contrary, the oxidising action gets the upper hand, then a tallowy flavour is the result.

A recognition of the influence of light and air does not necessarily imply that bacteria play no active part in rancidity. This latter view was maintained by H. SCHULZ (II.) in 1878, but was afterwards denied by E. RITSERT (I), ARATA (I), and others With reference to the careful work performed by Ritsert, it should be remarked that the main point is in the treatment of the question as to the occurrence of rancidity in the absence of micro-organisms The affirmative answer thereto does not, however, involve the conclusion that their activity is, under natural conditions, unimportant for the development of rancidity. The action of the lactic acid bacteria can be illustrated by a practical example afforded by the so-called *Paris butter*. This is a particularly stable product, and is prepared from sweet cream, which, before churning, is heated to nearly 100° C. and then rapidly cooled again, whereby the fission fungi just mentioned are killed The bacteria which split up the glycerides in butter include one which R. KRUEGER (I.) isolated from butter that had become cheesy, and which he named *Bacillus fluorescens non liquefaciens*

Investigations on the breaking up of fats by bacteria, which process is of medical interest in connection with the putrefaction of dead animals, were conducted by G. VON SOMMARUGA (I), chiefly with pathogenic species, only a few of which were found to possess this power Among these may be mentioned *Bacillus typhi abdominalis*, *B. pyocyaneus*, *Micrococcus tetragenus*, *Vibrio cholerae asiaticae*, *Denecke's spirillum*, and others. On the other hand, *Bacillus megatherium* and *B. subtilis* do not possess it.

CHAPTER XIX

THE PRESERVATION OF MILK.

§ 121.—Dirt- and Germ-Content in Milk.

THE sterilisation of milk is excessively difficult, because this liquid is particularly liable to infection by very hardy germs. Even when yielded by a healthy cow, the milk, on issuing from the udder, is already infested with bacteria. When milking is ended, a small quantity of milk is left behind in the lacteal ducts, and in this there settle a number of bacteria which make their way from outside, are favoured in their development by the high temperature, and become incorporated with the subsequent flow of milk. In addition to these are the innumerable bacteria originating in the dung and adhering to the udder. Both groups consist mainly of species very tenacious of life, derived from the soil and entering the alimentary canal along with the fodder. They pass through the intestines unhurt, are conveyed with the dung on to the udder and the hands of the milker, and then into the milk, where they flourish exceedingly. The dirt, adhering to the cows, and itself infested with bacteria, is partly disseminated as dust through the air of the cowhouse, so that this also is impregnated with bacteria, and yields up no small quantity to the milk. For investigations on this point we are indebted to G. J. LEUFVÉN (I), who held sterilised flat glass dishes open, for a second, above the edge of a milking-pail into which milk was being drawn from the cow, and then introduced into the dishes some liquefied nutrient gelatin, wherein the germs present in the basins developed into colonies, and could therefore be counted. In this manner it was proved that, in the space of one second, from 47 to 1210 germs, according to circumstances, were deposited in an area of 1 square decimetre (100 sq. cm., or about 16 square inches).

The amount of the germ-content is thus primarily determined by the degree of contamination prevailing in the cowshed, a criterion of which is afforded by the amount of dung constituents present in the milk. This estimation was first attempted by RENK (I), who found, for instance, in the market milk of Halle-on-Saale, some 75 mg. per litre; in Berlin milk, 10 mg.; and in Munich milk, 9 mg. of such **milk-dirt**, the highest quantity amounting, at Halle, to 0 362 grm. per litre. If the milk be treated in the centrifugal machine, in order to remove the cream, the dirt is separated, and collects, along with casein, bacteria, &c., as a coating, sometimes granular, at others mucinous, on the walls of the inner drum, and is generally known as **milk-sludge**. It consists of about 26 per cent. of albuminoids, 67 per cent of water, &c., and is relatively much richer in bacteria than the milk. O. WYSS (I) was the first to investigate this mud, and the quantity separated was found, in a case examined by NIEDERSTADT (I), to amount to 43 grms. per hectolitre (slightly more than 30 grains per gallon).

The connection between the content of dirt and of germs in milk was shown in particular by UHL (I), a few of whose figures are now given.

Sample No.	Dirt Mg per Litre	Number of Germs Per c c
1	36 8	12,897,600
3	20 7	7,079,820
6	5 2	3,338,775

Sundry researches on the **germ-content of cow-dung**, and on its dependence on the dietary, were carried out by E WÜTHRICH and E VON FREUDENREICH (I) They found up to as many as 375 millions of bacteria per 1 *gram* of fresh fæcal matter, the majority consisting of *Bacterium coli commune*, along with some 3 millions of hay bacilli and others   An equal weight of the hay used for fodder contained about 7½ millions of germs, of which about one-fourth were hay bacilli. Sour **brewers' grains** (forty-eight hours old) yielded 375 million colonies.

The germ-content of freshly drawn milk increases very rapidly during transport to the centres of consumption, as also during storage, *e g* in milk-shops   A number of estimations have been made in this connection, a few of which, by FREUDENREICH (II), are subjoined.   In these, the influence of the length of storage in conjunction with the prevailing temperature is taken into account.

Number of Germs per c.c.

On arrival in the laboratory (two and a half hours after milking), 9300

During	Stored at		
	15°	25°	35° C
3 hours	10,000	18,000	30,000
6 „	25,000	172 000	12,000,000
9 „	46,500	1,000,000	35,280 000
24 „	5,700,000	577,500,000	50,000,000

A milk with an initial germ-content of 9300 will be reckoned poor in bacteria when it is known that J. v GEUNS (I) found 2 5 million bacteria per c c in fresh samples of Amsterdam milk   The reproduction can be moderated, but not arrested, by cold, because, as we have seen in § 61, milk contains also sundry species of bacteria capable of development at 0° C.   Therefore if it be desired to prevent the decompositions set up by these organisms, it will be absolutely necessary to kill the germs.

§ 122.—The Part Played by Milk as a Carrier of Infectious Diseases.

The aforesaid requirement is really imperative in view of the fact that diseased cows—which the owners often fail (or refuse) to recognise as such—yield milk containing pathogenic bacteria   And this applies particularly to tuberculosis, from which complaint (on a moderate computation) one cow out of every five in Germany suffers.   As reported by J On BAY (I), on the authority of a compilation made by the Danish pathologist B Bang (to whom is due the honour of clearing up this question), out of 132,294 head of cattle examined in the Copenhagen slaughter-house between 1891 and 1893, no less than 23,305 (some 17.7 per cent.) were recognised as tuberculous by macroscopic examination alone According to the researches of Dr Martin, reported by R. CNOPF (I.), one out of every thirteen samples of milk exposed for sale in Paris contains tubercle bacilli, and from the results obtained by Dr. Schroeder in Washington, at least one in every nineteen samples of milk sold in that city contains a sufficient number of tubercle bacilli to produce infection.   For the microscopic investigation of the (specifically heavy) tubercle bacilli in milk, the sample, previously prepared by skimming and clarifying, is separated, by centrifugal force, in strong test-tubes, for which purpose special processes have been designed by THÖRNER (I.) and

ILKEWITSCH (I).   K. OBERMÜLLER (I) examined Berlin market milk in this way, and recognised it as infected with tubercle bacilli in a high degree.   In this connection it should be remarked that, according to determinations made in 1896 by A. BULLING (I.), goats are also liable to this disease, and therefore cannot be considered as immune.

The extent of the danger attendant on the consumption of unboiled milk is not sufficiently illustrated by the foregoing particulars, which are only concerned with the possibility of infection by such bacteria as are pathogenic for men *and* animals, *i e.* tuberculosis, anthrax, and so on.   Milk is, however, also a frequent carrier of typhus bacilli, which fission fungi (almost exclusively pathogenic for the human subject alone) find their way into the milk, either directly from diseased milkers or milk-dealers, or from the milk vessels being swilled out with water containing these microbes.   Farmyard wells are frequently very close to dunghills, cesspools, and closets, and if typhus breaks out on such farms, then the well-water very soon becomes impregnated with typhus bacilli by means of fæcal matter   Proofs of this exist by the dozen.   The first reliable observation on this subject was made by Ballard in 1870, when an epidemic of typhus broke out in Islington, 67 houses and 167 patients being infected.   A careful investigation of all the cases led to its being traced to a farm-house from whence the milk supplied to the infected families was derived, and there the closet cesspool was found to communicate (through rat-holes) with the well, the water from which was also used for cleansing the milk-pails.   To this first instance two others of recent date may be added   One of them was investigated by PAUL SCHMIDT (I.), and arouses interest because it treats of the inmates of a prison, where communication with the outer world is much easier to trace.   In two prisons in Strassburg (Alsace), where typhus had not recurred since the Franco-German War, it broke out again in 1890, and that, too, among a section of the inmates who had partaken of milk derived from a neighbouring village where the disease was rife   The epidemic died out when the supply of milk from this source was prohibited.   A second equally convincing instance was observed by REICH (I.) in 1892.   ROWLAND (I.) found living typhus bacilli in an Indian milk-comestible (known as " Dahi ") — The so-called explosive occurrence of this rapidly extending pestilence in a healthy neighbourhood is thus explained by the fact of its germs gaining access to the system with the food.   Finally, milk is also a carrier of certain diseases that are recognised as infectious, but whose exciting agent has not yet been discovered, *e.g.* scarlet fever—a case of which is recorded by W. H. POWER (I.)—and foot-and-mouth disease.

§ 123.—Boiling Milk.

The particulars given sufficiently evidence the necessity for killing the germs present in milk.   Experience teaches that a short boiling suffices to destroy the pathogenic organisms, the tubercle bacilli being—according to the researches of J. FORSTER and O. DE MAN (I), and of BONHOFF (I.)—killed by the action of a temperature of

55° C. in 4 hours.	80° C. in 5 minutes
60° C. „ 1 hour	90° C. „ 2 „
65° C. „ 15 minutes	95° C. „ 1 minute.
70° C „ 10 „	

The cholera bacteria and typhus bacilli are, as proved by GRUNS (II.), capable of still less resistance, and are therefore killed much more quickly than the tubercle bacilli by a treatment expressed by the above figures.   Only a single pathogenic species can withstand the short boiling to which milk is ordinarily subjected in domestic management, and this is the anthrax bacillus (spores).

The danger incurred on this account is, however, slight, since this microbe only forms spores in presence of oxygen, and therefore not within (the arterial circulatory system of) the animal body. Even in the worst case, therefore, only the vegetative forms (easily destroyed by boiling) of this microbe can find their way into the milk from the body of the cow, and, on the other hand, the introduction of these germs from external sources is hardly to be feared According to the researches of O. Caro (I.), the virulence of spore-free forms of growth of *Bacillus anthracis* in milk disappears within twenty-four hours at a temperature of 15° C, a circumstance attributable to the injurious influence of the lactic acid gradually formed in that liquid. The spores, however, completely retain their vitality under these conditions.

Among the bacteria present in unboiled milk, the species inducing lactic fermentation are never lacking, and it is to these that the souring of milk is due. The fact that they are destroyed by boiling explains why boiled milk will keep, without alteration, a much longer time than is the case with unboiled milk. In addition to the species already mentioned there is present a third group of *Schizomycetes* forming spores very tenacious of life, which withstand boiling, and germinate when the milk is kept at a moderately warm temperature. The resulting rods having, by the process of boiling, been freed from the presence of sundry inconvenient associates of other species, then develop and increase rapidly, setting up a brisk fermentation whereby a large volume of gas is liberated. The importance and extent of this fact first becomes clear in the case of suckling infants.

§ 124.—The Soxhlet Bottle.

With the continual extension of enervation the number of mothers unable or unwilling to suckle their infants increases from year to year. How far the natural nourishment thus withheld is superior, in point of chemical composition, in the various periods of lactation to any artificial medley cannot be expounded here. From the bacteriological standpoint it may be regarded as almost perfect. If the mother be healthy in body, then the milk absorbed by the child at the breast is, according to the researches of T. Ringel (I.) and others, almost entirely free from bacteria of any kind. The expression "almost free" is used advisedly, since the milk generally contains a small number, originating in the air and making their way into the lacteal ducts of the mammary glands, where they increase. The necessary hygienic treatment of this organ by the young mother will greatly contribute to the child receiving the best nourishment both bacteriologically and otherwise. To return, however, to those other matrons who bring up their infants on the bottle, filled with boiled and sufficiently cooled milk The stomach of the young child being small, whereas the amount of material required for the growing body is large, the infant requires frequent supplies of small quantities of nourishment. Generally, for the sake of convenience, a sufficient quantity of milk for the whole day is boiled at once, portions of this being taken from time to time as required Through ignorance on the part of the mother, or by the carelessness of the nurse, it often happens that this food is supplied to the infant in a partially decomposed condition. A particular fault, frequently committed, is that the bottle, which has been lying for two or three hours in the warm nursery, is refilled from the vessel containing the bulk, without the residual milk from the preceding meal having been removed. That such carelessness (frequent, though as constantly denied) must conduce to digestive disorders requires no further demonstration, the high rate of infant mortality from intestinal catarrh being sufficient evidence.

For this reason it has been attempted to render milk stable by boiling it in small bottles, holding just sufficient for a meal, and closing the same with a

stopper (impervious to bacteria), which is removed only just before use This is the fundamental idea of the so-called **Soxhlet method of sterilising milk**, in which several bottles are inserted in a movable frame and immersed in a tin pan containing water, which is thereupon kept on the boil for forty minutes SOXHLET (I.) employs latterly, as automatic stopper, an indiarubber disc resting on the ground mouth of the bottle, and prevented, by means of a loose-fitting tube, from becoming displaced laterally. The gases and steam given off from the milk in boiling escape into the air by forcing up the disc, and when the operation is finished and the apparatus is removed from the fire, this clack-valve is kept tight by the pressure of the outside air, the partial vacuum within the bottles being generally equivalent to 100 mm of mercury This stopper not only prevents access of air, but also debars dealers or purchasers from opening the bottle with fraudulent intent, since it cannot be closed again. This ensures the purchasing public receiving the milk in the same unadulterated condition in which it left the dairy. Similar to Soxhlet's method are those of Egli and Escherich, a short description of which (as also of those of Soltmann, Bertling, Gerber, and Stadtler) will be found in a comparative treatise by EMMA STRUB (I).

§ 125.—Germ-Content of Milk Treated by the Soxhlet Method.

The foregoing method would meet all requirements were the destruction of the pathogenic and the lactic acid bacteria alone in question. However, as has previously been mentioned, milk also contains very hardy bacterial spores, able to withstand such a course of boiling as that specified. The number of such spores varies, and is greater in proportion as the degree of uncleanliness in the attendance on the cows and in the operation of milking increases. These milk bacteria (which resist the ordinary means of sterilisation) were investigated with regard to their properties and action by C. FLÜGGE (II). A few of them are very widely diffused, but they do *not* develop below 18° C. Milk sterilised by mere boiling may therefore be rich in such bacteria and yet keep unaltered for a long time at room temperature, though, if introduced into the alimentary canal of the young infant, the hardy spores develop into rapidly-multiplying bacilli which decompose the constituents of the milk. In such case, not only are copious amounts of gas, giving rise to considerable flatulency, formed, but also poisonous decomposition products of albuminoid matter, which when fed to puppies produce diarrhœa attended with fatal results.

The danger incurred from this cause is much greater for the nursing infant than for the adult, not only because the latter organism is stronger, but also for the further reason that the dietary of adults is a mixed one, in consequence whereof numerous other bacteria, inimical to those in question, are introduced into the alimentary canal. On the other hand, the result of using such imperfectly and partially sterilised milk is that the digestive organs of the infant, nourished on milk alone, are converted into a veritable breeding-ground for these poisonous microbes.

These bacteria are closely allied to the *Bacillus mesentericus vulgatus*. Flugge himself described a number of such species, and S. STERLING (I.) added five new ones to the series, naming them—with reference to their chemical activity—as *Bacillus lactis peptonans a, β, γ, δ, ε* Attention was drawn at an earlier date, by LOEFFLER (III.) and Emma Strub, to the frequent occurrence of *B. mesentericus vulgatus* in milk.

Attempts have not been lacking on the part of dairy technicists and bacteriologists to arrive at a method for annihilating these pests as well A critical examination of these methods cannot, however, be made here, but any reader desiring fuller details is referred to a comprehensive exposition of the question

compiled by H. WEIGMANN (II)   At present, merely a single example will be given, namely

§ 126.—The Method of Neuhauss, Gronwald, and Oehlmann,

which was tested by PETRI and MAASSEN (II)   We have already, in a previous section, demonstrated that it is not easy to render milk sterile in the strict meaning of the word.   The high and long-continued heating necessary thereto is sufficient to alter the chemical constitution of the milk in such a manner that it becomes almost unsuitable for nutrition   The lactose, as P CAZENEUVE and HADDON (I) have shown, decomposes into dark brown fission products (containing formic acid), with an empyreumatic flavour, the fat loses its emulsified condition and separates out as cream, which cannot be made to diffuse again even by shaking ; and the albuminoids are converted into a form very difficult of digestion.

Neuhauss, Gronwald, and Oehlmann, calling to mind the information afforded by the fractional method of sterilisation, sought to induce the hardy spores to germinate, in order that the end in view might then be attained by moderate means   With this object the milk is placed in bottles with loose-fitting stoppers, which are then put into a specially constructed case, where they are surrounded by steam and allowed to remain for half an hour at a temperature of $80°-95°$ C This so-called preliminary sterilisation is once repeated, with the result that the pathogenic and the lactic acid bacteria are destroyed, and the milk is then left to cool gradually, whereby it passes through the degrees of temperature favourable to the germination of the surviving hardy spores   On the following day the samples are subjected (in the same bath) to the so-called chief sterilisation at $102°$ C., and when this is finished the stoppers of the bottles are immediately and simultaneously tightened up by means of an arrangement manipulated from the outside.   The instructions given to adhere to a temperature of $102°$ C prove that the germination of the spores is not numerically complete, since, if this were the case, a maximum of $100°$ C would suffice for the chief sterilisation, all the vegetative forms quickly perishing at this temperature.   If, however, spores be still present at the commencement of the chief sterilising process, the probability is by no means small that they will also be able to withstand the short exposure to $102°$ C, and it may be anticipated that even this method will not always accomplish its object.   As a matter of fact, reports are not wanting— e g that of M BLEISCH (I)—to the effect that milk samples assumed to have been sterilised by the method in question have been subsequently discovered to be in a state of decomposition, whilst Flugge was unable to confirm the favourable reports given by Petri and Maassen, and PIOTET and WEYL (I)

In short, there is at the present time no practicable and certain method for freeing milk (on a large scale) from germs without at the same time seriously prejudicing its flavour and nutritive value   Since, then, the annihilation of the hardy germs in this case is so difficult, attention is now directed to their exclusion from the milk, the greatest care is therefore taken—by washing the udder, hands, and milk vessels—to secure extreme cleanliness in the preparation of "nursery milk" intended for infant consumption.   The so-called sterilisation then becomes a much easier task, the milk, drawn with such precaution from the cow, being very poor in the above-mentioned gas-forming bacteria   As a ready means of detecting the presence of these organisms will often be useful to the scientific adviser of a Dairy Association, the fermentation flasks described by F. SCHAFFER (I), TH. SMITH (I and II), and others, are therefore recommended for the regular examination of the milk supplied by the individual farmers as regards its content of the pests under consideration.

§ 127.—The Content of Pathogenic Germs in Various Dairy Products.

A few words must be devoted to the description of the treatment of **skim-milk** in the factories of Dairy Associations   As is well known, a large portion of the fresh milk sent to the dairy is not sold as such, but is employed for butter-making.   The skim-milk formed in large quantities during this process is, in many instances, partly sold *per se*, and partly worked up into semi-fat and skim-cheese, milk-bread, and the like.   In other cases, the contracts made with the members of the association stipulate that each shall have returned to him, for feeding purposes, a quantity of skim-milk proportional to his deliveries of new.   Now, since in these large dairies the cream is removed from the milk by means of the centrifugal machine, it follows that the milk from all sources becomes intimately mixed up together, and consequently if any one parcel of the milk is contaminated, *the whole* of the skim-milk will become infected thereby. In this manner an epidemic hitherto confined to a single farm may, by means of the returned skim-milk, be rapidly disseminated to all the other cowkeepers. This has actually been frequently proved in respect of the foot-and-mouth disease   In this connection there is an increase in the number of supporters of legislative action in favour of a compulsory heating of the skim-milk returned by dairies to the farmers   As reported by P. VIETH (I.), a Ministerial ordinance has been in force in Prussia since 1894, prescribing that the skim-milk from cows suffering from infectious diseases shall be either kept at a temperature of 90° C for at least a quarter of an hour, or be heated up to 100° C. before being allowed to leave the dairy.

It is imperatively necessary that the cream destined for butter-making should be freed from pathogenic germs   According to the concordant results of the researches of L HEIM (IV.), G GASPERINI (II.), and O. ROTH (I.), the active organisms of cholera, typhus, and tuberculosis present in the butter long retain their vitality and power   Now, a large proportion of the butter made is consumed in a raw state in the form of bread and butter and the like, and if it has been derived from milk or cream infected with pathogenic bacteria, its consumption is attended with great danger.   Consequently a reliable preliminary treatment of the cream to ensure the removal of these germs is in the highest degree desirable.   That this can be practically accomplished will be shown in chapter xxiii. which treats of the artificial souring of cream

The same requisition should also be imposed in the case of milk designed for cheese-making, but at present this can hardly be effected, because the treatment required for killing the pathogenic germs lessens the suitability of the milk for the purpose in view, and also modifies the flora of the milk to such an extent as to unfavourably influence the ripening process of the cheese prepared therefrom. In fact, until we are in a position to introduce and carry on this process to its completion in a reliable manner by artificially added ferments, the above named requirement must necessarily remain unfulfilled.   Fortunately the acid produced by the ripening process forms an effective antidote, which checks the development of the pathogenic organisms.   H. WEIGMANN and G. ZIRN (I) proved that *Bacillus (vibrio) cholerae asiaticae* perished within twenty four hours when artificially inoculated on cheese.

As will be gathered from the preceding observations, the *sterilising* of milk samples destined for the cultivation of organisms in the laboratory is a very troublesome operation, since this necessitates an *absolute* freedom from germs In order to obtain this result, the samples are exposed for ten to fifteen minutes to steam under pressure, at a temperature of 120° C.   The decompositions

hereby induced have no injurious effect in some cases; nevertheless when delicate organisms are to be cultivated that would not thrive in milk thus altered, a method of mixed sterilisation must be practised. A little ether or chloroform is added to the sample, allowed to react for a short time, after being thoroughly shaken up, and, at the end of two or three days, disinfection is effected by placing the sample for twenty to thirty minutes in the steamer (at 100° C.). The statement, often met with in books, that milk may be sterilised by exposure to the action of a current of steam at 100° C. for twenty to thirty minutes on three successive days, is (according to the author's experience) deceptive. If such "sterilised" milk be placed in the incubator, an effluvial decomposition, with copious development of potato bacilli and the like, will be noticeable in nine cases out of ten.

A number of bacterium poisons—the suitability of which has been made the subject of comparative investigation by J. NEUMANN (I.) and M. KÜHN (I.)—have been proposed for preserving milk that is to be sent to a laboratory for the purpose of having its fat-content ascertained. The potassium permanganate, formerly recommended, behaved badly under the ordeal, whereas, on the other hand, potassium bichromate (for the use of which for the purpose in question Alén has taken out a patent) proved reliable in cases where it was a matter of preserving milk that was still sweet. The sample is treated with an admixture of 0.5 grm. of pulverised $K_2Cr_2O_7$ (or with 5 c.c. of a 10 per cent solution of this salt) per litre, the dilution produced in the latter case exercising no appreciable influence on the accuracy of the fat determination. If the milk at the moment the sample is drawn is already somewhat sour, then an addition of ammonia—3 c.c. of a 27 per cent. solution of ammonia per litre of milk—will be preferable.

Not infrequently milk intended for sale is qualified with substances acting as poisons towards bacteria, with the idea of increasing its keeping properties. Boracic acid, both in the free state and in the form of borax, is in great favour for this purpose, and recourse is occasionally had to salicylic acid. F. M. HORN (I.) records an instance where benzoic acid was used. All additions of this kind are contrary to law, and therefore punishable.

§ 128.—Condensed Milk.

It is not infrequently desirable to convert large quantities of milk into a permanently stable condition for use as food, e.g. for provisioning ships. In other cases, owing to local circumstances, the milk production of a district may be far in excess of the local requirements, and consequently the necessity arises for converting the product into a stable and readily portable condition for export. Many of the Swiss Cantons, for example, are in this position. Such milk must be capable of remaining entirely unaltered for any desired period (several years), even when exposed to tropical temperatures—a condition unattainable by any of the means already described. In former years the opinion was current that this could be effected by the method proposed by Appert, and in the middle of the seventies Nageli also attempted to attain this object by a similar (secret) method of treatment. However, the "preserved milk" produced by him (and not a few of his successors) failed to fulfil expectations, and equally unfavourable results attended the numerous attempts made to render milk stable by so-called "Pasteurisation," i.e. by the prolonged action of a temperature of about 60° to 65° C., in connection with which subject N. L. RUSSELL (III.) carried out a series of investigations (Fig. 51). All these methods, as well as numerous others of allied nature—such, for example, as that devised by Soxhlet, and which Loeflund attempted to technically utilise—have, however, been

I   L

recognised as unreliable   At present there is only one *single* way of arriving at the object in view, and that is by thickening the milk and adding sugar.

This so-called "condensed milk"—as manufactured in particular by the "Anglo-Swiss Condensed Milk Company" in their chief factory at Cham, on Lake Zug, and in a number of branch establishments outside Switzerland—is prepared in the following manner. The fresh new milk is purified by centrifugal force, and is then heated on a water-bath until nearly boiling, and mixed (in a wooden vat fitted with a steam coil) with 12 per cent. of cane-sugar. When this is dissolved the liquid is passed through a fine sieve and transferred to a vacuum pan, where the thickening process is effected at a temperature of $50°-60°$ C   As soon as the requisite degree of consistency is attained the milk is run off, rapidly cooled, and packed in clean tins, which are soldered air-tight. Commercial condensed milk contains about 25 per cent of water and 50 per cent. of sugar, the remainder consisting of albumen (12 per cent.), fat (11 per cent), and ash (2 per cent).

Some of the germs present in the new milk, especially the lactic acid bacteria, are already killed by the aforesaid heating before and during the thickening process. A few, however, survive this, and are found to be still alive in the finished product, but not in a condition to do any damage, since the high concentration plasmolyses the germs, retarding their development and so preventing decomposition   By reason of its high content of sugar, however, this condensed milk is unsuitable for the nourishment of infants.

FIG 51

Illustration of the effect of Pasteurising milk

The black square represents the germ-content of raw milk per unit of space, that of the same sample after Pasteurisation being shown by the small white square. (*After Russell*)

CHAPTER XX.

§ 129.—Storage in Cold Chambers.

IT has been established by the researches of MEISSNER and ROSENBACH (I), G. HAUSER (III.), F. ZAHN (I), J. VON FODOR (I.), and others, that the blood and flesh of healthy animals are entirely free from fungi. On the other hand, the contents of the digestive organs are exceedingly rich in *Schizomycetes*, higher fungi not being absent, though their number is quite subordinate to that of the former organisms. As was shown by D POPOFF (I.), the digestive canal of the healthy new-born animal is, at the moment of birth, free from bacteria. These, however, subsequently obtain access, principally in the food, and the contents of the bowels become extremely rich in microbes. According to the researches of NENCKI and FREY (I.), such species as decompose the carbohydrates predominate in the small intestine in man, whereas in the large intestine the microbes productive of albuminoid putrefaction exert their sway.

If, now, the carcase of a slaughtered animal be left without being disembowelled, these saprophytes will make their way through the capillary vessels of the intestinal villi into the arteries, the alkaline contents of which (rich in albumen), are unusually favourable for the development of these acid-shy putrefactive bacteria, so that the entire carcase quickly begins to undergo decomposition This can be prevented by the excision of the entire length of the alimentary canal from œsophagus to rectum inclusive, and if this long-known and practised precaution be adopted, then the remaining flesh, &c , will be perfectly free from fungi. If putrefaction subsequently arises, it is due to the bacteria from external sources (air, supports, butchers' hands, &c.) obtaining access to and settling in the flesh. Their gradual penetration by way of the blood-vessels into the interior of the flesh was studied by S. TROMBETTA (I.) and by Gartner. The latter found them only in the external layers in the case of meat three days old, but, at the end of another seven days, they had penetrated to a depth of 2 cm. below the surface. Since the sources of this bacterial infection cannot be entirely shut off, though they may be considerably reduced by cleanly procedure, attempts are made to prevent the increase of these parasites in the flesh.

The oldest known remedy is cold, but in order to realise expectations, the temperature must be kept several degrees below zero (C.), and this is the method pursued in the large (export) abattoirs in America (Chicago in particular) and Australia. Immediately a beast is killed and disembowelled, the carcase is placed in a refrigerating chamber and then transported in cooled railway trucks, and cold chambers on shipboard, to its destination in a frozen state. Thus, for instance, there appear daily in the London market hundreds of carcases of Australian sheep still frozen hard. In a similar manner Central Europe has been for several years supplied with haddocks prepared for shipment in the north of Norway (Vardo) by being frozen at - 40° R. (- 50° C) directly they are caught and cleaned, and being then shipped in this condition in specially built steamers. The freezing of meat does not kill the germs present, but merely hinders their

reproduction, and, as a matter of fact, A KOCH (III) found very many bacteria in fish that had been treated in this way.

If the meat be not stored at low temperatures, but merely put in the ice-chest or laid on ice, whereby it attains, in the most favourable instances, a temperature of o° C., then, as follows from the already reported labours of Forster and others, an increase of the initial number of germs ensues. To the activity of such cold-supporting organisms is attributable the peculiar, disagreeable taste and smell acquired by edibles after remaining in the ice-chest for a few days. Actual putrefaction is not, however, produced by these bacteria.

We must not lose the present opportunity of issuing a warning against bringing food-stuffs in immediate contact with natural ice, since this substance contains not only numerous putrefactive bacteria, but also, under certain circum stances, pathogenic germs (especially typhus bacilli) as well. In this connection we may refer to the researches into the bacterium content of ice that have been made by C. FRAENKEL (V), BORDONI-UFFREDUZZI (II.), F. PRUDDEN (I.), and A. HEYROTH (I.). In the cooling chambers of large abattoirs—of the arrange ment of which there is an excellent description in a work by OSTHOFF (I.)—the meat is not exposed to this source of infection.

The well-known fact that frozen meat, when thawed, undergoes decompo sition more rapidly than fresh meat is easily explained. The cellular structure is loosened by freezing, and access to the interior is thereby facilitated for any organisms present on the surface.

§ 130.—Dried Meat and Salted Meat.

The development and activity of the organisms exciting decomposition can also be prevented by depriving them of the water necessary for metabolism The drying of meat has been practised, particularly in hot countries, from th earliest times. The resulting conserve is known in South America under th names of pemmican, charque, and tassajo. The process is as simple as it is reliable, and has a great future in prospect, especially for the provisioning of armies in the field. In the method of treatment hitherto practised, the mea during drying suffers a great depreciation in flavour, but in recent years Hof mann and Meinert have devised and patented a process for the artificial dryin of meat without removing or destroying its flavouring matters. By this proces a Bremen firm manufactures a meat meal met with in commerce under the nam of *Carne pura*.

Under the same name inferior meat meals only fit for cattle food are shippe to Europe from Argentina ; these are prepared from waste materials and requi some care in handling. The drying of flesh does not, of course, result in th killing of all the bacteria present therein, and if the flesh of cattle suffering fro epidemic diseases has been employed, then, under the defective conditions live-stock inspection in South America, disease germs will be disseminat by means of such infected food. A case of this kind has been reported R. BURRI (I.).

The most important example of dried flesh is afforded by the dried cod-fi (stock-fish), which forms the chief article of export from the Scandinavi peninsula. It contains, in the dry state, nearly 80 per cent. of albumen, a constitutes a favourite and cheap article of food among the poorer classes Central Europe.

The salting and pickling of meat is generally credited with great effica but a closer examination reveals that it is really only the hygroscopicity of t salt that comes into play and that the sole power the latter possesses is that setting up plasmolysis in the germs present in, or subsequently conveyed to, t

flesh, and so preventing their reproduction. Consequently, the germs, especially those of a pathogenic nature, cannot be completely killed by these processes. O J. DE FREYTAG (I) has proved that the influence of concentrated solutions of common salt is resisted by tubercle bacilli for three months; by typhus bacilli for six months; and by the bacilli of swine erysipelas for two months, the organisms remaining alive and virulent during these periods. F. PEUCH (I) examined the effect of salting on the flesh of animals succumbing to anthrax, and found that a ham from such an animal, after lying in salt water for fourteen days, still contained virulent anthrax bacilli, as was proved by direct experiment on animals with the expressed juices of the meat. PETRI (II.) showed that virulent "*rothlauf*" (erysipelas) bacilli were still present in the pickled flesh from swine affected with swine erysipelas, after six months' immersion in brine.

If the inspection of meat is carried out with even only a moderate amount of care, it will not be easy for animals suffering from anthrax to be slaughtered for food, so that there is not much danger to be dreaded from that source. The case is, however, different as far as animals affected with tuberculosis or swine erysipelas are concerned.

In a former paragraph the prevalence of tubercular affections among cattle was mentioned, and this should be sufficient to deter the reader from indulging in uncooked beef, whether in the form of "*beefsteak à la tartare*" or uncooked pickled beef. It is well known that the flesh of swine that have been compulsorily slaughtered on account of swine erysipelas is offered for sale; hence it naturally follows that many kinds of ("*wurst*") sausages that are made from raw flesh, and eaten in an uncooked state, will contain pathogenic germs.

§ 131.—Smoked Meats and Corned Beef.

Smoking forms a more reliable means of preserving meat from putrefaction, the real active agents in the process being the vapours of phenol, creosote, and allied compounds present in the smoke. Beechwood being found to yield the smoke containing the largest quantities of these substances, is therefore held in particular esteem for this purpose. The volatile distillation products of heated wood chips are condensed on the pieces of flesh, and arrest the development of the bacteria. Since, however—as A. SERAFINI and G UNGARO (I.) have shown—these antiseptics do not penetrate far into the flesh, and are therefore unable to exert much action in the interior of the pieces, smoking can only be effectual when it is a question of preserving fresh meat (from healthy animals) which is only superficially infested with germs. The manner in which the process is carried out in practice very often leaves much to be desired; and thus it is—as shown by the exhaustive researches of H. BEU (I.) and A. SERAFINI (I.)—that the germ-content of commercial smoked meat varies considerably. The salting which precedes smoking, though of such little efficacy in itself, is nevertheless useful, and forms an essential part of the process, by withdrawing water from the meat, and thus facilitating the penetration of the smoke. The certain destruction of pathogenic germs is not effected by smoking, Petri having found that the flesh of swine affected with swine erysipelas contained erysipelas bacilli in a state of undiminished vigour, after immersion for a month in brine, followed by careful smoking for fourteen days. A similar unfavourable result was obtained by J FORSTER (III.) in the case of smoked meat from tuberculous animals.

The best method at present available for the preservation of meat consists in steaming the same in vessels which remain hermetically closed up to the time the meat is eaten Such a food is known as **preserved meat** (in the restricted sense), or as **tinned meat**, the quality in most demand being **corned beef** (chiefly obtained from Chicago). This *should* consist of the flesh of oxen, but, nerverthe-

less, occasionally originates at the horse-slaughterer's. As already explained in a former chapter, we are indebted to the French confectioner APPERT (I) for the fundamental practical experiments made in connection with preservation by steaming. In accordance with his process, the meat is boiled in any convenient vessel, and pressed into tins. These are then closed, with the exception of a small aperture, and placed in a bath of boiling water, the aperture being closed by means of a little liquid solder, after steam has been given off for a short time Appert's successors subjected his process to numerous modifications, Fastier proposing to heat the tins up to 110° C. in a bath of salt, and others recommending additions of boracic acid, &c. Up to the present, no known antiseptic possesses the dual property of, on the one hand, preserving flesh without depriving it of valuable nutritive constituents or flavouring matters, and, on the other, of having no injurious effect on health when the meat is eaten regularly.

Inventors have been, and are still, particularly active in this field, and the different processes for preserving meat that have been proposed are very numerous. To report exhaustively on these would, however, far exceed the limits of the present work ; any reader wishing to be more accurately informed on this subject is referred to a comprehensive treatise compiled by PLAGGE and TRAPP (I). Instructions intended for practical use in the preservation of meat, fruits, vegetables, &c., have been given by L E ANCLÉS (I.) and J DE BREVANS (I.).

§ 132.—Preserving Eggs.

The contents of the freshly laid eggs of birds, especially of poultry, are not in all cases perfectly free from fungi. In refutation of a widespread assumption to the contrary, it was shown by U. GAYON (II.) in 1875, and confirmed by O. E. R. ZIMMERMANN (I.) in 1878, that the eggs, even of healthy birds, are exposed, even during the time of their formation, to infection by bacteria These organisms, starting from the common anal duct of the bird, make their way into the ovary, where they become mixed with the albumen of the embryo egg, and reproduce themselves therein when the nutrient medium permits The new-laid egg is therefore already inhabited by bacteria, a circumstance that must be borne in mind when it is desired to utilise raw eggs for the cultivation of bacteria, according to the proposal made by F. HUEPPE (V.).

The obnoxious decomposition not infrequently set up in eggs is generally attributable to the development of these early intruders. Their pure cultivation was first attempted by J. SCHRANK (I), and then on a larger scale by C. ZÖRKEN-DÖRFER (I.), according to whom the so-called spontaneous stinking putrefaction of eggs goes on in two ways.

The *first* type is characterised by the albumen (white) changing colour through whitish-grey to grey-green, and by the yolk becoming gradually converted into a greasy, blackish-grey mass. At a subsequent stage the yolk becomes mixed up with the albumen, so that the entire contents of the egg form a pulpy ichor, smelling strongly of sulphuretted hydrogen, which gas is not infrequently produced in such quantity that the shell of the egg bursts with a report. Of the organisms taking part herein, Zorkendorfer isolated ten species, and distinguished them as *Bacillus oogenes hydrosulfureus* α, β, γ, δ, ϵ, ζ, η, θ, ι, κ, the first six of which liquefy gelatin.

In the *second* type of (bacterial) egg-putrefaction this gas is not detected. Here the yolk and the albumen quickly coalesce to form an initially thin, but subsequently pulpy, mass of a pale ochreous-yellow colour, and with an odour like that of human fæces. Zorkendorfer described five species of organisms causing this decomposition, and bestowed on them the name of *Bacillus oogenes fluorescens* α, β, γ, δ, ϵ. The first of these liquefies gelatin, and they all elabo-

rate a pale green pigment which imparts a beautiful blue fluorescence to the medium.

All these bacteria are exclusively aerobic, *i.e.* oxygen is essentially necessary to their development This needful gas obtains access from outside by passing through the eggshell, which is well known to be permeable thereto, since otherwise the development of the embryo chicken could not proceed. This necessity for air on the part of the egg-putrefying *Schizomycetes* supplies the explanation of the practice currently employed for preserving eggs, viz., by simply immersing them in milk of lime, which not only excludes air, but also—by its disinfectant properties—acts on the organisms present on the eggshell and ready to penetrate into the interior, killing some and restricting the development of others.

The aforesaid bacteria perish within the space of two days when exposed to temperatures above 40° C., but at lower temperatures and in damp air they develop rapidly. Bearing these facts in mind, eggs *could* be preserved by keeping them for one or two days at 50° C. and then storing them in a dry place, were it not that the quality is thereby depreciated. If, then, steeping in milk of lime is not determined upon, the eggs can be preserved by coating them with lacquer or varnish after a careful cleaning.

That bacteria penetrate through the unbroken eggshell has—contrary to an opposite opinion expressed by Gayon—been placed beyond doubt by the exhaustive researches of Zorkendorfer. WILM (I) showed that pathogenic bacteria are also capable of so doing, cholera bacilli being found, in his experiments, to penetrate to the interior of the egg within fifteen to sixteen hours. PIORKOWSKI (I) arrived at a similar conclusion with regard to typhus bacilli. On this account none but clean chaff, free from pathogenic organisms, should be used for packing eggs.

Bacteria are not to blame in all cases where eggs become spoiled during storage, but sometimes higher fungi (*Eumycetes*) come into play, penetrating the shell and growing freely in the interior. Further particulars on this point will be found in the second volume, in the chapter dealing with *Hormodendron cladosporioides.*

§ 133.—Desiccating and Preserving Vegetables and Fruit.

In contradiction of the erroneous assumptions of M GALIPPE (I.) and H BERNHEIM (II.), it has been shown by E. LAURENT (III), A. FERNBACH (I.), and H. BUCHNER (VIII) that—apart from the exceptions to be considered in chapter xxxiii —the cells and cellular tissues of the higher plants are, whilst in a healthy condition, free from fungi In the preservation of vegetable foodstuffs it is, therefore, merely a question of the destruction, or restriction of development, of the germs of extraneous origin inhabiting the surface. The oldest process for attaining this object is that of drying, and this is practised more particularly on certain fruits In warmer regions the rays of the sun suffice, *e g.* in the case of raisins and figs, but in colder climes recourse must be had to artificial warmth, and, consequently, so-called kilns or drying ovens are employed, wherein hot air at a temperature of 60°–65° C is allowed to stream over the fruit. American desiccated apples and Bosnian and Servian prunes are prepared in this way. A description of the individual systems of kilns for this purpose cannot be entered upon here. The dried fruit still contains a considerable proportion of moisture (some 30 per cent) and at least the same amount of sugar about 30 per cent. in the case of pears, some 40 per cent. in apples and damsons, 50 per cent. in figs, and 60 per cent. in raisins. From 1 to 3 per cent. of free acid is also present. Only some of the organisms present on the fruit are killed by drying, but the development and decomposing action of

the rest are checked by the plasmolytic influence of the high sugar-conte
The putrefactive bacteria also suffer through the action of the free acid prese
Sundry vegetables, especially those employed for Julienne soup, are preserv
by drying, for which purpose they are cut into small pieces, and exposed
special ovens to a hot air temperature of 50°–60° C. Not infrequently they a
then, in accordance with a proposal made by Masson, subjected to hydrau
pressure, compressed vegetables being thereby produced.

Drying is a comparatively inexpensive operation, but cannot be resorted
in every case, since many kinds of fruit and vegetables have their fine flavc
too much impaired thereby. Such articles are treated by the Appert proce
Green peas, cauliflower, asparagus, beans, and suchlike are manipulated
follows: After being carefully cleaned, they are placed in glass jars or in ti
which are then filled with water and set in a salt bath, the temperature of wh
is maintained at below 100° C. for one or two hours, and thereafter raised
boiling point (108° C), at which it must be allowed to remain for some time,
order to definitely destroy all the hardy spores of the hay and potato baci
The temperature is then allowed to sink to 60° C., whereupon the small blc
hole in the otherwise closed tin is sealed up by means of a drop of solder, gl
jars being closed air-tight by other suitable means. Preserves carefully p
pared in this manner are sterile in the strictest sense of the term; but if perf
sterility is, by reason of any oversight, not attained, the still-living germs su
sequently increase at a great rate. Their development is mostly attended w
the evolution of gas, in consequence of which the straight walls of the tin a
bulged outwards, and frequently even burst.

A drawback accompanying Appert's process is that the colour of t
vegetables so treated is generally destroyed. If the preservation of the colc
be desired, as is the case, e.g, with red beet, gherkins, and the like, then otl
means must be resorted to, and the antiseptic properties of the acids be utilis
The samples in question are boiled in vinegar, the liquor being then poured
and replaced by fresh unimpaired vinegar In this way mixed pickles,
instance, are prepared. In many cases the boiling is omitted, pickled gherki
for example, being preserved by simple immersion in cold vinegar.

That no protection against the development of bacteria is afforded
steeping in brine needs no further argument. As a matter of fact, an eas
observable decomposition occurs in the so-called salted gherkins prepared in t
way, the phenomenon proving to be lactic fermentation; and it is to the a
thereby produced, and not to the small proportion of common salt present, tl
the retardation of decomposition is due.

The boiling of fruits and fruit-juices is an operation too well known to ne
detailed description here The added sugar employed herein restricts deco
position by strongly plasmolysing and preventing the development of such ger
as are not destroyed by the boiling. In many cases, this action is assist
by the addition of a certain quantity of whortleberries. A few particul
respecting the high percentage of the strongly antiseptic benzoic acid present
the latter have already been given in an earlier chapter (§ 80). The glass j
destined to contain the finished jam, marmalade, &c., are sulphured previous
use.—The preservation of fruit is greatly facilitated by a careful prelimina
cleaning, a precaution that should, moreover, not be omitted when the fruit is
be eaten raw, since the usually sticky surface tenaciously retains the dust a
accompanying germs that are blown on to it. Thus, M. T. Schnirer (
discovered virulent tubercle bacilli on the surface of grapes sold in the Vien
market.

A word must be added with regard to the gelatinisation of fruit juice.
is well known, the cells of many fruits are rich in pectin, which, when the ce

are crushed, passes into the juice and causes it to coagulate. This phenomenon was explained by Frémy, in 1840, to be due to the action of an enzyme, viz., pectase, whereby the pectin is converted into pectic acid. This opinion was modified by the researches of G. BERTRAND and A. MALLÈVRE (I), in so far that they showed that the enzyme can accomplish the transformation referred to only when in presence of soluble salts of the alkaline earths (*e.g.* lime), with which the pectic acid enters into combination, and forms insoluble pectates. The presence of this gelatinising compound is indispensable for the preparation of pure fruit jellies; and the latter must not be too strongly boiled, or their setting properties will be diminished or completely destroyed. In the preparation of fruit juices, such as raspberry juice, it is necessary, on the other hand, to get rid of these pectin substances, because they detract from the utility of the juice, which should remain liquid. This end is attained by leaving the fresh juice to itself for a time, fermentation soon ensuing, by which the pectin or pectate is decomposed. The juice is then strained and boiled down after the addition of sugar.—A few references to the literature of the subject will be useful to the food-stuff chemist, who is not infrequently asked for advice concerning the best means of turning fruit to account. Full particulars on the treatment of fruit in general, as also of drying and preserving it, will be found in the handbooks of FR. LUCAS (I.), KARL BACH (I.), and H. TIMM (I.). A brief introduction to the preparation and treatment of fruit wines has been arranged by M. BARTH (I.), and a pamphlet written by W. TENSI (I.) deals chiefly with currant wine (as the finest of all fruit wines), as well as with gooseberry wine, &c.

The preservation of wine-must is practised on a large scale, particularly in Sicily. To render the juice highly suitable for transport, it is (after a preliminary filtration) concentrated *in vacuo* at 40° C. to about one-fourth of its original volume. In this manner a thick, syrupy mass is obtained, the composition of which can be deduced from the following analytical figures furnished by TH. OMEIS

Water	. 35 1	per cent
Dextrose + levulose	. 62 2	,,
Acid	1.2	.,
Ash.	. 0 7	,,
Albumen, gum, &c	. 0 8	,,

This concentrated wine-must, which is shipped in sealed tins, is not in any case sterile, though the still living germs (particularly yeasts) present therein are, by reason of the high concentration of the liquid, incapable of development. If, however, the mass be diluted with sterilised water, then fermentation ensues within a short time. According to the experience of J WORTMANN (II), and also of the author himself, the employment of this must can be recommended in laboratories dealing with Fermentation Physiology. The dilution of 1 part of must with 4 parts of water yields a nutrient medium exceedingly favourable for the cultivation of higher fungi (wine yeasts), the low percentage of nitrogen being improved by an addition of 1 per cent of ammonium tartrate.

The preservation of beer and wine by heating (Pasteurisation) will be dealt with in a subsequent section of the second volume.

LACTIC FERMENTATION AND ALLIED DECOMPOSITIONS.

CHAPTER XXI.

GENERAL CHARACTERISTICS.

§ 134.—Discovery of the Lactic Acid Bacteria.

IN chemical text-books acetic acid is generally characterised as being the first acid known to man. This assumption cannot, however, be considered as probable, since, in order to obtain acetic acid, the previous preparation of alcoholic liquids is necessary, and the human race in its earliest stage of civilisation, viz , nomadic life, would hardy have attained that skill—the production of wine and vinegar, even in the most primitive fashion, presupposing a settled mode of existence. On the other hand, the flock-owning nomadic races must, at a very early period, have noticed that the milk supplied by their animals very quickly underwent alteration, and turned sour when left to itself. Lactic acid must therefore be regarded as the earliest acid known to man, though not in a pure condition, since that condition necessitates the employment of methods for removing all the other constituents of the milk. This result was first accomplished by the Germano-Swede Scheele in 1780. The earliest complete chemical investigation of the souring of milk was instituted in 1833 by PELOUZE and GAY-LUSSAC (I.), but from that date fully twenty-five years elapsed before the knowledge that this process is a manifestation of vital activity on the part of sundry micro-organisms assumed definite shape It is true that already in 1701 Andry had noticed that sour milk contained such organised microcosms. Nevertheless, this observation remained at first as unproductive, as regards the comprehension of the question, as did also the labours of sundry other subsequent workers. Among these mention may be made of Blondeau, who in 1847 made a microscopic examination of milk, and distinguished therein two types of micro-organisms · the one (which he named *Torula*) was a yeast-like plant, the other, a mould fungus, which he assigned to *Penicillium* and held to be the cause of lactic fermentation.

We may recall to mind that Pasteur, in his treatise (against spontaneous generation) in 1862, pointed to the non-success experienced by the opponents of this theory, especially Schroder and Dusch, when they employed milk for their refuting experiments. Knowing, as we do, that, before Pasteur, no one had succeeded in rendering milk absolutely free from germs, it is therefore easy to understand that up till then nothing definite could be urged against the hypothesis, put forward by chemists, of the purely chemical nature of the process of lactic fermentation. Thus, for example, ROWLANDSON (I), under the influence of the Liebig and Gay-Lussac theories of fermentation, defined the preliminary conversion of lactose into lactic acid in the souring of milk as an oxidation process, and expressed the naïve opinion that a cow that had been running about (and therefore breathing rapidly) before milking would yield a milk rich in

oxygen, and consequently liable to turn sour with unusual rapidity The opinion of FRÉMY and BOUTRON-CHALARD (I.) (formed in 1841, under the influence of Liebig's theory), that casein was the cause ("ferment") of lactic fermentation, was revived, though with little success, by A. P. FOKKER (I.) in 1889.

Pasteur (X.) was the first to describe (1857) an organism characteristic of lactic fermentation, and to prove the same capable of producing acidification in a sweet, sterile milk. This organism, which Pasteur named the "ferment, or yeast, of lactic fermentation," was a bacterium. A pure culture of this, in the present meaning of the term, was at that time unattainable, no suitable method having then been devised. Pasteur demonstrated the difference existing between this "ferment" and that of alcoholic fermentation, and proved that in nutrient media containing sugar, the former organism always sets up lactic fermentation, whilst the other invariably gives rise to alcoholic fermentation.

This discovery formed an important and welcome support to the theory of specific ferments promulgated by Fr Kützing in 1837, and implying that chemically different fermentations are carried out by physiologically different species of organisms.

§ 135.—Bacterium lactis Lister, and Bacillus acidi lactici Hueppe.

The important work issued in 1873 by the English surgeon and founder of the antiseptic treatment of wounds has already been noticed (§ 68). In that paragraph the methods of working employed by him at that time were referred to as defective and misleading It was also stated that the name, *Bacterium lactis*, employed by him was erroneous, the bacterial culture to which it was applied not being a uniform species, but an indefinite (and very probably highly diversified) mixture of different species.

LISTER (II) himself very soon recognised the weakness of his arguments, and sought for a remedy. This he found in the so-called dilution method, by the aid of which, in 1877, he produced from sour milk a pure culture of a fission fungus to which he applied the name of *Bacterium lactis* as before—this time correctly. The twofold origin of this name should therefore always be remembered Lister was also the first to make the observation, subsequently confirmed by Cohn, that lactic acid bacteria, though of frequent occurrence in the rooms of dairies, are comparatively seldom found in the open air.

The introduction of gelatinised nutrient media into bacteriology also furthered the study of lactic fermentation. By means of this new method of pure culti- vation HUEPPE (IV.) in 1884 isolated from sour milk a microbe known as *Bacillus acidi lactici*, which, in so far as can be gathered from the description given, was identical with Lister's bacterium. Hueppe also made the more important discovery that several different species of bacteria are capable of setting up lactic fermentation. However, before noticing these other organisms, we will examine more closely the *Bacillus acidi lactici*, which occurs in the form of non-motile rods 1 0–1.7 μ long and 0 3–0.4 μ broad, mostly in pairs and but rarely united to form a four-cell chain; it is aerobic and forms endospores This ferment acidifies milk between the temperatures of 10° and 40° C , the reaction being accompanied by the precipitation of casein, and an evolution of gas On gelatin plates the organism forms white colonies which do not liquefy the nutrient medium.

In addition to the five species of lactic acid bacteria discovered by Hueppe— and to which *Micrococcus prodigiosus* belongs—many others possessed of the same property have been made known to us, by Maddox in England (1885), Beyer in North America (1886), and FOKKER (II) in Holland (1890). R. KRUEGER (I)

isolated his *Micrococcus acidi lactis* (which liquefies gelatin) from cheesy butter.
G. MARPMANN (I) discovered five species belonging to this group in Göttingen
milk, and named them *Bacterium lactis acidi, Bacillus lactis acidi, Bacterium
limbatum lactis acidi, Micrococcus lactis acidi,* and *Sphærococcus lactis acidi.*
G. GROTENFELT (II) isolated a lactic-acid-forming, anaerobic *Streptococcus acidi
lactici* from Finnish milk.   In his communication, issued from Hueppe's labora-
tory, there also occurs the remark that *Bacillus acidi lactici H.* can be perma-
nently deprived of its acidifying power by cultivating it for some time in media
free from sugar. This attenuation of the cultures is also often noticed in patho-
genic bacteria, many of which lose their virulence—*i.e.* poisonous nature, and
consequent capacity of producing disease—when kept for some time under
unaccustomed conditions of nutrition, viz., outside the animal body.   Bearing
this in-mind, Grotenfelt speaks of a **variable virulence** of *Bacillus acidi lactici,*
meaning thereby the possibility of reducing its fermentative power.

The fermentative properties of the *Bacterium lactis aerogenes,* found by
ESCHERICH (I) in the contents of the intestines of sucklings, and also in uncooked
cow's milk, were investigated by A. BAGINSKY (II.), who found that in artificial
media containing lactose it produces both acetic acid and lactic acid.   The gas
liberated during the reaction consisted of CO_2, 22 per cent.; H, 30 per cent.;
CH_4, 9 per cent.; N, 39 per cent. R. WURTZ and R. LEUDET (I.) considered
this microbe to be identical with Pasteur's lactic acid bacillus, but their opinion
does not seem to be well founded   According to J. DENYS and J MARTIN (I.),
B. lactis aerogenes is only a variety of the *Pneumobacillus* (§ 33) discovered by
Friedländer

Respecting the *Pediococcus acidi lactici* discovered by P. LINDNER (I.) a few
particulars will be given in chapter xxv.; and details concerning the part played
by the lactic acid bacteria in certain industrial fermentation processes, such as
distilling, dairying and cheese-making, tanning, &c., will be found in chapters
xxiii. to xxvii.

§ 136.—The Equation of Lactic Fermentation

is (when lactose or grape-sugar is presupposed as the raw material) generally
expressed in chemical text-books as follows :

$$C_{12}H_{22}O_{11} + H_2O = 4C_3H_6O_3$$
$$\text{Lactose} \qquad\qquad \text{Lactic Acid}$$

$$C_6H_{12}O_6 = 2C_3H_6O_3.$$
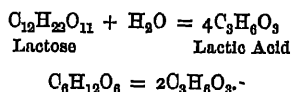
Actually the process is not so simple as here represented, a certain quantity
of the sugar employed being consumed by the organisms to enable them to dis-
charge their vital functions and bring about the fermentation in question. Con-
sequently the actual yield of lactic acid obtained is less than the theoretical
quantity calculated from the foregoing equations   Another proof of the complex
nature of the operation is afforded by the large quantity of gas liberated during
the fermentation, but which is not indicated in the reaction expressed by the
equations aforesaid.

According to the researches of R WARRINGTON (I.), the amount of acid pro-
duced varies greatly in different species, and is so small with some that (as noted
by Conn) it is insufficient to curdle the milk.   This difference is explicable by
the varying susceptibility of the individual species to the adverse influence of
the resulting acid. On this account alone, fermentation may come to a stand-
still notwithstanding the presence of sufficient unconsumed nutrient material.
The difficulty is easily met by opportunely neutralising the acid by an addition of

the carbonate of calcium, magnesium, or zinc. In the latter case, the high characteristic lustrous acicular crystals of zinc lactate ($[C_3H_5O_3]_2Zn + 3$ aq.) obtained.

A few quantitative experiments made by ADOLF MAYER (II) show that 100 parts of fermented lactose produce—

> 83.9 parts of lactic acid
> 3.7 „ „ acetic acid
> 12 4 „ „ unknown substances.

These results, however, were not obtained with pure cultures of lactic acid bacteria, and therefore are not fully conclusive.

Pure cultures of lactic ferments were first employed by E. KAYSER (I.) in 1894, in an investigation of fifteen different species of lactic acid bacteria isolated from French milk, Belgian beer, Danish cream, wine-must, rye infusion, sauerkraut, &c. Confirming the results of Mayer and Baginsky, he showed that volatile acids, also, are produced in the course of lactic fermentation, their amount depending on the composition of the nutrient medium as well as on the species of ferment. Thus, for example, a greater quantity of volatile acids was produced from a milk qualified with peptone than from a peptonised maltose solution. Cultures grown at the bottom of the nutrient liquid ("lactic bottom fermentation") yielded less than surface cultures. This fact had been already recorded in 1889 by OPPENHEIMER (I.), who found the ratio of acetic acid to lactic acid produced from milk fermented by *Bacterium lactis aerogenes* to be as 85 : 15; and in the case of *Bacterium coli commune* as 70 : 30. This ratio is, however, not invariable, but is chiefly determined by the amount of oxygen present. Hence, in the absence of air, only small quantities of the volatile acids are found, lactic acid being almost the only acid present.

Attempts were made by G. KABRHEL (I. and II.) and H. TIMPE (I.) to investigate the part played by casein and the phosphates in the lactic fermentation of milk. The optimum fermentation temperature is between 30° and 35° C., and the operation proceeds much more actively when air is excluded. Kayser was unable to detect any lactic *enzyme* excreted by the bacteria and capable of converting lactose into lactic acid.

In view of these results, it is hardly necessary to say that the establishment of a satisfactory equation to represent the reactions occurring in lactic fermentation is highly improbable.

THE PRODUCTION OF OPTICALLY ACTIVE ORGANIC COMPOUNDS BY FERMENTATION.

§ 137.—Isomers of Lactic Acid.

THE details given in the preceding chapter with regard to the physiological activity of the lactic ferments require supplementing in one important particular. Mention has been made of lactic acid, and always without qualification or reference to the fact that there are several isomeric lactic acids. This question can now be considered from a higher standpoint, and the occasion utilised for dealing with the production of optically active substances in general, through the activity of micro-organisms.

Stereo-chemistry teaches that all optically active organic substances contain in the molecule at least *one* asymmetric carbon atom, *i.e.* one whose four atom-fixing powers or affinities are connected with four different elements, or groups of atoms (radicals). On the other hand, experience shows that there are compounds (such as racemic acid and mesotartaric acid) which contain one or more asymmetric carbon atoms, but are nevertheless optically inactive. The structural formula of mesotartaric acid is COOH—CHOH—CHOH—COOH. This same formula, however, is also adopted for two other acids, named from their optical properties respectively *dextro-tartaric acid* and *levo-tartaric acid*. To explain this fact it is necessary to refer to the hypothesis promulgated in 1874 by VAN'T HOFF (I.), that the four equivalents of a carbon atom are arranged at the angles of a tetrahedron, in the centre of which the carbon atom itself is situated, whereas the atoms or radicals combined therewith occupy the angles. For example, let *Cabcd* be a compound containing *one* asymmetric carbon atom *C*, with which are combined the four (different) atoms or radicals *a*, *b*, *c*, *d*. By employing the tetrahedron scheme, the arrangement of these latter can be effected in two different ways:
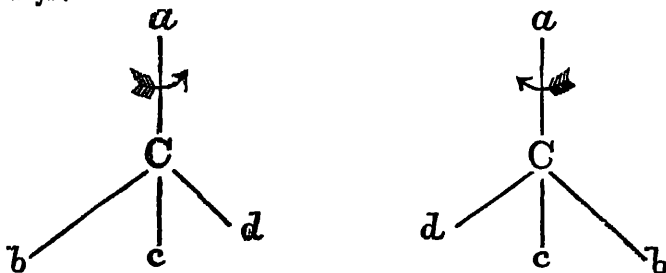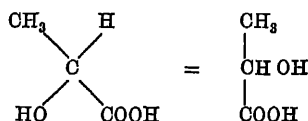

These two formulæ are not identical, but stand in the mutual relation of an image and its reflection. In order to get from *b* over *c* to *d*, it is necessary (looking from *a*) in the one instance to move in the same direction as the hands of a clock; but in the other case the movement is reversed.

The compound *Cabcd* is thus obtained in two modifications which have the

same structural formula, and behave similarly from a chemical point of view, but differ in a physical sense (crystalline habit, solubility, behaviour towards polarised light) Such compounds, whose different behaviour can only be explained by the assumption of a difference of grouping in the molecule, are designated stereo-isomers. Of the compound *Cabcd* there may exist one modification capable of deviating polarised light towards the right hand, and a second producing a left-handed rotation ; and one part by weight of the dextro-rotatory modification gives rise to just as great a deviation towards the right hand as one part by weight of the levo-modification does towards the left hand.

If now a compound containing such an asymmetric carbon atom be artificially prepared from substances devoid of asymmetric atoms, the probability is that just as many levo-rotatory as dextro-rotatory molecules will be produced; and assuming that each molecule of the one kind coalesces with a molecule of the other class to form a double molecule, then an optically inactive compound will result. There may therefore be presumably obtained from one combination, *Cabcd*, containing a single asymmetric carbon atom, *three* different modifications, two of which (one dextro-, the other levo-rotatory) are optically active and the third inactive. An example of such a compound is afforded by ethylidene lactic acid—

$$
\begin{array}{ccc}
\text{CH}_3 \quad \text{H} & & \text{CH}_3 \\
\diagdown \diagup & & | \\
\text{C} & = & \text{CH OH} \\
\diagup \diagdown & & | \\
\text{HO} \quad \text{COOH} & & \text{COOH}
\end{array}
$$

of which one optically inactive form is known, and in chemical text-books is generally named **fermentation lactic acid**. The dextro-rotatory modification has also long been known to chemists under the name of sarco-lactic or para-lactic acid, whilst the third modification, levo-lactic acid, was only discovered and produced a few years ago by fermentation physiologists. This will be again referred to later on.

§ 138.—The Isomeric Tartaric Acids.

Of the compounds containing *two* asymmetric carbon atoms in the molecule we will refer to only that group in which these two atoms are connected by a *single* bond, as is represented in the subjoined typical formula—

$$
\begin{array}{ccc}
a\diagdown & & \diagup a \\
b\!-\!\text{C}\!-\!\text{C}\!-\!\beta \\
\diagup & & \diagdown \\
c & & \gamma
\end{array}
$$
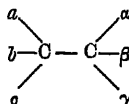
As regards the positional arrangement of the two groups of substitutes (*a*, *b*, *c*, or *a*, *β*, *γ*) in the molecule, four possibilities are conceivable (1) both grouped in the dextro- position; (2) both in the levo- position; (3) the one dextro- and the other levo-; (4) or, finally, *vice-versâ*.

Assuming the group $-\overset{a}{\underset{c}{\text{C}}}-$ to possess a (numerically) greater optical

rotatory power (D or L) than $-\overset{a}{\underset{\gamma}{\text{C}}}-\beta$, which may be expressed in letters thus—

$$D > d \quad L > l,$$

Then the **rotatory power** of the entire molecule will have the following values—

$$D + d, \qquad L + l, \qquad D - d, \quad \text{or} \quad L - l,$$

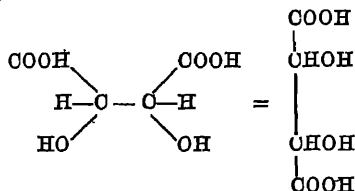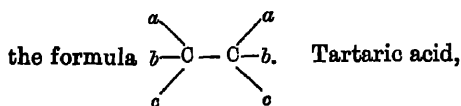according to which of the four possible compounds is present.

The optical effect of the one semi-molecule will therefore be strengthened by that of the other semi-molecule in the first two instances, or weakened thereby in the other two cases. Consequently there result *four* stereoisomeric active modifications: the first strongly dextro-rotatory; the second strongly levo-rotatory; the third faintly dextro-rotatory; and the fourth faintly levo-rotatory. Furthermore, one molecule apiece of the two strongly rotatory ($D + d$ and $L + l$), as also one apiece of the two faintly rotatory ($D - d$ and $L - l$) modifications, can coalesce to form inactive double molecules—

$$\left\{ \begin{array}{c} D + d \\ L + l \end{array} \right\} \quad \text{and} \quad \left\{ \begin{array}{c} D - d \\ L - l \end{array} \right\}$$

The **total number** of all conceivable stereoisomeric **modifications** of the compound $b-\overset{\displaystyle a}{\underset{\displaystyle o}{C}} - \overset{\displaystyle a}{\underset{\displaystyle \gamma}{C}}-\beta$ is therefore *six*.
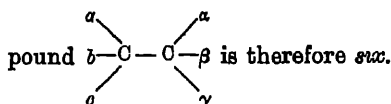
Particular interest attaches to the special instance wherein the two semi-molecules are equal, and which therefore comprises the compounds expressed by the formula $b-\overset{\displaystyle a}{\underset{\displaystyle o}{C}} - \overset{\displaystyle a}{\underset{\displaystyle o}{C}}-b.$ Tartaric acid,

$$H-\overset{\displaystyle COOH}{\underset{\displaystyle HO}{C}} - \overset{\displaystyle COOH}{\underset{\displaystyle OH}{C}}-H \quad = \quad \begin{array}{c} COOH \\ | \\ CHOH \\ | \\ CHOH \\ | \\ COOH \end{array}$$

is the type of this group.

In this case we have $D = d$ and $L = l$. The four above-named general expressions for the rotatory power of the individual active forms are, in this special case, resolved into

$$2D \qquad 2L \qquad 0 \qquad 0$$

The modifications $2D$ and $2L$ correspond to the dextro- and levo-tartaric acids; and, in place of the two faintly rotatory modifications, we have a single optically **inactive** form, which, in the tartaric acid group, is named meso-tartaric acid.

This last-named acid is inactive as a result of **intramolecular compensation**, and is therefore distinct from the modification produced by the coalescence of the two optically active forms $\left\{ \begin{array}{c} 2D \\ 2L \end{array} \right\}$, which double molecule is also optically inactive, and in the tartaric acid group is named racemic acid.

The existence of *two* fundamentally different groups of optically inactive compounds containing asymmetric carbon atoms is thus theoretically possible, viz.:

1. **Monomolecular**, indivisible, optically inactive in consequence of intramolecular compensation of their optically active atom groups. Type: $D - d = o$ or $L - l = o$. Example: meso-tartaric acid.

2. Bimolecular, optically inactive, appearing only as double molecule one of which belongs to the dextro- and the other to the levo- modificatio of the compound   Type : $2D - 2L = O$. The inactive double molecul can be split up into two optically active components, $2D$ and $2L$ Example : racemic acid (Fr. *acide racémique*, Ger. *Traubensäure*).

The first of these two modifications, viz., the monomolecular, indivisible kinc is termed the anti- combination , the second, or bimolecular modification, bein known as the para- form, or (from the best-known example) racemic modificatiol
On the other hand, it is found, both theoretically and in practice, that bodie containing but a single asymmetric carbon atom can appear in only *one* inactiv form, namely, the divisible, bimolecular para- form.

§ 139.—The Division of the Racemic Compounds.

The correctness of the assumption that the racemic modifications o stereoisomeric bodies are actually double compounds, consisting of an equa number of dextro-rotatory and levo-rotatory molecules, can be demonstratec in two ways. The first is by synthesis. If, for example, equal quantitie of equally strong (nearly saturated) solutions of dextro- and levo-tartari acid be mixed together, combination of the two, attended with the evolutioi of heat, occurs, and the mixture solidifies in consequence of the crystallisa tion of a racemic acid which can be proved to be identical with natural racemi acid. Again, if equal quantities of dextro-lactate and levo-lactate of zinc bc dissolved in water and mixed, the optically inactive zinc salt of " fermentatioi lactic acid "—possessing all the properties of this substance—will crystallisc out   This synthetic construction of the inactive double molecule from the twc optically active components is, however, of merely theoretical interest, but ii without any practical value, no new and hitherto unknown compounds being thereby obtained.
As a rule, it is the inactive para- form with which we have to deal, anc this has to be split up into its constituent molecules of the dextro- anc levo- modifications   This division can be effected in several ways. In somc cases the inactive substance decomposes spontaneously on crystallising out from solution, and the components—which differ in their crystalline habit— can be separated by hand (selecting the crystals according to their structure) The earliest example of this was given by PASTEUR (XI). If a solution oi sodium-ammonium racemate ($C_4H_4(NH_4)NaO_6 + 4$ aq.) be allowed to evaporatc slowly, hemihedral crystals are obtained. Pasteur found that the hemihedra. surfaces did not occupy the same position in all the crystals, but that the one class of crystals formed, as it were, the reflected image of the other, one being dextro-hemihedral but levo-rotatory, and the other levo-hemihedral but dextro-rotatory. On separating the two kinds, and preparing the acid from each, he obtained dextro-tartaric acid in the one case and levo-tartaric acid in the other, but in no instance was the inactive racemic acid produced
The most usual method of division consists in allowing the inactive sub stance to unite with an optically active one. If the substance to be split ur is an acid, then an active alkaloid (*e.g.* morphine, quinine, strychnine, &c.) ii used , if a base, it is combined with dextro-tartaric acid. Experience teachei that the resulting compounds have very different degrees of solubility, oi which account the two optically active modifications can be separated withoui much difficulty. Thus, it was shown by T. PURDIE and J. W. WALKER (I.) ii 1892, that by combining the optically inactive " fermentation lactic acid " witl

I   M

strychnine, it can be decomposed into its two optical components, viz., dextro-rotatory sarcolactic acid and levo-rotatory lactic acid.

However, the most important method of separation is that in which the activity of micro-organisms is called into play. This method, also, was proposed by Pasteur in 1860. He sowed certain lower fungi—the precise species cannot now be ascertained—in a solution of optically inactive ammonium racemate, and found that the levo-rotatory properties of the liquid gradually increased; and that after a certain lapse of time ammonium levo-tartrate alone was detectable. It must therefore be concluded that certain ferments are endowed with selective powers. In the present instance, the organism has separated the racemic acid into its two optically active components, one of which (the D-tartaric acid) it consumes, whilst the other (the L-acid) is liberated. Since Pasteur's time this separating power has been utilised in various ways, two examples of which are now given. In the first place, J. LEWKOWITSCH (I.) in 1882 dissociated the optically inactive mandelic acid, C_6H_5—CH OH—COOH, into its two active components in this manner. On the other hand, P. FRANKLAND and W FREW (III.) allowed their *Bacillus ethaceticus* to react on the calcium salt of the optically inactive glyceric acid, CH_2OH—CH.OH—COOH, whereby its dextro-rotatory component was obtained, the levo- component being consumed.

§ 140.—The Production of the Stereoisomeric Lactic Acids

by fermentation merits closer attention. In the first place, it should be mentioned that only the three isomers of *ethylidene lactic acid*, CH_3—CH.OH—COOH, are in question, since *ethylene lactic acid*, CH_2OH—CH_2—COOH, has hitherto been obtained by purely chemical means alone. The sub-title, **fermentation lactic acid**, so long borne solely by the inactive form of ethylidene lactic acid, is now recognised as also appertaining to both its stereoisomers, so that the term is now synonymous with ethylidene lactic acid generally. Of the two active forms, the so-called **paralactic acid** was the first to be prepared by the fermentation method, the discovery being due to M. VON NENCKI and N. SIEBER (I) in 1889. These observers found that certain tumours in an animal affected with symptomatic anthrax contained (in addition to the characteristic bacillus of this disease) an anaerobic fission fungus, which produces large quantities of paralactic acid (i e. dextro-lactic acid) in saccharine media, and is on that account named *Micrococcus acidi paralactici.* On the other hand, the aforesaid pathogenic bacillus, under the same conditions, produces inactive lactic acid.

Levo-lactic acid was first prepared in the year 1890 by FR. SCHARDINGER (II.), by means of the fermentative activity of a short-rod species found in a Hungarian well-water, and to which the name of *Bacillus acidi lævolactici* has been given. In size this organism greatly resembles Hueppe's *Bacillus acidi lactici*. It ferments dextrose, saccharose, lactose, and glycerin, the resulting products being levo-lactic acid, a little ethyl alcohol, a quantity of carbon dioxide, and an unspecified combustible gas. The zinc salt of inactive lactic acid is obtained by crystallisation from a warmed solution of the zinc salts of this levo-lactic acid and of paralactic acid.

So far as is known at present, the *Schizomycetes* species forming levo-lactic acid are of less frequent occurrence in nature than those producing either inactive or dextro-lactic acid. K. GÜNTHER and H. THIERFELDER (I) examined a large number of samples of sour milk, in none of which could they find levo-lactic acid. Neither did they succeed in isolating from the milk any fission fungus capable of forming this acid, but always obtained either inactive or dextro-lactic acid, or a mixture of both. This, however, does not imply that

Schizomycetes producing levo-lactic acid are rare. On the contrary, a considerable number of species (mostly pathogenic) endowed with this property are already known. Investigations on this point were first made by J. KUPRIANOW (I.) and repeated by B. GOSIO (I), and it was shown that the amount of this acid produced per unit of quantity of the fermented sugar varies according to the species employed

Vibrio cholerœ asiaticœ (in addition to other vibrios) was found to produce levo-lactic acid, whilst *Spirillum tyrogenum* (Deneke) produced dextro-lactic acid. On the other hand, G. LEICHMANN (I), in 1896, showed that when ordinary milk is kept at 44°-50° C.—instead of the lower temperatures employed by Gunther and Thierfelder—levo-lactic acid is invariably produced. The (long-rod) fission fungus concerned in this reaction was named by LEICH-MANN (II.) *Bacillus lactis acidi*, a name (as we have seen in § 135) already in use for another species of *Schizomycetes* as long ago as 1886

The kind of lactic acid produced under given circumstances by a certain bacterium affords, in many instances, a valuable means for differentiating allied species. For example, in the case of *Bacillus typhi abdominalis* and *Bacterium coli commune*, the latter—as shown by BLACHSTEIN (I.)—produces dextro-lactic acid from glucose, whilst the former, under identical conditions, gives rise to levo-lactic acid.

Nevertheless, the faculty of a given species of bacterium for producing a definite kind of lactic acid must not be regarded as unconditional. On the contrary, a good deal depends on the conditions of nutrition. The necessity for the maintenance of identical conditions during experiments of this kind has already been emphasised, and it should be also mentioned that the results are influenced not only by the kind of carbohydrate (sugar) subjected to fermentation, but also by the constitution of the nitrogenous nutrient material. For this discovery we are indebted to A. PÉRÉ (I), who showed that when ammonia salts alone (unaccompanied by peptone) are at the disposal of the microbe, both *B. typhi abdominalis* and *Bacterium coli commune* produce levo-rotatory lactic acid from glucose.

The separation of optically active compounds by means of *Eumycetes* will be frequently referred to in the second volume, so we will only mention a treatise by M VON NENOKI (II), which describes a valuable method for manipulating fermenting liquids and determining their content of optically active lactic acid. The characteristics of the salts of this acid have been described by T PURDIE and J. WALLACE WALKER (II.) According to F. HOPPE-SEYLER and FR. ARAKI (I), the lithium salts are the most suitable compounds to employ in experiments for determining the rotatory power of the various lactic acids.

CHAPTER XXIII.

THE ARTIFICIAL SOURING OF CREAM.

§ 141.—The Acid Generator.

THE preferences exhibited by the various families of the human race for different kinds of butter are very marked. China and Japan, for instance—to which countries Denmark ships large quantities of this food-stuff—prefer **sweet-cream butter**, *i.e.* that prepared from fresh, sweet cream, whereas, in Scandinavian countries, Denmark itself, North Germany, and England, a preference for **sour-cream butter** prevails.

In order to obtain the latter product; the cream is allowed to turn sour and undergo a fermentation, principally of a lactic character. Until within the last few years the general practice was simply to leave the cream to become sour spontaneously; hence, in view of the fluctuation to which the bacterial flora of milk and cream is exposed, it is not surprising that such a method of procedure frequently resulted in the production of defective butter.

A reliable means of combating this adverse tendency is, however, now available, namely, the process—introduced into the dairy industry in 1890 by H. WEIGMANN (III.-V.)—of artificially souring cream by the aid of **pure cultures** of selected races of lactic acid bacteria. This process is divided into two manipulations: the preparation of the acid generator and the preliminary treatment of the cream to be soured

The **acid generator** (or starter) brings the cream quickly into a state of fermentation. According to Weigmann's recipe, it is prepared as follows: Separated or skimmed milk—in the proportion of 2–3 per cent. of the cream to be acidified—is warmed up to about 60° C and then immediately re-cooled as quickly and as much as possible. This treatment kills some of the bacteria in the milk and weakens others to such an extent that they cannot offer more than a feeble opposition to the development of the lactic acid bacteria, which are then added to the treated skim-milk. F___r this purpose a pure culture of lactic ferment, obtained from a D___y experimental Station, is employed. The vessel is kept for twenty-four hours at a medium temperature (15° C.), by which time its contents will be converted into acid generator ready for use.

The cream, also, requires a preliminary treatment to prepare the way for the action of the acid generator. Sterilisation, or at least Pasteurisation, would afford the best results, but as these are generally difficult to effect in a reliable manner, a method of weakening the "wild" bacteria present, by cooling the cream down to a low temperature and then quickly warming it up again to 16°–20° C., has to be resorted to. The acid generator is then added and well incorporated by stirring, and the cream vat is left, at about 15°–20° C., until the following day, by which time the cream will be ripe for churning.

If it be desired to cultivate the acid generator further, a small portion is taken from the bulk before use and employed in the same way as a pure culture.

In spite of the adverse opinion of many practical men, the possibility of producing good butter from Pasteurised, or even sterilised cream, has been

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demonstrated by the researches reported by P ŠCHUPPAN (I); and BENNO MARTINY (II.) has drawn attention to the hygienic advantages attending such a method of working. Finally, it was proved by POPP and BECKER (I.) in 1893 that butter prepared from sterilised cream keeps better than that from Pasteurised cream, and far better than that from cream which has not been heated at all.

§ 142.—The Aroma of Butter.

The results of Weigmann's researches up to the present (according to a private communication) indicate that probably only a single species—though appearing as numerous varieties—of bacterium sets up the lactic fermentation now in question. This organism is a *coccus* (described by W. STOROH (I.)), measuring about 1 μ in diameter, and uniting to form chains. The varieties (also called *races*) of this coccus, which, from the point of view of the systematic botanist, do not differ sufficiently to be classified as separate species, generally exhibit one or other of the following good qualities they either give rise to a powerful aroma, which imparts a very fine flavour to the butter, or else the product, without exhibiting any marked excellence of flavour, is endowed with good keeping qualities. Consequently such races or varieties should be used in the manufacture of butters for export. Whether there are races in which both these advantages are combined cannot yet be definitely asserted. The aroma produced by the bacteria cannot have originated in the volatile acids of the butter, since it is also developed in cultures free from fat, and containing no nitrogenous nutriment other than peptone. Comparative experiments on the flavour of butters prepared by the aid of different varieties of lactic acid bacteria were made by ADAMETZ and WILCKENS (I.) in 1892.—Owing to a noteworthy discovery effected by H. W. CONN (I.) in 1895, the question has latterly taken a new turn. He succeeded in isolating from a sample of South American milk a fission fungus named *Bacillus No.* 41, which is not one of the acid bacteria, but produces in milk and cream a fine aroma, identical with the highly-prized flavour known in North America as " grass flavour" or " June flavour," because it is produced only in the month of June, at a time when the cows are foddered on tender grass rich with blossom and perfume. Cream inoculated with this bacillus yielded butter endowed with this fine grassy aroma. By means of this process butter can now be produced with a uniform degree of excellence and marketable value. The mode of working with this bacillus is simple. A culture of the organism in milk is procured, the usual volume being about ¼ litre (nearly half a pint), and is poured into about 6 litres (1 32 gall.) of Pasteurised and re-cooled cream. After a lapse of a couple of days the whole is transferred to the bulk of the (fresh) cream, which is left for twenty-four hours and will then be ripe for churning. About 6 litres of this ripe cream are reserved for inoculating the next batch in the same manner as before. It will be noticed that the bulk of the cream is not heated, and consequently the lactic acid bacteria therein will be still living and capable of souring the cream, whilst the *Bacillus No.* 41 acts concurrently and develops the aroma. The latter organism, however, retains the upper hand, having been initially present in excess. This flavour-developer has now been tested and proved in more than a hundred North American dairies, so that its employment can be recommended. Naturally, fresh cultures must be introduced into the dairy at intervals (two to three months), since otherwise the bacterium gradually loses its powers.

The discoverer of the organism attributes to it the additional faculty of remedying defective butters, but on this point the data at hand are insufficiently conclusive. It should be mentioned that the microbe appears in the form of non-motile short rods, 0.7 μ broad and 1.1 μ long, generally united in pairs, but

never as chains   The optimum temperature is 23° C   Its acid-producing powers are so slight that the milk is never coagulated.   The aroma developed in the milk is initially delicate, but becomes progressively stronger, and finally (after a lapse of several weeks) resembles that of fine cheese.   As a result of further researches published in 1896, CONN (II) was led to conclude that acidification and the production of aroma are independent phenomena   He considers that aroma is developed by the activity of peptonising bacteria which separate volatile bodies (of agreeable or offensive smell and taste) from the·albuminoid constituents of the cream.

§ 143.—Defects in Butter.

The advantages offered by this artificial method of souring cream are only appreciated at their true value when its application cures certain defects in butter, to which we will now refer, and which were formerly attributed exclusively to bad fodder.   In this case also bacteriologists have been able to confute erroneous opinions and render valuable assistance to practice.

There is probably not a single dairy in North Germany or Denmark whose butter has not at some time or other been "oily," i e. exhibited a flavour recalling that of mineral oil.   This malady appears with particular frequency in dairies deficient in appliances for keeping the souring cream and finished butter sufficiently cool.   Weigmann showed that both the acid generator (prepared by spontaneous acidification) and the butter-milk of such dairies are very impure, in a bacteriological sense, and he was invariably successful in remedying the complaint by the introduction of artificial souring

A second and not less injurious defect is the so-called **turnip flavour.**   Butter suffering from this complaint has a repulsive sweet taste, recalling that of turnips.   To throw the responsibility on the latter is an obvious, but not always justifiable, procedure, since cases are known where neither the cows nor any of the dairy appliances had come into contact with turnips, notwithstanding which the flavour still made its appearance in the butter.   O. O. JENSEN (I) discovered, in 1891, in the milk of several Jutland dairies where this complaint had long been rife, a microbe which he named *Bacillus fœtidus lactis*, and which was recognised as the cause of the malady.   This motile bacillus has a breadth of 0.4–0 6 μ, its length varying usually between 0 9 and 1.5 μ, and often attaining 5.5 μ.   No spore-formation has been detected, and the organism does not liquefy nutrient gelatin.   As its second name implies, this bacillus gives rise to stinking decomposition in milk, but is not the only species producing the same complaint, Jensen himself having grouped along with it a number of others possessing the same power, among them being several species of micrococcus (not more definitely named), a Merismopedium, &c.   The employment of pure cultures of lactic acid bacteria gave satisfactory results, producing a pure and fine-flavoured butter in place of the previously almost unsaleable article.

A third evil which is probably (though not yet indubitably) attributable to bacteria, is the so-called **fishy** or **train-oil flavour** in butter.   Other defects, such as greasy, tallowy, cheesy butter, have their origin in the inferior chemical com-position of the cream ; whilst for the third group of complaints, e.g stable smell and smoky smell, the uncleanliness of the milker is responsible

How firmly the injurious bacteria settle themselves in the rooms of the infested dairies is evident from the observations made by Ronneberg, according to whom the beneficial results accruing from the employment of pure cultures of acid bacteria in infested dairies are only temporary, and disappear if fresh supplies of the invigorating pure culture are not introduced in good time.

The advantages of this new method are so numerous that its employment should not be delayed until the maladies in question appear , in fact, the method

is designed as a protective rather than a remedial measure· The expense of applying the method is small, the supply of pure culture needs, under normal conditions, to be renewed only about once a fortnight, and the outlay at the same time ensures protection from unwelcome surprises. Even when the continuous employment of the method is not decided upon, it should at least be practised at such times as a change from dry to green fodder, or *vice versâ*, is made, this change often becoming very unpleasantly manifest, not only in the cream-pan, but also in the cheese-room, as will be explained in chapter xxxi.

The method is most extensively used in Denmark. From a report by FRIIS, LUNDE, and STORCH (I), it appears that whilst in 1891 only 4 per cent. of the samples of butter exhibited at the butter shows (held annually in various parts of the kingdom) had been prepared by the aid of pure cultures of acid generator, the number had increased in 1894 to 84 per cent This fact affords the best recommendation of the method.

CHAPTER XXIV.

THE COAGULATION (CURDLING) OF MILK.

§ 144.—Acid Curdling and Rennet Curdling.

THE amount of nitrogenous constituents in cow's milk fluctuates between 2.5 per cent. and 4.2 per cent. by weight, and is on the average 3 5 per cent   The chemical composition of these nitrogenous matters has not yet been satisfactorily determined, and can only be touched upon here so far as is requisite and useful for bacteriological purposes   More precise information, accompanied by copious bibliographical references, will be found in W. FLEISCHMANN's (I.) work on dairying.

In refutation of the opinion expressed by DUCLAUX (VII), that only a single albuminoid body is present in normal cow's milk, the Swedish chemist Olaf Hammarsten showed, in 1875, that at least three such compounds can be distinguished therein, viz., casein, lactalbumen, and globulin. The first forms about 80 per cent. of the total quantity, and the remainder is principally lactalbumen (free from phosphorus), globulin being present in but very small quantities , both of these latter are soluble in water. The casein (containing phosphorus) is acid in character, and consequently is not present in a free state in the milk, but occurs as a salt of lime containing 1 55 parts of CaO per 100 parts of casein. This compound of lime and casein is not dissolved in the milk, but is held in suspension as a swollen, colloid, finely divided mass. When the milk is acidified the casein is liberated, and—being insoluble and incapable of swelling—is precipitated in fine flakes ; in other words, the milk curdles. The acid may be either artificially added or generated by fermentation in the milk itself ; in either case the ensuing precipitate is known as acid curd (Ger *Quark*).

Milk can also be curdled by another means, namely, by lab or **rennet**, an enzyme secreted by special glands in the stomach of many animals. This rennet is very plentiful in the stomach of the calf, from which it is prepared by drying in the air and leaving to stand for a few months, then comminuting the mass and extracting with a weak (5 per cent.) solution of common salt.

On adding a small portion of such a solution of rennet to sweet, unboiled, lukewarm, milk, the latter gradually curdles, the coagulum thus formed being, however, not casein itself, but a derivative of that substance   Hammarsten found that the casein is in this case split up into two portions differing greatly in amount, viz., lacto-protein, small in quantity, soluble, and remaining in the whey, and the insoluble **paracasein**. The latter, therefore, forms the chief constituent of the coagulum separated (" set ") in cheese-making by the aid of rennet, and known as **rennet curd** (Ger. *Bruch*), or crude cheese.

Casein, or paracasein, though the sole nitrogenous constituent of the coagulum produced in any of these methods, is, however, by no means its sole component, a number of other substances being precipitated and carried down at the same time. If whole milk—*i.e.* unskimmed milk—is set for cheese, almost the whole of the fat will be found in the curd, which will then subsequently produce **rich cheese**—**skim-cheese** being the result in the converse case. Along with the fat, the calcium phosphate contained (in suspension) in the milk

will also be thrown down only in the case of rennet curd, not in the curd produced by acidification.

Not only are fat and (in this instance) calcium phosphate carried down by the coagulum, but also a large part of the organisms present in the milk will be found in the fresh curd, so that the latter is relatively as rich in organisms as the milk from which it was precipitated. Here, again, a considerable difference, from a biological point of view, exists between the two classes of curd, and exercises a decisive influence on their subsequent career. The flora of the rennet curd from sweet (*i.e.* almost neutral) milk is much more diversified than that of acid curd. The latter, having been thrown down from a sour milk in a state of vigorous lactic fermentation, consequently contains only a limited number of species, and these endowed with a particular fermentative power.

Acid curd differs, therefore, from rennet, both in the method of production and also in composition. Being devoid of flavour, both kinds are, however, unsuitable for food, their conversion into a form in which they both stimulate the appetite and are also themselves more readily digestible, is the task of the cheese-maker's art, fuller particulars of which, from the bacteriological point of view, will be found in chapter xxxi

§ 145.—Characteristics and Activity of Lab.

If the enzyme in question were exclusively a metabolic product of the animal body, the foregoing details would suffice. However, since it is excreted by many fungi as well, a few additional particulars will not be out of place in a work dealing with technical mycology.

When and in what manner the attention of mankind was first drawn to this enzyme cannot be determined, since even the oldest authorities, *e.g.* the Bible, speak of its employment as an ancient practice. Its method of action, however, was unknown even down to the commencement of the nineteenth century The prevalent opinion, based on the curdling of sour milk, was that the precipitation effected by rennet was indirect, an acid being first formed, which then caused the precipitation of the curd. The elucidation of the true state of the case was reserved for BERZELIUS (I.) in 1840, and his discovery was soon afterwards supplemented by the labours of FR. SELMI (I.), C. G. LEHMANN (I), HEINTZ (I), and VOELCKER (I.), who showed that the action of rennet is quite independent of the formation of acid.

The identity of this enzyme with the pepsin discovered by SCHWANN (§ 18) was disproved by O. HAMMARSTEN (I) in 1872. This observer was the first to successfully separate these two gastric secretions, an operation which DESCHAMPS (I.) had failed to effect thirty-two years earlier Hammarsten, however, did not make use of the name chymosin, proposed by his predecessor, but adopted the ancient appellation, lab We are also indebted to the Swedish chemist for deep researches into the activity of this substance. It acts on casein alone, not on lactose or lactalbumen.

A divergence of opinion still prevails as to the nature and course of this reaction, and we will therefore merely refer to the investigations made on these points by the under-named workers: A. DANILEWSKY and P RADENHAUSEN (I), W. EUGLING (I.), F. SCHAFFER (II.), E DUCLAUX (VIII), M. ARTHUS and O. PAGÈS (I.), A S. LEA and W. S DICKINSON (I.), S. RINGER (I), P. WALTHER (I.), and A. FICK (I.). SCHREINER (I.), in 1877, showed that milk when boiled is no longer coagulable by rennet, but the reason for this behaviour was not ascertained until eleven years later, when FR. SÖLDNER (I.) found that the lab reaction can only proceed in the presence of soluble salts of lime, which latter are precipitated by boiling. For the same reason coagulation does not

occur in milk that has been rendered alkaline; and the most favourable condition is one of very slight acidity. The constitution of this enzyme is still unknown   Its decomposing power is unusually high, one part by weight being (according to Soldner) sufficient to throw down at least one hundred million parts of casein.   As A. MAYER (III.) has shown, the optimum temperature for the reaction is 37° C., the coagulation taking three times as long at 25° C.   Above 45° C. the enzyme is paralysed, and is destroyed at 70° C.   Coagulation does not ensue immediately upon the addition of the lab, but only after a lapse of from several minutes to some hours, according to the temperature.

The occurrence of this enzyme in nature is by no means rare   It was found by Roberts in the stomach of birds, and in that of fishes by Benger, and is also present in the cell-sap of various plants, e.g. of butterwort (*Pinguicula vulgaris* and *P. alpina*), withania (*Puncerria coagulans*), fig-tree (*Ficus carica*), artichoke (*Cynara scolimus*), and others.   It is also excreted by several species of *Schizomycetes*, particulars of which will be given in the following paragraph.   For industrial purposes, however, there is only a single source (the richest) worthy of consideration, viz., the stomach of the calf   The method of production indicated above is now practised on a manufacturing scale, especially in Copenhagen, whence most of the German and Dutch cheese factories derive their supplies   The products are Rennet solution, containing boracic acid to improve the keeping quality; rennet powder; and, finally, rennet tabloids. The efficacy and germ-content of these preparations were investigated by FRITZ BAUMANN (I.).

§ 146.—Lab-Producing Bacteria.

Milk may also curdle without previous acidification or addition of rennet. HAUBNER (I.) in 1852 was the first to record this fact, and the first explanatory research was made by DUCLAUX (IX.) in 1882.   From his studies in this matter the latter concluded that this precipitation of casein (occurring with an alkaline reaction) is due to the activity of certain bacteria which excrete an enzyme resembling lab; and CONN (III), in 1892, succeeded in isolating this enzyme from cultures of such *Schizomycetes*.   At first sight the identity of this lab with the active ingredient in the rennet solution from the stomach of the calf appears probable, but the discovery that the bacteria in question (which include many of the species belonging to the potato bacillus group, § 111) are able to coagulate boiled sterilised milk, goes against this view, rennet being incapable of producing this reaction.

The presence or absence of this power affords, in many instances, a valuable means of differentiation between two species of bacteria.   This is particularly the case with the organism producing abdominal typhus in man, viz., *Bacillus typhi abdominalis*, discovered by Eberth in 1880, and already referred to in preceding chapters   This microbe is not endowed with the faculty in question, whereas, as JAK. URY (I) has shown, a number of the putrefactive bacteria collectively termed *Bacterium coli commune*, and greatly resembling the typhus bacillus in form, &c., rapidly produce coagulation in milk.   According to the researches of O. GORINI (I. and II.), *Micrococcus prodigiosus* also produces this enzyme in large quantities.

§ 147.—Casease.

Not infrequently the precipitation of casein effected by such bacteria disappears again after a short time.   Duclaux ascertained that this new alteration is due to a second (albumen-dissolving) enzyme, to which he gave the

name of casease. The same observer also discovered, in the case of several species of *Tyrothrix* isolated from Cantal cheese, and of which a description will be found in chapter xxxi , certain bacteria gifted with the faculty of excreting *both* enzymes, the casein precipitant and the casein solvent. R. WARINGTON (II) as well examined a number of such species, and observed that they all liquefy nutrient gelatin.

The ratio between these two enzymes differs in the various species. A few produce the casein solvent alone, and when sown in milk do not precipitate the casein, but decompose it direct into soluble fission products, among which leucin and tyrosin have already been identified. In proportion as the casein disappears the milk becomes clearer, and is finally quite transparent.

The production and activity of both these enzymes are variously dependent on external influences, the one resembling lab being able to act only within narrow limits of temperature, whilst the other—the proteolytic enzyme—t as a wider sphere of activity. The same applies to the methods of nutrition of the bacteria in question. A species examined on this point by Conn initially produced both enzymes, but subsequently—after prolonged cultivation on gelatin—yielded the proteolytic one only ; and by increased interference the production of the latter also can be restricted. Wood (I.) attained this object by adding, during several successive generations, a little carbolic acid to the nutrient bouillon employed.

The constitution of casease has not yet been accurately determined, neither has any one succeeded in ascertaining whether, and in what respect, this enzyme differs from pepsin and trypsin—which it greatly resembles in action—nor whether the casein-dissolving enzyme produced by different species of bacteria is the same in all cases. WEIGMANN (VI.) states that he has isolated casease from bacterial cultures, and that this substance favours and accelerates ripening when added to fresh cheese.

The spontaneous coagulation of milk without the co-operation of micro-organisms, the possibility of which was maintained by early workers, denied by Lister, and finally established as a fact by Meissner, was more closely examined in 1887 by A. LEVY (I), who found that a very faint coagulation can be detected in all milk that has been left to stand for some time. The sediment deposited by such milk contains, however, only a small quantity of coagulated casein, the bulk consisting of small fragments of decomposed colostrum As the cells of this latter substance die off a slight degree of acidification ensues, which causes the precipitation of a certain quantity of casein.

The rapid curdling of milk so frequently observed during thunder-storms has not yet been satisfactorily explained. The opinion expressed by J. LIEBIG (I.) in 1890 will not bear investigation, and the assumption put forward, on experimental grounds, by G. TOLOMEI (I.), that it is caused by the action of ozone produced by electrical discharges, rests on insufficient foundation. The same objection also applies to the views held by H. GERSTMANN (I) on this 'point.

CHAPTER XXV.

LACTIC ACID BACTERIA IN DISTILLING, BREWING, AND VINIFICATION.

§ 148.—The Spontaneous Acidification of Distillery Yeast-Mash.

THE preparation of the pitching yeast for distillery work is not such an easy matter as in the sister industry of brewing. In the regular course of the latter no special labour is required for the production of the necessary quantity of yeast, since in this case the yeast settles down, as soon as the fermentation is at an end, to the bottom of the tun, and can then—after the immature beer is racked off—be used at once for "pitching" (i.e. inducing fermentation in) a fresh quantity of wort. The case is different in distillery work, where the liquid to be fermented, instead of being thin and self-clarifying like wort, is a thick mash, ' in which the yeast cannot settle down. For this reason the distiller is obliged to prepare his pitching yeast in another way. He grows it artificially in special vats, and, on this account, terms it "artificial yeast." For this purpose a sweet mash is prepared in a small tun, the quantity amounting to about 10 per cent. of the principal mash to be fermented. A more detailed description of the preparation of this yeast-mash belongs to the domain of Chemical Technology, and we will here content ourselves with briefly mentioning that crushed green malt is mixed with water and gradually warmed to $67°$–$70°$ C., then mixed with a variable) amount of sweet "goods" from the principal mash tun, and the mixture left to saccharify for two hours at $70°$ C Before this medium is pitched with the yeast to be reproduced, it must, however, be subjected to another preliminary treatment known as "souring."

The green malt is infested with a copious flora of various kinds of bacteria, chief among which are the species of the hardy organisms causing butyric fermentation. These spores are not killed by the aforesaid mashing temperature—which, moreover, for reasons connected with the preservation of the diastase, must not be exceeded—and therefore they afterwards germinate and increase, and produce butyric acid. Now this acid, being a powerful yeast poison, would injure the development of the pitching yeast; but since the injurious bacteria are themselves very sensitive to high degrees of acidity, their development may be hindered by quickly making the fresh mash decidedly acid. To attain this end the lactic acid bacteria are called in aid.

The question now arises, How can the latter be cultivated without allowing the butyric ferments to gain the upper hand? This can be secured by maintaining the optimum temperature, which for the lactic acid ferments now under consideration is between $47°$ and $52°$ C , whereas the butyric ferments thrive best at about $40°$ C. The sweet yeast-mash is therefore kept at about $50°$ C. ; consequently the lactic acid bacteria develop with vigour, and the increase in their activity can be determined by titration.

When the operation progresses satisfactorily, the acid-content rises with increasing rapidity and attains 2.2–2.5 degrees of acidity; i.e. 20 c.c. of the filtered sour mash require 2.2 to 2 5 c.c. of normal alkali for complete neutralisation, a quantity corresponding to 1.0–1.1 per cent. of lactic acid. When this

point is reached, the mash is heated to 70° C. in order to kill the lactic acid bacteria, and is immediately re-cooled to 17°–20° C. and pitched with yeast. For the first yeast-mash of a new season a sufficient quantity (1 kilo. per hecto-litre of mash, i.e. at the rate of 1 lb. per 10 gallons) of a pure culture of a selected race of distillery yeast is employed. Such yeast can be obtained from the Berlin Experimental Distillery Station (Versuchsstation für Brennerei). At the expiration of some fourteen to sixteen hours the development of the yeast has so far progressed that the contents of the vat can be applied to their destined purpose, a portion (about one-tenth) being, however, reserved, under the name of mother-yeast, for pitching the soured yeast-mash on the following day. The remaining nine-tenths of the prepared yeast are then transferred to the principal mash, whereby the latter not only receives the requisite amount of active yeast, but is also rendered acid, and is thereby better enabled to resist bacterial infec-tion. This explains the whole dictum of the distiller, "The more acid in the yeast, the less in the fermenting tun," because the greater the acidity of the mature yeast-mash, the lower the possibility of injurious (acid-producing) germs developing in the principal mash during fermentation. The reason for this is that lactic acid reduces the vital activity of the microbes (butyric acid and acetic acid bacteria) now under consideration. The increase of acidity in the mash is employed as a measure of the progress of the fermentation. When the yeast is first added, the sweet mash exhibits an acidity of 0.5°–0.7°, corresponding to 0.2–0.3 per cent. of lactic acid, and this increases during fermentation by 0.2° when the management is first-class, 0.3° when good, and by as much as 0 4° and more when the process is not properly carried out.

§ 149.—Artificial Souring by the Aid of Pure Cultures of Lactic Acid Bacteria.

The credit of recognising the utility of souring the mash is due to practical distillers themselves, their experience on this point having been gained by repeated experiments. It is only in recent years, however, that a closer insight into the characteristics and actual value of this preliminary treatment of the yeast-mash has been obtained. Until lately the generally accepted opinion was that expressed by SCHULTE IM HOFE (I.), viz., that lactic acid is necessary, or at any rate favourable, to the conversion of the (insoluble and undiffusible) albumi-noids of the wort into peptones assimilable by yeast. Delbrück's researches on this point failed, however, to reveal the presence of any appreciable quantity of peptones in the soured yeast-mash, and it is now certain that the favourable result is solely due to the relative toxic action of lactic acid. This acid acts much more quickly and powerfully on the development of the bacteria than on yeast, the latter being able to stand a fairly large amount of the acid without appreciable injury.

The reader may well inquire from what source these lactic acid bacteria which cause the souring of the yeast-mash are derived. Until recently the answer was far from satisfactory, since it indicated that the matter was left to chance. The initial temperature of 70° C. in the yeast-mash kills the lactic acid bacteria already present therein, but not the spores of the butyric fer-ment; the subsequent development of the latter is, however, prevented by the restrictive temperature of 50° C. maintained during the souring process The active lactic acid bacteria must, therefore, make their way into the mash from outside sources, e.g. the air, the vessels, and utensils, &c., so that the inoculation of the mash is left entirely to chance. Consequently it is not surprising to learn that the operation frequently miscarries, failures being, under such circum-stances, inevitable.

We are indebted to Morawsky for the first improvement on this point. Instead of waiting for the yeast-mash to become infected spontaneously by lactic acid bacteria, as in the ordinary course, he proposed to set aside about one-tenth of the soured mash before applying heat, and to add this mother-acid to the next day's mash as soon as the latter has been saccharified and cooled down to 50° C. This modification, although constituting a valuable improvement on the older method when once operations are in full swing, nevertheless does not positively guarantee good souring; and its deficiencies are most apparent at a time when help is most essential, viz., at the commencement of a new season. During the first few days after work is resumed, it often becomes apparent from the odour permeating the yeast-room that the souring is not progressing satisfactorily, but that the mash is rich in butyric acid. This is due to the fact that the lactic acid bacteria in the distillery have more or less completely perished during the summer while the works were shut down.

To completely overcome the difficulty, nothing must be left to chance, and the souring must be properly regulated by inoculating the sterilised and cooled yeast-mash with a sufficiency of a pure culture of lactic acid bacteria. Such a method was first introduced by the author at the Hohenheim Distillery, where it was tried with great success. The further treatment of this artificially inoculated mash differs in no wise from the procedure already described, i.e. when the souring is completed the mother-acid is removed, the bulk of the mash is heated up to 70° C., then cooled, and pitched with the prepared mother-yeast. Next day a portion is taken to serve as mother-yeast for a succeeding mash, and the remainder is added to the principal mash. If, through any mischance (unskilfulness or carelessness on the part of the distiller), the souring of a given mash proves defective, then no mother-acid is reserved from it, but a pure culture is used for pitching the yeast-mash on the following day.

Although the species of bacterium now in question shares with the milk-souring bacteria the property of decomposing sugar and forming lactic acid, it nevertheless differs from them in more than one respect. For example, the various species of the lactic acid bacteria in milk, so far as they have been examined, are incapable of developing in mashes and worts under the conditions prescribed above. Morphological differences are also apparent at the first glance, the cells of the distillery-bacillus being long, almost invariably more than 2.5 μ, and very frequently attaining ten times this length, whilst the breadth remains uniformly about 1 μ. This microbe was isolated by the author in 1896 from a satisfactory yeast-mash prepared by the old souring method in the Lietzen Distillery (in the Mark Brandenburg), and received the name of *Bacillus acidificans longissimus*.

On account of its powerful fermentative activity, this bacillus can also be utilised to advantage in the preparation of lactic acid for technical purposes The dyeing and cloth-printing industries in particular require continually increasing quantities of this acid, the preparation of which by purely chemical means is at present a rather costly process, and can be more cheaply effected by means of lactic acid bacteria. For this purpose a sterilised unhopped beer wort, rich in maltose and qualified with a sufficient addition of calcium carbonate, is inoculated with a pure culture of the bacillus and maintained at 50° C. When the fermentation is ended the liquid is concentrated, and the lactic acid separated by decomposing the calcium lactate formed G. JACQUEMIN (I.) proposed a similar method, but gave no precise information concerning the nature of the ferment, and it is therefore uncertain whether the organism is allied to, or identical with, the above-mentioned bacillus. The method described by E. DELACROIX (I) utilises, by a similar course of treatment, the sweet whey formed as a waste product in dairies.

§ 150.—Effront's Hydrofluoric Acid Method.

It is found impracticable to protect the fermentation of distillery mash from injurious by-fermentations by sterilising the mash before adding the pitching yeast, since such treatment would also kill the diastase, the continuance of whose saccharifying action during the fermentation cannot be dispensed with.   More-over, such sterilising would not be of much value, since it is practically impossible to protect such large quantities of fermentable material from subsequent infec-tion by extraneous germs during the fermentation.   The only course, therefore, is to devise some means of restricting the development of the invading organisms. The souring of the yeast-mash is, as already explained, a method of this kind This method, however, was not based upon a recognition of the true nature of the evil to be overcome, but is rather the result of multifarious experiments, which finally demonstrated that a strongly acidified yeast-mash affords a guarantee for the satisfactory progress of fermentation in the mash proper, and protection from injurious bye-fermentations.   Consequently, as soon as the anti-bacterial action of lactic acid was recognised as the actual agency at work in this process, investigation into the suitability of other bacterium poisons for the purpose in question followed as a matter of course.   Thus in 1886, U. Gayon and G. Dupetit (I) ascertained that an addition of 0.1 gram of basic nitrate of bismuth per litre of mash was able to keep the fermentation free from contamina-tion.   Many other investigators have occupied themselves with the same subject, from which it is evident that the task is by no means an easy one.   As a matter of fact, the antiseptic sought must, to be suitable, unite in itself several properties. For one thing, if must be able to restrict the development of the *Schizomycetes* without injuring the yeast present at the same time.   Furthermore, it must not impart any evil odour or flavour to the alcohol produced, and must, therefore, be non-volatile and remain behind in the distillation residue (grains) without being—in its actual condition of dilution—dangerous to the animals subse-quently fed thereon.   Finally, the employment of the bacterium poison should not entail any great expense.   It is, however, difficult to find a substance capable of fulfilling the whole of these conditions   The metallic poisons, such as the aforesaid bismuth salt, must be at once dismissed from consideration.   The acid sulphite of lime (calcium bi-sulphite), which has been frequently recommended, is rendered unsatisfactory owing to the partial reduction of its sulphurous acid, by the fermentative organisms, to sulphuretted hydrogen, which spoils the odour of the alcohol.   An addition of artificially prepared lactic acid to the mash is too expensive, and its substitution by mineral acids is, with a *single* exception, impracticable, owing to their injurious action on the yeast.   Up to the present only a single reagent has proved useful, viz., hydrofluoric acid, which was intro-duced into distillery practice by J. Effront (II.).

This so-called hydrofluoric process—*i.e* the use of this acid, either in a free state or in the form of salts, especially as ammonium fluoride—has already passed through two stages of development and given rise to a number of investigations and treatises, which will be found epitomised in an essay by H Chatelineau and A. Lebrasseur (I.)   Effront (I) commenced his publications on the subject in 1890   His initial proposition was to add between 4 and 8 grams of HF per hectolitre (22 galls.) to the mash (treated in the usual manner) before pitching with the yeast, this quantity being sufficient to prevent the development of injurious bacteria.

Hydrofluoric acid surpasses all other mineral acids in its anti-bacterial powers, since, according to Effront, 25 mgrms. of this acid per 100 c c of wort will prevent the appearance of lactic or butyric fermentation, whereas 200 mgrms.

of hydrochloric acid or 300 mgrms of sulphuric acid are necessary to produce the same results. The butyric acid bacteria, being more susceptible to the influence of acids, can be repressed by as little as 10 mgrms. of HF per 100 c.c.

This original hydrofluoric acid process entailed no alteration in the customary method of preparing the yeast, and in particular the souring of the mash remained unchanged. Effront, however, endeavoured to render this preliminary treatment superfluous by modifying his method into the so-called **new hydro-fluoric process** by adding a sufficient quantity of hydrofluoric acid or fluorides to the sweet mash instead of leaving it to sour spontaneously.

Here naturally follows the question of the action of hydrofluoric acid on the **vital activity of yeast** It has been proved that the susceptibility of the various races of yeast to the influence of this acid differs, a circumstance which explains the irregular (sometimes good, sometimes bad) results yielded by the old process. In distilleries using very susceptible yeast the prescribed addition of HF to the mash might not only be without any good result, but probably even give rise to unfavourable symptoms, such as sluggish or imperfect fermentation.

The discovery that cell-reproduction on the one hand and fermentative activity on the other are affected in different degree is an important one. According to Effront (III), the former is completely arrested by the addition of 300 mgrms. of NH_4F per 100 c.c , whereas the fermentative energy is merely reduced, not stopped, by this quantity. The same authority has also showed (IV.) that yeast can be gradually accustomed to large additions of fluorine. In this manner a given yeast can be brought to withstand an addition of 300 mgrms. of HF per 100 c c. without losing its reproductive power. Doses below this limit—up to about 200 mgrms. per 100 c.c.—retard reproduction, but stimulate the decomposing energy of the organism, and therefore lead to a larger production of alcohol. The yeast becomes so much accustomed to this stimulant that it is subsequently rendered incapable of unfolding its energies except when pitched in a mash also containing fluorine, the yield of alcohol being otherwise far below the normal standard.

In practice the new hydrofluoric process is, in the main, carried out as follows · For the preparation of the yeast-mash 4 parts per cent. (by volume) are taken from the principal mash (previously saccharified and cooled down to 30° C), and a sufficient amount of hydrofluoric acid (or fluoride) added, this being followed by 1 volume of mother-yeast to each 4 volumes of mash taken. Of course, at the commencement of the season a sufficiency of pure culture yeast must be used instead. The amount of added acid is regulated by the kind of yeast in use, i.e. by its susceptibility, but 10 grams (say ⅓ oz) of HF per hectolitre (22 galls) of yeast-mash will generally suffice After the addition of the mother-yeast the mash, which was pitched at 26° C., quickly warms up to about 31° C., at which temperature it is maintained. In this procedure the older process described in the preceding paragraph is somewhat modified : the heating of the saccharified yeast-mash up to 70° C., which was there found advantageous, being, in the present instance, abandoned (since the injurious organisms are suppressed by the HF), as is also the separate addition of malt to the mash. In addition to the properties already mentioned, hydrofluoric acid is also credited with exercising a favourable influence on the diastatic action, in that in presence of this acid a much smaller amount of the said enzyme suffices to hydrolise a given amount of starch in a given time. The ratio of maltose and dextrin is also modified in favour of the former, as much as 96 parts of maltose being sometimes obtained per 100 parts of starch, whereas in presence of HCl (or H_2SO_4) the highest percentage of maltose amounts to 75 (or 76) per cent., and in the absence of mineral acids to 74 per cent. When the yeast-mash is matured (in about twenty hours time), one-fifth is set aside to serve as mother-yeast for

the succeeding mash. The residual four-fifths are incorporated with the fresh, saccharified principal mash, previously mixed with enough hydrofluoric acid (or ammonium fluoride) to make the (percentage) content thereof equal to one-half that of the yeast-mash   This proportion has been found to be sufficient; and since high patent royalties have to be paid—to the " Société Générale de Maltose à Bruxelles," of which M. Effront is director—for the use of hydrofluoric acid for this purpose, the distiller will naturally avoid employing more than is absolutely necessary.

Although hydrofluoric acid undoubtedly affords a reliable means for combating bacteria, and can be used with advantage to keep yeast free from these objectionable organisms, the case is different when the purification of a yeast from contaminating wild yeasts is in question. EFFRONT (V.) prescribed a method which he thought could effect this latter purpose, but the same was shown by A. JÖRGENSEN and J CH. HOLM to be unreliable. Some further particulars on this point will be given in a suitable place in the second volume. At present we will merely state that the hydrofluoric acid process in nowise supersedes the employment of pure culture yeast; on the contrary, the value of such yeast has here been revealed in a new light.

§ 151.—The Lactic Acidification ("Zickendwerden") of Wine.

The souring of wine and beer is by no means a uniform phenomenon, but may, on the contrary, appear in many forms.   The most frequent is the vinegar taint, i.e. a partial conversion of the alcohol into acetic acid by acetic acid bacteria.   Fuller particulars of this evil will be given in chapter xxxvii.   The subject of the present paragraph is the lactic acid taint, i.e. the production of acidity by lactic acid bacteria, and which is generally known in Germany as "Zickendwerden." This is a not infrequent malady, and usually makes its appearance in company with other injurious changes.   Even Pasteur classed it along with the so-called turning or breaking of wine, and the expression "Vin tourné" is still applied in France to both phenomena, other terms being "Vin monté" and "Vin qùi a la pousse." The course and characteristics of the malady are as follows   It mostly attacks young vintages, occasionally appearing even in the first year.   The wine turns turbid, and the odour and flavour gradually become irritating, like rancid butter   The turbidity increases by degrees to such an extent that the wine has the appearance of diluted milk, this white break passing over finally, in many instances, into the stage of black break, the wine then being in the condition of a brown to inky black liquid. Concurrently with this change of colour occurs a gradually increasing precipitation of dark slimy masses—a phenomenon characteristic of this malady.   The presence of lactic acid in "Vins tournés" was detected by A. BALLARD (I.) in 1861.   J. BERSCH (I) examined four samples of broken ("zickender") wine for their acid-content, and obtained the subjoined results .

—	No I	No II	No III	No IV
	Per Mil.	Pei Mil	Per Mil	Per Mil
Free acid, cal. as tartaric acid .	5 71	3 35	1.59	8 25
Carbonic acid .	0 09	0 05	0 05	0 19
Acetic acid . .	0 67	1.00	1 37	0 88
Lactic acid . .	2 37	0 86	1 73	0 98

The production of the two last-named acids was ascribed by PASTEUR (XII.) in 1866 to the action of fission fungi.   Experience shows that vintages poor in

I

acid, *e.g.* 1893 wines, are particularly liable to the malady. Müli
(I) found such wines were always infected with a bacillus 1 2–2
0.3 μ broad, capable of forming lactic acid, not merely from suga
also from tannic acid and another (unidentified) constituent of v
inoculated in must, this bacillus sets up lactic fermentation.

Musts that from any accidental cause have been deprived of th
great part of their acid-content are therefore very susceptible to
infection. Thus, MACH and PORTELE (II) report on a considerabl
of lactic acidity in South Tyrol, where, in the autumn of 1882 a.
vineyards in the lowlands of Etsch were flooded, and the grapes
crusted with the carbonates of lime and magnesia   Consequently a
portion of the acid in the must became neutralised in the process
the immediate result being complaints of the appearance of lactic
the other hand, those grapes that had been freed from the inc
carbonates, by treatment with dilute sulphuric acid before crushing
malady

Here, as also in most other maladies of wine, the true cause of
be sought in the defective constitution of the liquid. If the presenc
producing germs were the sole essential factor, there would be har
good wine at all, because all grapes—and therefore all fresh must—
with a variety of species of fission fungi, both harmless and inju
researches of MÜLLER-THURGAU (II –IV), MACH and PORTELE, and
and RIETSCH (I), conclusively proved this in many instances, a.
withstanding the (often considerable) infection thus produced, the
normal conditions resists its foes so effectually that the matured
sound wine is free from bacteria   This fact, demonstrated by Sc
FREUDENREICH (II.), is so decisive that it was regarded by both th
as an indication of purity, since the made wines examined by thei
exhibited a larger or smaller content of bacteria   In future inves
the subject of the diseases of wine, more attention will have to be p
hitherto been the case to the natural susceptibility of the wine
No known remedy exists for the lactic taint in wine, but Pasteurisa
recommended as a preventive measure.

§ 152.—The " Turning " of Beer.

Although the term " turning," as applied to wine, is not yet cle
still, in the case of beer, only a single malady is understood by th
viz., the undesirable appearance of lactic fermentation. PASTEUR
several observations on " bière tournée," and traced the cause to ce
fungi, which he described as long rods 1 μ broad and of variable
quently joined together in chains. For a closer investigation of t
indebted to H. VAN LAER (I.), who in 1892 obtained pure culti
ferment, and named it *Saccharobacillus pastorianus.*

The commencement of this malady in beer is evidenced by a grad
in the brightness of the (previously clear) liquid, which finally be
turbid, and gradually assumes an unpleasant smell and taste. If th
shaken, delicate waves of a fine thread-like character appear in
resembling in appearance the fine films produced at the plane of cont
two liquids of unequal densities   This appearance is so remark.
suffices of itself to characterise the malady. After a time there sep
deposit, which Pasteur reproduced in Plate II. of his above-ment
and which consists—apart from the yeast-cells, which may be disreg
nitrogenous precipitate thrown down by the lactic acid, and of sing

chains of _Saccharobacillus pastorianus_ The latter are the cause of the aforesaid optical phenomenon exhibited when the liquid is shaken.   Meat-broth gelatin is unsuitable for the pure cultivation of this fission fungus, and it develops but imperfectly in wort gelatin, so that slightly alcoholised malt extract agar-agar, in which the organism thrives, has to be employed.   The re-inoculations made by Van Laer into sound beer decisively proved the agency of _Saccharobacillus pastorianus_ in the production of "turning" in that beverage.   It is, however, incapable of developing or becoming injurious except when the percentage content of hop extract (i e. the hop resins inimical to bacteria) in the medium is small   This influence of the hop resins was, however, not further investigated by Van Laer.

As its generic name implies, _Saccharobacillus pastorianus_ ferments sugars, and especially saccharose, maltose, and dextrose, which it acts upon readily, but, on the other hand, ferments lactose with difficulty.   Saccharose is apparently transformed without inversion, since the presence of invertin could not be detected in the cultures   In media containing one of these sugars the bacillus chiefly produces inactive lactic acid, in addition to ethyl-alcohol and a small quantity of volatile acids (acetic and formic acids), the proportions varying with the kind of sugar and the conditions of cultivation.   Given a sufficiency of sugar, the degree of acidity produced is then solely dependent on the composition of the remainder of the medium , in unhopped wort it amounts to as much as 1 26 grams (calculated to lactic acid) per 100 c.c , whilst in hopped wort it does not exceed 0.27 gram   The development of the bacillus is not restricted by alcohol unless more than 7 per cent of this substance is present in the beer   It thrives better in the warm, and consequently the malady is of frequent occurrence in summer in countries where the cellar accommodation is defective. This explains the Flemish name "Zomerbier," applied to turned beer in general. The organism cannot survive continuous exposure to $55°$-$60°$ C. for a short time; consequently beer intended for export to tropical countries may be protected against risk of "turning" by Pasteurisation.

§ 153.—White Beer, Lambic, Ginger-Beer.

A low percentage of lactic acid is met with even in the best beers.   It is derived partly from the malt itself, which contains on an average o o5 per cent. of this acid, but is chiefly produced during the mashing process, the amount then developed being nevertheless small—ranging in normal beers between o o5 and 0.2 per cent. The nature and amount of the acids produced during the malting of barley, the kilning and mashing of the malt, and the boiling of the beer-wort, have been investigated by E. PRIOR (I.).   In addition to the rod-shaped species already described, lactic ferments in the form of globular cells developing into sheet colonies appear in the malt-mash.   An acid-producing species of this class was examined by P. LINDNER (I), who named it _Pediococcus acidi lactici_.   Its diameter is 0.6-1.0 μ, and the optimum temperature is about $40°$ C., but the organism is killed in two minutes by a temperature of $62°$ C., and it does not thrive either in hopped wort or beer.—The spontaneous lactic fermentation appearing under certain circumstances in malt-mashes has been investigated, from a chemical point of view, by M. HAYDUCK (II.).   The variety of the fission fungi developing in these mashes is very considerable, the first in point of size being the _Sarcina maxima_, described by P. LINDNER (II.), the individual cells of the packet-colonies of this organism measuring 3-4 μ in diameter.   Attempts to obtain pure cultures of this, the largest species of sarcina, have hitherto proved unsuccessful

In many instances the appearance of a vigorous lactic fermentation in beer-

wort is regarded with favour and its development encouraged   This applies to the so-called " Weissbier " (white beer)   No careful bacteriological investigation of the acidification process, which plays such an important part in the preparation of this refreshing beverage, has yet been made.   Possibly *Saccharobacillus pastorianus* is concerned therein, at present, however nothing definite can be stated on this point

A considerable amount of acidity is produced in the Belgian beers known as **Lambic, Faro, and Mars,** beverages prepared by spontaneous fermentation without any addition of yeast.   The boiled and re-cooled wort is placed in barrels which are only partly filled, the empty internal space communicating with the external air by a small aperture   Sufficient yeast-cells to set up fermentation are left adhering to the walls of the casks from the previous fermentation, so that after a lapse of twenty-four hours an evolution of gas is already noticeable. In addition to alcoholic fermentaton, lactic, and subsequently also acetic, fermentation sets in.   L. V. D HULLE and H VAN LAER (I) published in 1891 the results of a chemical investigation of this matter.   The more important of these are tabulated below

Age of the Lambic	Alcohol per Cent by Weight	Lactic Acid per Cent	Acetic Acid per Cent
10 months .	4 84	0 310	0 044
12 months .	4 07	0 900	0 121
36 months . .	5 59	1 051	0 098
47 months .	5 24	0 939	0 336

The beverage is consumed after a storage period of three to five years, and, in its matured condition, is known as " *gueuse lambic*."   The acidity then amounts to about 1 per cent., and is masked by an addition of sugar immediately before drinking.
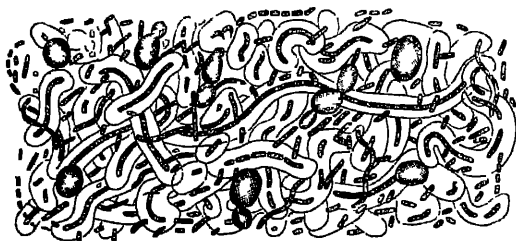

FIG   52 —Section through Ginger-beer Plant

The cells of Saccharomyces pyriformis are surrounded by the cells of Bacterium vermiforme, the membranes of which are very much thickened and swollen   Magn 680   (*After H M Ward*)

A spontaneous lactic fermentation also occurs in the case of ginger-beer (to which reference has already been made in § 64).   The preparation of this foaming acid beverage, which is largely consumed in England in the summer-time, is a very simple matter.   To a 10–20 per cent. solution of sugar are added a few pieces of ginger and a couple of granules of the **ginger-beer plant**   the whole being then left to stand uncovered.   The liquid soon begins to ferment briskly, is bottled at the end of twenty-four hours, and consumed within the next two days. The so-called **ginger-beer yeast** was more closely examined by H. M. WARD (II).   It consists of whitish translucent masses about the size of a hazelnut, and is a mixture (Fig. 52) of a yeast, *Saccharomyces pyriformis,* and a fission fungus.   The cell-walls of the latter organism are gelatinised in a manner with which we shall become acquainted later on, more particularly in the case of certain filamentous bacteria _ the greatly swollen outer layers of the cell-membrane

becoming detached, but only so far as to constitute an independent outer case or sheath enveloping the cells   This jacket either surrounds the cell along its
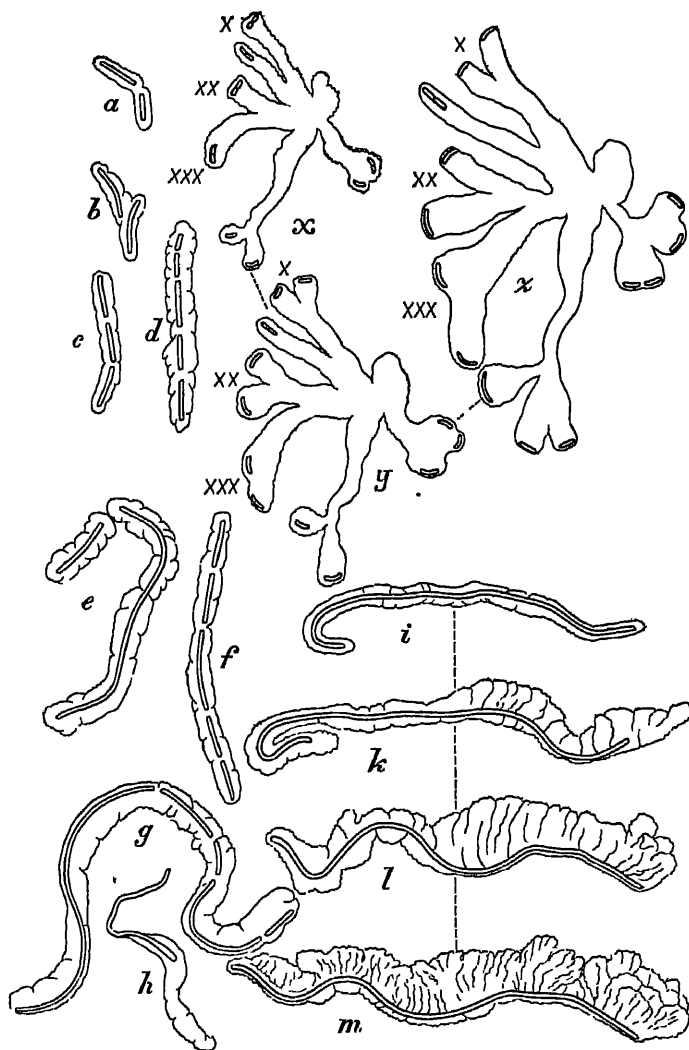

FIG 53 —Bacterium vermiforme

a–h The gradual evolution of the cells and chains into the worm form
i–m. Development of a sheath increasing on the one side, the cell-membrane thickens in certain directions only, and not equally all round. Magn 680
x–z Dissolution of such unilaterally thickened cell-membranes into branched forms, the cells being visible at the extremities of the branches   Culture in ginger-beer nutrient gelatin , *x*, observed at 10 A M , *y*, the same at 4 P M , and *z*, at 10 A M. the following day   The parts indicated by *x*, *xx*, *xxx*, correspond. Magn 420. (*After Ward*)

entire length or else only along one side thereof, and is in some cases absent altogether. The breadth of the cell itself measures about 0 5 μ, and the length

varies between 0 5 and 50 μ. The thickness of the sheath is often ten times greater than the diameter of the cell. When examined under the microscope, this thick envelope with its comparatively thin enclosure resembles a worm, and it is on this account that the name *Bacterium vermiforme* has been given to the fungus.

The bacterium is shown in Fig. 53 It stands in symbiotic relation with the *Saccharomyces pyriformis*, so that the development of the one is facilitated by the presence and vital activity of the other. Ward also succeeded in artificially constructing the ginger-beer plant from its two components. The chief products of this fermentation are carbonic acid and lactic acid, in addition to traces of alcohol and acetic acid. The lumps of the plant are kept in a state of saltatory motion by means of the carbonic acid gas, and increase considerably in size during the fermentation. They are able to withstand desiccation, and shrink up on drying to form a horny mass, in which condition they are stored for future use. The origin of the ginger-beer plant is unknown.

CHAPTER XXVI.

THE LACTIC ACID BACTERIA IN THE PREPARATION OF FODDER.

§ 154.—Brown Hay.

ONE of the processes wherein micro-organisms play an active part for the pre-servation of juicy fodder, viz., that dealing with **burnt hay**, has already been noticed in § 106; and we will now briefly sketch a second and more general practice, viz , the preparation of **brown hay.** As in the former case, the warmth necessary for driving off the water in the fodder has to be supplied by thermo-genic bacteria. In addition to these, however, another series of organisms, converting part of the carbohydrates into lactic acid, butyric acid, &c , comes into play. The percentage of water in the green fodder employed for making brown hay must be smaller than in that worked up into burnt hay. The materials (grass, &c.) are built up into a round or square rick from 16 to 24 feet in diameter, and 13 to 16 feet high, well trodden down, and thatched in the roof to prevent the incursion of rain-water. At the end of about three days, spon-taneous heating (" sweating ") will become manifest, and its progress can be conveniently followed by means of a metal pipe laid in the stack and containing a thermometer (provided with a couple of attached strings), which can be drawn out as required, for the purpose of reading off the temperature prevailing in the interior of the heap In proportion as the temperature rises (generally up to 70° C., and frequently still higher), the mass begins to steam, and this goes on for eight to fourteen days, a further four to eight weeks being allowed to pass before the **brown hay** can be considered as finished. The product forms a firm, dry mass, the colour of which, under normal conditions, is between pale and dark brown, but is black when overheating has occurred. In point of cohesion this brown hay is preferable to air-dried hay, being less brittle than the latter, but tough and suitable for fodder. Its odour is aromatic, and recalls that of freshly-baked bread or honeycomb. Comparative investigations into the chemical changes produced in this method of preparation have been made by Dietrich, Moser, Weiske, and others   The results obtained by Dietrich are given below (see Table, p. 200), since they afford material for judging the process from the Fermentation Physiologist's point of view   A parcel of aftermath was divided into two portions, the one being worked up into withered (air-dried) hay, and the other to brown hay , and an average sample of each was subjected to analysis.

A comparison of the first and third lines of this table at once reveals the high percentage of lactic acid and butyric acid in the brown hay, both of which are entirely absent from air-dried hay. It will be evident, upon mature con-sideration, that the production of these acids by bacterial activity does not occur in the centre of the stack, since the temperatures (70°–90° C) prevailing there are such as only the passive reproductive forms of these organisms are able to withstand   To obtain a correct idea, we must picture the changes occurring within the stack as proceeding in the following manner the thermo-bacteria develop in the centre of the mass and liberate heat, which radiates towards the outside. Between this hot central layer and the external strata exposed to the

Percentage Composition of	Water	Crude Protein		Fat	Extractive Non-Nitrogenous Matter		Woody Fibre	Ash	Citric Acid	Lactic Acid	Butyric Acid
		Total	Portion Soluble in Water (= Amides. &c.)		Total	Portion Soluble in Water					
a. Air-dried hay	15 0	9 8	3 0	2 3	40 9	17 5	24.6	6 7	0 66	—	—
b Brown hay	20 1	10 5	1.0	2 9	21 9	9 0	28 1	7 3	—	6 97	2 23
c The latter calculated to the same water-content as a	15 0	11 1	1 1	3 1	23 2	9 6	29 9	7 8	—	7 42	2 23

cool air lies a broad zone wherein the precise temperature (40°–50° C) most suitable for the development of the acid bacteria in question prevails. The metabolic products from these organisms then gradually permeate the entire mass. The chief material for these fermentations is afforded by carbohydrates (starch), as may be seen from the foregoing table, according to which the air-dried hay contains 40 9 per cent', whilst the brown hay contains only 23.2 per cent. of non-nitrogenous extractive matter (starch, sugar, &c) Of the nitrogenous constituents, those soluble in water, i e amides and kindred bodies—of which the brown hay contained 1.1 per cent and the air-dried hay 3.0 per cent —are for the most part consumed. The loss of matter attendant on the preparation of brown hay is calculated by Dietrich as about 14 per cent.

Brown hay exhibits one advantage over both air-dried hay and burnt hay, namely, that its preparation is much less dependent on the weather, a couple of fine days sufficing for protecting the finished hay. These days can, however, be selected at convenience, since the ricks of brown hay can be left untouched for a long time without dread of spontaneous ignition. Hence this method is frequently employed in rainy districts, e.g. the North Sea littoral and the Austrian Alps It is, however, inadvisable to resort to this practice where good air-dried hay can be made from the green fodder at disposal, because the feeding experiments performed by G. Kuhn and others, and reported by Fr Albert (I.) and Fr. Falke (I), concordantly demonstrate that the preparation of brown hay is attended with a considerable loss (amounting to as much as 50 per cent of the total) of digestible protein substances

§ 155.—Sweet Ensilage.

The preparation of brown hay is also partly dependent on the weather, in so far that a certain amount of dryness in the material before stacking is essential. Now, in many cases, it is either practically impossible or economically disadvantageous to remove from the green fodder even the small quantity of water that must be got rid of in making brown hay. One instance of this kind is afforded by the enormous quantities of beet leaves available for a few days only in each year (during the ingathering of the beet crop), and another is the drying of the de-sugared slices of beet, an operation impracticable in many places owing to the lack of the necessary costly drying apparatus. In such, and many other similar cases, putrefaction of the readily decomposable masses is prevented by subjecting them to an acid fermentation without any previous drying. Formerly

this was effected exclusively in silos—whence the term ensilage, current for this operation in England and France, is derived.

According to the composition of the raw materials, their water-content and method of treatment, two classes of durable fodder are obtained, viz., **green pressed fodder** and **sour fodder**. The main factor determining which of them shall be produced is the height of the temperature attained, as the result of spontaneous heating, in the mass. If this does not exceed 40° C., then the butyric ferments develop along with the lactic acid bacteria, and a sour-smelling product, known as **sour fodder**, results. More detailed particulars of this are given in the next paragraph.

If, on the other hand, the thermogenic bacteria develop vigorously in the heap, and thus cause the temperature to rise rapidly to 50° C. and remain there for some time, then the lactic acid bacteria develop by preference, overcome all their competitors, and exert a practically undivided sway In this case a durable fodder is obtained, which is almost entirely free from volatile acids and devoid of odour, or with a somewhat sweetish smell, on which account it is known as **sweet fodder**, though this name is hardly correct, owing to the strongly lactic acid character of the product. A more suitable appellation has latterly been bestowed on it, viz , **green pressed fodder**.

This process originated in England in 1885, under the name of **sweet ensilage**, but through the explanatory treatise written by G FRY (I) became known on the Continent, where it was at first styled **Fry's ensilage**. The numerous investigations to which it was there subjected led to important conclusions, both of a chemical and practical nature, which were fully reported by FR. ALBERT (II). Nevertheless, from the Fermentation Physiologist's point of view, no advance has been made beyond the general information already given by Fry On account of this deficiency we are obliged to dismiss this process (important though it is to agriculturists) with merely a very few remarks.

As the name itself implies, green vegetable substances, such as waste beet leaves, clover, green maize, &c , are used for the preparation of green pressed fodder. Silos are unnecessary, the materials being stacked in the form of a straw-thatched cottage, tightness of packing being an important feature By suitable means (e g horizontal beams with weighted ends, or similar stack presses) a continuous heavy pressure is exerted on the stack, the amount of the pressure being a predominant factor influencing the degree of spontaneous heating produced. Should the temperature not rise quickly enough, then the pressure is moderated to admit air more freely to the thermogenic bacteria On the other hand, if the temperature rises immoderately (beyond 70° C.), then the pressure is increased, the access of the air restricted, and the oxidising activity of the said organisms consequently diminished The state of the internal temperature is observed by means of an ensilage thermometer designed by E. Meissl, the scale of which projects from the side of the stack. In a word, the stack is regulated in such a manner as to ensure the predominance of the lactic acid bacteria, whereby, under normal conditions, a product of a green to olive-green colour, and of an aromatic sweet flavour, is obtained. The structure of the vegetable matters employed is still distinguishable A sample of this fodder, prepared from crimson clover, contained (according to Bohmer) 71 per cent of water and about o 36 per cent. of total acids, of which o 27 per cent. was lactic acid, and the remainder consisted of butyric acid, acetic acid, valeric acid, &c In the researches recorded by Fr. Albert the total acids (calculated to dry matter) in a sample of green pressed fodder, prepared from meadow grass and containing 68.4 per cent. of water, amounted to 2.49 per cent , of which 1 89 per cent. was composed of non-volatile acids (lactic acid).

With regard to the losses of matter inherent in this process, that consisting of carbohydrates need not be further dilated upon, since it is evidently unavoidable, being intimately connected with the production of heat on the one hand, and on the other with the formation of lactic acid. Greater importance attaches to the modifications effected in the albuminoids during the process, and these changes also afford a means for determining its value   The numerous researches made on this point all tend to prove that the conversion of green fodder into green pressed fodder is attended with a substantial loss of digestible albuminoids, the amount of the loss being in direct proportion to the water-content of the fresh material. The ferments consume a large amount of the albuminoids initially present, and decompose them into amide compounds, ammonia derivatives, and even ammonia—all substances of but little, if any, use for the nutrition of animals which are to be killed for food.   In the cases reported by Albert, the amount of these matters eliminated ranged from 13 to 31 per cent. of the total nitrogenous matter (crude protein) present.

§ 156.—Sour Fodder

is prepared in pits some 40 to 80 inches in depth, and 80 to 120 inches wide, the length depending on the quantity of fodder to be treated   The most important raw materials are the exhausted slices of beet-root from sugar-factories, fodder-beet, potatoes (previously steamed), frozen sugar-beet, chaffed maize stalks, &c. The silo is tightly filled with these and covered with a layer of chop (chaff), surmounted with a thick stratum of soil, and over this again are laid boards, weighted with sufficient stones to produce a pressure of about 1000 kilos. per square metre (nearly 2000 lbs per square yard). Since practically no oxygen can penetrate the interior of the compressed mass, the activity of the thermogenic bacteria is very much impeded.   Nevertheless, the temperature rises to some extent, as a result of bacterial activity, but not to anything like the degree attained in the case of green pressed fodder, and, in fact, generally remains below 35° C Observations on this point were made by R. Schatzmann in a silo of elliptical ground plan, and having a capacity of 37 cubic metres (1307 cubic feet).   On the 2nd day after filling, the internal temperature registered 26° C., and attained on the 16th day a maximum of 34° C., afterwards subsiding, so that on the 36th day 23° C, on the 56th day 19° C, on the 86th day 12° C., and on the 106th day 8° C. were recorded.   Under such conditions, the heat-loving lactic acid bacteria could not be expected to gain the upper hand ; and, as a matter of fact, a number of highly divergent fermentative organisms take part in the production of sour fodder, the percentage of volatile acids in the fodder being correspondingly high, and the smell consequently sour   In this process the loss of matter is still greater than was found in the case of green pressed fodder, and in an instance examined by Julius Kuhn amounted to 23 4 per cent. of the total dry substance. The loss is principally borne by the non-nitrogenous extractive matter (carbo-hydrates, &c.), then by fermentable woody fibres, and finally by the albuminoids, of which (in consequence of their conversion into amido-compounds) about 40 per cent. disappeared in the above-mentioned experiment (with soured green maize).   In a second instance (beet slices), the loss was 18–62 per cent., and in a third (soured beet leaves tested by O. Kellner) as much as 68 per cent. Although these high proportions of loss in the souring process are regrettable, it should not be forgotten that the fodder materials now in question would be entirely wasted unless utilised by means of this process, whereas, when so manipulated, a considerable proportion of their nutritive constituents can in any case be preserved.   This souring process can always be relied on to yield comparatively good results where all other methods for preserving a given fodder

are impracticable or disadvantageous. To this advantage must be added, in mar
cases, an improvement in composition effected by the process, *e.g.* the reduction (
the percentage of (purgative) oxalic acid in beet-leaves, observed by O. Kellne
Numerous researches on this subject have been published, and the reader ma
be referred for further information to the summary of these investigations whic
appears in a useful treatise by JULIUS KÜHN (I.), dealing with the matter fron
the Agricultural Chemist's point of view.

Granting that the sacrifice of a certain portion of the given substance, in orde
to retain the rest, is inherent in this process, it by no means follows that th
extent of the said sacrifice cannot be reduced   The great fluctuations exhibite
by the foregoing figures, referring to the amount of loss experienced, permit th
assumption that an excellent sour fodder can be prepared with a smaller pro
portion of loss than the existing average. This opens up to the Fermentatio
Physiologist a new and valuable field of work, still unexplored.   The fermentativ
nature of the souring process being acknowledged, it follows that the next objec
in view is the attainment of a clear insight into the physiology of this ferments
tion ; which knowledge will then facilitate the cultivation of the ferment
combining maximum efficiency with minimum consumption of material, an
consequently capable of yielding the greatest proportion of best quality sou
fodder   Attention should be directed to the artificial inoculation of the silos wit
selected ferments, since the resulting improvements are of great pecuniary valu
to the agricultural interest. (The author estimates them as represented by a
annual sum—for Germany alone—of several millions of marks, *i.e.* shillings
These researches should also be extended so as to include the kindred process (
sauerkraut fermentation, the preparation of which important food-stuff, on lin
practised from time immemorial, formed the prototype for the production of sou
fodder.   Little is known of the physiology of this method, the literature of th
subject being restricted to a couple of brief reports which ascribe the chie
agency in the process to the lactic acid bacteria.

CHAPTER XXVII.

THE PART PLAYED BY BACTERIA IN TANNING.

§ 157.—The Fermentation of the Plumping Soak.

THE preservation of animal hides by simple drying is feasible only when they are to be stored for some time and brought on the market in a dry state. To accelerate this drying, the fleshy side is in many instances rubbed over with arsenic or with common salt. A few investigations into the utility of the last-named substance were made by F HAENLEIN (I). The brittle solidity and fragility acquired by the hides in drying prevent their utilisation for practical purposes, and the more so because putrefaction sets in directly they are wetted. To remedy this defect is the object of **tanning**, $i\,e$ the conversion of hides into leather. Bacterial activity here comes into play at the outset of this operation, micro-organisms being necessary even in the preliminary treatment known as **unhairing**. As is well known, leather consists neither of the outer skin (epidermis) nor of the next layer of mucous cells (mucous membrane), but of the third layer (composed of closely interwoven cells), which, on this account, has been termed the leather (or true) skin (corium). Several methods of exposing this layer and simultaneously removing the hair exist, one of them being the so-called **sweating** process. In this operation the cleansed, soft hides are maintained at a moderate warmth in a chamber saturated with moisture—the sweating pit Putrefaction quickly sets in, but is only allowed to proceed so far as to loosen the hairs and enable them to be scraped off, along with the epidermis and mucous membrane. A second method of unhairing is by **liming** or **slackening**, the hides being repeatedly steeped in an initially weak, but progressively stronger, milk of lime. In the absence of investigation on the subject, it is still uncertain whether an active part is played in this operation—as is undoubtedly the case in the sweating process—by bacteria, certain species of which, capable of resisting the action of the milk of lime, have been discovered in the liquor by J. VON SCHRÖDER and W. SCHMITZ-DUMONT (I.). The result of both operations is substantially the same, viz, the hair, epidermis, and mucous membrane are loosened, and can then be removed.

In one respect, however, limed hides differ from those slackened by sweating. In the former case, calcium carbonate has been deposited in the intercellular spaces in the hide, which is thus rendered somewhat brittle and less pervious to the tanning liquor This carbonate precipitate is removed by steeping the hides in a pickle or "bate" consisting principally of a mixture of bran, barley, groats, and the excrement of various animals (dogs, fowls, pigeons, &c) in a state of acid fermentation. The chief, if not the sole *raison d'être* of this process, which has been gradually developed through tentative experiments alone, has only been brought to light in recent years. The first progressive step consisted in the discovery, by AUG. FREUND (I) in 1871, that lactic acid is the chief product of the spontaneous acid fermentation of wheat bran. This explained the favourable result of the bating of lime-slackened hides, the calcium carbonate being converted into the soluble calcium lactate, and thus washed out of the hides.

Pickling, however, effects a second and remarkable alteration in the hide,

causing it to swell up ("plump") to almost double its former thickness, thus loosening the cellular tissue and facilitating the subsequent penetration of the tannin. In order to make the most of this advantage, it is generally the custom to pickle even the hides that have been unhaired by the sweating process, and which consequently do not contain any lime that needs to be removed. The object in this case is to plump the hides, i.e. cause them to swell up, and this explains the term "plumping soak" applied to this acid liquor. This expansion of the skin is due to the action of the gases liberated in the fermenting liquor. The reactions occurring in this process are very diversified, as might be expected from the large number of bacterial species present in the bran itself and in the added fæcal matter Of these organisms, such as attack the carbohydrates develop most vigorously, these substances being present in large amount.

J. T. WOOD and W. WILLCOX (I) described one species of this kind and gave it the name of *Bacterium furfuris*. The starch in the bran is hydrolised and converted into glucoses by an enzyme, cerealin—isolated from bran extract by J. T. WOOD (I.)—and these sugars are then acted upon by the bacteria in question, organic acids and considerable quantities of gas being produced. In a sample examined by WOOD (II), 1 litre of the fermented bate contained o 8 gram of lactic acid, o.2 gram of acetic acid, o 03 gram of formic acid, and o o1 gram of butyric acid The disengaged gas was found, in different experiments, to consist of 22-42 per cent of CO_2, 28-53 per cent. of H, 24-26 per cent of N, and 1-4 per cent. of O, the percentage of carbon dioxide increasing as fermentation progresses. This fermentation is attributed by Wood and Willcox to the said bacterium, which they describe as short rods o 7 μ long and 1.3 μ broad, united to form chains, but not, so far as they could ascertain, producing endospores. The cell-walls exhibit a tendency to swell up. The organism is incapable of attacking solid or dissolved starch, and consequently can only come into play when the cerealin has completed its diastatic action and converted the starch of the bran, &c , into glucose.

These discoveries must be regarded as a preliminary step to be followed by many others The unappetising and (hygienically considered) objectionable use of fæces is shown to be unnecessary; and it would be advisable to endeavour to artificially "sour" the plumping soak by means of a sufficient quantity of actively fermenting leaven prepared from pure bran.

The next step in bacteriological investigation in this matter should be to ascertain whether—as the figures above given lead one to suppose—pure lactic fermentation is the best form of fermentation for this soak. It should be mentioned, moreover, that the removal of the lime from the slackened hides by the aid of dilute mineral acids is impracticable, since it seriously impairs the quality of the resulting leather.

§ 158.—The Souring of Bark Liquor.

For the actual tanning of the plumped hides there are, as is well known, three different systems available, viz., alum- or white-tawing; oil-tawing, or shamoying, and bark-tanning—the last of which will now be briefly considered from a bacteriological standpoint. The chief tanning material employed in this case is the bark of various trees (oak, pine, &c.), in addition to which gall-nuts, myrobolams, sumach, &c., are also used.

Hides intended for sole-leather are placed in a tanpit in such a manner that each hide is separated from its neighbour by a layer (a couple of fingers in thickness) of coarsely-broken fresh bark, mixed with powdered gall-nuts, &c. The empty corners and vacant spaces are packed with old spent tan, and the pit is then

filled up with water   At the end of eight or ten weeks the hides are taken out
and relaid in fresh strata of bark, and this operation is repeated (three or five
times) until the leather is impregnated with tan.   There is no doubt at all but
that the activity of microbes is also manifested in the tanpit, but at present no
hypothesis can be formulated on this point, there being no scientific foundations
to build upon.

Our knowledge of the reactions occurring in the bark liqour is, however, in
a more satisfactory state.   All the thinner hides, unsuitable for sole-leathers,
are steeped, not in the tanpit, but in an aqueous (cold-drawn) extract of tanning
substances, known as **bark liquor** or ooze (Fr. *jusée,* Ger. *Lohbrühe*)   The actual
process of tanning, *i.e.* the combination of tannic acid and phlobaphene with the
fibres of the skin, does not concern us at present, this being a purely chemico-
physical operation.   One circumstance, however, deserves brief mention here,
and that is the subsidiary phenomenon of the gradual souring of the **bark liquor.**
This change, well known to all tanners, was first chemically investigated by
H. BRACONNOT (I.), who, in 1832, discovered in soured bark liquor an acid which
—as little was then known of the properties of lactic acid—he considered to
be a new, hitherto undiscovered acid, and therefore named it "acide nancéique,"
after the town of Nancy, where the discovery was made.   This acid was, how-
ever, quickly recognised by L. Gmelin as lactic acid.   J. WLADIKA (I.) in 1890
showed that volatile, as well as non-volatile, acids are present in old spent bark
liquors, the former consisting chiefly of acetic acid and the latter of lactic acid.
The reader will have no doubt that the reduction of these acids (which are not
present in the "sweet" liquor) is attributable to the activity of micro-organisms,
which are here present in abundance, HAENLEIN (II.) having found no less than
60,000 bacteria (along with a few budding fungi) in the liquor prepared from,
and corresponding to 1 mgrm of, Silesian oak-bark.   The acids are produced
from the saccharine constituents of the bark, a fact already evidenced by the
researches of B. KOHNSTEIN (I.) and J. VON SCHROEDER (I), who showed that
in proportion as the sweet liquor becomes sour, so the percentage of these sugary
constituents decreases.   In practical working their initial quantity is found to
be from 0.3 to 0.8 gram per litre

A fission fungus named *Bacillus corticalis,* and recognised as an active partici-
pant in the souring of bark liquor, was discovered by HAENLEIN (III.) in large
quantities in sour pine-bark liquor, as also in the fresh pine-bark.   This microbe
appears in the form of short rods 0.7–1 μ broad and one and a half to twice these
dimensions in length ; at the period of active reproduction the individual cells
are connected into many-jointed chains   The bacillus thrives on nutrient gela-
tin, nutrient agar-agar, and slices of potato, and requires but very little nutri-
ment.   Being able to withstand desiccation, it remains alive in bark, where it is
found in large numbers.   It has a great affinity for light, and grows more freely
when illuminated than in the dark, though it is still capable of performing its
vital functions under the latter condition.   It thrives most vigorously at 30°–
40° C., and ceases to develop below 5° C.; in habit it is facultatively anaerobic,
i.e. oxygen is not essential to its growth.   Its most important property, so far as
we are now concerned, is its behaviour towards sugars, of which it acts upon
dextrose as well as saccharose and lactose, and produces, in addition to acids, a
considerable quantity of gas, composed (in one case investigated) of 95 per cent.
of hydrogen and 5 per cent. of carbon dioxide.   Tannin is not attacked by this
microbe, a fact confirmed by the observation made by J. VON SCHROEDER and A.
BARTEL (I.), that the percentage of tannin in the liquors undergoes no alteration
on standing or during storage.

The acidity of tannin bark liquors amounts to 0.25 gram (reckoned as acetic
acid) per litre and is, as the previously mentioned researches of Wladika have

shown, mainly due to lactic acid, the presence of which acid has a favourable influence on the quality of the leather. According to J. PÄSSLER (I) a leather tanned with pure tannin handles poor and hard, but is rendered soft and supple by the acid gradually formed in the bark liquor   It is therefore easy to understand why the tanner likes to see his bark liquor turn sour, and even attempts to favour and accelerate this state of things by mixing with the fresh liquor some of an older, already soured batch, thus unwittingly inoculating it with a culture (in any case impure) of acid bacilli   Whether this microbe also acts in other ways is at present unknown, but it is a subject worthy of investigation. This applies particularly to the liberation of gas in bark liquors, which, according to Haenlein's researches, amounts to 1–2 c.c per gram of pine-bark.   Important researches on the dependence of the normal progress of this souring operation on external conditions were made by F. ANDREASCH (I).

Although *Bacillus corticalis* is by no means the only species found on pine-bark, still the composition of the bark liquor is specially favourable to its development.   It is therefore probable that in the liquors prepared from other (and especially tropical) tanning materials, other (but allied) species will be found, not only because the bacterial flora of these materials (grown under other conditions) is different, but because these latter differ in chemical composition from our indigenous tanning barks, &c , and consequently favour the development of other species of bacteria   J. T. WOOD (III.) has described the micro-organisms present in sumach infusions —In conclusion, it may be remarked that the process of tanning still presents a very profitable field of research to the Technical Mycologist.

SECTION VII.

THE FORMATION OF MUCUS AND ALLIED PHENOMENA OF DECOMPOSITION.

CHAPTER XXVIII.

THE IMPORTANCE OF BACTERIA IN THE MANUFACTURE OF SUGAR.

§ 159.—The Zooglœa of Leuconostoc Mesenterioides.

In the manufacture of saccharose there are occasionally formed certain masses of gelatinous mucus, for the most part merely small colourless or reddish agglomerations, resembling frog spawn in appearance, but, under special conditions, increasing to masses exceeding a cubic foot in dimensions   Attention was first drawn to them by C. SCHEIBLER (I), who, in 1874, subjected them to chemical examination, the result of which indicated the presence of dextran, a carbohydrate first discovered by him in the plasma of unripe sugar-beet.   Scheibler, in fact, regarded this mucus as expressed beet plasma, but this opinion (which was also shared by E FELTZ (I)) was very soon opposed, P. JUBERT (I.) having shown a few months later, that these gelatinous lumps (known in France as "gomme de sucrerie") continue to develop in sugar solutions.   From this he concluded that they are not extravasated beet plasma, but a ferment (a "plant," as he expressly stated), whose reproductive power he succeeded in arresting by means of carbolic acid.   The microscopical examination of the mass, however, as first made by F. MENDES and J. BORSCHTSCHOFF (I.), led at the outset to no satisfactory elucidation.   For an accurate knowledge of the true state of the case we are indebted to L. CIENKOWSKI (I), who, in 1878, proved that the mucous masses in question are composed of bacteria with extraordinarily swollen and gelatinised cell-walls, which cause the individual organisms to adhere together and form the aforesaid large masses, whose superficial convolutions frequently resemble those of the mesentery.   Influenced by Billroth's publication on *Coccobacteria septica,* and in view of the name *Ascococcus Billrothii* given by COHN (II.) to a mucus-forming fission fungus, Cienkowski called his microbe *Ascococcus mesenterioides*   The forms of cell which he detected in these mucinous lumps he described as highly diversified. Coccus, Bacillus, Vibrio

In the same year VAN TIEGHEM (VII.) published a research on the organism producing these mucinous masses, agreeing with his Russian colleague as to its vegetable nature, though with respect to its position in the botanical system he held other views.   According to his observations, the development and reproduction of this microbe harmonised so well with those of the blue-green unicellular algæ of the genus *Nostoc,* that he felt obliged to call it "white" (chlorophyll-free) Nostoc, and bestowed on it the new generic name of *Leuconostoc*   Contrary to the observations of Cienkowski, who ascribed a copious polymorphism to this fission fungus, Van Tieghem could only discover globular

cells, as shown in Fig. 54 (which is made up from the drawings in the original treatise, and is here reproduced on account of its historic interest). Since, however, the researches of this French worker were not performed with pure cultures, we cannot go more closely into the details of this illustration.

It was not until 1891 that a thoroughly satisfactory bacteriological investigation was made—by O. LIESENBERG and W. ZOPF (I)—on this organism. In the interim, the same gelatinous masses had also been observed in Indian cane-sugar factories by H. WINTER (I.). Adhering to the existing nomenclature, the two first-named workers showed that *Leuconostoc mesenterioides* has only one morphological form, viz , a *coccus* of o 8 to 1.0 μ in diameter. This discovery in nowise detracts from the accuracy of Cienkowski's observations, for it must be remembered that he did not have pure cultures at his disposal, but examined a sample of the gelatinous mass, which from its nature was very likely to have become infected by any number of fission fungi during its removal from the sugar-works at Orlovetz to the laboratory of Charkow.

The discoveries made by Liesenberg and Zopf that this microbe, when grown in or upon nutrient media free from cane- or grape-sugar, does not

FIG 54 —Leuconostoc mesenterioides.

1–8 Various stages of development of the Zooglœa formation 9 Two chains of cocci, each exhibiting two enlarged (presumably sporogenic) members Magn about 500 (*After Van Tieghem*)

develop a mucinous envelope, but grows in chains, and consequently assumes the streptococcus form (Fig. 55), are entirely new. Such nutrient media are · *Solid*— potato slices, peptonised meat-broth gelatin, milk gelatin, maltose gelatin, on all of which it develops merely a thin pellicle without any formation of mucus , *Liquid* —milk and bouillon, in which merely a fine sediment of cells is deposited. If, however, a small portion of such a culture of non-mucinous cells be transferred to a medium containing saccharose or dextrose, the formation of mucus quickly ensues. Under these conditions there then develop (on slices of carrot, in particular) large zooglœa, at first dry, like cartilage, but afterwards becoming softer and resembling mesentery in appearance. Under the microscope it appears as shown in Fig 55. The cells, always globular, are invariably arranged in pairs, i e as diplococci The greatly swollen mucinous capsules of the individual cells gradually coalesce and form agglomerations of constantly increasing size, in which the diplococci are enclosed. Locomotion could not be detected in any case.

Not only was Cienkowski's work corrected by the researches of Liesenberg and Zopf, but the same fate also befell some of the statements made by Van Tieghem. The latter thought he had detected endospores in his *Leuconostoc*, and asserted that in unfavourable media some of the cells increased in size, and
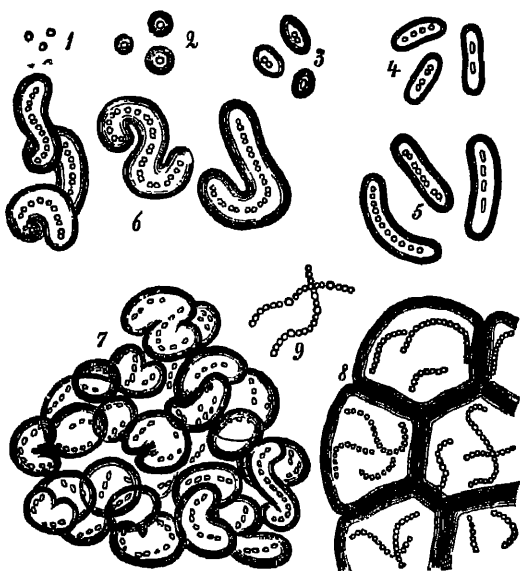
formed spores 1.8–2 0 μ in diameter, the walls of which coincided with those of the mother-cell. No. 9 of Fig. 54 shows two such cell-chains, each of which exhibits two enlarged members wherein spore-formation has just commenced. When transferred to a favourable medium, these spores were said to burst their solid membrane and then reproduce themselves by fission. Liesenberg and Zopf were, however, unable to discover such spores, and in any case their presence would be unimportant, since the organism already possesses in its mucinous envelope an excellent means of protection against adverse influences. Owing to this envelope it is able—so Liesenberg and Zopf found—to withstand three and a half years' desiccation in the air, and to resist the influence of *dry* heat at 100° C. for over five minutes ; whereas the naked modification, growing on sliced potatoes, succumbs after five minutes' exposure to a temperature of 75° C. Like other fungi, however, it is much less able to resist *moist* heat (steam, heating in liquids), the envelope being readily penetrable by moist warmth. On warming a culture of the gelatinous form in a nutrient solution up to 88° C. in forty-three minutes, and keeping it at that temperature for five minutes, all the cells were killed, whereas a temperature of 86°–87° C., under otherwise identical conditions, produced no injurious effect. The naked variety showed itself even somewhat more susceptible ; nevertheless *Leuconostoc mesenterioides* must be classified among the heat-resisting bacteria, by virtue of which property it is enabled to appear in the hot diffusion battery and juice conduits of the sugar-factory. The same faculty of resisting heat may
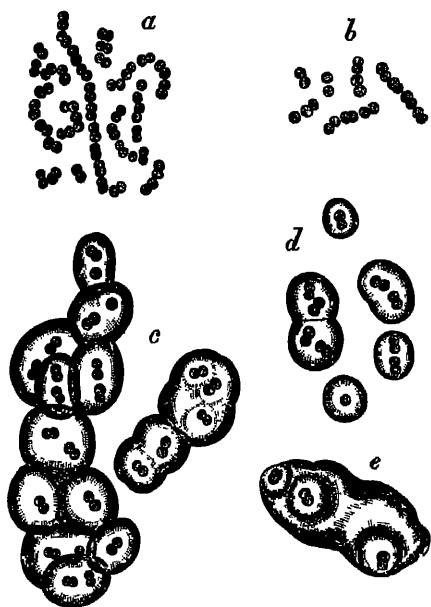
FIG 55—Leuconostoc mesenterioides.

a, b chains and bands of the non-capsuled variety, potato culture . *c, e,* cells with gelatinous capsule in various stages of development In *d* one pair of cells is viewed in the direction of the line joining their centres, and hence appears as a simple cell, not as a diplococcus The shading in *c–e* merely indicates the configuration of the agglomerations, and not a stratification of the capsule. Magn about 1200. (*After Liesenberg and Zopf*)

also be utilised in preparing a pure culture of the microbe : the sample (a gelatinous lump in a solution of sugar) destined for this purpose being kept for a quarter of an hour at 75° C., by which treatment most of the extraneous germs (adherent to the mucus) are killed, leaving the *Leuconostoc* unhurt.

§ 160.—Physiology of Leuconostoc.

The mucinous envelope is soluble in zinc iodochloride, concentrated sulphuric acid, strong caustic potash or soda, or in baryta water, but potassium iodide or iodosulphuric acid produce no noticeable alteration This fact by itself proves that we have not in this case (as was supposed by Van Tieghem) to deal with cellulose A beautiful double staining can be produced by first treating the cover-glass preparation with dahlia-violet, which stains the cocci alone, and then immersing it in an aqueous solution of rosolic acid, which is absorbed by the

mucinous envelope, the latter then surrounding the blued cells with a rose-red halo Preparations of this kind sometimes exhibit a scaly stratification of the envelope, the explanation of which is clear · the outer layer of the cell membrane swells up, detaches itself, and now encloses the cell on all sides, so that when this process has been repeated several times the envelope continually increases in size, whilst the dimensions of the bacterial cell itself remain unaltered. By softly pressing the cover-glass of such a zooglœa preparation the cells can be forced out of their mucinous envelopes

As already remarked, the substance of which this envelope consists was stated by Scheibler to be dextran, an opinion also shared (as the result of analytical experiments) by P. DAEUMICHEN (I.) If, however, we consider the means by which these chemists arrived at their discoveries, doubts will arise as to the accuracy of their conclusions. In all attempts, made by macrochemical means, to determine the composition of the vegetable cell membrane, the same difficulty is encountered, viz , the solution and removal of the cell contents. In order to attain their object, the reagents employed for this purpose must penetrate through the cell wall, and since they come into contact with it whilst still in all their pristine strength, they decompose it more or less effectually. When the lixiviation of the cell contents is completed, then the product remaining behind for ultimate analysis cannot be considered, in point of composition, as unaltered cell-membrane substance, though its form may be still unchanged. This applies to the case now under consideration. In order to obtain the substance of the mucinous membrane in a pure condition Scheibler boiled the gelatinous mass (freed from adherent sugar) with milk of lime, and found that only a small portion was dissolved. This fact of itself bears evidence against the (chemical) uniformity of the substance of the mucinous envelope of *Leuconostoc*. It is also probable that even the **dextran** recovered from the lime extract is a decomposition product of a more readily hydrolised constituent of the said membrane.

The behaviour of this fission fungus towards **sugars** merits special consideration. It has already been stated that the formation of mucus occurs only in such nutrient media as contain grape- or cane-sugar, the other carbohydrates, tested on this point by Liesenberg and Zopf, being found unsuitable. *Leuconostoc mesenterioides* produces **invertin**, which then splits up the cane-sugar ; so it may be surmised that the development of the gelatinous membrane can only occur in presence of grape-sugar (and perhaps also fructose !). This does not, however, imply that lactose, maltose, and dextrin are unaffected by this fission fungus ; on the contrary, it ferments them and forms lactic acid, a faint evolution of gas being at the same time noticeable. The presence of a small quantity (3-5 per cent.) of calcium chloride in the nutrient medium favours the production of mucus and the fermentative activity of the organism, the latter being brisker when oxygen is excluded. The optimum temperature for the development of this microbe being between 30° and 37° C., it is evident that in order to kill this pest the juice in sugar-factories must be kept at higher temperatures. *Leuconostoc* consumes a certain portion of the cane-sugar, and as it also produces invertin, which forms invert sugar—the presence of which is well known to seriously retard the crystallisation of the cane-sugar—another source of loss to the sugar-manufacturer arises. Molasses naturally forms a highly suitable nutrient medium for this microbe The speed at which it increases therein is reported (from practical experience) as follows by E DURIN (I.) . A wooden vat, previously used as a recipient for beet-juice, and the walls of which were covered with a thin film of mucus (i e. zooglœa of *Leuconostoc*) was charged with 50 h.l. (1100 galls) of neutral molasses. At the end of twelve hours the whole of this had become converted into a mucinous coherent mass. This microbe also gives rise to mischief in the refineries as well as in the raw-sugar works. F. STROHMER (I)

mentions such a gelatinous molasses derived from a colonial sugar-refinery
and which yielded a pasty sediment consisting of the zoogloea of *Leuconostoc*
mesenterioides on dilution with water.   It should be remembered, when deter
mining the sugar-content of a molasses by polarisation, that the mucinous
envelope of *Leuconostoc* is optically active and deflects the beam of polarised
light three times as much as an equal weight of saccharose

It may be mentioned as a curiosity that E Durin—who regarded the chie
component of the mucinous masses of *Leuconostoc* as cellulose—took out a paten
in France (Feb 14, 1876) for the "conversion of crystallisable sugar (= cane
sugar) into cellulose, and any uses (preparation of starch-sugar, dextrose, gun
cotton, oxalic acid, &c) to which this cellulose may be applied" (*Breveté san
Garantie du Gouvernement !*).

A fission fungus ranking along with *Leuconostoc* in so far as its importanc
to the sugar industry is concerned was examined by A Koch and H. Hosaeus (I.

In a certain sugar-works the syrup de
stined for working up into second pro
duct was found to contain gelatinou
masses resembling the zoogloea c
Leuconostoc, but consisting of anothe
species of bacterium, shown in Fig 5(
The special peculiarity of this microl
is that the swelling of the membrar
is unusually great and extensive c
one longitudinal side only, so that
long peduncular mucinous thread
gradually formed in this directio
On this account this fission fung
was named *Bacterium   pediculatun*
Unfortunately, it could not be o

FIG 56 —Bacterium pediculatum.

The mucinous envelope developed on one side only,
in the form of a peduncle   Magn 370. (*After
A. Koch and H Hosaeus*)

tained as a pure culture   In respect of this peculiar unilateral gelatinisatic
of the cell membrane it is not unique; the *Bacterium vermiforme* (the chi
constituent of ginger-beer yeast, shown in Fig 53), and also a fission fung
(*Nevskia ramosa*) discovered by A. Famintzin (I.) in aquarium water, havi
similar characteristics   Moreover, in the algae, forming the neighbouring gro
to the bacteria, and especially in the diatoms, many genera, e g. *Gomphonen*
exhibit well-developed and branched gelatinous stalks

§ 161.—Mucinous Fermentation and Inversion.

The faculty of rendering sugar-juice mucinous is not restricted to the t
microbes just described, a number of other species being now known to be capa
of working similar injury.   They, however, differ from the former in one cl
racteristic, which, though unimportant for the practical man, is neverthel
not without interest from a physiological point of view.   The gelatinisation
the nutrient media infested by the microbes described above must be char
terised as direct, since it is produced by the swollen cell-membranes of
organisms themselves   Conversely the gelatin-forming property of the spec
now to be described is an indirect one, it being here a question of the convers
of sugar (*outside* the cell) into the mucinous matter which A. Béchamp (I
proposed to call **Viscose.**   In fact, we have to do with the actual productior
mucus, whereas the former case was one of zoogloea formation.

E. Kramer (II.) in 1889 described a *Bacillus viscosus sacchari* which belo
to this second group.   The cells are rod-shaped, 1 μ thick and 2.5-4 μ lc
united into many-jointed chains; neither locomotion nor endospore formation cc

be detected.   The organism thrives only on neutral or faintly alkaline nutrient media, and in these it produces, in presence of cane-sugar, a mucus having the elementary formula $C_6H_{10}O_5$.   No swelling or gelatinisation of the cell membranes occurs   The optimum temperature for the reaction is 22° C., but beet-sugar juice will become changed to a viscid mass in one or two days at the ordinary temperature.

FRITZ GLASER (I.) described—as *Bacterium gelatinosum betæ*—a fission-fungus discovered by him in mucinous beet-juice.   Already by its active motility this species differs from the others we have described , and the same applies to several other characteristics.   It does *not* develop in neutral 10 per cent molasses, unless the medium has been previously qualified with a little of the precipitate thrown down by alcohol from beet-juice—*i.e.* phosphates, &c , of alkaline earths extracted from the molasses during the separation and saturation of the sugar-juice.   The chief products of the decomposition (preceded by inversion) of cane-sugar by this organism are mucus and amyl alcohol   The former is identical in properties with dextran, and is soluble in warm dilute acids and alkalies, but insoluble in baryta water or milk of lime.   An acid odour is evolved during this fermentation, but no lactic acid is formed.

The number of species of bacteria capable of interfering with the normal course of sugar manufacture is by no means exhausted with the examples mentioned above, but owing to the paucity of observations on this point no further reliable particulars can as yet be given   Consequently the subject presents an admirable field for bacteriological research in order to elucidate the causes and prescribe remedial measures for mucinous fermentation.   It is well known to sugar-makers that the percentage of **invert sugar** in molasses *increases* during storage (sometimes for months) in the so-called reserves, and they are also aware of the decomposition occurring in stored **raw sugar** and resulting in the formation of **invert sugar**.   Now the faculty of excreting an **inverting ferment** is not very widespread among bacteria   For a comprehensive investigation on this point we are indebted to C. FERMI and G. MONTESANO (I.), who examined about sixty (some of them pathogenic) species of bacteria, but found only four, viz., *Bacillus megatherium*, *Bacillus fluorescens liquefaciens*, the red *Kiel bacillus*, and *Proteus vulgaris*, capable of producing **invertin** in saccharified bouillon.   Experiments which have been made by A. HERZFELD and U PAETOW (I), on the prevention (by hydrofluoric acid and alkali fluorides) of inversion in molasses, lead to the hope that these antiseptics may prove useful in many cases.   Further researches on this subject are highly desirable.— The **nitric fermentation of molasses** will be briefly mentioned in chapter xxx

Sugar-juice and raw sugar are occasionally infested with higher fungi as well as with bacteria.   For instance, A HERZFELD (I.) and A. B. FRANK (I) report the occurrence of a red pigmentary fungus in raw sugar.   They found (in an after-product) red lumps, about as large as hazel-nuts, which, under the microscope, proved to be abundantly infested with a thread fungus, the protoplasm of which was stained by a red pigment, presumably generated by the bacteria present in large numbers in the mass   The development of pigment bacteria is also frequently noticeable in the saturation scum thrown out from the sugar-works and spread over the fields, this scum being often found covered with coloured (mostly red) patches, which are presumably zoogloea of *Micrococcus prodigiosus*.

Large though the number of injurious fission fungi in sugar may be, it is surpassed by the multitude of *Eumycetes* infesting the sugar-beet.   These, however, do not fall within our province, and readers who may be interested in them are referred to the various text-books on plant diseases.   The works compiled by A. B. FRANK (II) and P. SORAUER (II.) respectively, presuppose

a certain degree of (macroscopic) acquaintance with the individual maladie which they treat.   On the other hand, the young sugar-technicist, who will a rule, be mainly desirous of determining the nature of the disease brou under his notice, is advised to study O KIRCHNER's (I) "Handbuch Pflanzenkrankheiten" ("Handbook of Plant Diseases").   This work is admira supplemented by a good and cheap atlas (prepared by O. KIRCHNER and BOLTSHAUSEN (I)) of coloured plates showing the chief diseases attack industrial plants   With the information thus gained, the learner will then able to resort with advantage to the two first-named standard works.   A b review of the most important diseases set up in the sugar-beet by vegetable animal parasites has been written by A STIFT (I. and II), and particu attention is devoted to *Heterodera Schachtii* (the cause of the so-called nemat sickness) in a monograph by A. STRUBELL (I.), as also in a useful work J. VANHA and J. STOKLASA (I).   Investigations on the influence of these wor on the cellular activity of the beet, and on the resulting chemical chan thereby induced, were made by J STOKLASA (I), and may now be mention At present we will merely refer briefly to the **gummosis** (Fr. *gommose*) of sugar-beet, a complaint first described by SORAUER (II)   The symptoms of t disease are   extravasation of small drops of a gummy fluid from the unbrol surface, and a gradual blackening of the vascular bundles and parenchyma the beet, from the tip of the root upwards   It is still uncertain whether bacteria so abundant in this gum should be regarded as the actual cause of disease or merely as harmless saprophytes

ROPINESS IN MILK, WINE, BEER, AND OTHER LIQUIDS.

§ 162.—Ropy or Viscous Milk.

THE first attempt at a scientific study of this malady was made in 1847 b;
GIRARDIN (I.), who hoped to elucidate it by chemical analysis, and sought th:
cause in the defective composition of the fodder. This complaint may develoj
to a variable extent in milk. In the worst cases the thickened liquid can b:
drawn out to a thin thread a yard or so in length. J. LISTER (I.), in 1873, wa:
the first to reproduce this complaint by inoculation, and thus indicated th:
probability of a living source of infection. To ascertain this by microscopi:
examination was the task essayed by SCHMIDT-MÜHLHEIM (I.) in 1882, wh:
found that ropy sour milk contained an unusually large number of cocci 1 μ 11
diameter, frequently united as chains, but also in many cases isolated, and 11
the latter case apparently endowed with motile powers. Although at that tim:
suitable methods of pure culture were no longer lacking, this observer made n:
attempt to utilise them in his researches. This omission was, however, soor
remedied by E DUCLAUX (IX.), who prepared pure cultures of two species o!
bacteria from ropy milk, both of which belong, morphologically, to the so-calle:
capsule bacilli. The powerful lustre of the greatly swollen mucinous envelope:
surrounding these cells is the first thing to strike the assisted eye, on whic!
account the generic name, *Actinobacter* (lustrous bacterium, star bacterium)
was applied to both organisms Under their influence the milk yields alcohol
and acetic acid

To these two pests (known respectively as *Actinobacter du lait visqueux* and
A polymorphus) a large number of others possessing similar powers have been
added by different observers; *e g* a micrococcus discovered by HUEPPE (IV.) in
1884, the *Bacillus mesentericus vulgatus*, investigated by FLÜGGE (I), and the
Bacillus pituitosi, a thick, slightly curved rod, discovered by LOEFFLER (III.).
Other allied species are a streptococcus, described by HESS and BORGEAUD (I.),
and presumably identical with that observed by NOCARD and MOLLEREAU (I.) ,
and a bacillus, 1 2 μ broad and 2 μ long, obtained by Schütz from ropy milk,
and described by ST VON RATZ (I.). In 1890, L. ADAMETZ (IV.) found in the
Liesing brook (which runs into the Danube in the south of Vienna) a capsule
bacillus, 0.7–1 2 μ long and 0.7 μ broad, which he named *Bacillus lactis viscosus*,
and which is capable of turning both milk and cream ropy. It is fairly wide-
spread in nature, and was also detected by ADAMETZ (V.) in samples of milk
from the Sornthal (Switzerland) In addition to this, three other fission fungi
(named below) are found in Swiss soil, one of them being the *Bacillus Guille-
beau c.*, which is not only dangerous to the cows (giving rise to inflammation of
the udder), but also produces various disturbances in the dairy by making the
milk "ropy" and the ripening cheese "blown" The facultatively anaerobic
Micrococcus Freudenreichii, 2 μ in diameter, discovered by A. GUILLEBEAU (I.),
is still more injurious to milk, since, whereas the other organisms just mentioned
act only at high temperatures—approaching blood-heat, and therefore easily
avoidable in practice—this coccus is active even at a moderate temperature, and

turns milk ropy within five hours at 22° C. The optimum temperature of development is two degrees lower, and the microbe is destroyed by an exposure of two minutes to boiling heat. It has frequently been found in the district of Berne, and often causes considerable damage.

Simultaneously with this last-named organism, a third microbe, also endowed with the faculty of turning milk ropy, was introduced by Guillebeau under the name of *Bacterium Hessii*. This species, which appears in the form of actively motile rods, 3–5 μ long and 1.2 μ broad, is less injurious than the one just described, since the ropiness it produces in milk disappears directly acidification sets in

The substantive cause of the mucinous condition may be of three kinds. Either it is attributable to the swelling of the membrane of the bacteria in question—as is apparently the case in those already alluded to as capsule bacilli, e g. *Actinobacter*, *B lactis viscosus*, and also, according to the researches of W. VIGNAL (I.), with *B mesentericus vulgatus*—or, secondly, the milk-sugar is converted into a mucinous substance. This was asserted to be the case by Storch for two species of bacteria discovered by him, and was proved by G. LEICHMANN (III.) for a bacillus isolated from ropy milk. This latter organism acts on lactose, cane-sugar, maltose, galactose, levulose, and dextrose (but not on mannite, arabin, or starch) in such a manner that mucus and lactic acid are formed, together with a small quantity of ethyl alcohol In the third place, the ropy substance can also be produced from the casein of the milk. According to H WEIGMANN, this latter cause operates in the formation of the milk products known as

§ 163.—Ropy Whey (Lange Wei) and Thick Milk (Tættemælk).

The Swiss dairymen discard ropy milk for cheese-making, being afraid of its causing "nests," i.e places within the cheese where the ripening proceeds irregularly. They therefore devote particular attention to fumigating the stalls out with burning sulphur, scouring the milk vessels with soda solution, &c , in order to eradicate the evil as quickly as possible

On the other hand, the Dutch look on the bright side of this evil, and even derive benefit from it, the most palatable production of the Netherlands, viz., **Edam cheese**, being prepared with the aid of ropy whey (Dutch, *Wei*) The first observations on and experiments with this ropy whey were made in the "fifties" by a farmer (name unknown) of Assendelft, in Holland, but it did not come into general use in the manufacture of Edam cheese until 1887, when Boekel recommended it most emphatically.

WEIGMANN (VII.) examined such whey, and found in it large quantities of a fission fungus, which is mostly arranged in pairs, but frequently also in chains, and bears the name of *Streptococcus hollandicus*. Sterile milk inoculated with this organism becomes ropy and sour in twelve to fifteen hours at 25° C
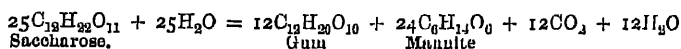
The same coccus was also found by Weigmann in the commercial products known as *Tættemælk* or *Tœtmælk* (thick milk) in Norway, and *Filmjölk* (stringy milk) in Finland and Sweden. This strongly sour, ropy, thick mass, the casein of which is in the condition of fine flakes, is a highly prized article of nourishment among the Scandinavian races, and is artificially prepared from normal milk by either rubbing the interior of the milk-pails over with butter-wort (*Pinguicula vulgaris*), called in Norway *Tættegræs*, or by feeding this plant to the milch-cows. The leaves are found to be infested with a fission fungus which turns milk ropy, and is presumably identical with the above-named streptococcus. As already remarked, the occurrence of ropiness in milk is usually accompanied by acidification, whereby the development of numerous other

species of bacteria is prevented This accounts for the circumstance that Tæt-mælk will keep for months without alteration if stored at a low temperature.

Herz was the first to record observations with regard to so-called **soapy milk**, a term applied by him to milk that exhibits a taste of soap and lye, and does not curdle, but only deposits a slimy sediment, even after prolonged standing. The cream from this milk froths up very strongly when churned. H. WEIGMANN and G. ZIRN (II) had occasion, in 1893, to examine a milk of this kind, and they succeeded in isolating therefrom a bacillus which is capable of converting normal milk into the soapy condition, and is therefore termed *Bacillus lactis saponacei*. It was afterwards discovered that the organism originated in the litter, which was in a damaged condition. When that was withdrawn and the cows littered on sound straw, the milk no longer suffered from this complaint.

§ 164.—Ropiness in Wine

was formerly attributed to a coagulation of the albuminoids, a hypothesis corrected in 1856 by G. MULDER (II.), who traced the chief source of this malady to the conversion of sugar into vegetable mucilage. Young white wines, in particular, fall victims to the disease, which in its incipient stage produces a faint opalescence, followed by gradually increasing turbidity, until, finally, the liquid becomes thick, and by degrees so viscid that it can be drawn out into threads a yard or so in length, and can scarcely be poured out of the bottle. The flavour is disagreeably slimy and insipid, though the odour (*bouquet*) is almost un-altered In France the malady is termed "*Maladie de la graisse*," or generally "*Vin filant*" or "*Vin huileux*," and the Italians style it "*Vino filante*." The earliest microscopical studies on this point were made in 1861 by PASTEUR (XII.), who found a very large number of fission fungi always present in ropy wine, and also that by transferring a little of the liquid to sound wine of the same class the disease was quickly communicated to the latter. He described two kinds of cell form small cocci united in chains (streptococci), and irregularly shaped cells somewhat larger in diameter than those of yeast. The chief products of the mucinous fermentation set up in wine by this mixture of organisms were found to be gum, mannite, and carbon dioxide. Their ratio was represented by Pasteur in the form of an equation as follows ·

$$25C_{12}H_{22}O_{11} + 25H_2O = 12C_{12}H_{20}O_{10} + 24C_6H_{14}O_6 + 12CO_2 + 12H_2O$$
Saccharose.   Gum   Mannite

These proportions were admittedly variable, but this was explained by Pasteur by the supposition that the one species of ferment produces more mannite, the other more gum , and Monoyer, in 1862, attempted to represent these reactions by splitting up the equation into two. Some observations on the aforesaid streptococcus have also been published by E. DUCLAUX (X.).

The thoroughgoing microscopical investigations performed by J. NESSLER (II.) showed that the streptococci described by Pasteur are frequently absent, or only present in very small numbers, in ropy wine, whereas, on the other hand, the presence of certain unusually plentiful, extremely minute round bodies can always be detected. Subsequently a few samples of ropy wines were examined by E. KRAMER (II.), mainly with the object of obtaining pure cultures of the organisms causing the malady, but this object has not yet been successfully accomplished. By means of the dilution method approximately pure cultures of such a fission fungus have been prepared , and the name of *Bacillus viscosus vini* has been given to the organism It occurs in the form of rods, 0.6–0 8 µ broad and 2–6 µ long, frequently united as many-jointed chains, and capable of producing ropiness in white wines in the absence of air. A thorough mycological

study of this malady has, however, still to be made. Neither the *Bacillus viscosus sacchari*, mentioned in chapter xxviii, nor other similar cause of mucinous fermentation, is capable of giving rise to ropiness in wine, since none of them is able to develop in acid media.

One point is perfectly clear, viz., that the presence of sugar is a *sine quâ non* for the occurrence of the malady, since it forms the material from which the mucus is produced. According to Nessler (an expert in the treatment of wines), wines containing over 10 per cent. of alcohol are proof against ropiness.

With regard to the **ropiness of cider**—the most frequent malady to which this beverage is subject—nothing reliable can at present be reported.

§ 165.—Ropiness in Infusions.

This was microscopically investigated as far back as 1834 by FR KÜTZING (I.), who ascertained that the lower orders of plants here in question are partly algæ and partly fungi, the schizomycetes being the most frequently found members of the latter group. A few examples are given below.

It is well known that *Infusum foliorum Digitalis* very often becomes ropy, to account for the occurrence of which divers hypotheses were formerly current. Thus, for instance, it was asserted that the mucic acid in the leaves of digitalis exerts a coagulating influence on the pectin bodies also present therein. W. BRÄUTIGAM (I) found in a ropy infusion of this kind a fission fungus, which he named *Micrococcus gelatinogenus*, endowed with the property of gelatinising vegetable infusions (*e.g. Ipecacuanhæ, Radix Althææ, Senegæ, Folia Farfaræ*, and especially *Folia Digitalis*), when mixed with sugar-cane, lactic acid being produced at the same time. The mucus is precipitable by alcohol In nutrient media devoid of sugar the micrococcus develops, but does not form mucus.

Of interest to the analytical chemist is the *Bacterium gummosum*, also obtained by E. RITSERT (II.) from a ropy infusion of *Digitalis*. This organism turns the nutrient medium ropy only when saccharose (but not dextrose or lactose) is present, and can therefore be employed as a **reagent for cane-sugar** to detect the latter in presence of large quantities of hexoses, *e.g.* in wine-must. It will develop in highly concentrated solutions of this sugar, its growth not being impeded until the concentration exceeds 60 per cent. The mucus produced by this fission fungus has received the name of **gummose**, a term likely to lead to error, since a somewhat widespread malady attacking the vine, the sugar-beet, and other plants, has long borne the name of gummosis or gummose This mucus is distinguishable from dextran chiefly by being optically inactive. In addition to mucus the organism produces an uninvestigated acid, and a compound of unknown constitution, which deviates the plane of polarised light to the right and reduces Fehling's solution. According to the conditions of cultivation, *Bacterium gummosum* appears as long or short rods, diplococcus or streptococcus, the first forms being motile and producing endospores. The addition of acetate of potash or soda or of yeast ash to the nutrient solution (*e.g.* sugar-beet juice, &c.) is highly favourable to development and to the production of mucus.

The *Bacillus gummosus*, isolated by C. HAPP (I) from a ropy infusion of *Digitalis*, is characterised by its large size, the length being 5.0–7.5 μ, and the breadth 0.6–2.0 μ. It exhibits an undulatory motion and forms endospores. In cultures on slices of potato and beet the cells are globular, with a diameter of 0.7–0.8 μ, but when transferred to gelatin or agar-agar they quickly become rod-shaped. Happ obtained from ropy Senega infusion a pure culture of *Micrococcus gummosus*, the diameter of which is about 0 4 μ. A notable difference exists between these two species with respect to their behaviour towards sugars, the first-named being able to set up ropy fermentation only in presence

of saccharose, whilst the *Micrococcus* also attacks maltose. The resulting mucus (soluble in water, but insoluble in alcohol and ether), which has the elementary formula $C_6H_{10}O_5$, is, although the chief, not the sole product of this fermentation, small amounts of mannite, butyric acid, lactic acid, and carbon dioxide being also formed; and a part of the saccharose is converted into glucose.

The so-called **distilled waters** (*e.g.* **orange-flower water**) often undergo mucinous decomposition, some particulars of which have been reported by L. VIRON (I.). As a remedy for this evil, P. Carles advised the shaking up of the affected water with 2–3 grams of basic nitrate of bismuth per litre, and filtering after standing. This is said to have answered particularly well with orange-flower water. Ordinary distilled water is often rendered mucinous by bacteria, especially when kept in wooden vessels; .A. GOLDBERG (I.) has reported an instance of this kind.

A fission fungus, *Bacterium gliscrogenum*, 0.57–1 1 μ long and 0.4 μ broad, has been isolated by P. MALERBA and G. SANNA-SALARIS (I.) from mucinous, viscid urine (which often exhibits this property as soon as voided), and has been recognised as the cause of this condition. According to a research of MALERBA's (I.), the mucus (gliscrin) thereby formed is nitrogenous

It is well known that ink frequently becomes mucinous and viscid. M. HÉRY (I.) investigated this matter and examined a bacterial species concerned therein. As a preventive measure he recommends an addition of not less than 0.5 gram of salicylic acid per litre of ink.

C. BOERSCH (I.) made an observation, interesting to the chemist, concerning a fission fungus, *Sarcina flava*, capable of producing ropiness in various liquids. This organism attacks fumaric acid (in acid solutions), but, on the other hand, leaves the isomeric maleic acid, $COOH—CH=CH—COOH$, untouched.

Ropiness in tan liquors is a phenomenon both well known and unwelcome to the tanner, to whom it causes considerable damage and loss, since not only is the liquor rendered worthless, but the hides steeped in it also suffer owing to the masses of mucus adhering so firmly to the leather that great difficulty is experienced in getting them off again. This mucinous coating retards, or even entirely prevents, the penetration of the tannin. Closer investigations regarding the best means and methods of prevention would be valuable to this industry.

§ 166.—Ropiness in Wort and Beer.

PASTEUR (III.) was the first to study this phenomenon with the aid of the microscope He traced the cause of this complaint, which has many points in common with ropiness in wine, to a fission fungus occurring abundantly in the form of long chains in the affected liquids, and known by the name of *Micrococcus viscosus* Morphologically, this organism greatly resembles a fission fungus observed by J. BERSCH (II.) in a beer wort, which, instead of fermenting normally when pitched with yeast, became thick, oily, and finally viscid and ropy.

P. LINDNER (III.) in 1889 was the first to obtain a pure culture of a viscous ferment. This was a pediococcus (not specifically named) occurring in large numbers in ropy white beers, a class of beverage that is particularly liable to the malady. The capacities of the microbe in question are restricted to the production of ropiness in white beer wort, it being unable to do so in hopped worts and beers. Hence it is perfectly innocuous and unimportant, so far as true brewing, in the narrow sense of the term, is concerned.

Other species appear in hopped beer. Two of these were found by H. VAN LAER (II.) in a number of samples of ropy beer, from which they were isolated to pure cultures, and named *Bacillus visoosus I.* and *II.* Both have several identical characteristics, *e g* the form and dimensions of the cells, which are rod-

shaped, 0.8 μ broad, 1.6–2.4 μ long, and mostly single, though not infrequently joined in pairs

In their behaviour towards beer-wort, however, they differ in a notable manner. It is true that both of them produce ropiness, but not of the same type. If *B. v I.* is in action, then, in proportion as the viscidity of the liquid increases, a number of mucinous, yellowish-white patches, terminating below in branches, appear on the surface   In this way a coating of mucus is formed, the surface of which is gradually covered with protuberances produced by bubbles of the carbon dioxide liberated during this fermentation   With *B. v. II.*, on the contrary, this coating is absent, moreover, the evolution of carbon dioxide is less copious, and the ultimate degree of ropiness less pronounced than in the first case. Whilst the malady is in progress, the colour of the wort changes to a chicory-brown, and at the same time an odour develops, which cannot be more closely defined, but which of itself suffices to reveal the presence of the complaint   A further characteristic affording a means of distinguishing between these two species of bacteria is their behaviour towards a sterilised solution of 3 grams of cane-sugar and 1 gram of peptone in 100 c c. of water. This medium is made viscid and ropy by *B. v I.* alone, the second species producing nothing more than a persistent turbidity, accompanied by the evolution of carbon dioxide. Milk is altered by both species in the same manner as wort.

The fact that both these organisms also cause ropiness in nutrient solutions, devoid of sugar and containing no organic matter beyond calcium lactate or ammonium tartrate, is also interesting   As a matter of fact, a high content of sugar is even injurious to the organisms   This discovery agrees with the experience gained in practice, that beers with a low attenuation (and therefore a higher sugar-content) are comparatively seldom ropy. The proximate cause of this alteration of the medium is a mucus excreted by the bacteria.   In the presence of sugar, carbon dioxide is liberated, and presumably a small quantity of another acid is also formed, since the acidity increases with the ropiness. The mucus is not a uniform substance, but consists of at least two constituents, one of which (insoluble in water) is characterised by its content of nitrogen. This fact harmonises with the circumstance that the malady sets in earlier in proportion as the nitrogen-content of the nutrient medium is greater.   It also explains the fact, noticed in practice, that worts rich in protein, peptones, and the like, are those most readily becoming ropy   A higher content of acid (0 15 per cent. reckoned as lactic acid) restricts the development of both these species of fission fungus; but alcohol, even in the proportion of 6 per cent by volume, is powerless to injure them   In both cases growth proceeds at all temperatures between 7° and 42° C., and is most vigorous at about 33° C.

A third viscous ferment, also discovered by Van Laer, differs from the other two in its property of liquefying gelatinised meat-juice.

L VANDAM (I) obtained from ropy English beer pure cultures of a fourth organism (*Bacillus viscosus III.*) in the form of rods 0.7 μ broad and 1.3–2 0 μ long, mostly isolated, but frequently also forming bands of two or three cells So far as can be gathered from the particulars given, ropiness is produced, not by any metabolic product excreted by the bacillus, but by the thickened cell membrane of the organism.   In other ways, too, this microbe differs from Van Laer's bacilli.   For instance, the development of the organism and the gelatinisation of the medium occur only in presence of sugar, and the degree of ropiness is proportional to the amount of sugar eliminated.   No evolution of gas could be detected in wort cultures.   The unrestricted access of air is essentially necessary to the growth and activity of this bacillus. The organism is incapable of injuring beer except when present in large numbers in the wort before the commencement of primary fermentation.

The number of organisms capable of rendering wort viscid is not exhausted by the *Schizomycetes* already mentioned   In the second volume we shall become acquainted with *Dematium pullulans*, a species of *Eumycetes* which is equally capable of producing damage of this kind.

§ 167.—The So-called Sarcina Turbidity in Beer

will now be referred to, although no mucinous ferments are here in question. Bottom-fermentation beer is required to be perfectly clear, and if it proves defective in this particular it is considered poor or bad, according to the nature of the turbidity. This may arise from several distinct causes · precipitated albuminoids = **gluten turbidity**, the presence of unsaccharified starch = **starch turbidity**; precipitated hop resins = **hop dimness**, a high content of yeast cells = **yeast turbidity**; or, finally, strong infection with fission fungi = **bacterial turbidity.** This latter, again, may be caused by different species of organisms, a few of which (*i e* those producing turned and ropy beer) have already been mentioned, the turbidity in their case being merely a secondary phenomenon attendant on another complaint.   In the following lines, however, we will confine ourselves to the turbidity caused by bacteria of the sarcina or pediococcus form of growth.   Very frequently these organisms (in enormous numbers) are the only ones observable in samples of turbid beer

The first observations on the subject were made by PASTEUR and J. BERSCH (II), and more minute researches were made by Julius Balcke, from whom these organisms first received the name of *Sarcina.* Francke afterwards found that this fission fungus always subdivides in two directions only (and not three), and consequently forms sheet colonies   On this account FRANCKE (I.) in 1884 applied the new generic name of *Pediococcus cerevisiæ* to this microbe.   Notwithstanding this, it is still customary to term the malady under consideration "sarcina turbidity"; which is, moreover, partly correct, since true sarcina in great numbers have also been found in turbid beers   The first successful attempt to obtain a pure culture of such a pediococcus was made by P LINDNER (II) in 1888.   The *Pediococcus cerevisiæ* isolated by him from "sarcina turbid" beer occurs as single cocci (0.9–1.5 μ diameter), diplococci, and tetrads   Still, though it is undoubtedly the fact that this fission fungus occurs in large numbers in such turbid beers, it by no means follows that the organism can be positively assumed to be the cause of sarcina turbidity, attempts to grow it in sterilised beer having proved unsuccessful   Moreover, as ANTON PETERSEN (I), E CHR. HANSEN (V.), and ALFRED JÖRGENSEN (I.) have shown, a considerable quantity of sarcina may be present in beer without any damage to the beverage (turbidity or unpleasant flavour) resulting therefrom.

Further particulars given by them render it highly probable, however, that "sarcina turbidity" is actually caused by fission fungi of the pediococcus and sarcina groups, but that the mere presence of these organisms is not sufficient to produce the malady, a special concurrent tendency thereto on the part of the beer being essential.   For the determination of the conditions under which the "sarcina organisms" are capable of producing "sarcina sickness" in beer, we are indebted to an instructive treatise by A REICHARD (I.).   He showed that this turbidity occurs only when the secondary fermentation of the beer goes on with vigour, and that, conversely, a similar degree of sarcina infection is innocuous if the primary fermentation has been carried so far that only a weak secondary fermentation ensues   Reichard attributes this behaviour (confirmed by searching experiments) to the avidity for oxygen (air-hunger) displayed by the pediococci   It is only when the microbes are continually brought up to the surface of the liquid by the bubbles of carbon dioxide given off during a brisk

secondary fermentation that this avidity for oxygen can be satisfied and the development of the organism proceed.

When no gas is liberated, and the pediococci consequently remain at the bottom of the liquid, then no turbidity or unsatisfactory alteration of the flavour or smell will occur   If, however, an infected beer be artificially brought into a state of active secondary fermentation by priming (*aufkrausen*) with fermenting wort, then sarcina turbidity will not be long in making its appearance.   This fact, determined by Reichard, indicates the necessity for caution in the employment of fermenting wort for priming beer.   This practice, as is well known is specially resorted to for livening up sluggish lager beers in the storage cask, and is of itself unobjectionable.   Care should, however, be taken to previously ascertain that no large amount of sarcina is present in the cask.   According to the researches of REICHARD and RIEHL (I.) hops are very useful in combating sarcina sickness.   To prevent the appearance of the malady 30–40 grams of hops per hectolitre of beer (or at the rate of 5 to 6 oz. per 100 galls.) should be placed in the storage cask, and the latter then closed (bunged)

The injurious organisms in question either find their way into the wort in the cooler, or—as stated by Balcke—may be transferred to the malt store on the boots of a workman (floor-sweeper) who has previously been working on the malting floor, where these organisms abound   It is, therefore, no wonder that the thick wort is also rich in these organisms, and may consequently become the source of acute troubles.   The evil reputation of the thick wort and thick beer is also easy to understand from a bacteriological standpoint   When such an infected wort is fermented, then, of course, the yeast crop will be contaminated with these injurious organisms and the malady will thus be perpetuated.   To purify such contaminated yeasts, S von HUTH (I), in 1888, proposed an addition of 5–7 grams of salicylic acid per hectolitre of beer (about 1 oz per 100 galls)   A second recipe of his, which was also approved by P. LINDNER (IV.) in 1895, reads as follows : To each kilo. (2 2 lbs.) of pulpy or liquid yeast take 6 grams of tartaric acid dissolved in water.   After stirring them thoroughly together, leave to stand for six to twelve hours, and then add the mixture to the wort in the tun.   The results of this treatment are said to be satisfactory.

It must, however, be expressly mentioned that this tartaric acid cure should not be employed unless the yeast under treatment is either almost or entirely free from wild yeasts, and is contaminated by sarcina alone.   Otherwise it is best to throw the batch away, since the tartaric acid treatment, by favouring the development of the wild yeasts, would only make it worse than ever.   This will be referred to again in a subsequent section of vol. 11.

DECOMPOSITIONS AND TRANSFORMATIONS OF ORGANIC NITROGENOUS COMPOUNDS.

CHAPTER XXX.

THE PHENOMENA OF PUTREFACTION.

§ 168.—The Degradation of the Albuminoids.

In § 15 of the Introduction it was stated that Liebig's differentiation between fermentation and putrefaction is untenable, and that no sharply defined limit between these terms exists. Enlarging the definition of the term fermentation beyond its usual limits, we there defined this phenomenon as the transformation of various chemical substances by the action of minute fungoid organisms.

Without prejudice to this general definition, we can nevertheless speak of putrefaction in particular, limiting the application of this term to such fermentations as chiefly effect the decomposition of albuminoid substances. Any further attempt to analyse this more restricted term is at once frustrated by our ignorance of the constitution of the albuminoids themselves. The multiplicity of contingencies here possible cannot be disregarded, and consequently no classification according to the final products obtained is feasible. On the other hand, no differentiation can be based on the composition of the bodies subjected to decomposition, since we are here encountered by a question, hitherto unsolved by chemists, viz, What are the albuminoids?

Obviously mycologists might postpone further researches on this point until the necessary preliminaries have been performed by their chemical colleagues. As a matter of fact, however, the opposite course has been adopted, and the determination of the nature of the putrefaction products of albumen has not only led to hypotheses regarding the composition of that substance, but will probably also indicate the means whereby the nature and synthetic preparation of these high-molecular nitrogen compounds can be established. Provided the results obtained are of value to the chemist, and, though in a minor degree, to the mycologist as well, the credit thus accruing to Fermentation Physiology is not necessarily injured by the remark that, owing to the employment of indefinite bacterial mixtures, these endeavours are not always free from objection from a bacteriological point of view.

In future researches into albuminoid decomposition or putrefaction, it should always be borne in mind that here also the co-operation and succession of various organisms (i.e. symbiosis and metabiosis) will have to be taken into calculation Until this is done, mycological text-books will have nothing better to offer than a varied collection of isolated observations, such as are given in the following chapters and paragraphs.

It has long been observed that the natural decomposition of albumen yields malodorous gases and vapours when proceeding in the absence of air. but that, on the other hand, these attendant phenomena are wanting when air is allowed

frœ access. Titular distinctions have been employed to express these differences, the natural inodorous decomposition of albuminoids being termed **decay**, whilst the name of **putrefaction** has, in a narrowed sense, been applied to the other set of phenomena. Formerly regarded from the chemical standpoint alone, the fundamental physiological basis of this differentiation has now been explained by the aid of mycological research as follows: **Decay** is the result of **aërobic** microbial activity; **putrefaction**, of the energy of **anaërobic** organisms. Of course both these processes may go on simultaneously in the same substance, the outer surface, exposed to the air, decaying, whilst the interior putrefies This fact alone sufficiently proves how little value attaches to researches wherein pure cultures are not employed. M. von NENCKI (III.) sought to explain the putrefactive decomposition of the albuminoids as a process of hydration, and cited in support of this view the observation that the products obtained are the same as those produced by the action of fusing caustic potash.

The bad smell characteristic of putrefaction is often attributable to several compounds of the aromatic series. One of these is **Indole**, $C_6H_4 \begin{smallmatrix} OH \\ \diagdown \\ NH \end{smallmatrix} OH$, which combines as an imide with nitrous acid to form the red nitroso-indole. This property is utilised for the detection of indole in cultures. Since a great many bacteria are capable of producing a small (though sufficient) quantity of nitrites in ordinary nutrient media, this characteristic red coloration can be developed (in presence of indole) by slightly acidifying the culture with sulphuric acid. Of the pathogenic bacteria, Koch's *Vibrio choleræ asiaticæ* was the first examined for this reaction. This accounts for the current use of the term " cholera red reaction," employed for this reaction by medical bacteriologists. β-**methyl indole** or **skatole**, which was first discovered in 1877 by L. BRIEGER (II) in human fæces, is almost invariably produced during the putrefaction of albumen; its smell is even more repulsive than that of indole. A closely allied derivative of skatole, viz., β-**methyl indole acetic acid**, was discovered by M. von NENCKI (IV.) among the putrefaction products occasioned by *Bacillus liquefaciens magnus* in the absence of air. **Phenol** was first recognised as a product of albumen putrefaction by E BAUMANN (I.) in 1877, and **orthocresol and paracresol** by E. BAUMANN and L. BRIEGER (I) in 1879 The capacity of a large number of (mostly pathogenic) species of bacteria for producing the above-named substances was investigated by A. LEWANDOWSKI (I)

M. von Nencki and his pupils made a series of investigations on the products of albuminoid putrefaction. Of their discoveries we will now briefly mention those referring to **leucine** and **tyrosine** These amido substances are secreted by the pancreatic glands, and are almost always present in fresh fæces. They are also produced, under certain conditions, in the putrefaction of various albuminoids. Now, according to NENCKI's (V.) researches, leucine is further decomposed by the activity of bacteria, the chief product being valeric acid, along with carbon dioxide, hydrogen, and ammonia. The reaction is approximately expressed by the equation—

$$CH_3—CH_2—CH_2—CH_2—CH\ NH_2—COOH + 2H_2O =$$
<div align="center">Leucine</div>

$$(CH_3)_2 = CH—CH_2—COOH + CO_2 + 2H_2 + NH_3.$$
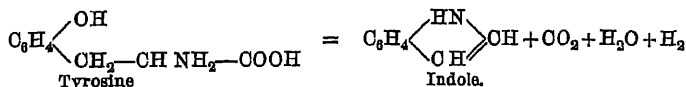<div align="center">Valeric acid</div>
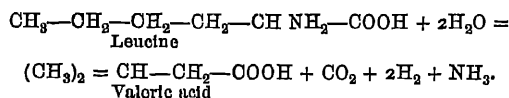
The decomposition of tyrosine may be effected in two different ways in presence of air—as was shown by E. BAUMANN (II.)—the NH_2 group is separated,

and hydroparacumaric acid, of which tyrosine may be regarded as the amine (alanine), is formed—

$$C_6H_4\left\langle\begin{array}{l}OH\\CH_2—CH.NH_2—COOH\end{array}\right. \quad \text{yields} \quad C_6H_4\left\langle\begin{array}{l}OH\\CH_2—CH_2—COOH.\end{array}\right.$$

Tyrosine.   Hydroparacumaric acid.
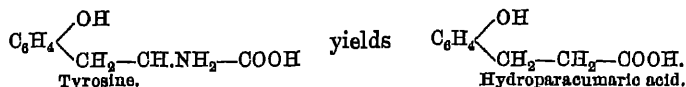
When air is excluded, the results are, however, very different, indole, together with carbon dioxide and hydrogen, being produced. This reaction is approximately represented by the equation—

$$C_6H_4\left\langle\begin{array}{l}OH\\CH_2—CH\ NH_2—COOH\end{array}\right. = C_6H_4\left\langle\begin{array}{l}HN\\C\ H\end{array}\right\rangle CH + CO_2 + H_2O + H_2$$

Tyrosine   Indole.

The evolution of **sulphuretted hydrogen** is a frequent accompaniment of putrefaction. A large number of bacteria are endowed with the power of liberating this gas, the production of which depends, however, not solely on the species of ferment, but also on the composition of the nutrient medium, a circumstance which explains the contradictory results obtained by different workers. Thus, for example, STAGNITTA-BALISTRERI (I.) denied that *Bacillus subtilis, Bacillus tetragenus*, the so-called *Wurzel bacillus*, and others could form sulphuretted hydrogen ; but PETRI and MAASSEN (III.) then showed this contention to be incorrect, and that, in presence of peptone, the gas in question is produced by these microbes. In other cases, again, this product may be masked, *e.g.* by combination with ammonia formed at the same time. A good deal of the sulphur present in the nutrient medium is utilised by the bacteria themselves for structural purposes, the amount so consumed having been found by M RUBNER (I.) to be equivalent to 23-40 per cent. of the total sulphur in the medium. The sulphur in organic combination is first occluded, a circumstance harmonising with the well-known fact that the sulphur in albuminoids is very easily removed. The more delicate processes leading finally to the evolution of sulphuretted hydrogen, still remain unelucidated. PETRI and MAASSEN (IV) are of opinion that the bacteria liberate hydrogen, which in the nascent state then extracts sulphur from the sulphur compounds and combines with it. They found that very little of the gas in question is produced when nitrates are present in the medium, but that these latter are thereby reduced to nitrites. With reference to the fact (put forward to refute this explanation) that sulphuretted hydrogen is liberated by aerobic bacteria in well "roused" (aerated) cultures, Petri and Maassen showed that hydrogen is also liberated under this treatment, and that consequently the presence of air favours the reducing action.

The faculty of producing sulphuretted hydrogen is very common among the pathogenic bacteria, being absent in not a single one out of thirty seven species examined, and in many of them—*e.g.* the bacilli of swine erysipelas—the inoculated nutrient solution fairly bubbles from the quantity of gas liberated. A convenient means of detecting and separating sulphuretted-hydrogen-generating microbes from a mixture of bacteria by the aid of plate cultures is afforded by the ferro-gelatin, recommended by A FROMME (I.) for this purpose; *i.e.* a peptonised meat-juice gelatin qualified by 3 per cent. of iron saccharate or tartrate. In such nutrient media each colony of the sulphuretted hydrogen bacteria will become surrounded by a black halo of FeS.

The conversion of sulphates into sulphides by bacterial agency is also a decisive indication of reducing power. The conditions of vitality of a particularly active species of fission fungus were investigated by BEYERINCK (II), who named the organism *Spirillum desulfuricans*. This strictly anaerobic microbe is utilised

in practice in so far that by skilfully encouraging its development pit-water very rich in gypsum has been entirely freed from sulphates ($CaSO_4$ being converted into CaS and FeS) and rendered suitable for various purposes, such as feed-water for steam-boilers, &c.   Further particulars on this matter will be found in the treatise referred to.   The sulphuretted hydrogen produced by the above-named bacteria is consumed by a special group of fission fungi which will be more closely considered in chapter xxxv.

Among the sulphurous products of albuminoid putrefaction mention must be made of mercaptan (C_2H_5 SH), which was first detected by M. von NENCKI and N. SIEBER (II.) in cultures of *Bacillus liquefaciens magnus*

§ 169.—The Putrefactive Bacteria.

In the course of his investigations (frequently alluded to in previous paragraphs) on the micro-organisms in putrescent liquids, Chr. Ehrenberg observed a variety of forms and dimensions   The smallest of them bordered on the limits (Lat. *termo*) of visibility, and was so minute as to be almost indistinguishable by the aid of the optical instruments then available.   On this account he, in 1830, gave it the name of *Bacterium termo*, and subsequently, in 1838, expressed the opinion that this species is identical with the *Vibrio lineola* already described by O. F. Muller.   However, when FELIX DUJARDIN (I), in 1841, undertook to critically examine Ehrenberg's discoveries, and classified all the (infusorial) micro-organisms devoid of visible organs of locomotion into the family *Vibrionia*, which comprised the three genera *Bacterium*, *Vibrio*, and *Spirillum*, the old name of *Bacterium termo* was re-applied to this organism   Dujardin also regarded this " infusorium " as the smallest of all living creatures (*le premier terme en quelque sort de la série animale*), and described it as follows. " Form, cylindrical, length, 2–3 μ; thickness, 1.0–1 2 μ; frequently united in couples; exhibiting a tremulous movement," the latter being ascribed to alternate contractions and re-expansions of the plasma.   To these characteristics PERTY (I) in 1852 added another, viz., the grape-like form peculiar to the zoogloea of this microbe.   One year later COHN (V) also described a like organism   Then when, towards the close of the sixth decade of the century, Pasteur fully explained the theory of specific ferments (originated by Kutzing), and proved its accuracy by a series of examples, of which lactic fermentation was the first, the inclination to regard putrefaction as the work of a specific fission fungus gradually spread   Hence it was that COHN (I.), in 1872, propounded the dictum that "putrefaction is a chemical process excited by rod-bacteria" (*Bacterium termo*)

The more accurate (physiological) investigation of this process long remained impossible owing to the lack of means for isolating and obtaining pure cultures of its active organism.   For this reason the results obtained by different investigators (e g. B. SANDERSON (I) in 1871, and E. EIDAM (I) in 1875) into the physiological conditions of the so-called *Bacterium termo* are now only of historical interest   On the introduction of plate-cultures into practical bacteriology, pure cultures of the supposed *Bacterium termo* were soon obtained, and it was then found that this term comprised a number of different species   ROSENBACH (II), in 1884, was the first to ascertain this fact, and described three distinct species of decidedly putrefactive bacteria, which he named respectively *Bacillus saprogenes I., II., and III.*   Rosenbach undertook these researches from a medical point of view, and consequently treated the morphological and physiological sides of the question in a perfunctory manner   Nevertheless, he deserves the credit of having finally banished the designation *Bacterium termo* from systematic botany; so that, though the name is still occasionally used, it has now no special import, but merely serves as a convenient synonym for the term " putrefactive

bacteria." In this general sense the term is used in Fig. 57. The figure itself represents a species of bacterium (not more specifically identified) isolated from a putrescent liquid.

G. HAUSER (I.) investigated this matter more thoroughly, and showed, especially, that *Bacterium termo*, in the sense implied by Cohn, does not exist. In 1885 he brought to our knowledge three putrefactive fission fungi, which are, moreover, bacteriologically important from their indisputable polymorphism, a peculiarity since recognised in many other species of bacteria, but at that time much disputed. Hauser's discovery was welcomed by the supporters of this
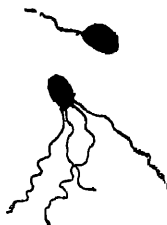
FIG 57 — Bacterium termo

Cilia staining   Magn about
1500   (*After photograms by
Fraenkel and Pfeiffer*)

FIG 58 — Proteus vulgaris

One long rod and one short rod. Cilia staining.
Magn about 1500   (*After photograms by Fraenkel
and Pfeiffer*)

theory, and the importance attached to it at the time was expressed in the name given to the organisms, Hauser having chosen the generic name *Proteus* for these extremely mutable *Schizomycetes* A short description of their characteristics is subjoined.

The cells of *Proteus vulgaris* are generally o 9–1.2 μ in length, o.4–o 6 μ broad, and almost always occur in couples In addition to these short rods, elongated forms, very frequently attaining a length of 3 7 μ, also occur. Some extremely vigorous but very rare cells will measure 6 μ long by o 9 μ broad. One of these is shown Fig 58 The large number of cilia indicates considerable locomotive activity, and in fact this power is possessed by the various species of *Proteus* in a high degree, manifesting itself both by a rapid forward movement and a con-current (longitudinal) axial rotation Hence, the coupled cells describe a kind of double cone, the vertex of which is at their point of junction. In addition to the above-named forms, gelatin cultures also yield spirilla, with two to four convolutions; thread-cells, which may grow to a length of 100 μ; and finally "spirulina," or threads bent in the form of a bow, with ends twisted into a *queue*. Under special circumstances involution forms are also produced : the cells swell up in the shape of a pear, and resemble spermatozoa, dumb-bells, &c. in form.

Proteus mirabilis exhibits a very decided tendency for producing such involu-tion forms. Globular or pear-shaped forms, 3–7 μ in diameter, are very frequently developed in the cultures of this microbe, which also exhibits polymorphism in a high degree, and in this particular greatly resembles the preceding species Here also we meet with short rods, long rods, spirilla, and thread-cells, rapidly moving one through another in varied alternation. At the same time small but unmistakable differences exist Thus for example, these threads not unfre-quently attain a length of 200 μ, i e. double the maximum size of the first-named species.

Proteus Zenkeri differs from the two preceding species mainly in its inability to liquefy gelatin, but resembles them in other particulars, though its cells ar

generally smaller, the least of them being globular in form and 0 4 μ in diameter. Short rods (0.8 μ long) joined in pairs are frequently encountered. These three species are unique in the bacterial kingdom in point of motile power, which they possess to such a high degree that a solid medium containing only 5 per cent of gelatin is unable to restrain them, and they make their way across it in all directions. In order to stop this roving motion the gelatin content must be increased to 10 per cent. This peculiarity is not only of physiological interest, but is also decidedly important so far as practical bacteriology is concerned, in that it indicates the futility of employing nutrient gelatin media containing less than 10 per cent of gelatin for the preparation of pure plate cultures of *Proteus* species. To complete the characterisation of these three species, it should be mentioned that none of them forms endospores, and that their growth may be arrested by depriving them of oxygen, though they do not necessarily die in consequence. They will not thrive in mineral nutrient media, such as those of Cohn and Nageli. When grown in albuminous media, they produce stinking decomposition. A. BRODMEIER (I.) proved that in neutral or alkaline solutions *Proteus vulgaris* is able to convert urea into ammonium carbonate. He thus refuted the assertions of Leube to the contrary, and confirmed the discovery of Schnitzler and Hofmeister.

No pretension can be made in the present work of giving a complete description of all known forms of putrefactive bacteria, and therefore the examples already cited, being the species most frequently met with, must suffice. Moreover, we have already mentioned others of this class in previous paragraphs. One of these, viz, the *Bacterium Zopfii*, discovered by KURTH (I) in the stomachs of fowls and shown in Fig. 31, is, according Czaplewski, identical with *Proteus Zenkeri*. This note appears in an abstract of a work by CH MOUGINET (I), who, also, minutely examined a number of putrefactive bacteria. HOLSCHEWNIKOFF (I.) described a fission fungus closely allied to *Proteus vulgaris*, which, from its faculty of producing sulphurretted hydrogen, has been named *Proteus sulfureus*.

Only one more species will be dealt with here, and that briefly, viz, *Bacterium coli commune*, which is an invariable inhabitant of the alimentary canal of the human subject (and of all the higher animals hitherto examined), and constitutes the most important of the bacteria present in fæces. This parasite was first described by TH. ESCHERICH (I.) as a slender short rod, 0 4 μ broad, the length varying with the conditions of nutrition and cultivation, but mostly measuring 2–3 μ, though occasionally it decreases to 0.5 μ. By some authors this fission fungus is named *Bacillus coli communis* and *Colon bacillus*. Like the *Proteus* species, it generally appears as double rods, but its movements are sluggish and laboured. It does not liquefy gelatin. In media containing sugar it can develop even in the absence of oxygen, and liberates a gas which—according to FRÆMLIN (I.)—consists of two-thirds carbon dioxide and one-third hydrogen. No development of endospores has hitherto been detected In its manner of growth in artificial media this organism agrees in many particulars with *Bacillus typhi abdominalis*. Consequently they are extremely hard to differentiate, and this makes the bacteriological examination of water a particularly difficult operation when the presence of typhus bacilli has to be quantitatively determined. A further complication is imparted by the extreme sensitivity of *B coli commune* to modifications in the conditions of cultivation, and by its great tendency to form varieties. For instance, a number of *races* of *B. coli commune* are now known, which, under certain circumstances, are not merely saprophytic, but also pathogenic. A more detailed treatment of this question would occupy too much of our space, and besides, the matter is fully recorded in Tiemann-Gartner's work on Water Analysis. A synopsis of the most important researches of

Escherich, Kohler, Baginsky, Bischler, and others, on the methods of nutrition of *B. coli commune* and its powers of decomposition, was prepared by M. Ide (I.) in 1891. The facts brought to light since that date will be found in the several yearly volumes of A. Koch's "Jahresbericht"

We will now briefly refer to the subject of **intestinal putrefaction**. Mention has been made in a previous paragraph of the fundamental difference between the processes of decomposition effected in the small intestine on the one hand and in the colon on the other, in man. On issuing from the stomach—where, by the action of the pepsin and hydrochloric acid secreted by the gastric glands, a more or less extensive peptonisation of the digestive albuminoids in the food has been effected—the pulpy food, now known as **chyme**, has a strongly acid reaction (equivalent to o.1–o 3 per cent. of hydrochloric acid) Immediately on its arrival in the upper division of the alimentary canal (small intestine), it becomes mixed with bile and pancreatic juice, under the influence of which the fat is emulsified and the insoluble carbohydrates (starch) are hydrolysed. Both secretions have an alkaline reaction, which, however, is not sufficiently strong to immediately neutralise the acidity of the contents of the intestine This slightly acid nutrient medium, rich in sugar, offers a favourable field for the activity of the lactic acid and allied bacteria introduced along with the food; and, moreover, the acidity restricts the development of the competitive putrefactive bacteria In proportion, however, as the contents of the intestine are forced onward and approach the colon, the acid reaction is neutralised by the alkaline mucus secreted by the intestinal glands. At the same time the composition of the mass has become changed, since the products of the hydrolysis of starch, which have also to some extent been converted by the aforesaid bacteria, have been absorbed into the blood-vessels. Therefore in the contents of the colon it is the (undigested or indigestible) albuminoids and biliary constituents which are decomposed by the putrefactive bacteria now coming into action, and it is here that the malodorous products (indole, skatole, volatile acids, sulphuretted hydrogen, &c.), to which the intestinal contents (finally issuing from the rectum as fæces) owe their repulsive smell, are produced.

The researches of MACFADYEN, NENCKI, and SIEBER (III) revealed both the actual course of the process just described, and the fact that, contrary to the view expressed by Pasteur, the putrefaction occurring in the colon is not essential to digestion The above-named workers performed their experiments on a patient suffering from a strangulated hernia at the junction of the ileum and the cæcum This portion of the intestine was removed by an operation, and the subsequent surgical treatment necessitated the construction of an artificial evacuatory passage (*anus præternaturalis*) at the extremity of the small intestine, until complete union of the severed portions was restored, an affair of six months' duration. Meanwhile the contents of the intestine were discharged through this artificial passage, and, though no digestive functions were performed by the colon, the patient nevertheless kept in good health, and even increased in weight. This will explain why Nencki regarded the development of antiseptic digestion as the goal of the physiology of nutrition, *i.e.* digestion in which the putrefaction occurring in the colon is either abolished, or at least reduced to a minimum, in order to prevent the formation of decomposition products that are not only useless to the body, but even troublesome and dangerous. As a matter of fact, GEORGE NUTTALL and H THIERFELDER (I.) recently afforded a convincing proof of Nencki's theory by rearing some young porpoises, born by the aid of the Cæsarean operation, and nourished in a suitable sterilised chamber. On examination at the close of the experiment, they were found perfectly healthy, though entirely free from bacteria. Pasteur's assumption (which was also supported by Soxhlet with reference to his incomplete process of milk sterilising) was thus shown

to be erroneous. A few observations on this point were also made by E. DUCLAUX (XI.).

§ 170.—Proteolytic Enzymes.

All the fission fungi (with the few exceptions given in chapter xxxiii.) require nitrogenous nutriment for the construction of their cells. Such of these nitrogenous materials as are soluble in water, and therefore diffusible through the cell-wall by osmosis, need not be referred to here Mostly, however, the nutriment presented to the bacteria is insoluble in water, and this is particularly the case with the protein albuminoids. To enable these latter to supply the nitrogen required for the elaboration of the bacterial plasma they must first be converted into soluble compounds, a task which is effected by the proteolytic enzymes. So far no comprehensive study of these active bacterial secretions has been made, and at present our knowledge is chiefly confined to the enzymes dissolving gelatin and fibrin. A new classification of the bacteria into two groups, the liquefactive and non-liquefactive towards gelatin, according to the presence or absence of a proteolytic enzyme, has obtained currency in practical bacteriology since the introduction of the Koch system of plate-cultures.

We are indebted to CL. FERMI (II.) for the first extensive series of pure culture investigations on this point.   He proved that a gelatin-dissolving enzyme is formed in cultures of the following species of *Schizomycetes* :— *Bacillus subtilis*, *B. anthracis*, *B megatherium*, *B pyocyaneus*, *Vibrio choleræ asiaticæ*, *Vibrio Finkler-Prior*, *Micrococcus prodigiosus*, *M. ascoformis*, *M. ramosus*, *spirilla from cheese*, &c.   Fibrin is dissolved as well as gelatin, but less readily than the latter.   Egg-albumen and coagulated blood-serum offer greater resistance to these bacteria, thus indicating that pepsin is not present   Reasons exist for assuming that the enzymes produced by the said microbes are *not all* of the same kind, one conclusive indication being afforded by their behaviour under different temperatures.   Thus, for example, the proteolytic enzyme produced by *Micrococcus prodigiosus* is rendered inactive (in solution) by a temperature of 55° C., that from *B pyocyaneus* by 60° C., that from *B. anthracis* by 65° C., and that from *Vibrio Finkler-Prior* not below 70° C. Similar differences of behaviour are observed towards acids, bases, and poisons. A fundamental difference exists between these enzymes and pepsin, since whereas the latter is extremely sensitive towards alkalies, and is absolutely incapable of dissolving albumin except in presence of free hydrochloric acid, the bacterial enzymes in question act on fibrin in neutral or faintly alkaline solutions only, though they will attack gelatin even when the liquid is slightly acid (0.5 per cent HCl). On this latter account they more nearly resemble trypsin, *i.e.* the enzyme secreted by the gastric glands. None of the *Schizomycetes* under examination was found eapable of producing an enzyme able (like pepsin) to dissolve fibrin in presence of an acid.   According to FERMI's (III.) results, the excretion of the proteolytic enzyme occurs, as a rule, only when albumen is present in the nutrient medium. Two only, of all the species examined by him, exhibited any variation in this respect, viz., *Micrococcus prodigiosus* and *B pyocyaneus*, which yielded a proteolytic enzyme when cultivated in a mineral nutrient solution qualified with glycerin or mannite.

It has long been known that antiseptics in small doses exert no injurious influence on the action of enzymes.   On this point some conclusive investigations were published by FERMI and PERNOSSI (I), and use is made of this property in testing for the presence of a proteolytic enzyme in samples of liquids or bacterium cultures, an easy method proposed by FERMI (IV) being employed.   A so-

called **Thymol-gelatin** is prepared in the following manner ·—Water saturated with thymol is qualified with 5–10 per cent. of purest gelatin, and after being warmed on the water-bath is poured into test-tubes (10 c.c. in each). The tubes are kept in a vertical position, and are ready for immediate use as soon as the contents have set. The thymol present therein will prevent any development of bacteria. A large stock of these tubes can be prepared, and the contents preserved from desiccation by placing the (open) tubes, mouth downwards, in a covered glass vessel containing a little distilled water. The liquid to be examined is filtered to remove any solid particles. A few c c. are then placed in one of the thymol-gelatin tubes, and a little thymol is added to prevent the development of any bacteria already present in the sample. The tube being then left to stand at room temperature, the presence of any proteolytic enzyme in the sample will be revealed in a few days by the liquefaction of an appreciable stratum of the gelatin. To enable this change to be reliably ascertained a mark is made on the tube at the time of filling, to denote the level of the gelatin. The risk of the gelatin becoming dissolved by any large percentage of acid or alkali present should be obviated by neutralising the sample before commencing the experiment. Liquids containing substances such as tannin, glycerin, &c, capable of preventing or retarding the solution of the gelatin, are unsuitable for use This simple method may also be employed as an approximate quantitative test for determining the relative strength of two solutions of a proteolytic enzyme, since the amount of gelatin dissolved per unit of time under identical conditions may be regarded as a measure of the concentration or potency of the samples. If tubes of equal diameter are used, then this relation is simply expressed by the height (thickness) of the two liquefied strata Fermi claims that his method is more reliable than those proposed (for the same purpose) by Grunhagen, Grutzner, Brucke, and Schütz, and which consist chiefly in determining the amount of *fibrin* dissolved by the sample under certain definite conditions. As we have already mentioned that this latter substance is attacked with greater difficulty than gelatin, it will be at once evident that Fermi's method is the more delicate

With regard to **casease**, *i e* the enzyme decomposing the casein of milk into soluble products, the chief particulars have already been given in § 147. Many bacterial species are, however, capable of dissolving this albuminoid without any trace of casease being found in the cultures. One of these is the *Bacterium peptofaciens*, isolated from milk by AL BERNSTEIN (I), which is particularly active in converting casein into peptone and albumoses, a little (0 2 per cent.) lactic acid being also formed. If, now, the milk be boiled after the bacterium has been in action for a short time, the unconverted casein will be thrown down, and, when filtered off, leaves behind a liquid which is rich in readily digestible peptones, and has been named " galactone " by its inventor. The milk-sugar present in this liquid may be fermented by the addition of suitable yeasts, and then yields " galactone wine."

The bacteriological researches of the past few years have resulted in an important modification of the opinions held regarding the so-called **carnivorous plants**. According to earlier statements, the glands of the parts of the plant acting as a snare secreted a dissolving albumen enzyme, which digested the captured prey, *i e* converted its albuminoids into assimilable peptones, &c Hoppe-Seyler in 1876 threw doubts on the presence of this enzyme in *Drosera rotundifolia*, and in 1889 N. TISCHUTKIN (I) ascribed the phenomenon to bacterial activity

This observer ascertained that the juice collecting on the surface of the leaves of *Pinguicula* is rendered inactive by painting the leaves over with bactericidal media. The same conclusion was arrived at by R DUBOIS (II.) in

1890, in his experiments on the contents of the urns of *Nepenthes* ; and two years later the matter was again examined by TISCHUTKIN (II.) in the following plants :—*Drosera rotundifolia*, L., *D. Longifolia*, L., *Dionæa muscipula*, Ell., *Nepenthes Mastersi*, the results confirming the hypothesis expressed above, viz., that the digestion of the albuminoid bodies falling or introduced into the juice excreted by these plants is exclusively due to the activity of bacteria settling in the said liquid and there producing a proteolytic enzyme. According to an analysis by Volker, the juice collecting in the cups of *Nepenthes* contains about 0.8–0.9 per cent. of dry matter, about 39 per cent. of which consists of malic acid and 50 per cent. of potassium chloride, *i.e.* the two substances already mentioned in § 41 as powerful bacterium stimulants. The juice in the unopened young cups of *Nepenthes* contains neither proteolytic enzyme nor bacteria, the latter falling out of the air into the liquid only after the cups are opened. Ample opportunity is soon afforded for the exertion of their decomposing power on the insects caught in these traps and prevented by special contrivances from escaping. For the preparation of this nutrient material the organisms elaborate enzymes, the proteolytic properties of which are utilised by the plant. These so-called **carnivorous plants** consequently present a beautiful example of symbiosis existing between higher plants and bacteria.

§ 171.—Ptomaines and Leucomaines.

The first step towards the elucidation of the regrettable fact that putrefying albuminoids, when introduced into the blood-vessels of man or the higher animals, set up violent reactions (*sepsis, septicæmia*), which may, under certain circumstances, prove fatal, was made by P L. PANUM (I) in 1856, who proved that putrescent albumen contains a poisonous fission product which cannot be destroyed by boiling, treatment with alcohol, or similar methods, and is consequently not an organised creature, but a chemical compound (known as "extractive putrescent poison") This discovery, which was tested and confirmed by M. HEMMER (I.) and F. SCHWENINGER (I.), is also of historical importance in Pathological Bacteriology, since thenceforward medical views and researches concerning the nature of the diseases engendered by bacteria pursued two divergent paths the one school holding these diseases to be toxic phenomena produced by the poisonous metabolic products (**toxins**) of parasites growing within the body, whilst the other regarded the vital activity of the organisms themselves as the immediate cause of the malady There is no occasion for us to follow this conflict of opinions, which is still rife; so we may confine our attention to the efforts of Panum's successors in the narrower field of albuminoid putrefaction Among these E. BERGMANN (I) and O. SCHMIEDEBERG (I.) chiefly deserve mention as being the first to obtain (1868) a poison of this group—by precipitation as sulphate (the so-called sepsin sulphate) from putrescent beer-yeast—in a crystalline form, and therefore available for closer chemical investigation and characterisation. M. VON NENCKI (V.) was the first, in 1876, to successfully prepare such a poison in the pure state, viz , the alkaloid *collidine* (isolated from putrid albumen), having the formula $C_8H_{11}N$, and being (according to its constitution) trimethyl pyridine, C_5H_2N. $(CH_3)_3$. Such alkaloids, are also formed, as a matter of course, during the decomposition of the human cadaver (Gr *ptoma*), and on this account F SELMI (II.) in 1878 gave the name **ptomaines** to putrefaction alkaloids in general.

This newly discovered group was gradually enlarged, and now includes more than fifty substances. Comparatively speaking, the majority of these new bodies were discovered by L. BRIEGER (III.), to whom we are also indebted for new methods for the separation of these poisons from putrescent liquids. Cf the

ptomaines prepared by him, viz., choline, saprine ($C_5H_{16}N_2$), putrescine ($C_{14}H_{12}N_2$), neuridine ($C_5H_{14}N_2$), and cadaverine, peculiar interest attaches to the last-named from its having been the first putrefaction alkaloid prepared by synthetic methods. The first to accomplish this was Ladenburg, who determined its formula as $NH_2.CH_2$—CH_2—CH_2—CH_2—$CH_2.NH_2$, i e, pentamethylene diamine. Putrescine and cadaverine were detected by F. OBERMAYER and R. KERRY (I) in considerable quantities in the putrefaction of yeast Choline (CH_2 OH—CH_2—$N(CH_3)_3.OH$) may be separated from lecithin, which forms an important con-stituent of nerve and brain. By substituting hydroxyl for one of the hydrogen atoms of the central CH_2 group, we obtain muscarine, $CH_2.OH$—CH OH—$N.(OH_3)_3.OH$, which O SCHMIEDEBERG and E HARNACK (I) recognised as the powerful poison of red agaric (*Amanita muscaria*), and to which must be ascribed the intoxication resulting from the consumption of this fungus, or of the beverage prepared therefrom, by the natives of Eastern Siberia. According to L BRIEGER (IV.) the same poison also results from the putrefaction of choline and certain albuminoids, and it was also found in 1878 by Gautier in putrid fish. By separating a hydrogen atom from the central CH_2 group in choline and the hydroxyl adherent to the adjacent carbon, and combining these liberated equi-valents to form water, we then have left behind neurine, $CH_2 = CH$—$N (CH_3)_3$ OH, a vinyl derivative which may also be formed in the putrefaction of nerve tissue and brain. According to the researches of P. JESERICH and F NIEMANN (I.), choline undergoes this conversion under the action of *Bacterium coli commune* Hydrocollidine, $C_8H_{15}N$, is regularly produced during the putrefaction of the flesh of horses and cattle, and is generally accompanied by the nearest homo-logue of collidine, viz., parvoline, $C_9H_{13}N$. A more detailed characterisation of these ptomaines must be omitted here, but the reader desiring instruction in this particular will be able to obtain it from the concise monograph by F. JACQUEMART (I.) Not every ptomaine is poisonous,—cadaverine, putrescine, and saprine being devoid of this property.

The composition of tyrotoxicon, or cheese-poison, which was first discovered by V. VAUGHAN (I.), is still unknown, but from its chemical behaviour it appears to consist principally of a diazo body (diazobenzene ?) It is formed (under con-ditions still uninvestigated) in stored cheese by the action of bacteria, and when eaten in such cheese produces symptoms of violent poisoning. A case of this kind, in which fifty persons were simultaneously attacked, is recorded by SCH. WALLACE (I) The same poison is also occasionally formed in milk Thus, VAUGHAN (II.) reported an instance of eighteen persons being rendered ill by eating vanilla ice, from which substance (chiefly composed of milk) crystals of tyrotoxicon were obtained L. DOKKUM (I.) extracted from a cheese recognised as dangerous to health a ptomaine-like substance which he termed tyrotoxin, but which is not identical with tyrotoxicon. In America such cases of cheese-poisoning are more frequent than in Europe, Vaughan having enumerated three hundred within two years.

It is not essentially necessary that the food should contain ready-formed ptomaines for symptoms of poisoning to appear. On the contrary, the ptomaines may be formed in the body itself if the food contain bacteria capable of pro-ducing them, and provided that the composition of the substances present in the intestines is favourable at the moment. In such event the poisons are called leucomaines, and most of the cases of so-called meat-poisoning are due to this cause. Thus A GÄRTNER (I) reported a case wherein he succeeded in identifying a fission fungus, *Bacillus enteritidis*, as the cause of the poison, and the same microbe was discovered by J KARLINSKI (II.) in a case of meat-poisoning in Herzegovina, where sun-dried meat (" suché mieso ") is an ordinary article of trade, and is frequently eaten raw by the natives. Many of the cases

of so-called fish-poisoning, *i.e.* illness produced by eating fish, also belong to this category. On the other hand, these ill effects may also be brought about by ptomaines produced during the storage of this (readily decomposable) food-stuff, a remark which applies equally to the so-called sausage-poisoning. Researches on this point have been conducted by H. MAAS (I.) The poisonous decomposition products developed by the activity of fission fungi in eggs, and also cases of poisoning ensuing from the consumption of eggs so spoiled, have been investigated by GLASMACHER (I.), BONHOFF (II), and GRIGORIEW (I).

§ 172.—The Albuminous Poisons.

To attribute the poisonous effects of bacteria, in all cases, to the formation of products of the ptomaine group would be incorrect As a matter of fact, the injury is frequently caused, not by these alkaloids at all, but by certain true albuminoids, which, on account of their decomposing power, have been named **active albumen.** We have to thank CHRISTMAS and HANKIN (I.) for the first proof of this fact, though Pflüger was cognisant of it as long ago as 1875 We have already stated in § 82 that certain pathogenic fission fungi will develop on nutrient media destitute of albumen and there elaborate poisons synthetically.

The fundamental differences between **active albuminoids** and **ptomaines** are not confined to their production and composition, but extend also to their mode of action the former behaving like enzymes, and acting as a result of the lability of their atoms, so that a small quantity of the active substance is able to induce decomposition in a comparatively enormous mass of decomposable material. On the other hand, the poisonous effects of the ptomaines depends on the quantity coming into play, and increases therewith As in the case of the enzymes, the active albumen is completely deprived of its powers by moist heat (100° C.), by which it is converted into non-poisonous **passive albumen**; whereas the ptomaines remain undecomposed and undebilitated by the same treatment. This fact is also of importance to the food-stuff chemist, since it will restrain him from certifying a sample of suspected meat to be innocuous merely because a negative result has been obtained with the current alkaloid reactions.

Many cases of meat poisoning are probably due to the presence and action of active albumen A fuller insight into this matter must first, however, be gained by investigation. Thus we find it recorded by M. ARUSTAMOFF (I) that in the Lower Volga district the opinion prevails that only the consumption of uncooked fish (salted sturgeon and salmon) is harmful In view of the remarks already made on the influence of heat on active albumen this observation becomes intelligible. The danger resulting from the presence of living bacteria in incompletely sterilised milk, and their developing in the intestines of the nursing infant (see § 125), is probably in many cases due to active albumen formed by the organisms. The author puts this interpretation on the results of the experiments made by A. LÜBBERT (I.) on this point.

As was first established by MITCHELL and REICHERT (I) in 1886, it is to the presence of such active albumen that the effects of snake-poison are due. Moreover, albuminous poisons are found in the normal blood of different animals, a circumstance first established by A. Mosso (I) in the case of *Murænidæ*, to which family the common eel belongs. A list of fishes naturally containing poison has been drawn up by J. POHL (I.) Poisonous albuminoids are likewise found in various plants, *e g.* abrin in the seeds of the paternoster pea (seeds of the wild liquorice, *Abrus precatorius*), ricin in the seeds of *Ricinus communis*, and many others.

The reaction between the animal body and bacteria is reciprocal. Just as the latter are able to excrete noxious metabolic products, the effect of which on

the infected animal body is manifested as disease, so also the former can elaborate substances having a poisonous effect on the parasitic micro-organisms. The normal and continuous presence of such protective albuminoids, or alexines, as they are called, in the blood, is the cause of the natural immunity enjoyed by certain animals against certain pathogenic species of bacteria. A closer consideration of this matter would, however, be beyond the scope of the present work, though it must be referred to, as throwing new light on the connection between Bacteriology and Physiological Chemistry. Full information on the subject of protective inoculation and serum therapeutics can be gathered from the concise text-book prepared by HUEPPE (VI.), which at the same time provides an introduction to the study of *Pathological Mycology*. On this latter subject P. BAUMGARTEN (I.) has written a reliable handbook which is hereby recommended to food-stuff chemists and agriculturists.

§ 173.—The Liberation of Nitrogen, and De-nitrification.

The interest with which the farmer regards the decomposition of nitrogenous substances, both in the manure heap and in the soil, always proceeds from the same desire : to know what becomes of the nitrogen, and whether it is retained in the soil.

The alterations suffered by nitrogenous manurial constituents derived from urine will be described in chapters xxxii and xxxvi, and at present we are concerned merely with the putrefaction of the albuminoids, &c., evacuated in the fæces.

In the first place, it must be remarked that the loss of nitrogen may occur, not only as a result of its liberation in a free gaseous state, but also in consequence of the volatilisation of ammonia produced by the action of micro-organisms on the albuminoid matter of the manure. We are indebted to E. MARCHAL (I.) for proving that the faculty of eliminating ammonia from albuminoids is common to a great many fungi (both *Schizomycetes* and *Eumycetes*), occurring in large numbers in the soil, and quite distinct from the *Schizomycetes* effecting the conversion of urea. Among the fungi (widely distributed and frequently discovered in the soil) examined and recognised by MARCHAL (II.) as powerful ammonia-producers, may be mentioned in the *Schizomycetes* group — *Bacillus mycoides*, Flügge ; *B. fluorescens liquefaciens*, Fl., *B. fluorescens putidus*, Fl., *B. subtilis*, *B. arborescens*, *B. mesentericus vulgatus*, Fl., *B. mesentericus ruber*, Fl., *B. janthinus*, Zopf, *Proteus vulgaris*, H., *Bacterium coli commune*, *Sarcina lutea*, *Micrococcus roseus*, Fl., *M. flavus*, Fl., *M. candicans*, Fl., &c., and in the *Eumycetes* group —*Aspergillus terricola*, *Penicillium glaucum*, *P. cladosporioides*, *Mucor mucedo*, *M. racemosus*, *Botrytis cinerea*, *B. vulgaris*, *Cephalothecium roseum*, and others. The potency of the different species varies, the largest quantity of ammonia (0.8 gram per litre of nutrient solution) being produced by *Bacillus mycoides*. This last-named fission fungus, which was minutely examined by Marchal, decomposes both albumen, leucine, and tyrosine, but does not attack urea. The losses occasioned by the volatilisation of ammonia produced in this manner may be very considerable, but will not be further considered here. We will now turn to the liberation of uncombined nitrogen.

The first researches on this point were undertaken by JULES REISET (I.) in 1854 and 1855. He asserted that free nitrogen is always evolved during the putrefaction of manure, whilst G. HÜFNER (I.) arrived at the contrary opinion, being unable to discover any liberation of free nitrogen when atmospheric air or pure oxygen was led through the putrefying substances. The same result was obtained by ALEXANDER EHRENBERG (I.), O. KELLNER and T. YOSHII (I.), and BR. TACKE (I.) ; and this view was also held by H. IMMENDORFF (II.) in 1893

Although these discoveries may justify the conclusion that no free nitrogen is disengaged during the putrefaction of albuminoids, it must not, however, be assumed that the same also applies to the decomposition of manures in general under natural conditions; since, under these circumstances, very considerable quantities of this element can be liberated and become lost to the soil. This result is, however, due to the reduction of nitric salts, and not to the putrefaction of albuminoids.

This de-nitrification in arable soil was first noticed by GOPPELSRÖDER (I.) in 1862, and was long regarded as a purely chemical process The first reference to the agency of bacteria in this decomposition was made by E. MEUSEL (I.) in 1875, and the earliest pure cultures of such organisms were obtained by U. GAYON and G. DUPETIT (II.) in 1882. In succeeding years a large number of species, all capable of reducing nitrates, was made known; *e.g.* by W. HERAEUS (I.) in 1886. Two years later P. FRANKLAND (II.) was able to associate with the group in question 17 out of 32 species, and R WARINGTON (I.) 16 out of 25 species examined, among them being *Bacillus ramosus*, the so-called "Wurzel-bacillus." All these reduce nitrates into nitrites, but these two chemists do not say whether the latter substances in turn may be still further reduced by the bacteria. For this reason we must revert to the labours of GAYON and DUPETIT (III.), who made pure cultures of two bacterial species, named *Bacillus denitrificans* a and β, which exhibit a noteworthy difference in their behaviour towards nitrates. Species a is the more energetic, decomposing as much nitrate as is presented to its action, and reducing the same to nitric oxide and free nitrogen. The β species, on the other hand, forms nitrites, and ceases to act before the whole of the nitrate is destroyed, free nitrogen being the only gaseous fermentation product. Quite distinct from these two species is the *Bacillus denitrificans*, isolated from arable soil by E GILTAY and J H. ABERSON (I.), which reduces the nitrates to free nitrogen in an almost quantitative degree. When grown on nutrient gelatin the rods measure 0.5 μ in breadth and 1.5–3 μ in length, but in liquids they assume a somewhat more elongated form. Closely allied to these three species is the *Bacillus denitrificans II*, discovered by R. BURRI and A. STUTZER (II.) on old straw, but differing from them in that it liberates as gas only some 80 per cent of the nitrogen in the decomposed nitrates, the remainder being elaborated into an organic compound (still uninvestigated), which is precipitated in large flakes. The same observation was made (though not with pure cultures) by E BRÉAL (I.) in 1892. Like the aforesaid three *Schizomycetes, Bacillus denitrificans II* is anaerobic, and decomposes nitrates only when oxygen is excluded Another (sporogenic) de-nitrifying bacillus, isolated by J SCHIROKIKH (I.) from horse-dung, may also be mentioned

The facultatively anaërobic *Bacterium coli commune* exhibits a peculiarity worthy of special consideration. When kept in a nutrient solution by itself and with exclusion of air, it reduces nitrates to the condition of nitrites; but the decomposition proceeds in quite a different manner when the organism is grown in symbiosis with a second species of bacterium, invariably found in horse-dung by both the above-named workers, and named *Bacillus denitrificans I*. In such case, even when air is admitted, the nitrogen of the nitrate is set at liberty, though neither species is able to produce the same effect by itself. *Bacterium coli commune* can, however, be replaced by *Bacillus typhi abdominalis*. The potassium or sodium present in the nitrates or nitrites is converted into a hydroxide, which accumulates in the medium, and eventually arrests the vital activity of the bacteria in question. For this reason not more than 5 or 6 grams of saltpetre (potassium nitrate) can be fermented per litre The fact that *Bacterium coli commune* in the absence of air (*e g.* in the intestines) converts nitrates

into the exceedingly poisonous nitrites is also of interest to Patholog Mycology, but we cannot further discuss the matter here. The important pc so far as we are now concerned, is, that the disengagement of free nitrogen f nitric salts can go on even in the presence of air. The de-nitrification occurr in stored manure and in arable soil appears to be a twofold process : anaёrobic nitrate destroyers acting in the lower strata away from the air, wh the symbiotic activity of the *Bacterium coli commune* (so plentiful in ani. excreta) and the *Bacillus denitrificans I.* comes into play at the surface. Fi this it is evident that the theory which assumes the possibility of preventing destruction of nitrates by thoroughly loosening, and consequently aerating soil, is of little value.—The bacteria in question are (for some unexplai reason) present in enormous numbers in the excrement of various anim. First in this respect is horse-dung, which has always been regarded by practi men as a **hot** manure, a property which is explained by the foregoing obser tions Consequently such manure should not be applied, especially when fre to soil that has recently received a dressing of nitrate of soda, otherwis serious loss of nitrogen will result This injurious action is, however, not limi merely to such fields as have been artificially manured with nitrate, since (as shall see in chapter xxxvi) the ammonia salts in the soil are, under favoura conditions, oxidised into nitrates by the activity of a special group of bacter such nitrates then forming a welcome food for the organisms dealt with in t present paragraph That it is a question of more than insignificant quantit will be evident from the discovery reported by PAUL WAGNER (I)—a discove which led to the aforesaid researches of Burri and Stutzer—viz., that out of 1 parts by weight of nitrogen applied in the form of stall-manure to the soil, or 25 parts are, on an average, recovered in the crop, whilst the remaining 75 pai are entirely lost. These figures do not fully represent the extent of the l occasioned in the soil and manures by the activity of the de-nitrifying bacter and there still remains another phenomenon for consideration. We must rec that the fission fungus known as *Bacillus denitrificans* (and probably also a numl of allied species not hitherto investigated) separates nitric oxide as well as nitr gen from nitrates. This oxide then escapes into the outer layers of the manu heap or soil, where it is brought into contact with oxygen, and combines thei with to form nitrogen trioxide—

$$2NO + O = N_2O_3$$
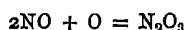
This latter then reacts on the ammonia and ammonia derivatives (urea, &(in the soil, in such a manner as to liberate both the nitrogen of the trioxide ai that of the ammonia as well—

$$N_2O_3 + 2NH_3 = 2N_2 + 3H_2O.$$
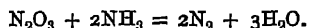
Consequently the nitrogen compounds insusceptible to the direct action of tl microbes in question are also included in the wasteful reaction set up. It w. on this account that the production of ammonia during the decomposition manure was casually referred to at the commencement of this paragraph. Tl present is a fitting opportunity for referring to the statements of several worke —*e g.* H B. GIBSON (I.)—who, like Reiset, thought they had observed a liber tion of nitrogen in their researches on putrefaction. Their results were a obtained by the use of complex bacterial mixtures, and therefore cannot l considered as reliable. In this case, also, those experiments alone are decisive i which pure cultures have been employed.

By the activity of these bacteria an enormous quantity of combined nitroge is daily set at liberty in the soil. To replace this loss, and to restore the coi

tinuity of the nitrogen cycle, is the task of a separate group of bacteria, which will be dealt with in chapter xxxiii.

The reduction of nitric acid by bacteria does not always stop short at the liberation of free nitrogen, but in many instances extends to the formation of ammonia. Several investigations on this point were made by O. LOEW (III.), but, unfortunately, not with pure cultures He found that "ordinary putrefactive bacteria," grown in a solution of 1 per cent. of peptone, 0.2 per cent. of KNO_3, and 0.2 per cent. of K_2HPO_4, cause the potash and carbon dioxide to combine, whereas the nitrogen of the nitric acid is converted into ammonium carbonate. When 0.2 per cent. of ethyl alcohol is also present (in anaërobic cultures) the acetate is formed instead of the carbonate.

What has already been detailed will explain the so-called nitric fermentation of molasses The cell sap of the sugar-beet contains a quantity—generally small, but occasionally larger—of nitrates, principally potassium nitrate. This is not separated during the saturation process, but remains in the mass in undiminished quantity, a portion crystallising out, and being then found in the raw sugar from the centrifugal machine, whilst the rest remains in the mother liquor, *i.e.* the separated syrup. If this syrup is then boiled up for the manufacture of second product, and again passed through the centrifugal machine, the proportion of nitrates in the mass will be still larger,—Pellet having found 1.9 per cent. of KNO_3 in one sample examined by him. At this stage the molasses has a faintly alkaline reaction, and is rich in organic and inorganic nutrient substances of various kinds Hence it is no wonder if bacteria rapidly develop therein. Under special conditions the upper hand is gained by such organisms as reduce potassium nitrate and eject its nitrogen in the form of NO, which compound, on coming in contact with air, is oxidised into the dioxide NO_2. The latter hangs as a dense red-brown vapour over the surface of the molasses, and the sugar-maker then says his molasses is in a state of nitric fermentation. This phenomenon is of less frequent occurrence in the "reserves" in the sugar-factories than in the dilute molasses of the molasses distilleries. Certainly, the activity of these reducing bacteria can be arrested by souring, but this treatment liberates organic acids inimical to the yeast. Bearing this in mind, Czeczetka proposed to remedy the evil by boiling the molasses directly the malady is observed. According to a report by DUBRUNFAUT (I) in 1868, nitric fermentation was first noticed by Tilloy at his distillery in Dijon, and was successfully suppressed by him by boiling the molasses along with sulphuric acid An explanation (characteristic of the state of knowledge in the domain of Fermentation Physiology at that time) of the favourable influence of this treatment was made in the same year by J. REISET (II), who stated that the NO or NO_2 formed during the so-called nitric fermentation proceeds from the oxidation of ammonia in the molasses, this being attacked only when present in combination with a *weak* acid, whilst when in the form of sulphate it resists the action of oxygen , consequently the molasses treated in the manner adopted by Tilloy was exempt from this decomposition. This view was left uncontradicted by BÉCHAMP (III.), although he had already ascribed de-nitrification to the agency of micro-organisms. A closer investigation (embodying modern methods of working) of this nitric fermentation of molasses is highly desirable. To be thoroughly satisfactory, such research must trace the course followed by the potassium nitrate in the juices of the sugar-works, and more narrowly examine the quantitative dependence of the nitrate in molasses on the method of preparation employed, very little being as yet known on these points

The nitric decomposition in question is also of frequent occurrence in the fermentation of tobacco in heaps. SCHLÖSING (III.) reported in 1868 on the first observation of this phenomenon by Ch. Ray.

§ 174.—The Loss of Colour (Umschlagen, Brechen) in Wine

was first examined chemically by G. MULDER (II) in 1855. Of this complaint which is known in France as *vin tourné*, and in Italy as *vino girato*, he gives the following explanation.—"This alteration of wine consists in a decomposition of the tartaric acid, but how this decomposition is induced is unknown The cream of tartar is converted into potassium carbonate, whereby the colour of red wine is altered and becomes brown The decomposition begins at the bottom of the cask, and is hence undoubtedly a result of the decomposition of the organic matter of wine-yeast, which contains a substance acting destructively on the tartaric acid, and, in co-operation with air, oxidising it to carbon dioxide and water. As the malady progresses, the alcohol is converted into acetic acid, and a putrefactive fermentation ensues." The commencement of this malady, which appears more frequently in red wines than in white ones, manifests itself by a slight evolution of carbon dioxide, which preliminary symptom is known in practice as "boiling away" (*versieden*). Tartaric acid is not the only substance eliminated, glycerin also—according to the researches of P. CARLES (I)—being slowly decomposed. Simultaneously, the amount of volatile acids increases to an unusual extent (up to 4 grams per litre), a fact observed by SCHULTZ (I.), and afterwards confirmed by J. MACAGNO (I).

Ten years after Mulder's observations, PASTEUR (XII.) undertook the task of discovering the cause of this malady and proving that here also the activity of a still unknown micro-organism was in question. He showed that in wines affected with this complaint bacteria are always detectable in large numbers their length being 3–5 μ, with a breadth of 1–1 5 μ Greater probability was imparted to this assumption by the observation made by SCHULTZ (I.), who, in 1877, succeeded in artificially imparting the malady to sound wine by inoculating it with a small portion of a wine already infected. A closer study of the organism could not at that time be made, owing to the lack of methods of pure culture, a defect that, in this connection, was first overcome by E KRAMER (I with the organisms from a number of samples of Styrian and Croatian wines affected with loss of colour. This malady, as is well known, is exceedingly prevalent in southern countries, and causes great loss to the agricultural interest every year. Kramer examined nine various species, all aerobic and liquefying gelatin. The first seven of them he named *Bacillus saprogenes vini I.–VII.*, and the other two *Micrococcus saprogenes vini I. and II.* Details and experiments to prove whether these species are capable of producing loss of colour in sound wines are still wanting, and consequently the *Schizomycetes* in question possess a merely morphological interest The actively motile *Bacillus saprogenes vini I.* which is found in nearly every sample examined, is probably identical with Pasteur's "*Bacillus du vin tourné.*" It attains a breadth of 1 μ and a length of 2 5–6 μ, and bands composed of two or three cells are not rare. *Bacillus sapr v. III and VI* form endospores, and the cells of *Micrococcus saprogenes vini II* have a diameter of 1–1 4 μ. A pure culture of a bacillus, which, however, was recognised as innocuous, was obtained, from Italian wine suffering from loss of colour, by J. GALEAZZI (I.) in 1894.

These remarks sum up all that has hitherto been discovered by fermentation physiologists respecting the loss of colour in wines. Consequently, knowledge of the subject is still only in a very early stage, and we can only hope that future researches will succeed in affording us further enlightenment. This wine malady is so diversified in its mode of development and so changeable in its course, that we are obliged to ascribe it to a very fine example of metabiosis, i e that a single bacterial species is insufficient to occasion the complaint, the successive action of

a number of species being essential. In fact, the number of decomposable constituents in unaltered sound wine is so great as to preclude the possibility of a single species effecting all the changes involved. Consequently, investigations on this point will need to be carried out to a somewhat comprehensive scale. Several purely chemical researches into the changes produced were made by J. König, and abstracts of them are given in BABO and MACH'S (I.) "Handbuch des Weinbaues" (Handbook of Viticulture). Similar researches should now be made with pure cultures of bacteria isolated from wines that have lost their colour, and such researches should also include the examination of the changes produced by the different species of these organisms, in each of the most important constituents of wine.

This malady is also known as the putrefactive fermentation or "decaying" of wine, from the final condition attained by the liquid. Wines rich in albumen, e.g. even the Hungarian red wines, according to M. PREYSS (I.), are found to have a special tendency to loss of colour. In order to understand why southern wines are so prone to this malady, it is necessary to recall the fact—already mentioned in previous chapters, and first quantitatively investigated by N. SIEBER (I.)—that putrefaction does not ensue in strongly acid liquids, whereas these wines are *poor in acid*. FONSECA and CHIAROMONTE (I) recommended the addition of citric acid to increase their power of resisting the complaint. The destruction of the acids of wine must therefore precede its final putrefactive fermentation; hence the primary object of research must be the discovery of the changes produced in these acids. Here, again, everything still remains to be done, since all the information at present available is derived almost exclusively from experiments in which pure cultures were not employed.

According to the discoveries of PASTEUR (IX.) and A. FITZ (IV.), tartaric acid (in the form of its calcium salt) can be decomposed by bacterial agency in three ways: viz., either into propionic acid (along with a little acetic acid), to butyric acid; or, finally, to acetic acid, small quantities of ethyl alcohol, succinic acid, and butyric acid being also produced. Malic acid also may yield very different fermentation products, among which BÉCHAMP (IV) mentions acetic acid, propionic acid, butyric acid, carbon dioxide, and hydrogen. According to the researches of A. FITZ (IV.), malic acid (combined with lime) may be split up by different species of ferments in three different ways. In the first case, succinic acid, acetic acid, and carbon dioxide are formed, the relative proportions being approximately represented by the equation

$$3COOH—CH_2—CH\ OH—COOH = 2COOH—CH_2—CH_2—COOH +$$
$$CH_3—COOH + 2CO_2 + H_2O$$
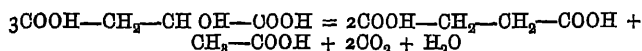
In a second case, propionic acid, acetic acid, and carbon dioxide may be found; or, thirdly, butyric acid may be the chief product, along with a small quantity of carbon dioxide. With regard to succinic acid, BÉCHAMP (V.) asserts that this also may be split up (by a bacterial mixture not more precisely specified) into propionic acid and carbon dioxide, the following equation—

$$COOH—CH_2—CH_2—COOH = CH_3—CH_2—COOH + CO_2.$$
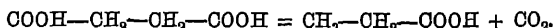
approximately expressing the reaction. The succeeding homologue of this acid, viz., pyrotartaric acid, breaks up, under similar conditions, into carbon dioxide and methane, according to the equation—

$$2COOH—CH_2—CH.CH_3—COOH + 2H_2O = 5CH_4 + 5CO_2.$$

According to the researches of Fitz, citric acid is converted, by an unspecified bacterial mixture, into acetic acid and small quantities of ethyl alcohol and

succinic acid. A series of experiments on the behaviour of fifty-two specie
bacteria towards twenty-one different organic acids was performed by
MAASSEN (I.), principally on medical grounds. One result of this research ·
the discovery of a new characteristic—valuable in the bacteriological analysi
water—for the differentiation of *Bacillus typhi abdominalis* from *Bacterium*
commune—viz., *tricarballylic acid*, COOH—CH₂—CH.COOH—CH₂—COC
which is attacked and partly destroyed by the first-named organism, but is]
altogether untouched by the second.

More minute investigations into the fermentation of the above-named orga
acids would be of value, not only in solving the preliminary questions involved
the study of loss of colour in wine, but also in connection with the **decrease**
the acidity of wines and fruit wines during storage, a phenomenon well kno
in practice and one quantitatively examined by PAUL BEHREND (I.) and
P. KULISCH (I.). This decrease—so long as it remains within narrow limits
is looked upon with favour, as contributing to the rounding and improvement
the flavour of the maturing wine. If, however, it proceeds too far and t
acidity falls too low, then a proportionate decrease in the power of the bevera
to withstand disease (especially loss of colour) ensues. This fermentation of t
acids is, as already stated, principally effected by fission fungi, on which poin
few particulars have been given by MÜLLER-THURGAU (V.). To a small exte
these acids are consumed by the yeast in the primary fermentation, so that t
quantity present in the young wine is less than in the must. Consequently,
the total acidity in the former is found greater than that of the fresh grape-ju
and fruit-must, the excess is due to the carbonic acid held in solution.

Grapes from vines infested with mildew, whereby both development a
sugar formation are retarded, yield wine poor in alcohol and consequently of l
resisting power. Such wine frequently becomes diseased, and is then known
France as *vin mildiousé* Here again bacterial agency is at work, the rod-shap
organisms forming many-jointed chains and reproducing so abundantly, th
they finally accumulate as a thick sediment. U. GAYON (II.) regards this mala
as identical with that causing the loss of colour, because he identified in vi
mildiousés the same volatile acids (acetic and propionic acids) as have be
discovered by others, e g. E DUCLAUX (XII), in *vins tournés*.

The **mannitic fermentation of wine**, which presents a certain oppositeness
character to the malady known as loss of colour, will be described now, becau
otherwise no suitable occasion would arise. This complaint does not wait
attack the finished wine, but even makes its appearance at the stage of prima
fermentation. If the surrounding temperature keeps above 30° C., th
alcoholic fermentation is confined within narrow limits, and an opportunity
thus afforded for the development of certain species of bacteria which conve
the sugar of the must into mannite. Of this hexatomic alcohol there will
produced, according to circumstances, from 1 to 30 grams per litre of wine,
addition to a little acetic acid. A knowledge of this fact is useful to the analytic
chemist as well. Attention was first drawn to the mannite content, of Algeri
wines in particular, by P. CARLES (II.) in 1891. Figs, as is well known, ve
often contain considerable quantities of this alcohol; hence Carles thought th
the presence of mannite in any wine indicated adulteration by fig-wine. Ho
ever, as reported by J. BEHRENS (IV.), the presence of mannite in reliably pu
natural wines (e g. Bordeaux, Château-Yquém) had been proved a year previous
by Portes and Lafauric; and very soon afterwards JÉGON (I) showed that
wines of reliable purity, but imperfectly fermented, as much as eight grams
mannite could be found per litre. L. ROOS (I.) then proved that this result w
due to bacterial activity, a discovery confirmed by U. GAYON and E DUBOURG (I
who isolated from such wine a pure culture of a non-motile short-rod fissi

fungus, capable of converting sugar into mannite (up to 50 grams per litre). In nutrient solutions devoid of sugar this species fails to develop, a circumstance sufficient to distinguish it from the bacteria (presumably) causing the loss of colour of wine. Moreover, these latter—as already observed by Mulder—attack cream of tartar first of all, whilst the bacteria of mannitic fermentation leave this salt completely untouched. The fact, now firmly established, that a high temperature (36° C or over) favours the appearance of the last-named microbes, explains the defective fermentation (familiar to Sicilian and Algerian wine-growers) of wine must during the prevalence of the hot south wine (sirocco or simoom), the red wines, in particular, being greatly affected According to G. BASILE (I), this wine disease is as frequent in Sicily as it is dreaded, and in some years affects the greater part of the vintage.

The bacteria here coming into action can be destroyed by heating up to 60° C , a treatment impossible to apply hitherto on account of its fatal effect on yeast-cells However, by artificially inoculating (pitching) with strong, pure yeast, and by cooling the mash down to 15°–20° C., the liquid could be rapidly brought into a state of alcoholic fermentation, which could be controlled by suitably regulating the temperature. In this way the desired result would be ensured, and would amply repay the increased outlay required. In this connection the experience gained by M. RIETSCH and M. HERSELIN (I) should also be borne in mind, viz , that the injurious influence of an excessively high temperature (36° C) can be reduced by aerating the fermenting liquid.

In conclusion, it will be useful to remember that mannite is also formed during the mucinous fermentation of sugar, and that this hexavalent alcohol is also excreted as a metabolic product by certain *Eumycetes, e.g. Penicillium glaucum.*

CHAPTER XXXI.

THE FERMENTATION OF CHEESE, AND ALLIED DECOMPOSITIONS.

§ 175.—The Composition of Ripe Cheese.

THE conversion of the fresh curd into finished cheese is termed ripening. We will, in the first place, consider this process from the purely chemical side. It was explained in § 144 that fresh curd can be obtained from milk in two different ways, either by precipitating with acids or by setting with rennet. In both cases nitrogenous compounds are present in the coagulum, only in the acid curd ("Quark") they consist of casein, and in the rennet curd ("Bruch") of paracasein. The cheeses obtained from the acid coagulum are, with the sole exception of "Glarner Schabziger," of inferior quality, and only suitable for early consumption. These have hitherto received but little attention from fermentation physiologists; consequently the following particulars are restricted to cheeses obtained from rennet coagulum, which to emphasise the point once more—contain only one nitrogenous compound, namely, paracasein.

In contrast to this uniformity stands the variety of the nitrogenous compounds present in ripe cheese. The first observation on this point was made in 1818 by J. L. PROUST (I.), who isolated leucine from ripe cheese. Some sixty years later (1880) N. SIEBER (II.) detected the presence of tyrosine in Roquefort cheese. Nevertheless, these discoveries, as also those of two French workers who will be mentioned later, were of a casual nature, the first thorough attempt to follow the ripening process in a quantitatively analytical manner being made in 1882 by A WEIDMANN (I.), whose results, especially as regards the qualitative composition of the cheese, were admirably supplemented by a later (1888) research undertaken by B. RÖSE and E. SCHULZE (I) These investigations were made in Switzerland on Emmenthal cheese, in which the above-named chemists discovered a considerable amount of leucine. On the other hand, comparatively little tyrosine was found, and other amido-compounds, as well as bodies of the xanthine group, were altogether lacking. The presence of ammonia, however, was readily proved, different samples being found to contain from 0.16–0.44 per cent., calculated to the dry weight The amount of nitrogen found in the form of ammonia, amido-acids, and other compounds distinct from albumen and peptone, in three kinds of this cheese, ranged between 1.22 and 1.48 per cent. , i.e. about one-fifth of the total quantity of this element present. Among the albuminoid constituents special mention must be made of caseo-glutin, a body allied to the peptones, and one that must be considered as among the chief products of the ripening process, since it constitutes 20 per cent , and even more, of the total dry matter. In addition, there was found (along with a small quantity of peptones) another albuminoid body, recognised as paracasein To the list of the constituents of ripe Emmenthal cheese still another unit phenylamidopropionic acid, was added in 1887 by the labours of F. BENECKE and E SCHULZE (I)

Several other kinds of cheese were included in the scope of these investigations. In harmony with the similarity existing between the methods employed in their preparation the qualitative composition of Spalen cheese was found to resemble that of the Emmenthal product. Peptone was also detected in Gruyère, Vacherin,

and Bellalay cheese, as also in Schabzig cheese; the latter, however, differs from the other kinds just named by not containing any ponderable quantity of caseo-glutin.

The comparative examination of the constitution of hard and soft cheeses was undertaken by BONDZYNSKI (I.)

If cheese be allowed to become over-ripe, then the percentage of albuminoids falls off still more. Thus, A. MAGGIORA (II) found in a sample of over-ripe Stracchino (Gorgonzola) cheese only one-seventh of the initial nitrogen in the form of protein, the remaining six-sevenths being in the form of amido- and ammonia-compounds.

We will now briefly consider the amount of fatty-matter in cheese. When whole-milk is set for cheese, the whole of the fat passes into the coagulum, which then contains almost as much fatty matter as albuminoids, the former constituting about 45 per cent. of the total dry matter of the curd. So far as the observations hitherto made extend, it would appear that the fat suffers no great alteration, whether of quality or quantity, during the ripening process. Bacteriologically exact investigations are, however, still wanting. Reference has already been made in § 120 to the influence exerted on the fat by light and air, and this influence also makes itself felt during the ripening of the cheese. The saponification of the glycerides mentioned in the said paragraph occurs in cheese to a still greater extent than in butter. The course of this operation has been traced by E. DUCLAUX (VI), who found that, in one instance, about one-third of the glycerin butyrate originally present was broken up into its two components.

The conversion of albumen into fat, the development of which question has been reviewed by S. SOSKIN (I) in a prize essay, is not only of the greatest importance in the study of chemical alterations in the animal body, but also comes under consideration in the ripening of cheese. BLONDEAU (I.), in 1864, was the first to remark, in his researchs on Roquefort cheese, that in this process fat was produced at the expense of albumen. The same has also lately been asserted by H. JACOBSTHAL (I), but was denied by the majority of subsequent workers, e g. inter alia, BRASSIER (I.) in 1865, N SIEBER (II) and O KELLNER (I.) in 1880. NÄGELI and O LOEW (I), however, proved beyond doubt, in the case of certain Eumycetes, that lower fungi are able to convert albumen into fat. The above-cited researches of Weidmann show that no remarkable quantitative increase of fat occurs in the ripening of cheese, but this does not disprove the possibility of the formation of fat from albumen during the process. On the other hand, G. Musso and A. Menozzi, on the basis of their researches on Stracchino cheese, believe that such a formation of fat must be assumed to occur.

The probability of such a conversion of albumen into fat cannot be rejected if we recall another process—very different, it is true, from an æsthetic standpoint, but, nevertheless, very similar from a chemical and bacteriological point of view—namely, the formation of adipocere Fatty concretions, which in many cases can only have originated in albuminoids (muscular substance, &c.), are frequently found in bodies which have undergone decomposition in the grave This question, which chiefly concerns the medical profession, we need not dwell upon. Various proofs will be found in a treatise on this subject by ERMANN (I.).

§ 176.—E. Duclaux' Studies on Cantal Cheese.

As ripenening progresses, the amount of the aforesaid amido-compounds continually increases, whilst the paracasein concurrently decreases. This transformation may be due to two causes: one entirely chemical, the other physiological

The earliest worker who believed the ripening of cheese to be due to microbia activity was FERD. COHN (II.), who, in 1875—from the researches by which he controverted the hypotheses of Bastian on spontaneous generation—arrived a

the following conclusion .—"The ripening of cheese I hold to be a true fermen tation." This fermentation he ascribed to the organisms ("lab-bacilli") present in the rennet liquid, and which he associated with the bacteria then grouped under the name *Bacillus subtilis*, a general term not to be confounded with that at present applied to an entirely distinct species. Cohn's decision was based exclusively on the microscopical examination of rennet and cheese; and the same applies also to the statements of F. BENECKE (I)

It was not until 1878, however, that attempts were made to obtain pure cultures of the presumptive cause of the ripening of cheese, and to test the influence of the organism. This was effected by E. DUCLAUX (VII. and XIII) in his studies on Cantal cheese, from which he isolated ten species of *Schizomycetes* and classified these under the common generic name of *Tyrothrix* belonging to the large sub-group of the so-called hay and potato bacilli. Out of these ten species, one, *T. virgula*, being unable to grow in milk, will be omitted from further considera-tion. Each of the remaining nine produces *two* classes of enzymes, one resembling lab and coagulating milk, whilst the other, casease (§ 147), dissolves and splits up the albumin thus precipitated. This proteolytic enzyme can be thrown down from the bacterial cultures by means of alcohol. A particularly abundant yield is obtained from *Tyrothrix tenuis*, an actively motile, sporogenic rod about 0.6 μ broad and 3 μ long, and often growing in the form of filaments; hence the name Tyrothrix (= cheese-thread, cheese-hair). This species is aerobic, as are also *T. filiformis*, *T. distortus*, *T. geniculatus*, *T. turgidus*, and *T. scaber*, whilst *T. urocephalum*, *T. claviformis*, and *T. catenula* are, on the other hand, anaerobic Cultures of *Tyrothrix tenuis* obtained from Duclaux' laboratory were investigated in 1895 by W. WINKLER (I.), who formed the opinion that this species can be modified, by cultivation, into three varieties or races. This was, however, contradicted by J. WITTLIN (I.) in 1896.

The metabolic products, e g. leucine, tyrosine, and the ammonia salts of acetic, valeric, and carbonic acids yielded by the *Tyrothrix* species are the identical substances we have seen to be produced in the ripening of cheese. This concordance necessarily strengthens Cohn's hypothesis, that the ripening of cheese is effected by the vital activity of micro-organisms. On this point, how·ever, Duclaux was unable to afford any certain proof. Later workers attempted to arrive by various ways at a solution of this highly important matter, and mostly by endeavouring to ascertain whether the ripening of cheese could be accomplished in the absence of any fermentative organisms. F. SCHAFFER and St BONDZYNSKI (I.) showed that curd prepared from boiled milk does not ripen; and, according to FREUDENREICH (III.), the same applies equally to Pasteurised milk Moreover, L. ADAMETZ (VI) found that neither does ripening occur when bactericidal substances, such as thymol or creoline, are added to the fresh curd; and the same result was attained by L. PAMMEL (I) by the use of hydrogen peroxide

§ 177.—Changes in the Bacterial Flora of Ripening Cheese.

L. Adametz also attempted to ascertain the active cause of the ripening process in a new way, namely, by tracing the quantitative and qualitative alterations occurring in the bacterial content of the ripening curd These researches—made on a Sornthal (Switzerland) soft "household" cheese, in addition to Emmenthal cheese—led to the following results·—

(1) The freshly precipitated curd, moulded in the press and freed from excess of whey, contains between 90,000 and 140,000 bacteria per 1 gram, a comparatively large number of these being able to liquefy gelatin, and conse-quently excreting a peptic ferment.

(2) During the period of ripening, the germ content gradually rose to 850,000 in Emmenthal cheese and up to 5,600,000 in the "household" cheese, but only a small share in this increase fell to the liquefactive species, since whilst the quantitative ratio of these to the other (non-liquefactive) kinds was in the fresh curd 1 : 40, only one colony of liquefactive bacteria was found in 150 to 180 in the gelatin plate cultures prepared from the ripe cheese

The expectation of finding the liquefactive bacteria assume the upper hand during the ripening process was thus dispelled, and the same result was attained in a later research published by E. VON FREUDENREICH (IV) in 1894, according to whom the number of lactic acid bacteria in cheese increases with the age of the latter. Hence the chief, if not the sole, share in the ripening of Emmenthal cheese must be ascribed to these lactic ferments   E. J. LLOYD (I) also came to the same opinion in his researches on the ripening of Cheddar cheese.

The reason why Duclaux, in his earlier investigations, arrived at a contrary result will be readily understood when it is remembered that he prepared his pure cultures exclusively by the dilution method, and therefore by the aid of liquid nutrient media (bouillon in particular), since in this last-named liquid the organisms of the genus *Tyrothrix* thrive exceedingly, whereas the lactic acid bacteria grow badly, if at all, therein. Consequently mixed sowings in this medium result in a preponderance of the liquefactive species described by Duclaux. From the discovery made by FREUDENREICH and SCHAFFER (I) that the ripening of hard Swiss cheese also goes on uniformly throughout the mass when air is excluded, it follows that the said lactic acid bacteria are (facultatively) anaerobic.

The harmonious results of the researches of M. LANG and FREUDENREICH (I,) with Swiss, and of E. MARCHAL (III) with Belgian (Limburg), samples show that a principal part in the ripening of soft cheese is taken by *Oidium lactis*, further particulars of which member of the *Eumycetes* group will be found in the second volume. Various budding fungi also seem to aid in the process, but more detailed information on this point is absent   According to the statements of JUL. HENRICI (I.), Swiss cheeses are poor in yeast-like fungi and rich in bacteria; but the converse ratio has been shown to exist in American cheeses.

Odour is one of the characteristics peculiar to individual kinds of cheese; it is but slightly developed in many, but is prominent in others. In isolated instances it is produced by the mechanical incorporation of added flavouring substances to the fresh cheese mass. This applies, for instance, to the already mentioned " Glarner Schabziger " or herb-cheese (*Krauter Kase*), which owes its characteristic aroma to the addition of dried *Melilotus cærulea* (blue or Swiss melilot). The English " sage-cheese " and American " clover-cheese" may also be mentioned as examples.

In many cases the odorous principle is, on the other hand, produced spontaneously in ripening, i.e. by the activity of micro-organisms, of which nothing is as yet definitely known. L. PAMMEL (II.) discovered on cabbage leaves a *Bacillus aromaticus* which, when inoculated into fresh curd, produces during the ripening process an aroma similar to that of " clover-cheese."

§ 178.—Pure Culture Ferments.

The results briefly recorded in the preceding paragraph must be regarded as first steps inspiring us to further progress towards the goal of all methods relating to the practical application of Fermentation Physiology, viz, the establishment of control over the progress of fermentation   In the matter of cheese-making the attainment of this desire is still remote, and Mycologists are not yet able to recommend this or that particular microbe with any assurance of

success. On the contrary, practice has also in this respect taken the lead by employing in special cases such additions as, without being pure cultures (in a bacteriological sense), nevertheless contain a predominating proportion of the organism most suitable for the object in view. One of the two classes of cheese to which this applies is the Roquefort, the other being Edam cheese.

Any one examining for the first time the said French cheese (originally prepared in the village of Roquefort (Dep. Aveyron), from unskimmed sheep's milk) will notice the green growth of mould occupying all the cracks abundantly intersecting this brittle, sharp-flavoured mass. This filamentous fungus, whose presence is by no means a sign of unsound composition, has been shown to be the organism known as *Penicillium glaucum* (described in vol. ii.), which settles in the fissured cheese mass and there consumes the acid which has been produced by the lactic acid bacteria and is retarding the development of the albumin-degrading organisms. The favourable influence of this thread fungus is so indubitably established by experience, that the practice is now common to sow it purposely in the fresh cheese mass. To this end bread is allowed to become covered with a luxuriant growth of mould, and is then dried and ground, the resulting powder (rich in mould spores) being then strewn between the separate layers of the sliced curd. In order to favour the development of this aerobic assistant, some 60 to 100 fine holes are pierced by a needle in each cylinder of ripening cheese.

The coatings of mould appearing and tolerated in Gorgonzola, Brie, and Stilton cheese seem to have a similar action. In other cheeses, again, such as Emmenthal and Gouda cheese, the formation of a mould coating in the ripening cheese is prevented as far as possible, since it would unfavourably influence their specific flavour. For this purpose the surface of the cheese is repeatedly wiped over with salt water or strewn with dry salt. A comparison of the surface of Gouda cheese with that of Brie cheese will show the remarkable difference between them

In the above-mentioned instance a *thread fungus* is employed, whereas in the case of Edam cheese a *bacterium* is mixed with the milk to be made into cheese. This is the *Streptococcus hollandicus*, whose acquaintance we have already made in § 163, as a microbe capable of making milk or whey ropy. It is employed by adding 2 per cent. by volume of ropy whey to the milk to be set for cheese

§ 179.—Natto and Miso.

The process of fermentation known as the ripening of cheese both improves the flavour and increases the digestibility of the albuminoids by degrading them into more readily assimilable products. On this account the ripening of cheese might be termed a preliminary digestion of the casein.

Similar to fresh whole-milk curd in the nature and proportional ratio of its chief constituents is the **Soja bean,** i e. the seed which replaces a meat diet among the natives of Eastern Asia. This bean was first introduced into Europe at the Vienna Universal Exhibition in 1873, and was shortly afterwards more minutely described by FR. HABERLANDT (I). It contains 35–40 per cent of albuminoids and about 15 per cent. of fat; and, consequently, a dish of soja beans prepared in the ordinary manner forms a heavy, tough food-stuff How-ever, it can be made more attractive to the palate, and better suitable to the stomach, by boiling the beans for five hours in salt water, then forming the mass into balls from 4 to 18 ounces in weight, packing these in straw, and leaving them in a warmed cellar for a few days. Under these conditions they undergo a fermentation which loosens the cellular tissue, and effects a partial conversion of the protein into amides, peptones, guanine, xanthine, and tyrosine. The

mass is then sold (in Japan) under the name of natto. The nature of the species of bacteria taking part in this fermentation has been studied by K. YABE (I.) and O. LOEW (IV.).

In the preparation of the second kind of vegetable cheese, viz., **Miso**, recourse is had to a substance known as **Koji** (described in vol. ii.), which is added to the boiled bean pulp before allowing the latter to ferment. Full particulars respecting the production of this and several other Japanese articles of diet (e g **Shojou** prepared from soja beans) have been published by O KELLNER (II.), and a few details of the last-named sauce, also highly appreciated in England under the name of soy or shoyn, have been furnished by A. BÉLOHOUBEK (I.). For the preparation of **Tofu** and **Nukamiso** reference should be made to two treatises by M. INOUYE (I. and II.); and H. C. PRINSEN-GEERLIGS (I.) has reported, *inter alia*, on the preparation (by the aid of fungoid ferments) of other dishes from soja beans in Chinese cookery, such as **Taohu** or bean-cheese, the sauce **Tao-yu,** &c.

§ 180.—The Normal Pitting of Cheese.

The ripening process does not always progress satisfactorily, but very often results in a defective, or spoiled, inferior, or quite unsaleable product. Thus E. VON FREUDENREICH (V.) reports that about 40 per cent. of Emmenthal cheeses ripen imperfectly. The pecuniary loss accruing to cheese-makers from this cause is estimated to amount, in Switzerland alone, to about a quarter of a million of francs (£10,000) per annum.

The defects here in question are of various kinds. L. ADAMETZ (III.), in his comprehensive monograph on the subject, enumerates the following — (1) Defects caused by the unfavourable constitution of the milk employed (2) Inflation ("blown cheese"). (3) Bitter cheese. (4) Discolorations. (5) Poisonous cheese. Of these, the first named is beyond the scope of the present work, the fourth has already been discussed in §§ 89, 95, and 98, and the fifth in § 171. More frequent, however, than any of these is the malady known as inflation, puffiness, or "blown" cheese (Fr. *boursouflement*, Ger. *Blähen*), which will now be briefly mentioned.

In addition to the constituents detailed in § 144, the curd from sweet milk contains a certain quantity of lactose. This is dissolved in the whey remaining in the coagulum, and which cannot entirely be removed by pressing Consequently, sugar is always present, and is decomposed in various ways by the organisms existing in the curd. Some of them, for example, form lactic acid, whilst others consume it and liberate an abundance of gas. This causes holes (bubbles or eyes) in the mass of the ripening cheese, and these holes manifest themselves as *pittings* in the cut suface of the ripe product Both the dimensions of these holes and the manner of their distribution throughout the mass are very characteristically developed in individual classes of cheese. In order to render this clear by examples, reference may be made to two main types, **Emmenthal** cheese on the one hand, and **Edam** cheese on the other, the former exhibiting a few holes of large size, and the latter a greater number, but of small dimensions. The appearance of small holes, regarded as indispensable in the said Dutch fatty cheese, is looked upon as a defect in the Swiss cheese, which, if it contains many holes of small size, is characterised as "Nissler," and considered as inferior. Still, the other extreme of immoderately large cavities is also undesirable. This last condition reveals itself, in the course of its development, by the bulging of the surface of the ripening cheese, which, in particularly bad cases, is even split open. This malady, known as inflation or puffiness, has already formed the subject of several investigations, as a result of which both the cause of the

complaint and the means of preventing and combating it have been dis-covered

§ 181.—The Cause of Puffy ("Blown") Cheese.

The naturally obvious hypothesis that pitting is due to the gas-producing powers of microbes was experimentally confirmed by H WEIGMANN (VIII.) in 1890. Undoubted though it be that inflation is also a result of microbial activity, it is, nevertheless, equally undecided whether the malady is caused by specific ferments, or only differs from normal pitting in degree, *i.e.* arises from the same cause.

Of the two possible methods of explanation here indicated, the *first* is championed by ADAMETZ and FREUDENREICH (VI) in particular. The latter in 1890 established, in the case of three species of bacteria—recognised as setting up inflammation of the udder in cows, and named *Bacillus Guillebeau, a, b, c,* after their discoverer—that when inoculated into fresh curd they produce inflation and bad flavour. The fermentative activity and products of these three microbes were minutely examined by A. MACFAYDEN (I.) and L. NENCKI (I.). The gas evolved by *B. G. c* is a mixture of carbon dioxide and hydrogen, their relative proportions at the climax of fermentation being about 76 23, but afterwards becoming so far modified that, at the close of fermentation, only 0.72 per cent by volume of hydrogen is found in the gas. To these three injurious organisms (pathogenic in cows and goats) a fourth was soon afterwards added by FREUDENREICH (VII.), viz., *Bacillus Schafferi* (bacteriologically very similar to *Bacterium coli commune*) which was first obtained as a pure culture from "puffy" cheese, and subsequently also found in "Nissler" cheese. Freudenreich consequently regarded this bacillus as the cause of both these maladies in cheese, and explained its dual manner of working by stating that puffiness is produced when the fresh cheese curd contains comparatively few colonies of this bacillus, at considerable distances asunder, but of large size, and therefore capable of liberating much gas, whereas, on the other hand, "Nissler" cheese results when the microbe is distributed abundantly, as individual cells, throughout the ripening mass. ADAMETZ (VII.) found in the milk and cheese of a Sornthal dairy a fission fungus which he named *Micrococcus Sornthalii,* and which proved capable of causing puffiness in cheese. This microbe is shown in Plate I. Fig. 1.

The *second* method is founded on a different basis. Whereas Freudenreich devoted his attention to the discovery of specific inflation ferments, FR BAUMANN (I), on the other hand, endeavoured to ascertain the external conditions under which a microbe producing the normal pitting of cheese would become the cause of inflation. An example of this is afforded by the *Bacillus diatrypeticus casei,* discovered by him. This organism, when sparsely inoculated in curd prepared from Pasteurised milk, produces a "blind" cheese, *i.e.* one containing merely a few holes; but when it is added in large amount, it gives rise to puffiness. This facultatively anaerobic fission fungus is a non-motile capsule bacillus generally 1.5 μ in length and 0.7 μ in breadth; a photographic representation is given in Plate I. Fig. 2. In media containing sugar it liberates a gas chiefly consisting of carbon dioxide along with a not inconsiderable portion of hydrogen; and in addition to these products alcohol and lactic acid are formed.

If this bacillus be inoculated into fresh curd prepared from non-Pasteurised milk, and consequently rich in a variety of bacteria, a struggle ensues between them. In presence of a superior force of species that do not generate gas, the *B. diatrypeticus casei* is suppressed, and "blind" cheese will result On the other hand, when the restrictive power of the other organisms is merely sufficient to moderate, but not prevent, the development (and consequently gas-producing

power) of our bacillus, then the pitting will be normal; whilst, if finally these adverse influences be almost entirely lacking, "puffy" cheese results. No rejection of this explanation is implied by the mention of the error into which its author has fallen in attributing to his bacillus *alone* the capacity of producing pitting—the inaccuracy of which assumption has been noted, *inter alia*, by ADAMETZ (VIII.).

§ 182.—Cheese-makers' Recipes.

In the light of Baumann's discovery, the reason for a number of (apparently pedantic) rules current among cheese-makers both for the production and subsequent treatment of the curd is made clear. The careful adherence to certain temperatures during and after setting; the time of exposure to their influence; the nature of the mechanical treatment, and even the extent of the pressure applied to the moulded curd in the press,—all these conduce to the result—the cause of which is certainly unknown to the operators—that a certain bacterial species attains pre-eminence.

The researches published by FR. SCHAFFER (III.) and FREUDENREICH (VIII.) in 1895 tend to elucidate the processes occurring during the after-warming of the curd. As has already been indicated, the freshly precipitated curd is kept for a short time at a certain constant temperature, the degree and duration of exposure varying in different kinds of cheese. In the case of Emmenthal cheese, Schaffer showed that a merely gentle after-warming of the curd results in a quicker and more perfect ripening, so that the finished cheese contains a large proportion of products formed by the decomposition of albumin. Freudenreich investigated this condition from a bacteriological point of view, and explained the fact already recorded in § 177—viz., that the ripening of hard cheese is almost exclusively brought about by bacteria, whilst that of soft cheese is chiefly occasioned by higher fungi (budding fungi, oïdium)—as due to the inferior powers of resistance to high temperatures exhibited by the latter organisms. Thus the cheese-maker's old rule that "curd for soft cheese should be only gently warmed" is shown to be well founded.

Certain prescriptive methods of preparation for a large number of different kinds of cheese have been gradually built up as the outcome of practical experience. The possibility of such a result is a proof that the bacterial species necessary in the ripening of cheese are present everywhere and at all times. The exact observance of these recipes can, however, only continue to indefinitely yield uniform results where the composition of the bacterial flora of the milk set for cheese undergoes merely unimportant fluctuations. This is the case in the Alpine dairies, where the grazing grounds seldom, if ever, receive any application of manure from external sources, and consequently the same species of bacteria are continually returned to the ground anew in the dung of the grazing cattle. For this reason the cheese-dairying industry necessarily developed first in the High Alps, since there the business is, in a bacteriological sense, exposed to the minimum of danger. The case is different in the lowlands, where the cows are fed with fodder of highly diversified origin, frequently consisting of the residues of agricultural industries (grains, distillery residue, grape skins, &c.). The bacterial flora of the dung of such animals will be liable to frequent changes; and since the bacterial content in the milk is for the most part derived from the dung, it will be evident that the cheese-making process will be affected by this change. Greater difficulties consequently attend the pursuit of the industry in lowland districts, and much less reliance can be placed on recipes. To explain this more clearly by an example, reference may be made to an observation which chemists have been unable to explain, but which

—regarded from a bacteriological standpoint—seems almost self-evident, vi the difficulty experienced in the working up of milk at such times of the ye as a change is made from dry to green fodder, and *vice versâ*. The bacter flora of fresh grass is of a much more diversified character than that on d hay ; only a few species remaining alive and capable of development in t latter.

§ 183.—Counteracting Puffiness in Cheese.

The reader will now probably inquire whether any method exists where milk that will produce puffy cheese may be recognised as dangerous before is worked up and rejected by the cheese-maker   This course will be advisal when the gas-forming bacteria greatly preponderate, a condition ascertainal by the so-called fermentation test   A sample of the milk to be examined kept in a fermentation flask (§ 126) for twelve hours at 40° C., a conclusi based on experience being then formed as to its suitability or the rever: according to the changes occurring during this period.   Fuller particulars · this point will be found in Adametz's monograph, as also in the highly coi mendable text-book of W. FLEISCHMANN (I).

As a means of preventing the malady, FREUDENREICH (IX) recommends t addition of 3 per cent of common salt to the freshly precipitated curd, fre from the main bulk of the whey.   For restricting incipient puffiness, Adam« counsels setting the cheese to cool, since the ferment is found, by experience, be violent and injurious solely at higher temperatures.

From the results of an investigation made by H. L. BOLLEY and C.] HALL (I) it must be concluded that gas-forming bacteria are not present in mi at the moment it leaves the udder   If this observation is confirmed by renewe (highly desirable) researches in other places and under different conditions, ar thus become a general law, it will indicate the means of preventing puffiness, vi: by taking care to keep the fresh-drawn milk free from dirt and dung, which ai the vehicles by which the gas-forming bacteria are introduced.

§ 184.—Bitter Milk and Bitter Cheese.

According to a rule based on experience, and observed by all housewiv skilled in cookery, boiled milk must be stored in uncovered vessels, otherwi it is liable to turn bitter.   The attention of Pasteur was also directed to tl matter in the course of his studies on spontaneous generation.   We have alreai seen, in a previous section, that the French investigator here made the importa discovery that, though the lactic acid bacteria are thus destroyed, the more high resistant spores of butyric acid bacteria can withstand such a brief exposure boiling heat.   Now, since the majority of these latter are anaerobic, they ci then only manifest their activity provided the admission of oxygen is eith entirely prevented, or at least restricted, a condition ensured by covering t milk-pan with a lid.   There then gradually accumulates within the pan ; atmosphere of carbon dioxide, &c., produced by the vital activity of bacteria ai preventing the access of oxygen to the strongly fermenting milk.   The existen of this gaseous stratum can be detected by the sense of smell on carefully raisii the lid.

The bitter flavour developed in the milk was formerly ascribed—*e g*] R. KRUEGER (II.)—to the chief product formed by these bacteria, viz., buty: acid, until WEIGMANN (IX) in 1890 showed that no bitter taste is produced milk by the addition of butyric acid.   Like HUEPPE (VII.), he attributes t bitter flavour to the peptone formed from the albuminoids in milk   Later r searches on this point have led, in the main, to the same results.   Consequent

the subject falls within the present section, which deals with the decomposition of albumin.

Nageli was the first to attribute the development of bitter flavour in milk to bacterial activity, and since then many attempts have been made to find and prepare pure cultures of bacteria possessing such properties. Some of these are capable of producing bitter principles both in milk and cheese, whilst others are injurious solely to the former; but all liquefy gelatin, and consequently produce a peptic ferment.

Of the first group two species are known up to the present, viz., (1) *Tyrothrix geniculatus*—obtained as a pure culture by DUCLAUX (XIII.) from Cantal cheese, and already noticed in § 176—produces a bitter substance in both milk and soft cheese (2) *Micrococcus casei amari* was isolated to a pure culture by FREUDEN-REICH (X.) in 1894 from bitter, hard, Swiss cheese. This organism, which measures about 1 μ in diameter, is endowed with the somewhat rare dual property of forming lactic acid and liquefying gelatin. In milk and cheese—but not in bouillon—it gives rise to a strongly bitter flavour, which Freudenreich only partly ascribes to the peptone produced, since after the latter has been thrown down by alcohol from milk cultures of the coccus, the filtered liquid leaves, on evaporation, a bitter residue. Here the widespread experience of practical cheese-makers, viz., that the bitter taste generally makes its appearance at the stage of semi-ripeness, vanishing again as the cheese increases in age, may be mentioned

The capacity of developing a bitter flavour in milk alone is possessed by the following organisms.—1. Weigmann described in his above-named treatise a sporogenic bacillus, 1.5–1.8 μ long and o 9–1.1 μ broad, which does not produce gas, but gives rise to a casein-dissolving enzyme and a volatile acid (differing from butyric acid). 2. **The micrococcus of bitter milk**, of CONN (IV.), is aerobic and non-motile, forms butyric acid, and develops a repulsive bitter flavour in milk, cream, and butter. 3. M. BLEISCH (I) obtained from milk, which had become decomposed after sterilisation by the Neuhauss process, a pure culture of a facultatively anaerobic motile bacillus, whose endogenous spores were able to stand exposure for six hours at a temperature of 100° C. in milk. When inoculated into sterilised milk it produces a strongly bitter flavour, and must, from its behaviour towards casein, be grouped with Duclaux' *Tyrothrix* species. 4. *Bacillus liquefaciens lactis amari* was found by FREUDENREICH (X) in cream which had turned bitter spontaneously. The relative dimensions of this motile bacillus vary considerably the most usual measurements are o 5 μ for the breadth and 1.5 μ for the length, but the latter may extend to 6 μ. It induces coagulation in milk—which it also makes very bitter—but no formation of acid takes place; and it liquefies gelatin.

CHAPTER XXXII.

THE FERMENTATION OF UREA, URIC ACID, AND HIPPURIC ACID.

§ 185.—Urea, the Final Product of Animal Metabolism.

THE natural cycle pursued by the elements normally present in the vegetable or animal body is, with a few exceptions, very simple and easy to follow. Those resisting the action of fire, and therefore found in the ash—viz., K_2O, Na_2O, CaO, MgO, SiO_2, SO_3, P_2O_5, Fe_2O_3—are taken up by the plant from the soil (where they are generally present in sufficient quantity) and are returned thereto in manures. Hydrogen and oxygen are, in the form of water, always plentifully at hand. Carbon is absorbed from the air as carbon dioxide by the plant, and is given up again by the animal in the same form.

The natural circulation of **nitrogen** is much more complex. This element, which is indispensable for the construction of albuminoid substances, is an object of solicitude not only to the farmer, who balances the incomings and outgoings of his soil, but also to the bacteriologist, who carefully watches the changes of form nitrogen undergoes during its passage from the plant through the body of the animal and back to the earth, where it is again gradually enabled to renew the cycle.

Only a portion of the nitrogen consumed by man and animals in the food—chiefly in the form of albuminoids, but also as amido-compounds, &c.—and transformed and again excreted, leaves the body *viâ* the intestinal canal, *i.e.* in the fæces. This portion consists, on the one hand, of indigestible or undigested food constituents, and, on the other, of nitrogenous metabolic products, such, for example, as glycocholic acid and taurocholic acid from the bile, leucine and tyrosine from the gastric juices, &c. &c. The subsequent fate of these amido-compounds passing into the excrement has already been dealt with in § 168.

The residual nitrogen takes another route in order to make its exit from the body, namely, *viâ* the kidneys, and is excreted in the urine. The most important constituent of this substance is **urea**, but uric acid, hippuric acid, allantoin, &c., are also present, though in much smaller quantities. The qualitative content of these bodies differs in the various kinds of animals, whilst quantitatively they depend on the amount and composition of the food.

In human urine and that of the carnivorous mammalia, birds and reptiles—which has an acid reaction owing to the presence of acid sodium sulphate—uric acid is present to a greater extent than hippuric acid. The total amount voided by a man of normal size is :—

Urea	35–50 grams.
Uric acid	0 5– 75 gram
Hippuric acid	0 3 gram.

On the other hand, the urine of the herbivorous mammals and birds—which has an alkaline reaction owing to its content of $KHCO_3$—exhibits a different substantive ratio, uric acid being in very minute proportion ($\frac{1}{100}$th per cent.), whilst hippuric acid is comparatively plentiful, *e.g*, in cows' urine up to $\frac{1}{2}$ per

cent, in horses' urine up to 2 per cent. (in combination with lime). Urea is present to the extent of 2-5 per cent. in cows' urine, and 3 per cent. in that of the horse.

§ 186.—Urea Unassimilable by Higher Plants.

Assuming the total number of inhabitants in the world to be 1500 millions, and estimating the diurnal excretion of urea at an average of only 25 grams per individual, then there results a total daily production of 37,500 tons of urea by the human race alone. This quantity in the solid state would occupy a space of 50,000 cubic metres (65,400 cubic yards), and contain 17,000 tons of combined nitrogen The excretions of the animal kingdom must be estimated at a much higher figure.

The amount of nitrogen daily excreted in urine and passing into manure is consequently enormous, and the question naturally arises. What becomes of it afterwards? Is urea (and also uric acid, &c) adapted to serve immediately and directly for the nutrition of plants, and of cultivated plants in particular?

Agricultural practice answers this query by a decided negative, knowing from experience that manuring with fresh urine is at first either entirely useless or actually injurious. No satisfactory explanation of this fact has yet been discovered by scientific research. O. KELLNER (III.), for example, sought it in the circumstance that urea is not absorbed (retained) by the soil. We must therefore turn away from research, and fall back on the fact that urea is unsuitable for the nutrition of the higher plants, and that consequently its nitrogen, not being available for this purpose, is lost If this element is to continue its cycle in the organic world, it must first be converted into other forms and modes of combination ; and the question arises as to which of these involves the least labour and smallest expenditure of energy.

It must be borne in mind that urea is a derivative of carbon dioxide on the one hand and ammonia on the other—*i.e.* two compounds which are known to be suitable for the nutrition of plants—and may be regarded as a condensation product from these two atomic groups by dehydration. If their coherence can be loosened, and the carbamide split up by hydrolysis to form carbon dioxide and ammonia—

$$CO(NH_2)_2 + 2H_2O = CO_2 + (NH_4)_2O,$$
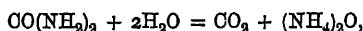
this prevents the danger of the said quantity of urea remaining undecomposed and accumulating, in consequence of which its nitrogen would be withheld from, instead of restored to, the vegetable kingdom

There is, however, no need to seek far for an instrument for this conversion, Nature herself having already provided the implements for this work in the micro-organisms known as urea bacteria. The next three paragraphs will be devoted to the consideration of their character and capabilities; a fourth dealing with the decomposition of uric acid and hippuric acid.

§ 187.—Discovery of the Urea-Ferment by Pasteur.

The natural process of the decomposition of urea was discovered some two years after the first successful attempt at the artificial preparation of this substance. The well-known fact that urine, which is clear when first voided by healthy individuals, becomes turbid on prolonged standing, while with increasing age it turns more and more alkaline, and exhibits an increasing smell of ammonia, found an explanation in 1830 at the hands of Dumas, who regarded this modification (the ammoniacal fermentation of urine) as a conversion of urea

into ammonium carbonate by the absorption of water. This hydrolysis of urea was considered as a purely chemical process, a readjustment of the atoms in the molecule.

It was reserved for PASTEUR (I.) in 1862 to show the incorrectness of this view. He discovered in fermented urine a micrococcus (0.8–1 0 μ in diameter, and frequently united as diplococci, tetrads, and chains) which is capable of inducing the change in question in sterilised urine. This organism was shortly afterwards (in 1864) also described by VAN TIEGHEM (VIII.), and called *Bacterium ureæ*, being subsequently named by Cohn *Micrococcus ureæ*.

The next researches on this important fermentation appeared in 1879, and afforded proof that this capacity for converting urea into ammonium carbonate is not restricted to one single species of microbe, but that, on the contrary, Pasteur's micrococcus has competitors, not only in many bacteria, but also in a few of the higher fungi. This work was performed by P. MIQUEL (V.), to whom we owe most of our present knowledge of the fermentation of urea. Before turning to his more recent labours on the subject, we will, however, briefly review the endeavours made by his colleagues in the same direction

R. VON JAKSCH (I.) in 1881 published a thoroughgoing investigation, the morphological part of which was also instrumental in founding the theory of bacterial pleomorphism; the physiological results will be given in the next paragraph The urine-bacterium discovered by him throve best in a liquid containing the following dissolved salts per litre of water. acid potassium phosphate, 0.12 gram, magnesium sulphate, 0.06 gram; Seignette salt, 5 grams; and urea, 3 grams. This liquid is known in the literature of the subject as Jaksch's nutrient solution.

LEUBE (I) in 1885 added four new species of bacteria to the group of urea ferments already known. One of them, called *Bacterium uricæ*, appeared in the form of plump rods, 2 μ long and 1 μ broad, and of the remaining three, one belongs to the sarcina group.

In contrast to the bacteria (forming solid colonies) mentioned above is the urea-fermenting micrococcus discovered by FLÜGGE (I.), and known from its liquefying influence on gelatin as *Micrococcus ureæ liquefaciens*.

The report drawn up by C. LUNDSTRÖM (I.) and R. CAMBIER (I) also made known a few new species of urea-fermenting bacteria, and the same applies to a research by R. BURRI, E. HERFELDT, and A. STUTZER (I), which we will deal with briefly below. We can now turn our attention to the above-mentioned newer

§ 188.—Researches of P. Miquel (VI.).

This author isolated from air, soil, liquid manure, water, &c., some sixty different species of bacteria, all of them capable of fermenting urea. Out of these he selected seventeen as particularly worthy of interest, and has more closely investigated and described them. Morphologically he distinguishes three genera, *Urobacillus, Urococcus, Urosarcina*. In the further subdivision within these three groups two principal factors of a chemico-physiological nature are adopted as criteria, viz., the rapidity of fermentation, *i.e.* the amount of urea fermented per unit of time, and on the other hand, the fermentative power, expressed by the maximum quantity of urea completely fermented by the species in question per unit volume of nutrient solution. These two indications acquire almost the character of mathematical constants for any determined species.

The most powerful as well as the most energetic is the *Urobacillus Pasteurii*, so frequent in both natural and drainage waters This organism ferments 3 grams of urea per hour in a 2 per cent. peptonised urea-bouillon, and

completes its task even when the nutrient solution contains 140 grams of urea
per litre. Morphologically similar to this, but differing greatly in physiological
character, is the *Urobacillus Freudenreichii*, found with particular frequency in
the sweepings of the streets of Paris. This bacillus can only hydrolise o 3 gram
of urea per hour, and cannot ferment a larger quantity than 45 grams per litre
of nutrient solution. It is an actively motile rod, 1 0–1.3 μ broad and of
variable length.

High degrees of speed and power of fermentation are not always found in
association. This is well exemplified by *Urobacillus Schutzenbergii*, a rod 1 μ
long and 0.5 μ broad, incapable of producing spores It was found by Miquel
both in natural and drainage waters, but never in the air. This species is very
energetic, *i.e.* transforms a large amount of urea per unit of time, but its activity
ceases as soon as the liquid has become somewhat enriched with ammonium
carbonate. That this is actually the cause of the cessation follows from the fact
that the fermentation proceeds further when this salt is removed by aerating the
medium.

To this injurious influence of ammonium carbonate—towards which different
degrees of susceptibility are exhibited by the various species—is due the rapid
dying of cultures of the bacteria under consideration, in liquids containing urea.
If it is desired to prolong their existence or to refresh debilitated cultures, they
must be transferred to nutrient media that are free from urea, and consequently
enable growth to proceed only at a slow rate, but, just for this reason, ensure a
longer life.

Even greater than their sensitiveness towards ammonium carbonate, of which
even the most delicate species can support a very considerable quantity, is the
susceptibility of the urea bacteria to the presence of free acid in the nutrient
medium. Burri and his collaborators ascertained (in their above-mentioned
researches) that 0.4 per cent. of sulphuric acid produces a fatal effect. This
observation can be practically utilised in the protection of stall manure from
early decomposition.

Into the remaining distinguishing characteristics of Miquel's urobacteria—
especially the degree of resistance to heat exhibited both by the vegetative forms
and the spores produced by all but the last-named species—we cannot enter
further. One common characteristic peculiar to the group must not, however,
be omitted from mention, and that is the aureole with which the colonies
surround themselves when grown on 2 per cent. urea-gelatin, and whereby they
are distinguishable from all other bacterial species, even at an early age, by
macroscopical examination or under a low power. The colonies are closely
surrounded, for a distance exceeding their diameter, with numerous biscuit-
shaped bodies, embedded in the gelatin and for the most part so close together
that they envelop the colony as in a cloud. These bodies are facetted crystals,
each composed of two combined globules, containing lime, carbonic acid, and
phosphoric acid, and formed by the reaction of ammonium carbonate (liberated
by the bacteria), on the salts of the alkaline earths contained in the medium.

Whether and how far the nitrogen in the urea contributes to the structure of
these bacteria is also an interesting point. Jaksch in his treatise asserted that
his bacillus preferentially takes up nitrogen from urea, other sources, such as
peptone, being less suitable; but Miquel came to exactly the opposite conclusion,
the species examined by him greatly preferring peptone, or any similar
substance, before urea as a source of nitrogen.

Urobacteria are of very frequent occurrence in nature. For quantitative
determinations on this point we are indebted to Miquel, according to whom the
average number of such bacteria in the air of Paris was (in 1891) 151 germs per
cubic metre. The smallest number (90) was found in autumn and the highest

in spring (197) and summer (202). The relative content of these in nat
waters increases with the degree of impurity   A good example is afforded
the Seine: before reaching Paris 103 urobacteria were found per 10,
microbes, whereas a sample taken within the city limits gave 204, or twice
many as before.   According to Miquel, 1–2 per cent. of the bacteria presen
the soil, and 15 per cent of those in cowhouse manure, are capable of hydro
ing urea.   It is therefore evident that Nature provides for the conversion
urea into a more readily assimilable compound, which we have already found
be essential

The ammoniacal fermentation of the urine of herbivorous domestic anin
(horses, horned cattle) begins soon after its evacuation from the body.   '
resulting ammonium carbonate partly volatilises, and thereby leads to a
of nitrogen, the extent of which—as A. MÜNTZ and A. GIRARD (I.) sho·
in 1893—was formerly under-estimated.   Out of the many methods propc
and attempted for the prevention of this loss and the combination of
ammonia, a few (e.g sulphuric acid) have proved unfit for application in
stall, and others (such as peat litter, gypsum, kainit) insufficient, only a sir
one being actually suitable   the **superphosphate** recommended by E. HEIDEN
in 1887, viz , the acid phosphate prepared by means of sulphuric acid, ·
capable not only of chemically fixing ammonia, but also, by reason of its ·
reaction, preventing the inception of uric fermentation.

§ 189.—Urase.

In 1874 MUSCULUS (I) expressed the opinion that the conversion of urea 1
ammonium carbonate is merely an indirect result of bacterial activity,
hydrolysis of the urea being effected not by the organisms themselves, but by
enzyme they excrete   This enzyme was said to be particularly plentiful in, ·
recoverable from, the urine of patients suffering from catarrh of the bladde
circumstance which would also account for the alkaline reaction of this ur
when in a freshly voided condition   This statement was investigated and c
firmed by PASTEUR and JOUBERT (II) in 1876.   On the other hand, the attem
made by LEUBE (I) to separate the enzyme from the bacteria by filtrat
through a clay cylinder failed   P MIQUEL (VII.) then showed, in 1890, t
these conflicting results are due to the extreme decomposability of this enzy
which he proposed to name **urase**, and which, being very easily oxidised, ou
to be filtered in an atmosphere devoid of oxygen, a precaution neglected
Leube   Urase decomposes in three to four hours at 50° C , and in a very
minutes at 80° C.   With regard to its chemical composition nothing is at pres
known , not even whether it is a single body or a mixture of several substar
(varying in constitution according to the conditions of fermentation)   (
thing, however, Miquel placed beyond doubt, viz , its capacity to hydrolyse ·
rapidly convert urea (in a sterilised solution) into ammonium carbonate.   I
therefore a true enzyme.

§ 190.—The Decomposition of Uric Acid and Hippuric Acid.

With regard to the disruption of the uric acid molecule by microbial ager
F. and L SESTINI (I.) instituted an investigation, according to which
decomposition corresponds to the equation—

$$\begin{matrix} NH-C-NH \\ \| \\ C=O \quad C-NH \\ | \\ NH-C=O \end{matrix} \bigg\rangle C=O + 8H_2O + 3O = 4\frac{NH_4}{H}\bigg\rangle CO_3 + CO_2$$
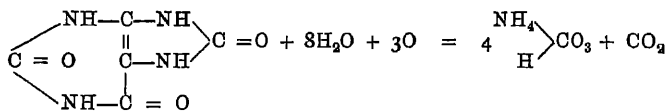
Unfortunately, pure cultures were not employed in this research, which was published in 1890, and the same defect attaches to an investigation made by E. GÉRARD (I.) in 1896.

In a chemical sense, the statement just recorded was confirmed by the treatise of Burri and his co-workers, mentioned in § 187. These workers also included the decomposition of hippuric acid in the scope of their labours. Like their Italian colleagues, however, they did not employ pure cultures of ferments, but used "a drop of manure drainings" for inoculating the media. They found that hippuric acid was not attacked *per se*, but only when in combination with lime, the decomposition, moreover, being more difficult to effect than was the case with uric acid or urea—which last named is the easiest of all to convert into ammonium carbonate. Both for the sake of completeness and also to show the necessity for a more accurate investigation of the decomposition of hippuric acid, we must refer to a remark made by Van Tieghem in his above-mentioned treatise, namely, that his *B. ureæ* is capable of splitting up hippuric acid into its two components, glycocoll and benzoic acid, according to the equation—

$$\underset{\text{COOH}}{\text{CH}_2}\ \underset{\text{C}_6\text{H}_5}{\text{NH—CO}} + \text{H}_2\text{O} = \underset{\text{COOH}}{\text{CH}_2 \cdot \text{NH}_2} + \underset{\text{C}_6\text{H}_5}{\text{COOH}}$$
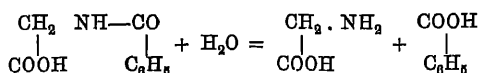
Analytical data to prove that this decomposition actually goes on so smoothly are, however, lacking.

So far as the destiny of hippuric acid in the soil is concerned, K. YOSHIMURA (I.) has observed that its fermentation proceeds much more rapidly in the upper layers than in the subsoil

CHAPTER XXXIII.

THE FIXATION OF FREE NITROGEN BY BACTERIA.

§ 191.—Accumulators and Consumers of Nitrogen.

IF seeds of any of the leguminous plants, *e g.* peas, lupins, clover, &c , be sown in a soil containing all the food-stuffs (K_2O, P_2O_5, &c), except nitrogen, necessary for the growth of plants, then, given sufficient moisture, germination will soon be observed At the outset the young plant develops just as well in the absence of nitrogen as if that substance were present in the soil. it feeds upon the stores of nutrient substances (carbohydrates, albumen, fat, &c.) accumulated in the cotyledons or seed-leaves. When, however, this store is exhausted, a complete cessation in the growth of the plant visibly ensues, the leaves turning yellow and becoming partly dried up, and the whole plant presenting a moribund appearance The cause of this condition can only be sought in the dearth of combined nitrogen that has now set in, since the plant has all the other essential food-stuffs at its disposal. The condition itself is consequently termed **nitrogen-hunger**. All other kinds of higher plants hitherto examined suffer in the same way when grown under these identical conditions. Differences, however, are noticeable in their subsequent behaviour If left unprovided with assimilable nitrogen, the representatives of all other families of phanerogamic plants—apart from a few exceptions to be enumerated later—persist in this state of starvation and finally die away. Not so the *Leguminosæ*. These will be observed to remain for some time— a few days to three weeks, according to circumstances—in this debilitated condition, but will then revive almost instantaneously, rapidly turning green, throwing up thick, juicy stalks, embellishing themselves with luxuriant foliage, putting forth a large number of blooms, and producing a good crop of seed. Such a plant may then overtake others that have been provided with nitrogenous food (manure), and have not had to pass through the famine period, and may, finally, at harvest yield a quantity of haulm and seed as great, and containing just as much nitrogen, as its more highly favoured fellows Hence the leguminosæ, in contrast to (nearly) all the other cultivated plants (cereals, such as wheat, oats, &c.; hoed crops, such as beet, potatoes, &c ; oilseeds, and so forth) that have been examined on this point, are characterised by the capacity for growing and ripening in a soil perfectly devoid of nitrogen and without the application of nitrogenous manure. This circumstance is so much the more remarkable since both the leaves and seed of pulse contain an unusually large proportion of combined nitrogen, and are, in fact, richer in this element than any other vegetable food-stuffs. This fact will be best displayed by the subjoined table, giving the average figures, obtained from a large number of analyses, of the percentage of nitrogen in the dry matter of the seeds of—

Maize	.	.	.	1.8	Peas	.	.	. 4 3
Buckwheat	.	.		1 9	Beans	.	.	. 4 6
Oats	.	.		1 9	Lentils	.	.	. 4 7
Wheat	.	.	.	. 2.3	Soja beans	.	.	. 6 1

From the large number of researches instituted on the assimilation of nitrogen, by plants, the following example, given by EMIL WOLFF (I.), may be selected, and may easily be repeated on a small scale by the reader for his own information. A number of zinc boxes were filled with 24 kilos. (53 lbs.) of washed calcareous river-sand destitute of nitrogen, the necessary mineral nutrient substances (P_2O_5, SO_3, K_2O, MgO) being then added and the seeds mentioned in the following table sown   A certain number of these boxes contained 0.83 gram of nitrogen apiece in the form of nitre.   The total dry matter and total nitrogen in the crop were determined, with the following results —

Per Box	Oats		Buckwheat		Rape		Peas		Vetches.	
	With-out N	With N	With-out N	With N	With-out N	With N	With-out N	With N	With-out N	With N.
	Grms.	Grms.	Grms	Grms	Grms	Grms	Grms	Gims	Grms	Grms
Dry matter in the crop	24	91	12	44	13	50	352	330	250	241
Nitrogen therein	0 15	0 44	0.14	0 43	0 13	0 50	6 74	6 45	6 01	5 95

This table shows that, in the case of peas and vetches, a high yield containing much nitrogen can be obtained, without the soil (initially destitute of nitrogen) having received any application of this element in the form of manure.   Such manuring is consequently unnecessary to these plants.

The results of these experiments on a small scale are confirmed by the observation of large farmers.   Schultz of Lupitz, a land-owner in the Mark (Brandenburg), states that he has gathered 227 kilos of nitrogen per hectare (about 200 lbs. per acre) annually from his "lupin meadows," for fifteen years without any nitrogenous manuring.   Similar results are reported by Dehérain from experiments with sainfoin, by the cultivation of which from 205 to 237 kilos. of (combined) nitrogen were obtained per hectare (180-208 lbs per acre).

For this reason agricultural chemists have termed the *Leguminosæ* collectors or accumulators of nitrogen, and have set them up as a class distinct from all other cultivated plants, the latter being grouped under the name of nitrogen consumers.

This does not, however, imply that the leguminous plants are averse to such manuring, on the contrary, they readily absorb any nitrogenous food present in or added to the soil, this food assisting the plant to tide over the stage of nitrogen-hunger.   So soon, however, as this period of stagnation is passed, they no longer require nitrogenous manure, and the latter, when applied, does not influence the size of the crop.   Practical agriculturists utilise this observation by planting soils which are poor in nitrogen with such nitrogen accumulators (especially lupins) as a first crop, which is ploughed in when sufficiently developed.   This constitutes the practice known as **green manuring**   A field treated in this fashion will then contain a much larger amount of nitrogen (in organic combination) than before, and is rendered capable of properly developing other cultivated plants (cereals, hoed crops, &c.), which, by reason of their high nitrogen requirements, would otherwise have yielded only a miserable crop in such poor soils.

§ 192.—The Discovery of the Leguminous Nodules.

The source of the free nitrogen accumulated by this class of plants must be sought in the atmosphere alone.

Formerly the ammonia compounds of carbonic acid, nitrous acid, and nitric acid, always present in the air (i.e. the rainfall), were considered as likely sources of nitrogen. The quantities of the last-named acid brought down in the rain, in temperate and tropical climates respectively, are given in the following table, drawn up (partly from personal experience) by A. MÜNTZ and V. MARCANO (I.) :—

	Liebfrauenberg in Elsass (*Boussingault*)	Rothamsted (*Lawes and Gilbert*)	Caracas, in Venezuela (*Müntz and Marcano*)	Island of Réunion (*Raimbault*)
HNO₃ per litre of rain-water	Mg 0 18	Mg 0 42	Mg 2 23	Mg 2.67
Amount of HNO₃ thus brought into the soil per hectare per annum .	Kilos 0 33	Kilos 0 83	Kilos 5 78	Kilos [1] 9 93

The researches and calculations made by HELLRIEGEL (I.), in particular, showed, however, this addition of nitrogen is much too small to deserve all the credit of the enrichment of the soil. Moreover, since the supply is delivered in approximately regular quantity to all the fields in a given district, it would afford no explanation of the fact that, of all these fields (without nitrogenous manure), it is only just those that have been planted with leguminous crops that yield such a surplus of nitrogenous matter.

Consequently, only one other feasible explanation remains, viz., that the *Leguminosæ* possess the inherent property of absorbing nitrogen from the air, and elaborating it into nitrogenous compounds (albumen, &c.).

However compulsory this conclusion may be, its recognition by the majority of vegetable physiologists and agricultural chemists was slow. Both classes were under the influence of Boussingault, whose experiments (1837 to 1858) in the cultivation of leguminous and other plants under bell-glasses in soils destitute of nitrogen gave results—confirmed by the check experiments of Lawes and Gilbert—which seemed to deny the absorption of free atmospheric nitrogen by plants.

Shortly afterwards, however, attention was directed to the bodies now termed *leguminous nodules* These are lateral appendages or swellings on the roots (as shown in Fig 59), and occur both on the younger and older portions of same, the former being the most thickly infested. In one and the same plant all the inter-mediate stages of this formation, ranging from such as are just barely perceptible to those as large as a pea or hazel-nut, can be found Their form (Fig 60) differs in different species, observations on which point have been published by A. TSCHIRCH (I.) and by LAWES and GILBERT (I).

The earliest description of the leguminous nodules was given by MALPIGHI (I) in his book published as far back as 1687, and this observer referred to them as galls, i.e. diseased excrescences, an opinion also shared by P. DE CANDOLLE (I)

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[1] 1 kilo per hectare = 0 89 lb per acre

in 1825. TREVIRANUS (I.), in 1853, was the first to regard these nodules as normal growths, and thirteen years later they were studied by WORONIN (I.), who made the (subsequently important) observation, that the formation contains entirely closed cells filled with living bacteria   In the seventies ERIKSSON (I.) and CORNU (I.) recognised these appendages as metamorphosed lateral roots of perfectly unique structure.

When regarded in section (Fig. 61), a nodule of this kind is seen at the first glance to consist of two different portions—a white or colourless external zone

FIG 60 —Root-nodule of Vicia sativa

mw main root , sw lateral root , the meaning of
    the other letters is given in Figs 61 and 63
    Magn 3   (After Beyerinck)
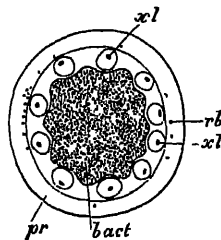
FIG 61 —Cross-section through a nodule of Vicia
sativa, cut along the line c in Fig 60
pr the primary integument with a few epidermal
    bacteria (1 b)
xl the vascular bundles, each with a xylem fibre
bact the strongly developed bacteroidal tissue
    Magn. 10   (After Beyerinck)

FIG 59 —Root of Vicia Faba

With young nodules on most of the lateral roots
and on the tap root   Somewhat reduced
(After Strasburger)

and an interior layer, pale red in the young nodule, but afterwards greenish grey, the line of demarcation between them being somewhat sharply defined, and the outline indented like that of a blackberry.   It is in the cells of this inner layer that the bacteria now under consideration, and more fully described below, are sheltered; and the layer itself is known as the **bacteroidal tissue**   On account of these enclosures the said root-nodules received from A B FRANK (III) in 1879 the name of **mycodomatia** (1 e **fungus** chambers), an expression that has, however, been abandoned

§ 193.—Formation and Functions of the Nodules.

The first to take under consideration the physiological importance of these nodules was LACHMANN (I.), who in 1858 defined them as stores of alburnen.

One-and-twenty years later FRANK (III.) showed that the formation of nodules does not occur when the plants are grown in sterilised soil, thus proving that the co-operation of microbes existing in the soil is a necessary factor. This result, conjoined with Woronin's observations, led to the conclusion that the production of the nodules is effected by soil bacteria. Frank's observation, and the conclusion deduced therefrom, were subsequently confirmed by H M WARD (V.), who showed that the nodules are absent in water-cultures of *Vicia Faba* in sterilised nutrient solutions, but, on the other hand, appear in large numbers if chopped nodules, grown in the ordinary soil, be inserted amongst the root-hairs. This discovery threw a little more light upon the manner in which the nodules are produced, and increased the probability of the assumption that they result from the activity of bacteria which gain access to the root, and there exert a certain stimulance inducing a luxuriant cell-growth. A more intelligent investigation of the importance and mode of action of the nodules thus became possible, and it was then remembered that the *Leguminosæ* are precisely the plants found capable of growing in soil destitute of nitrogen. Hence the obvious idea sprang up that possibly these nodules should be regarded as organs facilitating the absorption of uncombined nitrogen from the air.

It naturally follows that if this assumed faculty is actually possessed by these growths, a direct connection between the formation of the nodules and the development of the plant as a whole should be traceable, and this was accomplished by HELLRIEGEL (I.), in conjunction with WILFARTH, in the years 1884 to 1886. These workers, as a result of exhaustive investigation of plant-roots, arrived at the conviction that the development of the root-nodules stands in most intimate relation to the growth and assimilation of the whole plant. The number of nodules per plant was found to be the greater in proportion as the development of the latter was more perfect   Papilionaceous seeds (*e.g.* peas) sown in boxes of sterilised soil devoid of nitrogen, and protected from subsequent infection, perished after nitrogen-hunger set in, but, on the other hand, throve and ripened when the boxes were supplied with a small quantity of an aqueous extract from fertile soil   When the non-nitrogenous soil was watered with a little of the said extract, and then sterilised and covered with a layer of sterilised cotton-wool before planting the seeds, the result was identical with that of the first experiment, *i.e.* the plants started well, arrived at the stage of nitrogen-hunger after the unfolding of the sixth leaf, and then gradually fell into a consumptive state and perished, barren. By these and many other experiments Hellriegel arrived at the incontrovertible conclusion that the absorption of atmospheric nitrogen by the *Papilionaceæ* is directly connected with the development (or the presence and activity) of the so-called leguminous nodules, which latter are produced solely by the action of certain bacteria on the roots

Hellriegel confined his researches entirely to the *Papilionaceæ*, and left out of consideration the other two families, viz , *Cæsalpinaceæ* and *Mimosaceæ*, which form with it the order *Leguminosæ*. The formation of nodules in these two families was subsequently investigated by D. MORCK (I.), with the result that the faculty was discovered in each one of sixty-five species (from thirty-eight genera) examined   These discoveries were supplemented by H. LECOMTE (I), who, in 1894, proved that nodules are formed in *Arachis hypogæa*, the earth-nut, a fact already noted by Poiteau in 1852, but afterwards denied by Eriksson. The nodule bacteria of the Soja bean (*Soja hispida*) have been described by O. KIRCHNER (II).   On the other hand, neither Morck nor FR NOBBE (I) was able to discover the presence of root-nodules on *Gleditschia ,* and it is found impossible to inoculate this plant by the nodule bacteria of other *Leguminosæ*. Apart from *Leguminosæ*, the organs in question are found (so far as is at present known) only on *Alnus, Elæagnus angustifolius, Hippophae,* and *Podocarpus*, all of which

are able to thrive in soils destitute of nitrogen   The faculty of nodule formation has also been ascribed to other plants.   A. B Frank (IV.) goes the farthest in this respect, and, indeed, assumes that all plants are in a position to take up free nitrogen, an opinion also recently expressed by J Stoklasa (II.)   Liebscher (I) confines his opinion within narrower limits, but ascribes the power of fixing free nitrogen to oats and mustard as well.   The statements of Frank and Liebscher have, however, been disproved by the searching criticisms of, e g. Wilfarth (I.), U Kreusler, P. Wagner, F. Nobbe, and L. Hiltner (I), and J. H Aeby (I.) has shown that mustard does not possess the faculty with which it is credited by Liebscher.   The same results were obtained by Ch E. Coates and W R Dodson (I) in 1896, in their experiments on the cultivation of the cotton plant (Gossypium).

§ 194.—The Nodule Bacteria.

The discoveries reported in the foregoing paragraph, and for which we have chiefly to thank Hellriegel and Wilfarth, lead up to the question whether this proved absorption of free nitrogen is effected in the root-nodule itself, or whether, by the influence of this formation, the entire plant—particularly the foliage—becomes capable of taking up this gas from the atmosphere and fixing it in combination ?

This latter opinion, which was chiefly supported by A B. Frank, has been investigated by P Kossowitsch (I.), who was, however, unable to discern any absorption of atmospheric nitrogen by the parts of the plants above ground. Attention must, therefore, be concentrated on the root-nodules themselves, but before going into the question whether and in what manner the nitrogen is fixed by them, it will be necessary to become more closely acquainted with the living organisms they contain, viz., the nodule bacteria.

The discovery of these bacteria by Woronin in 1866 was not followed immediately by their general recognition in scientific circles   For example, H. De Vries (II.) in 1877 looked upon them as non-essential.   Moreover, when J Brunchorst (I), in 1885, examined them more closely, he came to quite a different conclusion, and defined the supposed bacteria as organised albuminoids collected in the interior of the nodule cells, and therefore termed them bacteroids (on account of their external resemblance to bacteria)   Hence the term bacteroidal tissue, applied to the internal portion of the nodules in which these organisms appear in large numbers.   Remarkably enough, Brunchorst's opinion found favour in the eyes of A. B Frank (V.), although conflicting with his own discoveries made in 1879   A. Tschirch (I) also ranged himself on the side of Brunchorst.

A complete revolution of opinion took place in 1888, when Beyerinck (XIV.) indubitably established the fungoid nature of these supposed pseudo-bacteria, by isolating them from the nodules, and cultivating them further in artificial media   The pure culture obtained from the individual species of Papilionaceæ exhibited certain slight but undeniable differences, which, however, were not so extensive as to make their discoverer feel justified in classifying the organisms as separate species, so he defined them as varieties of a single species, for which he proposed the name of Bacillus radicicola.   The artificial formation of nodules induced by inoculating the roots of Leguminosæ with such pure cultures was successfully attempted a year later by Piazmowski, and will be noticed in § 195. The bacillus in question develops either feebly or not at all on ordinary nutrient gelatin, this substratum being too rich in nutrient materials   Beyerinck recommends a decoction of the leaves of Papilionaceæ, with the addition of 7 per cent of gelatin, ¼ per cent. of asparagin, and ½ per cent of cane-sugar   The requisite

degree of acidity in the medium is represented by about 0.6 c.c. of normal acid per 100 c c   The plates coated with this nutrient medium are inoculated with an infusion prepared by mixing a few c c. of sterilised water with a small portion of the contents of the bacteroidal tissue of a fresh, young nodule that has been previously washed with water, then steeped a short time in alcohol, freed from the latter by means of ether, and finally cut open with a sterilised knife.   The small quantity of inoculating liquid will be absorbed by the gelatin, leaving the bacteria on the surface, where their growth progresses in the most favourable manner.   Here they develop to small mucinous colonies that do not liquefy the gelatin

Two forms of cells will be immediately noticeable in preparations made from such a culture   Beyerinck distinguishes them as rods and rovers.   The former have a breadth of $1\ \mu$ and a length of 4 to $5\ \mu$, and wander eagerly towards the edge of the cover-glass, where fresh oxygen obtains access   As veritable dwarfs in comparison with these are the rovers, which are only $0.9\ \mu$ long and $0.18\ \mu$ broad, and therefore belong to the smallest of known micro-organisms   Even the Chamberland filter cannot restrain them, and they escape through its pores   As the name implies, they are endowed with motile power, which is frequently so strong that individual rovers are able to escape from the parent colony, make their way across the gelatin plate, and found a daughter colony at a distance The rod cells are not invariably of the ordinary cylindrical shape ; on the contrary, variously bulged or lobed forms appear in larger or smaller number according to circumstances, and a forked branching, resembling the Greek γ, is very frequent This peculiarity, be it remarked en passant, is also shared by other bacterial species, e g. the Pasteuria ramosa already mentioned in an earlier paragraph. Bacillus radicicola does not produce an enzyme capable of dissolving gelatin, starch, or cellulose, or of inverting saccharose, neither has spore formation been detected, a circumstance harmonising with the fact that a temperature of $60°$–$70°$ C suffices to destroy this fission fungus   On the other hand, the organism appears to support drought and frost without sustaining any injury

A few words must be devoted to the varieties exhibited by the nodule bacteria   Hellriegel established (though without employing pure cultures) that the nodule bacteria of peas cannot develop nodules on lupins and Seradella (Ornithopus sativus).   This observation, which was challenged by A. B Frank, was confirmed by NOBBE, working with pure cultures, in conjunction with SCHMID, HILTNER, and HOTTER (I)   Whether the species should be divided merely into races or varieties, as advocated by the observers just named ; or whether we should here speak of different species in the sense used by naturalists, and consequently express them by different specific names, as was attempted, e g. by A. SCHNEIDER (I.), is a point of remote importance.   BEYERINCK (XV.) has also become a convert to this view, having been unsuccessful in inducing the formation of nodules in Vicia Faba by means of bacterial cultures from those of Ornithopus.   On this point an interesting discovery was made by NOBBE, HILTNER, and SCHMID (I), according to whom the bacteria from any given species of Leguminosæ produce the most plentiful development of root-nodules in the shortest time when applied to other plants of the same species, the potency diminishing in the case of plants of merely allied species, and finally becoming nil when a greater specific difference exists between the original plant and the one inoculated   The various separate species (or varieties) of the nodule bacteria can, to a certain extent, therefore replace one another.   Thus, those of the pea are also efficacious for all the examined species of the genera Vicia and Phaseolus, but, on the other hand, without effect on Robinia, Ornithopus, red clover, kidney vetch, and other clovers   Those of Robinia form nodules only on Phaseolus and a few species of the genus Trifolium.   Finally, according to the researches

of F. NOBBE, E. SCHMID, L. HILTNER, and E. HOTTER (II), the nodule bacteria of *Elæagnus* differ greatly from those of *Leguminosæ*.

These observations are also of importance to practical agriculture. Already, for several years past, soils intended to be brought under cultivation (*e g.* high moorland soils) for the growth of nitrogen-collecting plants are previously inoculated, *i e.* strewn with a little earth from fields that have borne leguminous plants for a long time, and are consequently rich in nodule-forming bacteria. For this inoculation to have the desired result, it is necessary to use earth containing the bacterial species most efficient for the kind of *Leguminosæ* to be afterwards grown. Practical experience on the importance of this consideration is already available Thus SALFELD (I) has reported that a similar soil intended for peas could not be rendered capable of yielding a crop unless strewn with a little soil obtained from a good pea-field, soil from a lupin-field failing to produce the desired effect. A similar discovery was made by M. FLEISCHER (I). This treatment must, of course, be preceded by any improvement found necessary in the chemical composition of the soil. Thus, for example, sour moorland must previously be limed, in order to neutralise the acids preventing the development of the nodule bacteria. Moreover, this operation must be performed with discretion, an excessive addition of lime being avoided Reference may be made on this point to a communication by TACKE, IMMENDORF, HESSENLAND, SCHÜTTE and MINSSEN (I.).

§ 195.—The Bacteroids.

The bacteria in question are often met with in air and water, and very frequently in the soil. NOBBE, SCHMID, HILTNER, and HOTTER (I.) made several quantitative bacteriological investigations on this point. The bacteria pass from the soil into the roots of such plants as will admit them. The first successful, artificial production of nodules by the aid of pure cultures was made by A PRAZMOWSKI (IV.). This worker, in view of the absence of the sporogenic faculty in these organisms, changed the name of *Bacillus radicicola*, bestowed on them by Beyerinck, into *Bacterium radicicola* According to his observations, this fission fungus penetrates the epidermal cells of the root-hairs, and there develops to a colony which then surrounds itself with a tough membrane. From this original position there branches out a lustrous sac, filled with bacteria, which turns towards the bark cells and branches out amongst them. As a result of this advance towards the centre of the root-hair, the cells thereof are incited to rapid increase and become densely crowded, in consequence of which they assume a polygonal outline, and constitute the bacteroidal tissue already mentioned (Fig 62) The plasma of these cells, with its fungoid enclosures, has been termed mycoplasma by A. B. FRANK (VI).

Fig 62 —Section through the bacteroidal tissue of Lathyrus sylvestris

Nuclear staining with 1 per cent chromic acid and methylene blue, *k* the nucleus with its nucleolus (lying in a vacuole), *f* the infection-threads, *mi* the microsomes of the cytoplasma, *am.* starch granules, *v* large central vacuole, *bact* the bacteroids Magn 400 (*After Beyerinck*)

Before tracing the career of the bacteria any further, a few explanatory

words must be added concerning their mode of arrangement (already referred to as a " sac ") at the time of penetration into the cells The-e branching bacterial colonies, enveloped, as has been stated, in a membrane, were for a long time misunderstood. Beyerinck at first considered them as the surplus matter from the division of the nucleus of the nodule-cells (*Kerntonnenfaden*) By other workers they were styled mucus threads, fungoid hyphæ, plasmodial cords, &c. Frank, for a long time, held them to be the mycelium of an independent higher fungus, differing from the nodule bacteria and belonging to the genus *Schinzia*, and consequently named them *Schinzia leguminosarum*. Subsequently Frank, by the new name **infection threads**, indicated their true nature, first recognised by Prazmowski. Frank, moreover, in his studies on the immigration of the nodule bacteria in the root, arrived at results differing in several points from those of Prazmowski. We cannot, however, go further into this matter, which
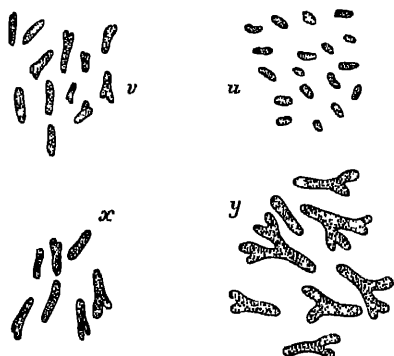
FIG 63 —Development of bacteria to bacteroids from the meristem of a root-nodule of Vicia sativa Explanation in the text

Magn 700 (*After Beyerinck*)
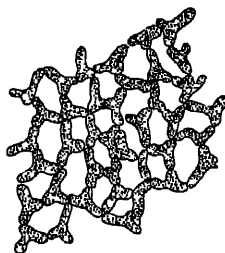
FIG 64.—Reticulated band of bacteroids from the nodules of Vicia Faba

Magn 700 (*After Beyerinck*)

is one more of botanical than mycological interest. He also discarded the name bestowed on these root-dwelling organisms by Beyerinck in favour of the term *Rhizobium leguminosarum* The integument of the threads is not, as erroneously opined by Frank, a product of the plasma of the nodule-cells, but is formed by the union of the swollen membranes of the outer layers of the bacteria constituting these filamentous colonies (or zooglœa). As was shown by A. KOCH (IV.) and M. W. BEYERINCK (XVI), it is stained *blue* by zinc iodo-chloride, and therefore consists of a substance allied to cellulose. The structure and progress of the infection threads in the nodule-cells can be readily recognised in sectional preparations, stained by a solution of equal parts of fuchsine and methyl violet in 1 per cent acetic acid This colours the plasmal contents and membrane of the nodule-cells *blue*, the bacteria of the infection threads being stained *red*, whilst the membrane of the latter remains uncoloured It should be mentioned, in conclusion, that these threads of capsuled bacterial colonies are but rarely found in the nodules of lupins.

The bacteria which have gained access now develop in the cells of the bacteroidal tissue, and, finally, under the influence of the surrounding protoplasm, become modified into involution forms termed **bacteroids**, rich in albumen and no longer capable of reproduction This morphological change is represented in Fig 63, in an example taken from the nodule of *Vicia sativa* shown in Fig 60.

A section cut near the lower extremity meets the youngest meristem, where only long rods (*u*) in a high state of development are to be seen. A little higher

up, in older layers (v), the commencement of branching is already discernible, and is found in a more forward state in a still higher position (x)   Finally, in a section across the cells of the internal tissue of the nodule in the direction y (Fig. 60), none but variously shaped bacteroids (y) for the most part united to form reticulated bands (shown in Fig 64) occur   After attaining this condition the bacteroids are soon dissolved by the surrounding cell plasma and disappear. However, before this occurs, small globular vesicles of an unknown nature, which, however, should not be regarded as endospores, not infrequently appear in the interior of this formation   Little can as yet be said of the chemical composition of the contents of these bacteroids   Micro-chemical reactions, however, indicate that the greater part is composed of albumen.   Certain enclosures are also frequently observed, A. B. FRANK (VII.), for instance, having noticed such bodies in the bacteroids of individual nodules of peas   He regarded them as amylodextrin, the discovery leading him to the opinion that two kinds of nodules develop on these *Papilionaceæ*. albumen nodules and amylodextrin nodules   A subsequent investigation of this matter by H MÖLLER (I) showed, however, that these doubtful enclosures do not consist of carbohydrates, but of waxy or fatty substances, that they are also to be found in the bacteroids of the "albumen nodules", and that, moreover, there is but little probability of the existence of dimorphism in the root-nodules of the pea, since these enclosures are also occasionally met with in the bacteroids of the nodules of other *Leguminosæ* (*e.g. Trifolium repens*)

Concurrently with the reproduction and transformation of the bacteria marches the development of the nodule, which not only increases in size, but also becomes richer in nitrogenous compounds   This gradual increase in the percentage of combined nitrogen in the nodules and the relative proportion of this substance there present, as compared with other parts of the root, were quantitatively investigated by J. STOKLASA (II.)   From his results a few figures have been collected into the subjoined tables, which refer to yellow lupins —

Nitrogen Content in the Dry Matter from —	At Flowering Time	At Fructification.	In Fully Ripe Fruit
	Per Cent.	Per Cent	Per Cent.
Root-nodules .	5 2	2 6	1 7
Roots free from nodules . .	1 6	1 8	1 4

That this nitrogen of the nodules was chiefly in the form of albumen is revealed by the following table —

Percentage Content of the Dry Substance of the Nodules	In Nitrogen as —		
	Albumen.	Amides	Asparagin
	Per Cent	Per Cent	Per Cent
Flowering time . . .	3 99	0 35	0 34
Ripened fruit	1 54	0 15	traces

The figures just given are surpassed in two analyses made by A. B Frank according to which a few of the pea nodules examined by him contained 6.94 per cent., and those from the dwarf bean 7.44 per cent. of nitrogen, corresponding—

on the basis of the factor 6.25—to an albumen content of 43 4 and 46.5 per cent. As already remarked, the bacteroids—which, when they exhibit the aforesaid vesicles, are frequently termed vesicular bacteroids—are finally dissolved by the surrounding plasma, which is thus enriched with albuminoids, and is then diffused through the plant The cell contents of the (formerly reddish but now greenish grey) bacteroidal tissue gradually vanish and are dispersed into other parts of the plant, and the nodule consequently shrivels up. The commencement of this process of evacuation of the cells is indicated by the appearance of a central vacuole of gradually increasing size (Fig. 62).

Neither the transition of the nodule bacteria into bacteroids, nor the final dissolution of the latter, goes on simultaneously in all parts of the individual nodules, which, moreover, are themselves of different ages. Some of the bacteria escape the converting influence of the cell plasma by remaining within the protecting mucinous capsule (membrane) of the infection threads. Hence, it happens that in the autumn large numbers of the bacteria are still present in an active condition within the nodules In the subsequent putrefaction of the latter the organisms are set at liberty, pass the winter in the soil, and then act again as nodule-formers in the following spring.

The bacteroid stage is not reached in every case, the plasma of the nodule cells being unable in many instances to utilise the microbes. In such event it swarms with bacteria alone, which then act solely as parasites towards their host, and consequently the latter derives little or no benefit from the formation of nodules. This phenomenon is termed by Beyerinck "an **overgrowth of bacteria**" According to the observations of Nobbe and Hiltner (I), it occurs when the inoculation is performed with bacteria that have been grown on artificial media for a long time

§ 196.—Clostridium Pasteurianum.

The fact recorded in § 194, that absorption of nitrogen is not effected by the superior (aerial) parts of the *Leguminosæ*, led us to investigate the root nodules more closely. We then observed that the possession of these appendages enables the plant to grow well and ripen even in soils destitute of nitrogen, and we furthermore learnt that the production of these nodules is directly connected with the activity of certain special bacteria—the nodule bacteria However important and satisfactory this result may be to agricultural practice, it still leaves unsolved the (from a theoretical standpoint) main question, "By what is the free nitrogen fixed?" Is this effected by the nodule bacteria themselves, or do they merely exert a stimulative action on the plasma they inhabit, which is thereby empowered or spurred to unwonted activity?

The latter question cannot be answered by experimental means, since for that purpose the chemical activity of the bacteria would need to be eliminated and only their assumed stimulating action allowed to operate. However, the wished-for decision may be expected from the experimental solution of the previous question, and attempts may therefore be made to ascertain whether the nodule bacteria are of themselves capable of fixing free nitrogen. On this point Beyerinck (XVII.) has conducted researches in nutrient solutions with pure cultures of *Bacillus radicicola*, and in this manner ascertained that during a period of two months an increase of 9–18 mgrms. of combined nitrogen occurred per litre. Nevertheless he considers that this discovery leaves the question still undecided, and consequently a repetition and extension of his researches in this direction is highly desirable.

The exercise of the still imperfectly proved capacity of *Bacillus radicicola* for fixing free nitrogen is opposed by a considerable obstacle consisting in the

difficult accessibility of the interior of the nodule (the chief seat of this fission fungus) to the gas to be fixed. A. B FRANK (VII.) has ascertained that the air passages (intercellular spaces) of the nodules lead only as far as the cambium layer, but not into the bacteroidal tissue; consequently atmospheric nitrogen cannot penetrate directly to this tissue. On this point R. BOUQUET (I) has expressed an opinion worthy of further investigation. According to him, it is the water absorbed by the roots and exhaled through the leaves which conducts and gives up free nitrogen in solution to the root cells, where it is then combined by the plasma.

If, then, we must consider the question, whether the fixation of free atmospheric nitrogen is effected *within* the nodules, to be still imperfectly solved, it is, on the other hand, clearly proved that such an operation goes on in (uncultivated) soil. This was first observed by M. BERTHELOT (I.) in 1885, and he subsequently proved that this phenomenon is not occasioned by exclusively chemical affinity, but is due to the activity of micro-organisms. Opinions on the nature of the organisms were at first divided. TH SCHLOESING, jun, and EM. LAURENT (I) ascribed the fixation of nitrogen to certain lower algæ (*Conferva, Oscillaria, Nitzschia*), and mosses (*Bryum, Leptobryum*) Doubt was cast on this hypothesis by A GAUTIER and R. DROUIN (I.), and P KOSSOWITSCH (II) disproved it—so far as the algæ are concerned—by the aid of pure cultures. BERTHELOT (II.) then showed that the activity of fungi—both *Eumycetes* (such as *Aspergillus niger, Alternaria tenuis,* &c.) and *Schizomycetes*— is in question.

For more precise investigations on this matter we have to thank S. WINO GRADSKY (II.), who described a fission fungus belonging to the group of butyric acid bacteria, and bearing the name of *Clostridium Pasteurianum* This occurs in the form of rods, 1 2 μ broad and 5 μ long, each producing an endospore the cells thereby swelling up to the clostridium form, and storing up in their interior (though not at both poles) substances that are stained a deep blue-black by iodine. The ripe spore escapes through the wall of the mother-cell in a longitudinal direction. The great resemblance between this clostridium and the butyric acid bacteria described in a previous section is not only morphological but also extends to the fermentative capacity For example, *Clostridium Pasteurianum* acts on sugar in such a manner that both volatile acids butyric acid and acetic acid (4 1), and gases carbon dioxide and hydrogen (60–75 per cent of H by volume) are formed The importance to us of this fission fungus on the present occasion consists in its behaviour towards nitrogen which gas it absorbs from the atmosphere, fixes it and employs it in the elaboration of organic substances. The energy necessary thereto is supplied and liberated by the decomposition of sugar; consequently it is easy to understand that a definite relation exists between the amounts of sugar fermented and of nitrogen combined. This ratio was determined by Winogradsky as 2 5–3 mgrms. of nitrogen to 1000 mgrms. of dextrose. This element (N.) when in a state of combination is not only valueless to *Clostridium Pasteurianum*, but when present in large quantity even injurious thereto For the cultivation of the microbe—which cannot be carried out on the ordinary nutrient media (gelatin bouillon) in use, though it grows on sliced potatoes—use is made, preferably of an aqueous solution containing 1 gram K_2PO_4, 0 5 gram $MgSO_4$, 0 01–0 02 gram NaCl, $FeSO_4$, $MnSO_4$, a little $CaCO_3$ (for fixing the acids), and 20–40 grams of dextrose per litre. *Clostridium Pasteurianum* is strictly anaerobic, and in the soil is therefore obliged to rely on the co-operation of aerobic fission fungi which remove the injurious oxygen from its sphere of influence, surround it with an atmosphere of nitrogen, and as a reward for this service have the opportunity of consuming the nitrogenous substances elaborated and excreted

by the *Clostridium*. Winogradsky observed two species of such assistant organisms, detailed mention of which can, however, be omitted, it being sufficient for our purpose to have referred to this new case of symbiosis.

Whether the just-named faculty of fixing nitrogen is also possessed by the organisms akin to *Clostridium Pasteurianum*, e g Prazmowski's *Clostridium butyricum*, still remains undermined. WINOGRADSKY (III.) examined fifteen species of soil bacteria in this connection, with only negative results   In the higher fungi K. PURIEWITSCH (I.), in an improved continuation of Berthelot's researches, showed that both *Aspergillus niger* and *Penicillium glaucum* fix free nitrogen. Their potency is, however, but slight, and is not to be compared with that of *Clostridium Pasteurianum*, since it is not manifested in media devoid of nitrogen . According to H. JUMELLE (I.), *Spirillum luteum*, also, is capable of thriving in media free from nitrogen

The proof of the fact that the fixation of free nitrogen occurs in the soil affords a new *possible* explanation of the activity of the nodule bacteria of the *Leguminosæ*, viz , that the actual absorption of the free nitrogen goes on *outside* these root formations ; substances unassimilable by higher plants being formed, and then converted into an assimilable form by the nodule bacteria. This provisional interpretation, which will not encroach on future research, is not without its analogies; one need only recall the *Mycorhiza*, discovered by Frank on the rootlets of the majority of forest trees (*Cupuliferæ*, *Coniferæ*), heaths (*Ericaceæ*), &c , and in regard to which the most important discoveries made up to the year 1888 will be found briefly reviewed in a treatise by F. BENEOKE (II).

The great importance to General Physiology of the researches reported above will be readily appreciated, since they have brought to our knowledge organisms which dispense with combined nitrogen as a food-stuff, and are consequently of the greatest importance in the economy of Nature, by revealing the means for maintaining the circulation of nitrogen. Large quantities of this element are daily liberated by the activity of both de-nitrifying and nitrifying bacteria, and restored to the atmosphere, the result being that the stock of nitrogenous compounds, so essential to the nutrition of all other plants and all animals, becomes reduced   To compensate and reverse this loss is the task of the nitrogen-fixing fungi, which, for this reason, must be regarded as the benefactors and foster-mothers of all other living creatures

SECTION IX.

OXIDISING FERMENTATIONS.

CHAPTER XXXIV.

THE IRON BACTERIA.

§ 197.—Morphology of the Genera Crenothrix and Cladothrix.

THE group of *Schizomycetes* known as thread bacteria can be divided into tv
sub-groups. To the one of these belong the two genera (described in the follov
ing chapter) which store up globules of free sulphur in their cells. Tl
appearance of these organisms is so characteristic that a skilled eye can dete
them with ease. On the other hand, the five genera of the second sub-grou
lack this peculiarity In three of these latter, viz, *Streptothrix, Leptothrix,* ai
Cladothrix, the fission of the cell takes place in one direction only, co
sequently, rods are formed. In the other two, however, viz, *Crenothrix* ai
Phragmidiothrix, the extremity of the thread is broken up, by division in a
three directions of space, into coccus-like cells No perfectly satisfactory morph
logical description, or definite separation of these five genera into species, h
yet been drawn up on this basis. This defect—to which reference has also be
made by C. SAUVAGEAU and M RADAIS (I)—therefore restricts us to the co
sideration of individual examples A typical one is afforded by *Crenothi*
polyspora. This thread bacterium was first described by F COHN (XI) in 18;
and was subsequently also named *Crenothrix Kuhniana* It is illustrated
Fig. 65, which shows that the threads are sessile, each of them consisting of
single row of short cells held together by a common tubular integument, call
a sheath. This latter is formed by the splitting of the cell membrane into t
layers, the outer one of each cell becoming merged into the adjacent membra
above and below, and thus forming a uniform tube in which the cells can m
up and down. The threads are not cylindrical, but increase in diameter fr
about half-way along their length towards the free end, so that the cross-sectio
diameter varies from 1.5 to 5μ. Moreover, as may be seen from the figure, 1
various segments of the thread are of unequal length As the cells multi
rapidly, the upper members are forced out of the sheath, but are, for the m
part, already subdivided—by the development of partition walls in all th
directions of space—into small rounded cells, which thereupon make th
escape. The new cells vary in size according to the rapidity of this proces
subdivision, and are correspondingly distinguished by Zopf as micrococci
and macrococci (q) (Fig 65). Cohn proposed the terms microgonidia a
macrogonidia, because their method of formation bears a slight apparent
semblance to the endospores (known as gonidia) of certain Eumycetes, w
which we shall become acquainted in the second volume The dimensions of tl
cocci vary between 1μ and 6μ. Their cell-walls swell up readily, and unit
form zoogloea (f) up to 1 cm. in diameter Under favourable conditions
cocci then grow, by repeated subdivision and sheath-formation, into the thr

forms already described. ZOPF (VI.) observed that in many cases this occurs before they have quitted the sheath of the parent thread, and in this manner tufted forms, similar to that shown in Fig 66, are produced. Otherwise, the sheath is gradually emptied of its contents, and then collapses limply and withers up.

If we disregard the exceptions just named, wherein the germination of the cocci proceeds within the parent thread, and so causes it to present a branched appearance, it may be said that the genus *Crenothrix* appears only in the form of single, unbranched, filamentous chains of cells. This characteristic suffices to distinguish this genus from that bearing the name of *Cladothrix*, the best-known species of which is *Cladothrix dichotoma* As this name implies, we have here to do with a forked thread, such as shown in Fig. 67 This false branching is produced in the following manner —One of the rod-shaped joints of the thread turns aside, and, growing beyond its next higher neighbouring cell, repeatedly subdivides and forms a new thread, on which a similar false branching may also develop. Hence there ensues a formation the internal structure of which is represented diagrammatically in Fig 68. In many species (not depicted here) the sheath becomes greatly thickened at the base, where it attains a diameter many times exceeding that of the cells it encloses, but tapers off gradually towards the free extremity *Cladothrix dichotoma* also differs from the above-mentioned thread bacteria in another important particular,

FIG 65 —Crenothrix polyspora

i–i thread forms of different diameter
n–q macrococci, and *r* micrococci splitting off   Magn about 600
a–e reproduction of the cocci, *f* colony (zoogloea) of cocci, *g* same, natural size, *h* same beginning to germinate   Magn 600.   (*After Zopf*)

viz., by the production of rod-shaped roving cells, called **rod-gonidia**, which develop at the extremity of the threads, and, after being initially embedded in the swollen sheath (Fig 69), are liberated, wander about, and finally settle down to form new threads by subdivision and sheath-formation.

Allied to *Cladothrix dichotoma*—though not, as ZOPF (VII) opined, belonging to the morphological cycle of this organism—is *Leptothrix ochracea*, which was

I   S

first described by Kutzing. A second sheath-forming thread bacterium, allied to the genus *Cladothrix*, was also examined by him, and named *Sphærotilus natans* It is still too imperfectly known to be dilated upon here, although ED EIDAM (II)
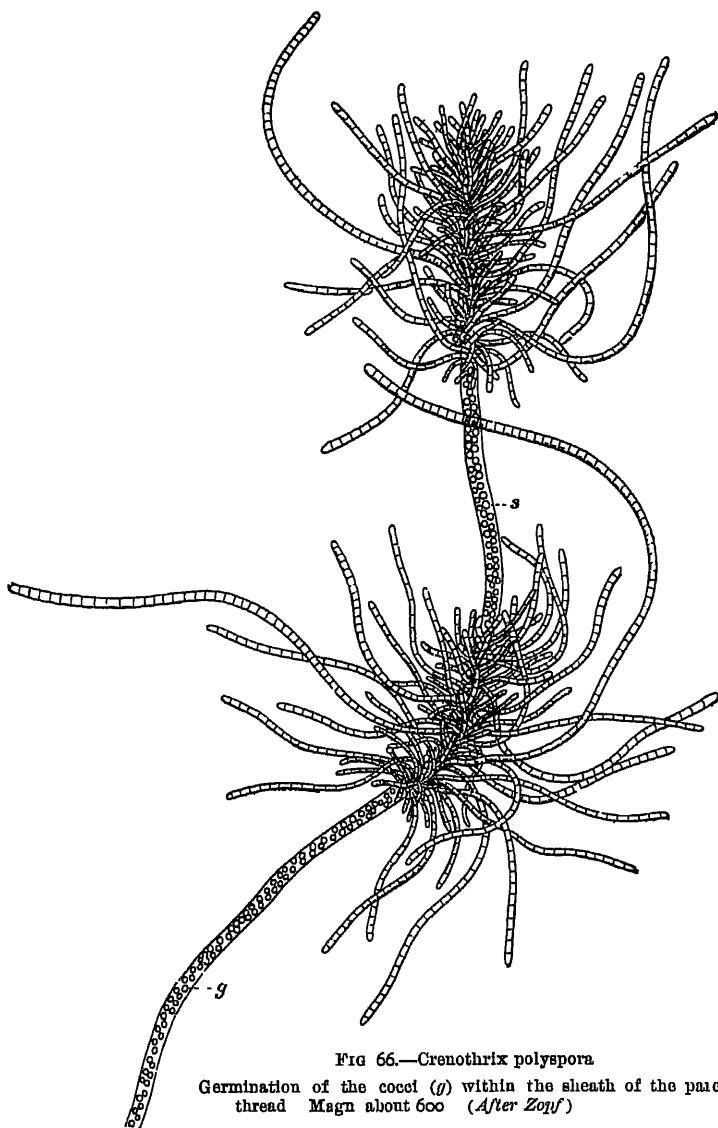
FIG 66.—Crenothrix polyspora
Germination of the cocci (*g*) within the sheath of the parent thread   Magn about 600   (*After Zopf*)

also occupied himself with it   Associated with this colourless species is a secol (coloured) species, discovered by W. ZOPF (VIII.) in a Silesian river receivii the drainage from a sugar-works. The 'cells of this, *Sphærotilus roseus*, conta a yellow and a red colouring-matter, which circumstance is of itself sufficient distinguish it from all other (colourless) species of thread bacteria hither mentioned.

The genus *Phragmidiothrix*, one species of which—*Ph. multiseptata*—was discovered by ENGLER (I.) in the so-called "dead ground" of the Bay of Kiel, differs from all the foregoing in the absence of sheath formation

§ 198.—Physiology of the Iron Bacteria.

It is not always possible to discern the structure of these thread bacteria without some preliminary treatment, because in most cases the sheaths are
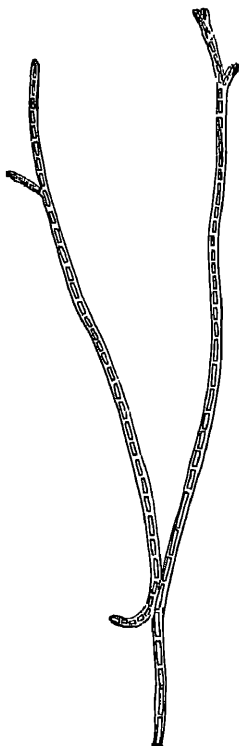
FIG 67 —Cladothrix dichotoma.
Portion of a thread with several branching forks Stained with fuchsine solution, and thus reveal-ing the articulation into long rods. Magn. 540. (*After Zopf*)
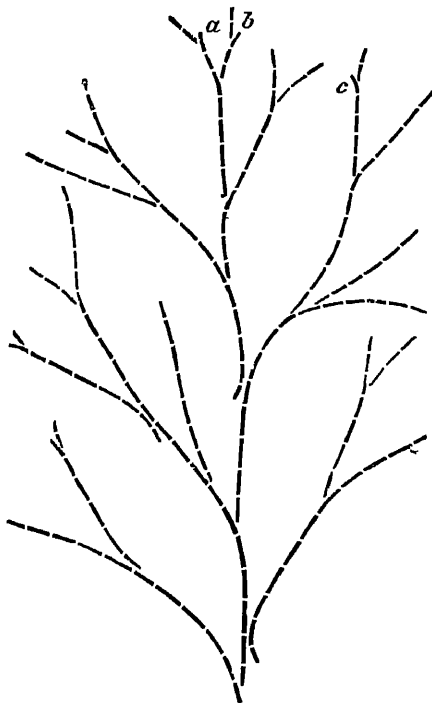
FIG 68
Diagram of the false branching of Cladothrix.

surrounded and permeated by red-brown masses of ferric oxide. These deposits and accumulations are characteristic of these plants, and facilitate their detection and discovery. Since other fungi exposed to the same conditions do not exhibit this peculiarity, Cohn formed the opinion that its occurrence is intimately connected with the vital activity of the thread bacteria, the ferric oxide being deposited in their sheathing in the same way that silica is accumulated in the plates of the diatoms We are indebted to S WINOGRADSKY (IV) for proving the correctness of this view, and for refuting the opinion of Zopf that the deposition is purely mechanical, and we have to thank the same observer for the more intimate investigation of the process in question.

The species *Crenothrix polyspora, Cladothrix dichotoma, Leptothrix ochracea,* &c ,

occur in particular abundance in such waters as are rich in iron, not in the form of oxide, but as the soluble bicarbonate of the protoxide, $FeH_2(CO_3)_2$ Ferruginous springs, ascending from the deeper strata of the rocks, bring up this substance in a ready-formed state, and in the water of the upper strata it is produced by the decomposition of vegetable matter, the iron, both in this and in the water itself, being converted during cellulose fermentation into the hydrocarbonate. This compound is then absorbed by osmosis into the bacterial cell, where it is split up by the plasma and oxidised, according to the equation—

$$2FeCO_3 + 3H_2O + O = Fe_2(OH)_6 + 2CO_2$$

The ferric oxide is then stored up in the sheath, to which it imparts a coloration, initially pale yellow but gradually changing to dark brown. Freshly precipi tated ferric hydroxide is, as we know, somewha soluble in water, but afterwards gradually passe into a condition in which it is only attackable b weak acids   This change can be traced in the youn bacteria, the colouring-matter in the yellow sheat being at first extractible by washing with wate containing CO_2 in solution   Subsequently, howeve dilute hydrochloric acid must be resorted to, an at a still later stage even this solvent is powerless to extract the brown deposi A very fine and fast blue stain can be produced in young sheaths (the iron i which is still soluble in acid) by exposing them to a *mixture* of hydrochloric aci and yellow prussiate (potassium ferrocyanide), whereby the hydroxide is dissolve immediately converted into Berlin blue, and re-precipitated. In older threac the deposits of ferric oxide increase to a thick incrustation, and entirely conce the structure of the cells

FIG 69

Cladothrix dichotoma

Subdivision into roving rods at the extremity of a thread   *s* the loosened sheath , *g* the roving rods with their (lateral) cilia (*c*) Magn 1000. (*After A Fischer*) Cilia staining

Winogradsky discovered that these bacteria thrive only when ferrous carbona is available, and that growth is arrested directly the nutrient medium contain no iron, or only iron in the condition of oxide   This fact entails the conclusic that the life of these bacteria is mainly sustained by the energy liberated durin the oxidation of ferrous oxide to ferric oxide. Consequently, these organism rightly deserve their name of " iron bacteria."   According to the discoveries H MOLISCH (I.), iron can be replaced in this oxidation process by the chemical allied metal manganese. These bacteria require but a very small quantity other nutrient materials, an addition of, *e g* a few thousandths of 1 per cent. sodium acetate to ferruginous water being entirely sufficient to bring them a state of perfect development. This inexigency is also indicated by t observation, made by O. RÖSSLER (I), that *Cladothrix polyspora* can be grov on bricks moistened with a little ferrous sulphate solution. In 1894 M. BUSG (I.) succeeded in obtaining pure cultures of *Cladothrix dichotoma* on gelatin.

The decomposing power of these organisms is very great, the amount ferrous oxide oxidised by the cells being a high multiple of their own weigl This high chemical energy on the one hand, and the inexacting demands in t shape of food on the other, secure to these bacteria an important part in t economy of Nature , the enormous deposits of ferruginous ochre and bog-ir ore, and probably certain manganese ores as well, being the result of the activi of the iron bacteria

Moreover, they make their presence evident not only in natural water basi but in all other places where water rich in iron is to be found in quanti

Consequently, these organisms may develop into an actual nuisance to water-technicists by penetrating into the clarifying reservoirs and delivery pipes, and there growing so vigorously as to completely obstruct the passage of the water, and thus interrupt the service of distribution   Many towns deriving their water-supply from a soil or river water rich in iron have suffered from this nuisance ; Lille, for example, as reported by GIARD (III), and Berlin, as mentioned by W. Zopf in his treatise already referred to. In the waterworks at Lake Tegel, from which the greater part of Berlin derives its supply, these bacteria (and especially the " well-pest," *Crenothrix polyspora*) flourished so luxuriantly that they constituted more than one-half of the layer of sediment (about forty inches in depth) gradually collecting at the bottom of the reservoirs One means of obviating this nuisance (although not practicable on a small scale) is by freeing the water from its content of ferrous oxide, for which purpose P. WOLTERING and A. SASSEN (I.) recommended a method (which is said 1 answer) consisting in passing the water through coke towers where the ferroi oxide is converted into ferric oxide, the latter being. then removed by suitable strainers.

Finally, *Cladothrix odorifera* merits brief consideration.   Every one is acquainted with the peculiar smell of the soil, more particularly when moist, *e.g.* after a brief shower of rain.   According to the researches M BERTHELOI and G ANDRÉ (I.), this odour is due to a neutral organic compound, present in the soil and volatilising at the same time as water vapour.   The producer of this (not yet precisely identified) compound has now been recognised by RULLMANN (I.) in a new species of bacterium, viz , *Cladothrix odorifera*.   It occurs along with *Cl. dichotoma* in the soil, and, like the latter organism, can be cultivated on nutrient gelatin ; but whereas the colonies of *Cl. dichotoma* are inodorous, liquefactive, and turn the substratum brown in a short time (two days), those of *Cl. odorifera*, on the other hand, retain their chalky white appearance and evolve the aforesaid earthy smell   RULLMANN (II) also found that this species is capable of withstanding the influence of drought and poisons, being able to bear exposure for twenty-four hours to a 1   1000 solution of corrosive sublimate. Like its aforesaid congener, *Cl. odorifera* possesses considerable oxidising power, though this is manifested by the transformation of ammonia into nitric acid, and not by the conversion of ferrous into ferric oxide.   This mode of action is not peculiar to this organism alone, but is shared in a still higher degree by a group of bacteria whose acquaintance we shall make in chapter xxxvi.

The iron bacteria are not the only *Schizomycetes* capable of liberating the energy necessary for the maintenance of their existence from inorganic bodies. In the next two chapters we shall make the acquaintance of fresh natural groups and other processes similar to those described ; thus justifying the title of this concluding section.

CHAPTER XXXV.

THE SULPHUR BACTERIA.

§ 199.—Morphology of the Genus Beggiatoa.

THE sulphur bacteria, so called on account of their peculiar properties, diff
both in structure and external appearance from the filamentous bacteria describe
in the preceding chapter. They may be divided into two sub-groups, one
which forms the species classified by Engelmann as purple bacteria, and alread
noticed in chapter XIII. on account of their behaviour towards light. The oth
sub-group of the sulphur bacteria, which assume the form of long threads, wi
now be described.

It will be useful to preface this description with a few hints concerning art
ficial cultivation and reproduction for the purposes of investigation. The sulph
bacteria are seldom absent in marsh water, although their number is frequent
so small as to elude the inquiring eye of the microscopist. In order to cau
them to increase, the conditions of the environment must be rendered favourabl
and with this object the simple method proposed by their careful observer,
WINOGRADSKY (V), is employed. A few cuttings of the fresh root-stock of t
flowering rush, *Butomus umbellatus* (found in every pond, and by no means rai
on river banks), are placed, along with the adherent mud, in a deep vessel co
taining 3–5 litres (about a gallon) of water, a couple of grams of gypsum bein
added, and the whole left to stand uncovered at room temperature. After fi
to seven days the liberation of sulphuretted hydrogen will already be noticeabl
the gas being disengaged by various species of fission fungi present in the mi
and acting on the gypsum. In this manner the ground is prepared for t
sulphur bacteria also present, and the latter then develop rapidly. At the ei
of three to six weeks their presence can be ascertained by the aid of the micr
scope, and they gradually increase to such an extent as to be recognisable by t
unassisted eye. Generally, this diversified mixture of sulphur bacteria is n
deficient in the red species as well, but the colourless long thread forms are mc
plentiful.

Two genera were more closely investigated by Winogradsky The one
these bears the name of *Beggiatoa*, given by TREVISAN (I) in 1842 in honour
the Italian physician F. S. Beggiato of Vicenza, who, in 1838, published a coi
munication on the flora of the sulphur springs of the Euganean Hills, near Padi
The species of this genus occur as actively motile cylindrical filaments, whi
may attain a length of 1 c m and more The breadth is always constant in ea
separate species, and thus affords a means for differentiating between the
Under favourable conditions of nutrition, and especially in presence of s
phuretted hydrogen, the interior of the individual threads (Fig. 70 *a*) is seen
be well stocked with roundish, highly refractive granules, *i.e.* the sulph
granules described later on In this condition the transverse cell walls a
indiscernible or only detected with difficulty, as will be gathered from Fig. 7c
which shows the same thread after it has lost its enclosed sulphur granules by
long sojourn in water devoid of sulphuretted hydrogen Moreover, the leng
of the cells varies in the different species If this organism be deprived of t

said gas, which is indispensable to its continued existence, then the threads begin to break up (Fig. 71), the contents—except a thin coating attached to the walls—vanish, and they finally perish.   No success has attended the search for spore formation in the *Beggiatoa*.   The most abundant species of this genus is *Beggiatoa alba*, the threads of which are 2.8–2 9 μ in thickness, whilst the length of the individual members varies between 2 9 and 5 8 μ, the shortest of them being thus symmetrical.   A second species, with a diameter of 1 6–1 7 μ, the length of the separate cells being 4–8.5 μ, has been named *Beggiatoa media ,* and

FIG 71.—Beggiatoa alba.

Moribund through lack of H_2S Thread falling apart into its short members, which thereupon assume a rounded form.   Magn 900   (*After Winogradsky*)

FIG 70.—Beggiatoa alba

The same portion of thread under different conditions of existence

a in a medium rich in H_2S , the thread is densely packed with sulphur granules , *b* after twenty-four hours' sojourn in a liquid devoid of H_2S , only a few sulphur granules remain , *c* at the end of a further forty-eight hours , sulphur totally disappeared, transverse walls now visible, contents of individual cells granulated   Magn 900   (*After Winogradsky*)

FIG 72.—Terminal portion of threads of (*x*) Beggiatoa media and (*y*) B minima

Magn 900   (*After Winogradsky*)

a third kind, whose diameter is only 0 8 μ, is called *Beggiatoa minima*   Both these species are shown in Fig 72, magnified to the same extent as the first-named species   In addition to these there is still a large number of species whose threads vary in diameter between the above limits.   Compared with all these the *Beggiatoa mirabilis* noted by COHN (XII), Warming, and Engler, but not yet more minutely examined, the threads of which are said to attain a breadth of 30 μ, is gigantic.   According to Winogradsky, the breadth of the cells of any given species is—to emphasise this point once more—unalterable.

The growth of these *Schizomycetes* is very slow, a thread requiring at least twenty-four hours to double its length.   They are extremely susceptible, even merely the grip of the forceps being fatal.   For this reason they have to be sucked up by means of a small tube, for purposes of examination, and protected from the pressure of the cover-glass by introducing splinters of glass, &c., into the liquid.

§ 200.—The Species of the Genus Thiothrix,

which has been newly established by Winogradsky, differ from *Beggiatoa* by the absence of free motility, they being sessile, *i.e.* attaching themselves at one extremity by means of a mucinous sucker to the walls of the culture vessel, the cover-glass of the microscopical preparation, to stones, remains of plants, and similar quiescent substrata in the situations where they occur naturally; whilst the other end extends into and grows in the liquid. Such a one is shown in Fig. 73. In this genus, also, the articulation of the threads is ordinarily concealed by the abundant content of sulphur, but if the latter be washed out with absolute alcohol and the cells stained, *e.g.* with fuchsine, the transverse walls are plainly revealed. The length of the joints gradually increases towards the free end, as will be seen from the subjoined measurements given by Winogradsky —Length of joint near the point of attachment, 4–8.5 μ; at the apex 8–15 μ. However, there is no scarcity of considerably shorter cells So far as the breadth of the threads is concerned the above conditions are reversed, the threads tapering off towards the free end, where, for example their diameter is only 1.5 μ, compared with 2.0 μ at the base. Consequently the cells are more slender toward the tip.

Fig. 73 —Thiotrix nivea Group of young threads with one end firmly attached to the substratum by means of the sucker (indicated by dots) Magn 900 (*After Winogradsky.*)

A second characteristic point of difference from the genus previously described is the appearance of a (merely slight) sheath, whereby the moribund members are partly held together, whereas the *Beggiatoa* threads at this stage break into short fragments and finally into separate cells

A third characteristic of the genus *Thiothrix* is the dislocation (termed conidia-formation by Winogradsky) of the uppermost joint of the thread The rod-shaped cell, thus loosened from the chain, crawls a short distance along the solid substratum, then develops a mucinous sucker and grows into a new thread from which in turn conidia subsequently wander and settle in the vicinity, the result being the formation of the whitish, tufted, thread colonies characteristic of *Thiothrix*

Here also the thickness of the threads constitutes a criterion for the classification of species One of them, named by Winogradsky *Thiothrix nivea*, has diameter of 2–2.5 μ near the base, 1.7 μ in the middle, and 1.4–1.5 μ at the tip In a second species the diameter is almost uniformly 1 0–1.1 μ throughout the whole extent of the thread. It is known as *Thiothrix tenuis*, and is probably identical with a fission fungus discovered by ENGLER (I), in the so-called " deep ground " of the Bay of Kiel, and which he held to be a *Beggiatoa* and called by the specific name *B alba var. universalis*. The threads of a third species (*Thiothrix tenuissima*), from a sulphur spring at Adelboden (Switzerland), measure only 0.4–0 5 μ in breadth. W. ZOPF (VII) regarded the sessile sulphur bacteria as belonging to the morphological cycle of the *Beggiatoa*, and named them " sessile Beggiatoa," until Winogradsky proved that two distinct genera are here in question.

As will be shown later on, the life of the sulphur bacteria is indissolubly connected with the presence and availability of free oxygen In the mode of satisfying their needs in this respect the two genera differ. The *Beggiatoa* being endowed with the power of locomotion, can more readily accomplish the

object by their ability to proceed at will to the surface of the liquid Consequently this species gains the upper hand in stagnant or quietly flowing waters, in which they search about so eagerly that very little of the oxygen diffusing into the water can reach the bottom where the *Thiothrix* species rest. The latter, however, have the advantage in rapid running water, the loose *Beggiatoa* species being washed away by the current. In either event, whitish mucinous masses highly characteristic of sulphur springs accumulate in time—*e.g* those of Barèges in the French Pyrenees—and are known in France as *barégine* or *glairine*

§ 201.—Morphology of the Non-Filamentous Sulphur Bacteria.

Several red species of these organisms are already known to us (§ 91), viz , *Chromatium* (*Monas*) *Okenii, Monas Warmingii, Spirillum rubrum, Sp volutans, Ophidomonas sanguinea, Rhabdomonas rosea* These are again shown in Figs 74 to 78 It was remarked in § 68 that Ray Lankester had assumed all these

FIG. 74.
Chromatium Okenii
Magn 600 (*After F Cohn*)
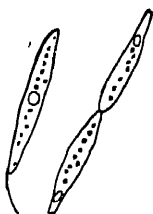
FIG 75
Rhabdomonas rosea
Magn 600 (*After F Cohn*)

FIG 76
Monas Warmingii.
Magn 600 (*After F. Cohn*)
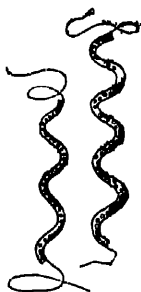
FIG 77 —Spirillum volutans
Magn 600 (*After F Cohn*)
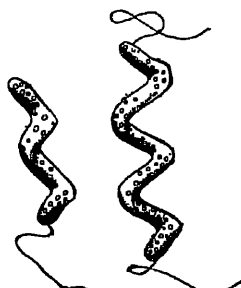
FIG 78.—Ophidomonas sanguinea
Magn 600 (*After F Cohn*)

organisms to be merely special forms of one species for which he proposed the name *Bacterium rubescens.* The basis for this assumption was, however, a very insufficient one, since it rested principally on the identity (which, moreover, was not satisfactorily demonstrated) of the red colouring-matter, peculiar to these organisms, and which received from Lankester the name bacterio-purpurin This investigator was supported in his views by Warming (in 1875), who on his part classified a large number of the red sulphur bacteria examined by him into a single species, viz., *Bacterium sulfuratum* Zopf (in 1882) went still farther than either by defining all these organisms as special forms of growth

of a single species of thread bacterium, viz, *Beggiatoa roseo-persicina*, which, under certain circumstances, was said to appear as long threads (*Leptothrix*), and under others as fractions of such threads, viz, as *Monas*, *Spirillum*, &c, capable of developing once more into threads.

The re-investigation of these discoveries (which were not made with pure cultures) by Winogradsky led to the refutation of this assumed variability of form, and also to the discovery that the above-named red sulphur bacteria are not capable of **progressive** development, *i.e* of growing into thread form. Some little doubt still prevails as to the existence of **retrogressive** development, *i.e.* the dismemberment of short cells from filamentous red *Beggiatoa* species In contradiction of Winogradsky's statement that the filamentous sulphur bacteria (*Beggiatoa* and *Thiothrix*) are invariably colourless, and consequently cannot throw off coloured cells, W. ZOPF (VIII), in a subsequent communication (1895), reported the existence of red *Beggiatoa* species which become dismembered into short (sulphur-bearing) cells The question must consequently be considered as requiring further investigation. The results will, however, be chiefly of botanico-morphological interest, and will not affect either the firmly established theory of the pleomorphism of the *Schizomycetes*, or touch the physiology of the sulphur bacteria, which latter is the sole property meriting consideration, so far as we are concerned   So long, however, as Winogradsky's discoveries remain uncontroverted by any thoroughly reliable investigations, his deductions must be allowed to stand, viz., that the sulphur bacteria are *not* pleomorphic—neither the colourless, filamentous genera nor the non-filamentous red genera   The Russian physiologist described a long series of species of the latter type, which, as they are devoid of special physiological importance, we need not examine more minutely   It will be sufficient to mention the chief forms

The aforesaid purple bacteria are only a single sub-group comprising all those sulphur bacteria whose living cells are free and capable of locomotion. It is divided into three genera, *Chromatium*, *Rhabdochromatium*, and *Thiospirillum* Of these terms the penultimate one is synonymous with Cohn's *Rhabdomonas*, whilst the last one comprises all red sulphur-bearing spirilla, and consequently includes Ehrenberg's *Ophidomonas*. A contrast to this sub-group of free cells is afforded by the species of red sulphur bacteria which are generally united as colonies   In the genera *Thiocystis* and *Thiocapsa* this union is effected by a mucinous sheath, which is absent in *Thiosarcina*.   In all three cases reproduction occurs by fission in three directions, and the same behaviour is exhibited by the genus *Lamprocystis*, which principally differs from the other three in the structure of its bag-shaped zoogloea, which is hollow internally, and consists solely of a lattice-like network. A good representation of this species was given by COHN (II) who described it, along with other organisms, as *Clathrocystis roseo-persicina*. The genus *Thiopedia* is characterised by the division of the cells in *two* directions of space, and by the consequent flat colonies   In the remaining species cell fission occurs in *one* direction only. The *Amoebobacter* species are distinguished by an amoeboid movement, those of *Thiodictyon* owe their name to the reticular conjugation of their spindle shaped cells, whilst *Thiopolycoccus* forms zoogloea of closely crowded cocci. The genus *Thiothece* is distinguishable from all other sulphur bacteria by its particularly thick gelatinous sheath.

A few remarks with regard to the properties of **bacterio-purpurin** will be opportune in this place. The difficulties in the way of preparing a quantity sufficient for the performance of a chemical analysis have not even yet been overcome ; consequently its chemical composition is still entirely undefined, and we cannot yet say with certainty whether the colouring-matter is the same in all red sulphur bacteria. This is, however, assumed to be the case, on the

ground of the concordant results yielded in separate instances by chemical reactions, a few of which are now given. The pigment is insoluble in water or ether, but is soluble in cold alcohol (as found by Winogradsky in contradiction of Lankester's report). It is converted, by warming with water and by chloroform, into a golden-brown compound, which is changed into brown by hot alcohol, hydrochloric acid or acetic acid; whereas ammonia or caustic potash produces no visible change at first, but finally gradually develops a dirty shade of colour Concentrated sulphuric acid changes the red almost instantly into a deep blue, which afterwards gradually tones into a brownish green. This reaction resembles that set up by the same acid with the lipochromes. Bacterio-purpurin is very quickly destroyed by oxidising agents (e g. dilute nitric acid or bromine water) Iron and manganese appear to favour its production, a conclusion deduced from the fact that the addition of the protosulphide of either of these metals to the medium results in a much stronger coloration of the cells. The sensitiveness of bacterio-purpurin to chemical influences explains the varied change of tone produced in the colour in one and the same cell under different external conditions, causing it to assume all shades, from pure violet to purple, peach-blossom red, rose, orange, brown-red, and brown. With regard to the spectrum of bacterio-purpurin, examined by Lankester, Warming, and Englemann, details have already been given in § 92

The classification of the non-filamentous sulphur bacteria, drawn up by Winogradsky and briefly outlined above, received an important extension by M. Jegunow's (III) discovery that, in addition to the red species already described, certain colourless non-filamentous sulphur bacteria also occur in Nature. Two of these he subjected to a closer physiological examination, which will be referred to in the succeeding paragraph. The one of them, indicated as species α, occurs as slightly curved motile rods, their breadth varying from 1.4 to 2.3 μ, and the length between 4.5 and 9 μ For the second species, known as β, the dimensions are 0.6–0.8 μ and 2 5–5 μ respectively

§ 202.—Physiology of the Sulphur Bacteria.

The true nature of the rounded, highly refractive enclosures present in these fission fungi, and attracting the eye of the microscopist, was first recognised by Cramer, whose discoveries are noticed in a treatise by C Müller (I). It was shown in these experiments that these granules behaved exactly like sulphur in presence of solvents, and they were therefore thought to consist of that element. F. Cohn's (II) extension of these investigations (which were confined to *Beggiatoa*, and were confirmed by J. Mayer-Ahrens) to the red sulphur bacteria as well, led to the same result the granules appearing in these coloured *Schizomycetes*, under certain—as yet undefined—conditions, are composed of pure sulphur. The term granules applied to these forms is unsuitable, inasmuch as they consist not of solid granular, but (as Winogradsky afterwards proved) of oily, amorphous sulphur, the greater part of which is soluble in CS_2. However, when the enveloping cells are killed, the sulphur granules are gradually changed into the crystalline modification of this element. If a few *Beggiatoa* threads rich in these droplets be immersed in concentrated picric acid and left in water, a number of very fine monoclinic prismatic plates and rhombic octahedra will be found in the threads after a lapse of twenty-four hours, and it will at the same time be noticeable that the growing crystals have penetrated the adjacent cell walls.

F. Cohn was the first to investigate the origin of these internal constituents, which occasionally fill the cell to such an extent as to exceed 90 per cent of its weight. Starting from the fact that the sulphur bacteria are only found in

abundance in natural waters containing sulphuretted hydrogen, and are, on the other hand, almost entirely lacking elsewhere in Nature, he came to the opinion that this gas is produced by the reducing action of these fission fungi on the sulphates in the water, and that they subsequently reoxidise the gas, sulphur being left as a deposit in the cells. In forming this opinion he was chiefly influenced by the result of an investigation made by LOTHAR MEYER (I.), who kept a sample of sulphur-spring water (rich in *Beggiatoa*) from Landeck in Silesia for four months in a stoppered flask, and found that at the end of that time it contained five times as much H_2S as at first. The same conclusion as deduced by Cohn was also arrived at by E. PLAUCHU (I), and by A ETARD and L OLIVIER (I.). This hypothesis, which credited the sulphur bacteria with both a reducing and an oxidising capacity, was first thoroughly investigated in 1886 by S WINOGRADSKY (VI), who showed that the sulphur bacteria consume (instead of producing) sulphuretted hydrogen, oxidising it and storing up the separated sulphur in their cells The amount of these enclosures in the cell is larger or smaller according as this process can be carried on with a greater or lesser degree of vigour. It depends, therefore, on external conditions, and consequently cannot be relied on—as was done previously by various observers *inter alia*, Winter in Rabenhorst's "*Kryptogamen Flora*" and by Engler—as a characteristic for the differentiation of species. The sulphur does not permanently remain in the cells, but is oxidised by them to sulphuric acid, the latter being then absorbed by the carbonates—usually $CaH_2(CO_3)_2$—in the water, and converted into sulphates.

If these *Schizomycetes* are deprived of sulphuretted hydrogen for a long time, they consume their internal store of sulphur (which will be exhausted in twenty-four to forty-eight hours), and then perish of hunger. This fact demonstrates that the sulphur bacteria cannot permanently dispense with sulphuretted hydrogen, but that this gas is actually their special (and almost exclusive) source of energy. Sulphur, or rather its compound with hydrogen, plays the same part towards these organisms as the carbohydrates do towards the majority of *Schizomycetes*, its combustion liberates the energy necessary to the maintenance of their vitality According to the observations of Winogradsky, the individual threads of *Beggiatoa* daily consume from two to four times their own weight of the gas. These *Schizomycetes* require but little other (organic) nutriment, and in fact will not stand very much. This explains, on the one hand, their unusually slow rate of growth in proportion to the amount of sulphur separated, and, on the other, their inability to grow in the ordinary nutrient media employed in bacteriology e g. on gelatin they perish in a very few minutes Attempts to grow them as pure cultures on a large scale have hitherto failed, and the physiological facts determined concerning them have all had to be ascertained very laboriously by cultivating single organisms in sulphur-water on microscope slides.

The optimum, *i.e.* the maximum supportable, quantity of sulphuretted hydrogen in the water is higher in the case of the red sulphur bacteria than with the colourless, filamentous species. These latter require less, and in fact die instantly if placed in water saturated with the gas, whereas the red kinds will stand this degree of concentration very well Consequently, under natural conditions, these latter will gain the upper hand in such places where large quantities of sulphuretted hydrogen are evolved, either as a result of the decomposition of an abundance of organic matters (albumen) or by the powerful reduction of sulphates. This is the case, for example, in the stagnant shallow bays on the Danish Zeeland coast, and the same conditions obtain in the Limanes so plentiful along the coast of the Black Sea (e g near Odessa). These latter are shallow salt lakes, separated from the open sea merely by a low,

narrow tongue of land.   Their bottom is covered by a thick mud, which owes its
black colour to the FeS thrown down from the iron compounds in the water
(and in the plants rotting therein) by the sulphuretted hydrogen generated from
sulphates by the reducing action of bacteria investigated by E. BRUSILOWSKY (I.).
The red sulphur bacteria are but rarely found in mineral sulphur springs.
According to Cohn, they have been detected by Morren in the sulphur spring at
Ougrée, on the Maas; by Fontane and Jaly, in that at Sales, in the P
by Meneghini, in that of the Euganean Hills, near Padua; and by Cohr
in that of Tivoli, near Rome.

The existence of the sulphur bacteria is often a very hard one, be
requires the simultaneous presence and availability of two gases which ne
one another and become converted into sulphur and water—

$$H_2S + O = H_2O + S.$$
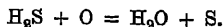
So that actually the surface of liquids wherein H_2S is produced in abundar'
the activity of reducing bacteria becomes coated with sulphur formed by '
chemical means, in accordance with the foregoing equation.   Now, in ord
the sulphur bacteria may be in a position to exert their powers of oxid
becomes necessary for them to inhabit certain strata of the liquid betr
limits where the oxygen can gain access from above and sulphuretted h
reach them from below   If the liberation of the latter gas goes on bris
level rises, and may ascend to the surface of the liquid, otherwise it s
approaches the bottom, where the sulphuretted hydrogen is generate_
change of feeding-ground cannot, however, be followed by all species of sul
bacteria, since—just in the same way as has been explained with regard _
sulphuretted hydrogen—these organisms are adapted to a certain tension of
oxygen, which varies in the different species, i.e they cannot stand the presence
of more than a certain quantity per unit of volume of the liquid.   In the case
of oxygen, this tension is naturally greatest at the surface and smaller at greater
depths.   It will be evident that even the fluctuations of atmospheric pressure
will suffice to produce a change in the predominating species of a diversified
mixture of sulphur bacteria in their natural haunts.   The same applies to the
rate at which the sulphuretted hydrogen is disengaged.

For an instructive insight into these conditions we are indebted to the re-
searches of M. JEGUNOW (I.) on the colourless non-filamentous species referred to
at the close of the last paragraph   As already stated, the habitat of the sulphur
bacteria is in those strata of the liquid where the oxygen from above comes
into contact with the sulphuretted hydrogen from below.   At this level the
organisms congregate to form an assemblage visible to the naked eye, and which
the above-named Russian physiologist termed the **bacterial plane**, the structure
of which he examined minutely.   He artificially induced the processes going on
in the Limanes to repeat themselves—so far as necessary to the purpose in view
—on a small scale in the laboratory, by placing a certain quantity of the black
mud in suitable vessels containing water, and then leaving the whole to stand
uncovered   We will not go further into the matter of the rise and fall of the
bacterial plane as observed by him, because BEYERINCK (I.) had made similar
experiments two years earlier, and applied to the phenomenon a term (*Bakterien-
Niveau*) having the same significance as that used by Jegunow.

The discoveries made by JEGUNOW (II.) with regard to the construction of
this bacterial plane, in the case of the organisms now in question, must, however,
be considered as novel   When cultivated in higher and broader, but thinner,
strata of liquid, the plane assumes the form reproduced on a reduced scale in
Fig 79, i e the bacteria do not form a simple plane, but become piled up in
places into tuft-like projections—each about 3–4 mm long—four of these being

shown (enlarged) in Fig 80   The examination of these tufts by the aid of
a horizontal microscope shows that they are formed by the movement of the
individual bacteria, in a manner similar to the gushing of a spring, they ascend
in the axis of the tuft, and then describe an arc in their return to the plane.
When inverted by the microscope this resemblance is still more striking, so
that Jegunow has styled the planes " fountain planes." The velocity of the
individual cells he found to be 0.02 mm. per second.   In tracing the chemical
activity of the bacteria, he made use of a simple and reliable reagent for sulphu-
retted hydrogen   a fine (woollen or similar) thread treated first with ferric
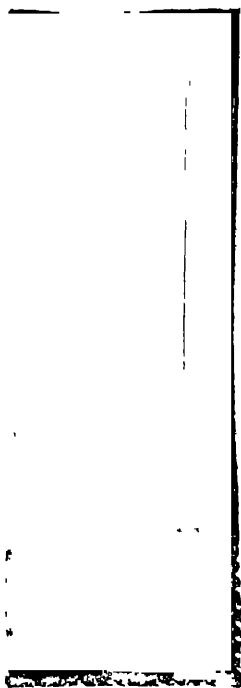
FIG 80 —A portion of the Bacterial plane from the preceding Fig
showing the arched construction of the plane itself, as well as
four of its fountains. Magn 11. (*After Jegunow*)

FIG 79 —Culture of Sulphur Bacteria from the Limanes (in a small vessel, reduced scale)

The figures on the label give the dimensions, the thickness of the layer of liquid being 09 mm   The
bottom is occupied with black Limanes mud, above this is the liquid, the meniscus of which is
visible at the top of the Fig, and in between is the bacterial plane with five "fountains" (*After
Jegunow*)

chloride and then with ammonia, both in such a very dilute condition that the
thread is stained merely a pale yellow. A glass weight is then attached to the
thread and let down into the liquid, whereupon the lower part of the thread, as
far as the summit of the tufts on the fountain plane, quickly turns black, from
the formation of FeS   From that point onwards, however, the colour gradually
changes to white. This experiment shows that in the summits of the tufts the
sulphuretted hydrogen arising from below is first oxidised to sulphur, and stored
up in the cell, which conveys it to a higher level (the actual plane), and there
oxidises it to sulphuric acid   This acid then dissolves the ferric oxide on the
upper part of the thread, which is consequently decolorised at this level   The
time occupied by the cells in making a single trip—and therefore the total

period required for the conversion of H_2S into SO_3 and the expulsion of the latter from the cell—was ascertained by Jegunow to be about five minutes.

The importance of the sulphur bacteria in the economy of Nature is unmistakable in co-operation with the sulphate-reducing bacteria they ensure that the sulphur cycle pursues an uninterrupted course, the element being taken up by the higher plants in the condition of sulphates, and deposited in the cells in the form of organic compounds, from which, in the course of putrefaction, it is liberated as sulphuretted hydrogen, and is finally then reconverted into sulphates by the sulphur bacteria and recommences its course through the higher plants.

CHAPTER XXXVI.

THE NITRIFYING BACTERIA.

§ 203.—The Recognition of Nitrification as a Physiological Process.

THE nitrogen excreted from the animal body as urea has not, when converted into ammonium carbonate (*see* chap xxxii), yet attained the form in which it is usually taken up by plants. Although it is indubitable that plants in general can obtain their requirements of nitrogen from the ammonia salts, it is nevertheless certain—both as a result of manuring experiments on the small scale and also from the experience of agricultural practice—that the majority of cultivated plants absorb the element in question more rapidly and abundantly when it is offered them in the form of nitrates In fact, for some of them, *e g.* maize, buckwheat, and tobacco, JUL. LEHMANN (I) puts forward the well-grounded assumption that they derive their nitrogen exclusively from nitrates. Here again Nature has made provision for the necessities of the case by converting into nitrates the ammonia salts which—partly as a result of decomposition and partly as artificial manures—find their way into the soil

This process, long known and briefly termed nitrification, was defined in 1846—on the basis of an experiment by J. DUMAS (II)—as a purely chemical process of oxidation This observer regarded chalk as the intermediary facilitating the intimate combination of ammonia and atmospheric oxygen Fifteen years later this *rôle* of " go-between " was ascribed by MILLON (I.) to the porous humic bodies in the soil—a view that still remained destitute of any convincing proofs when revived in 1863 by BLONDEAU (II.)

Ten years later other opinions began to arise. The first adverse hypothesis was expounded in 1873 by ALEX. MÜLLER (I), but was not based on any solid foundation, nor was it followed up any farther. Four years afterwards SCHLOESING and MÜNTZ (I.), relying on the results of their researches in this direction, hazarded the opinion that the formation of nitre in the soil is due to the vital activity of organised ferments (soil bacteria) In a subsequent communication these two workers detailed some of the conditions requisite for the inception and course of nitrification. The operation is almost stagnant below 5° C., but becomes apparent at 12° C , and attains its maximum at 37° C. As the temperature rises still higher the reaction becomes weaker, and ceases altogether at 55° C. It proceeds the more rapidly as the degree of moisture in the soil increases, provided aeration is not thereby impeded. A faintly alkaline reaction facilitates the progress of nitrification, which, moreover, may not always result in the production of nitrates, but at times does not extend beyond the formation of nitrites, especially at low temperatures (below 20° C.) and with a restricted admission of air.

Both workers also endeavoured to obtain pure cultures of the organisms under investigation The result of their endeavours will not be judged too harshly when the existing lack of any reliable method of pure cultivation at that time is remembered. When the introduction of the Koch gelatin plate afforded a new appliance for this purpose, it was pressed into the service now under

consideration by several workers, *inter alia* by ADAMETZ (IX.) and A. B. Frank; nevertheless, the result did not fulfil expectations. The last-named German mycologist then contradicted the assumptions of the two French agricultural chemists, and championed the views held by Dumas. To this revival of an old hypothesis we owe the production of a comprehensive work by H. PLATH (I.), which is commended to the attention of the reader not only on account of the new discoveries it mentions, but also because the first part contains a collection—rich in information for the chemist—of all the then known methods for the production of nitric acid from ammonia by oxidation In the second part of this treatise it was stated, on the basis of new experiments, that completely sterilised soil no longer possesses the faculty of converting ammonia into nitric acid. It was furthermore shown that, when organisms are entirely excluded, neither the soil as a whole, nor any one of its constituents, is capable of transforming ammonia into nitric or nitrous acid by occluding atmospheric oxygen. A re-examination of this work by H. LANDOLT (I.), who undertook the task in consequence of an objection raised by A. B. FRANK (IX.), led to a complete confirmation of Plath's discovery on all points. It was thus ascertained (in 1888) by the exclusion method that in the oxidation process now under our notice the *rôle* of oxygen-carrier is played by living organisms, and that consequently nitrification is a physiological process.

§ 204.—Nitroso-Bacteria and Nitro-Bacteria.

The discovery and closer investigation of these unknown organisms was shortly afterwards effected by S WINOGRADSKY (VII) It is not surprising that their preparation as pure cultures was so long delayed, when we remember that these bacteria do not thrive on media rich in organic nutrient substances. The above-named Russian physiologist successfully employed for this purpose the gelatinous inorganic substance, viz, precipitated silica, recommended by W. KÜHNE (I.). When prepared by precipitation from water-glass (alkali silicate) by hydrochloric acid, and purified by dialysis, concentrated by boiling, and then sterilised in the steamer, this silica forms a vitreous mucinous mass This is then incorporated with a sterilised solution of the sulphates of potash, magnesia, ammonia, and carbonate of soda, inoculated with the bacterial sample. These salts cause the silica to set, so that the germs in the sowing are fixed separately, and thus may be kept apart, even when they have developed into colonies. In this manner Winogradsky primarily succeeded in obtaining cultures of assured purity, by means of which he was enabled to arrive at conclusions unattainable by the fractional sowings and dilution method employed by previous workers, *e.g* W. HERAEUS (I.), P. FRANKLAND (III), and R. WARINGTON (III.)

One of the weightiest of these results is the fact determined by WINOGRADSKY (VIII.) that the numerous species of the group of nitrifying bacteria are classifiable into two sharply divided sub-groups: nitroso-bacteria and nitro-bacteria.

The nitroso-bacteria oxidise ammonia to nitrous acid, in accordance with the equation—

$$(NH_4)_2O + 3O_2 = N_2O_3 + 4H_2O,$$
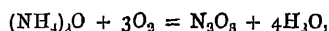
but no farther. For this reason nitrites are not altered by these bacteria.

On the other hand, the nitro-bacteria lack the faculty of attacking ammonia, but perform the task of converting nitrous acid into nitric acid, in accordance with the equation—

I   T

$$O\left\langle\begin{matrix}N=O\\N=O\end{matrix}\right. + O_2 = O\left\langle\begin{matrix}N=O\\=O\\=O\\N=O\end{matrix}\right.$$
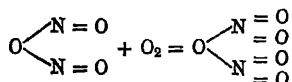
As is apparent from this equation, their powers differ from that of the nitroso-bacteria, inasmuch as the latter convert the pentavalent nitrogen of ammonia into the trivalent nitrogen of nitrous acid, whilst the nitro-bacteria re-convert the element into the pentad condition.

It is evident that these oxidation processes can be effected only in the presence of bases which take up the acids with which the ammonia was initially combined, and also neutralise the resulting nitrous or nitric acid—thus protecting the bacteria from injury from this source. This task is excellently performed in the soil by calcium carbonate. The favourable influence exercised on the course of nitrification by the presence of this salt is therefore readily explainable without dragging in any hypothesis about the condensation of oxygen Free alkali is unsuitable here for the fixation of the acids, since the presence of this reagent in quantity would be injurious to the bacteria. In artificial cultures calcium carbonate can be replaced by magnesium carbonate, a practice adopted by Winogradsky.

§ 205.—Nitrosomonas and Nitrosococcus.

Two main types of nitroso-bacteria can be differentiated in consequence of the results of existing investigations. One of them (in several species) is found in all the soils of the Old World (Europe, Asia, Africa) hitherto examined, and is known as *Nitrosomonas*. The second is peculiar to the soil of the two remaining continents, and has received the name of *Nitrosococcus*. The individual organisms of the first-named type are each provided with a single cilium, and exhibit powers of locomotion which are manifested at an early stage in the cultures, and cause these to become decidedly opalescent. Subsequently the cells become quiescent and collect as zoogloea, which rest in the form of greyish gelatinous clouds on the carbonate at the bottom of the liquid. We will describe this (*Nitrosomonas* genus first.

Only a single species of nitroso-bacterium has been discovered in European soils, viz., *Nitrosomonas europaea*. At the opalescent stage of the culture this organism appears as briskly motile cells (fitted with a short flagellum) in the shape of short rods 1.2–1.8 μ long and 0 9–1.0 μ broad. The cells of *Nitroso monas javanica*, cultivated from the soil of the Botanical Garden at Buitenzorg near Batavia, are globular, and only attain a diameter of 0.5–0 6 μ, but their flagellum is very long—as much as 30 μ. The *Nitrosomonas japonica*, found in soil from Tokio, is—like the *Nitrosomonas africana*, isolated from samples of soil from Tunis and from La Reghaïa, in Algeria—very similar to the European species, only somewhat smaller.

Differing from these species are those of the genus *Nitrosococcus*, found in South American and Australian soils. They do not form zoogloea, neither are they ciliated. That obtained from Quito (Ecuador) is a coccus 1.5–1.7 μ in diameter. A similar species, except in point of size, is the *Nitrosococcus brasil ensis*, obtained as a pure culture from the soil of Campinas (Brazil), and attaining a diameter of 2 μ; and the species grown from Melbourne soil is undistinguishable from this latter The nitroso-bacteria are, as observed by Winogradsky (IX. very susceptible to desiccation, and consequently the amount of such organism in the soil decreases as drying progresses. They are almost entirely lacking in the air.

§ 206.—The Nitro-Bacteria.

differ from the species already described, not only from a chemico-physiological,
but also from a morphological point of view, being smaller and more slender
The cells are an elongated oval, mostly pear-shaped, 0.5μ in length and 0.15–
0.25μ in breadth, and are therefore among the smallest of all known organisms.
In liquid cultures they develop and congregate to form a thin, mucinous skin
adhering firmly to the walls of the vessel. Compared with their powerful
oxidising action, the vegetative development of these organisms is astonishingly
slight. Spore formation has not been found either in these or in the nitroso-
bacteria; and up to the present no subdivision of the genus *Nitrobacter* into
species has been made.

BURRI and STUTZER (III) in 1895 obtained from Hanoverian soil a nitro-
bacterium which they assert will thrive both on nutrient gelatin and in bouillon,
but (so it is said) exhibits no nitrifying action in nutrient media of this kind, as
a rule, and, indeed, loses this power entirely, so that when re-transferred into
mineral nutrient solutions it does not attack the nitrites placed at its disposal
A careful examination of such a culture, obtained direct from the above-named
chemists, was made in 1896 by S. WINOGRADSKY (X.), who showed that the
alleged pure culture contained, not only the nitro-bacterium, but also *three* other
species of (saprophytic) bacteria which thrive well in bouillon, a medium in which
the nitro-bacterium will not grow. Winogradsky's treatise is recommended
to the reader, more particularly because it mentions numerous contingencies
likely to arise in working, and render of no avail the trouble bestowed on the
nitrifying bacteria by the bacteriologist. Furthermore, he gives a new recipe
for a medium for the pure cultivation of nitro-bacteria, more convenient in use
than gelatinous silica, viz., nitrite agar-agar, *i.e.* a mineral solution containing
nitrites and qualified by 1.5 per cent. of agar-agar.

If the amount of nitrogen oxidised per unit of time be taken as the standard
for measuring the chemical energy of these organisms, then—as Winogradsky
ascertained by comparative investigations—the nitroso-bacteria will be found
the more active of the two. From this fact it is permissible to draw the further
deduction that the conversion of the trivalent nitrogen of nitrous acid into
pentavalent nitric nitrogen requires the expenditure of a greater amount of
internal force than is needed for the converse operation in the oxidation of
ammonia to nitrous acid.

Both nitroso- and nitro-bacteria are always present in the soil, the second
type of organism immediately oxidising the nitrous acid generated (from ammonia
salts) by the first. Whether nitrification begins already in the dung-heap, or
has its first inception in the field, is dependent on various circumstances. It
will proceed whenever a sufficient quantity of ammonia salts has been produced
by the fermentation of urea, provided air has ready access. Thus, H. IMMEN-
DORFF (III.) showed that in the outer layers of manure heaps (especially horse-
dung), the production of nitrous acid will set in briskly in a few days. There
are ample reasons why the formation of the easily lixiviable nitrates, which may,
moreover, expose the materials to wasteful reduction processes, should be pre-
vented in the manure heap. On this account endeavours should be made to
minimise the aeration of the manure by battening the heaps well down.

§ 207.—Assimilation in the Dark.

The incapacity (recorded in § 204) of the nitrifying bacteria to grow on
nutrient gelatin is, in the main, attributable to their general distaste for organic

nutriment, a peculiarity noted by MUNRO (I.) in 1886. The smaller the quantity of organic food present, the more energetically do growth and oxidation proceed; and the latter effect is most powerful in solutions containing exclusively inorganic matters. For nitroso-bacteria Winogradsky recommends a mixture of 2–2.5 grams of ammonia sulphate, 2 grams of common salt, and a sufficient quantity of magnesium carbonate per litre of well-water. For nitro-bacteria the ammonia salt is replaced by sodium nitrite.

When such a nutrient solution containing solely inorganic matters is inoculated with a few nitroso- or nitro-bacteria, energetic oxidation occurs, accompanied—as was first brought into notice by W HERAEUS (I.) in 1886—by a rapid reproduction of the bacteria. When development is concluded, and the available quantity of ammonia or nitrite oxidised, then the bacterium crop grown in this manner contains a certain quantity of organic matter, the carbon of which has been exclusively derived from inorganic sources—in this case carbon dioxide. The amount was ascertained by Winogradsky, by four quantitative analyses, as 0 020–0 022 gram per 100 c.c of liquid. Consequently the nitroso- and nitro-bacteria are able to abstract from carbon dioxide, *in the absence of light*, the carbon necessary for the construction of their cells, and are therefore able to assimilate carbon dioxide *in the dark*.

Two sources of carbon dioxide are available to the nitrifying bacteria. One of them is the **carbonate** present in the nutrient solution (or soil), and which is also necessary for other reasons already given in § 204 According to Winogradsky, this carbonate supplies carbon to the newly formed bacteria, which are assumed to decompose it by means of the acids they produce, and then utilise the carbon in the construction of new cells He considers that the function of these organisms is to liberate and restore into general circulation the carbon that, by any means, has been converted into carbonates, and so withdrawn therefrom. On the other hand, E. GODLEWSKI (I.) showed that it is chiefly from the atmosphere that the carbon dioxide requisite for the construction of new cellular substance is derived. He found that development did not occur in cultures containing magnesium carbonate when only air free from carbon dioxide was admitted. Now the atmosphere contains not only carbon dioxide, oxygen, and water, but also ammonium carbonate, with which substances the nutrient requirements—ash constituents apart—of the nitrifying bacteria are satisfied. These organisms will therefore be able to develop in places where there is nothing present but bare rock, the cracks and fissures of which afford them a shelter against the desiccating action of the winds. In fact, it was in such arid places that A. MÜNTZ (I.) constantly found nitrifying bacteria. It can very easily be shown that friable ("rotten") stone, especially that from the Faulhorn, is thickly impregnated with these organisms.

In order that the carbon of the carbon dioxide may be prepared for its ultimate purpose, it must first be freed from the two attached atoms of oxygen. In green plants the force requisite for this purpose is supplied by the thermal power of the sun's rays ; but in the nitrifying bacteria, which also assimilate in the dark, it is the energy liberated during the oxidation of nitrogen that effects the dissociation of the carbon dioxide molecule. Consequently, the assimilation of carbon is dependent on the oxidation of nitrogen, a fact quantitatively proved by Winogradsky. According to this authority, about 35 mgrms. of nitrogen are oxidised for *each* milligram of carbon assimilated, the atomic ratio being—

$$C : N = 1 : 30$$

More accurate knowledge of the progress of this assimilation—especially on the thermo-chemical side of the question—is at present lacking. F. HUEPPE (VIII) and O. LOEW (V) constructed equations to represent the

changes occurring in the reaction, but these can merely be alluded to here. Godlewski ascertained that by no means the whole of the ammoniacal nitrogen eliminated during the nitrification is recovered as nitrous or nitric acid, but that a portion is liberated in its elementary condition, and escapes from the solution undergoing nitrification. It may be opined that this loss is not immediately connected with the action of the nitrifying bacteria, but is only an associated phenomenon produced by the reaction of the N_2O_3 on the still undecomposed NH_3, in accordance with the equation—

$$N_2O_3 + 2NH_3 = 3H_2O + 2N_2.$$
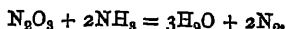
The reason for this is that the nitrous acid liberated does not in every part of the liquid come into immediate contact with the carbonate which would protect it from the action of the ammonia.

§ 208.—Wall-Saltpetre and Plantation-Saltpetre.

The particulars already given of the life-conditions of the nitrifying bacteria will explain the origin of wall-saltpetre, i e. the corroding efflorescence of salt-petre on masonry. This substance is a white snow-like mass, consisting principally of crystals of calcium nitrate, and occurring with particular frequency on the walls of stables and closets. It is precisely in such places that the fission fungi under discussion find an abundance of the food-stuffs they require : the ammonia salt is supplied by the urea absorbed by and hydrated in the walls ; calcium carbonate and a little alkali are present in the brickwork, and there is no lack of the necessary oxygen. Consequently all the preliminary conditions favouring the activity of the nitrifying bacteria introduced in dust, &c., are fulfilled. However welcome this activity may be when restricted to the soil, it is entirely undesirable in brickwork, the latter being gradually corroded and rendered brittle by the calcium nitrate produced. Sprinkling the walls with powerful antiseptics, such as antinonnin, may, however, afford a remedy. That the phenomenon is really due to the nitrifying bacteria has been proved by the researches of O. HELM (II.) and G TOLOMEI (II)

A few words must also be devoted to the saltpetre plantations. Since the discovery of the South American deposits of nitrate of soda, which substance can be converted into saltpetre by treatment with potassium salts, the production of plantation-saltpetre has decreased. It will, however, come to the front again whenever the Chilian beds are exhausted. In fact, the production of saltpetre for agricultural purposes by this method is even now worthy of consideration. The quantity of Chili saltpetre imported by European countries is very considerable, and large sums of money are annually disbursed to South America which might be retained by producing the saltpetre at home. The accomplishment of this project necessitates a searching investigation of all the conditions of nitrification, in order to ascertain how the reaction may be suitably controlled. The result would be that, instead of using expensive foreign nitrate, the ground would be manured with cheap sulphate of ammonia, now formed as a waste product in home gas-works and coke-factories, and put upon the market in constantly increasing quantities. The consequent freedom from the hands of Chilian speculators would be a great gain from the point of view of national economy. Moreover, this method of manuring presents another advantage from the standpoint of the agricultural economist As is well known, the soil has no power of fixing nitrates, a certain portion of the added saltpetre invariably—as P. DEHÉRAIN (III. and IV.) and others have shown—escaping in the drainage-water, so that more has to be added to the soil than is recovered in the crop. This disadvantage does not attach to manuring with salts of ammonia, since they

are fixed by the soil and protected from wasteful lixiviation, the nitrifying bacteria then oxidising the ammonia and supplying the plant with nitrates according to its requirements.

So far as plantation-saltpetre is concerned, the external conditions favouring the rapid formation of this compound have been gradually ascertained by means of tentative experiments. A pyramidal heap, resting on an impervious clay foundation, is prepared by mixing chalky soil with various kinds of organic matter, and is frequently watered with liquid manure, an admixture of brushwood in the heap imparting porosity and facilitating aeration. The nitrates, &c., formed in the interior appear—like wall-saltpetre—on the surface of the mass, and gradually increase to form a crust which is richer in nitrates than the interior of the heap The crude lye obtained therefrom by lixiviation is treated by adding a potassium salt in order to convert the nitrates of calcium, magnesium, and sodium into potassium nitrate, the crude saltpetre thus produced being then purified in refineries.

The elucidation of the optimum external conditions for influencing nitrification has been attempted by numerous investigators, and a few of their results will now be given. J. Dumont and J. Crochetelle (II. and III.) found that the chlorides of potassium injuriously affect nitrification, whereas the carbonates of these metals, and also potassium sulphate, act beneficially. From what has already been stated it will be evident that the merely faint (or altogether inoperative) activity of the nitrifying bacteria in soils poor in calcium carbonate (e.g. sour meadow-land) can be stimulated by the addition of the said carbonate. On this point a few experiments have been made by J. Dumont and J. Crochetelle (I.). The kind of acid with which the ammonia is combined must not be regarded as unimportant, Hueppe and Winogradsky having noticed that—as afterwards shown by the special experiments of O. Loew (VI.)—nitrifying bacteria do not attack ammonium formate at all, and that the oxalate is acted upon only very imperfectly, and with great difficulty.

CHAPTER XXXVII.

ACETIC FERMENTATION.

§ 209.—Discovery of the Acetic Acid Bacteria.

IF beer, wine, or other similar alcoholic liquids, are left to stand exposed to the air, they will, at the end a few days, become covered with a tough, mucinous (usually smooth) skin or film. The alcohol gradually disappears, and, in approximately the same ratio, the presence of acetic acid makes itself evident the beer, &c., is converted into vinegar. It has been known from the earliest times that an unsoured sample of beer, wine, or the like can be quickly turned into vinegar by the addition of a small quantity of such skin. This latter was regarded as the carrier of the vinegar fermentation, and consequently received the name of "mother of vinegar" (Fr. *mère de vinaigre*, Ger. *Essigmutter*). The first botanical investigation of this substance was made in 1822 by PERSOON (I.), who described the organised skin developing on various liquids, and gave it the general name of *Mycoderma*, i.e. mucinous skin or fungoid skin, but never contemplated the existence of any direct connection between acetic fermentation and the development of such a structure.

This was reserved for the German algologist FR. KÜTZING (I.). In his treatise on this subject, published in 1837, he showed, without, apparently, being acquainted with the labours of his predecessor—that the "mother of vinegar" is constructed of a number of minute dot-like organisms (which we now call bacteria), arranged together in the form of chains. These he classified as algæ, and named them *Ulvina aceti*, and asserted quite positively that alcohol is converted into acetic acid by the vital activity of these organisms.

Kützing's results, however, attracted but little notice, because, two years after their publication, LIEBIG (III.) appeared on the scene with his theory of acetic fermentation (which will be described in a subsequent paragraph), in which no mention was made of the potency of living organisms, but the "mother of vinegar" was asserted to be a formation devoid of life a structureless precipitate of albuminous matter. Only one of the reasons put forward by the German chemist in support of this view, which he stubbornly upheld, will be mentioned here, and that merely as a curiosity. The Dutch chemist, G. MULDER (III.), celebrated as a chemical expert on wine, subjected the "mother of vinegar" to chemical analysis, and, because he failed to discover the presence of any ash constituents, thought that it must be regarded as a compound of protein and cellulose. Mulder's statement was refuted in 1852 by R. THOMSON (I.), who showed that a sample (but by no means a pure culture) of "mother of vinegar" contained 94.53 per cent. water, 5.134 per cent. organic matter, and 0.336 per cent. ash.

The diffusion of new light on this matter was reserved for PASTEUR (XIII.) Taking up anew the question of the origin of acetic fermentation—examined by Kützing merely from the purely botanical side, and that only cursorily—he controverted the opinions of the chemists, and proved, in 1864, that this fermentation also is a physiological process, whose inception and maintenance is bound up with the vital activity of minute fungoid organisms, to which he

applied the specific name *Mycoderma aceti*, first employed by Thomson. Of course, at that time, PASTEUR (XIV) was not in a position to prepare or use pure cultures, consequently the results of his experiments cannot now be credited with more than the single value of having unimpeachably proved the dependence of acetic fermentation on the vital activity of certain micro-organisms. Pasteur did not determine to what group of living organisms *Mycoderma aceti* belongs, the botanical, and especially the morphological side of the question concerning him but little ; only, in one place in his treatise, he states that he cannot regard the organism as a bacterium, as was done by Stack in 1863. Nevertheless, we, at the present day, must agree with the opinion of the last-named · the cause of acetic fermentation studied and described by Pasteur can only have been a fission fungus

The property of forming mucinous skins on the surface of liquids is not peculiar to the acetic acid bacteria alone, but, on the contrary, is a very general vital phenomenon among fungi. It is particularly noticeable among a group (which will be considered in the second volume) of budding fungi, which have been named, according to the nature of the medium in which they are found, *Mycoderma cerevisiæ*, *Mycoderma vini* (Fr. *fleur de la bière* and *fleur du vin* respectively) Pasteur denied that any of these skin-forming budding fungi have the power of producing acetic acid, but the author refuted this opinion by proving, in 1893, the existence of at least *one* such species endowed with this · faculty. More particulars concerning this will be given in one of the chapters of the second volume. At present we are only concerned with the fact that acetic fermentation is a vital manifestation not peculiar to fission fungi alone.

§ 210.—Morphology of the Acetic Acid Bacteria.

Strictly speaking, Pasteur's publication did not advance our knowledge of the morphology of the organisms in question beyond the discoveries made by Kutzing; and the case remained *in statu quo* for another fifteen years, until taken up by Emil Christian Hansen, whose researches on the acetic acid bacteria not only threw new light upon the organisms themselves, but were also—and that in a dual sense—important to the subject of Fermentation Physiology generally.

Until then the opinion was current that any given fermentation was carried through by merely a single species of ferment   HANSEN (VI.), however, showed in 1878 that, in the spontaneous souring of beer at least two different species of bacteria can come into action, one of which he named *Mycoderma aceti* and the other *Mycoderma Pasteurianum*, in honour of his predecessor. At the suggestion of W. Zopf he afterwards changed these names to *Bacterium aceti* and *Bacterium Pasteurianum* respectively. This important discovery was subsequently extended, partly by HANSEN (VII.) himself—who afterwards introduced into the literature of the subject a third species under the name of *Bacterium Kutzingianum*—and partly by A. J. BROWN (I.), W. PETERS (I.), A. ZEIDLER (I.), WERMISCHEFF (I.) and the author. Of all these species, only those described by Hansen have been thoroughly investigated morphologically, and for this reason they alone will be more closely considered in the following lines.

When inoculated into lager-beer or the so-called " doppel-bier "—a Danish high fermentation beer rich in extract and poor in alcohol—and kept at a temperature of about 34° C., these three species—provided air is freely admitted —will develop on the surface of the beer (which remains bright) to a pellicle within twenty-four hours. In the case of *B. aceti*, this skin is moist and mucinous, smooth and veined, but *B. Pasteurianum* is, on the other hand, dry,

and soon develops fine implications. That of *B. Kutzingianum* resembles the first species, but differs therefrom in raising itself high above the surface of the liquid by gradually climbing up the walls of the vessel. Fresh differences make their appearance when a small portion of the skin is examined under the microscope. Whilst the cells of *B. Kutzingianum* are, for the most part, single, and are only rarely seen joined together as chains, those of the other two species are seldom found as separate cells. The cells of *B. aceti* (Fig. 81) are somewhat more slender, and frequently exhibit the sand-glass or figure 8 form ("*en huit*") noticed by Pasteur. In *B. Pasteurianum* (Fig. 82) they are mostly rather longer and considerably broader (plumper) than those of the other two species.

These bacterial pellicles are true zoogloea, *i.e* the individual cells are attached
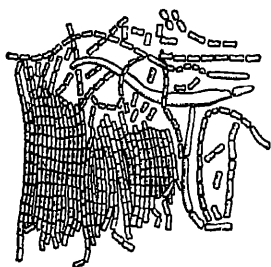
FIG 81 —Bacterium aceti

Cells from a freshly formed skin on
" doppel-bier "

Magn 1000 (*After Hansen*)

FIG 83 —Bacterium Kützingianum

Cells from a freshly formed skin grown
at 34° C on "doppel-bier"

Magn. 1000 (*After Hansen*)
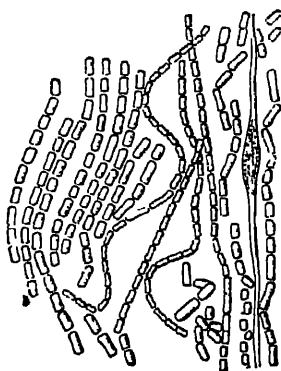
FIG 82 —Bacterium Pasteurianum.

Cells from a freshly formed skin grown at 34° C.
on ' doppel-bier."

Magn 1000. (*After Hansen*)

together by a mucinous envelope, formed by the swelling and mutual fusion of the external layers of the cell membranes, in which the cells then become embedded In ordinary (unstained) preparations the presence of this envelope is only deducible from the mutual cohesion of the cells; it can, however, be rendered visible by suitable treating and staining, *e.g.* by Loeffler's method. Fig. 8 (p. 31) was drawn from a preparation of this kind.

The behaviour of the mucinous envelopes of these three species towards iodine solution (iodine in water or alcohol or potassium iodide) is worthy of notice; those of *B. Pasteurianum* and *B. Kutzingianum* are thereby stained *blue*, whilst that of *B. aceti* remains unaltered. The point must be emphasised that it is the mucinous envelopes and *not* the true cell membranes that are stained in this manner. The cell plasma is in all three cases coloured yellow, consequently the preparations all exhibit yellow cells after the iodine treatment, these cells being embedded in the case of *B. aceti* in a colourless matrix. In *B. Pasteurianum* and *B. Kutzingianum* this latter is blue, and to the unaided eye the appearance of the whole

varies from green to bluish green, according to the proportion of the matri
It was this difference in the behaviour of the mucinous envelopes—which, hov
ever, is noticeable only in young and vigorous pellicles—that first directed tl
attention of Hansen to the existence of two species of acetic acid bacteria. Tl
chemical composition of the envelope has not yet been determined, but that it
not cellulose must be concluded from the negative results obtained from the tes
made with various reagents (iodosulphuric acid, zinc iodochloride) for th
substance. Already in this characteristic these three species differ from t'
acetic acid bacterium introduced into the literature of the subject by A. J Brov
under the name of *Bacterium xylinum*. The tough, leathery skin of zoogl
(measuring as much as one inch in thickness), formed by this bacterium on t
surface of the nutrient solution, and generally known in England as the vinega
plant, consists principally of the extensively developed mucinous envelope of t
cells. If the contents be extracted by suitable means, a mass is left whi
answers to the cellulose reactions (*e.g.* solubility in ammoniacal copper oxic
and on ultimate analysis exhibits a composition agreeing with the formu
$(C_6H_{10}O_5)n$.

Moreover, the three Hansen species differ notably in the appearance a
development of their colonies, prepared by the transference of droplets (rich
cells) from a pure culture grown at $25°$ C. on to solid nutrient media (wo
gelatin or doppel-bier gelatin). Those from *B. aceti* assume the form of excee
ingly pretty, many-rayed stars or rosettes, those from *B Pasteurianum* have
almost perfectly smooth periphery (without dentations) and exhibit convolutic
of the surface resembling those of the brain; whilst those of *B. Kützin
anium* are readily recognisable by the absence of both the stellar form a
convolutions.

§ 211.—The Morphological Influence of Temperature.

Hansen's researches into the acetic acid bacteria also afford an importa
support to the theory of bacterial pleomorphism, as will now be shown. T
cell forms described and illustrated in the previous paragraph are not the on
ones assumed by the fission fungi under consideration. On the contrary, t
pleomorphic variations are exceedingly plentiful, though they may all be group
under three main types, viz., chains of short rods (as already described), lo
threads, and, finally, distended or bulged forms. The conditions ascertained
HANSEN (VII.) as influencing the development of one of these forms, its grade
conversion into the others, and, finally, its restoration to the original shape, v
now be briefly referred to. It must be premised that the minimum limit
temperature at which development can proceed is for *B. aceti*, $4°-5°$ C.;
B Pasteurianum, $5°-6°$ C ; and for *B. Kützingianum*, $6°-7°$ C, the maxim
being about $42°$ C., and the optimum temperature about $34°$ C.

Cultures of *Bacterium Pasteurianum* on doppel-bier have shown that, at
temperatures higher than $5°$ C. (but not greatly exceeding $34°$ C.), chains
short rods develop, which, when grown at temperatures below $15°$ C, of
attain extraordinary dimensions, especially in the direction of the breadth. T
formation of chains proceeds most abundantly at about $34°$ C, the individ
short rods then having the ordinary form and being filled with firm, sligh
lustrous plasma.

If a small portion of such a skin, cultivated at $34°$ C., be transferred t
fresh nutrient medium, and maintained at $40°-40.5°$ C., a morphological alte
tion of the cells (Fig. 84) occurs, and is already noticeable at the end of a f
hours. The short rods about 2μ long and 1μ broad, of which the chains of
seed were composed, begin to elongate, and at the end of eight to nine ho
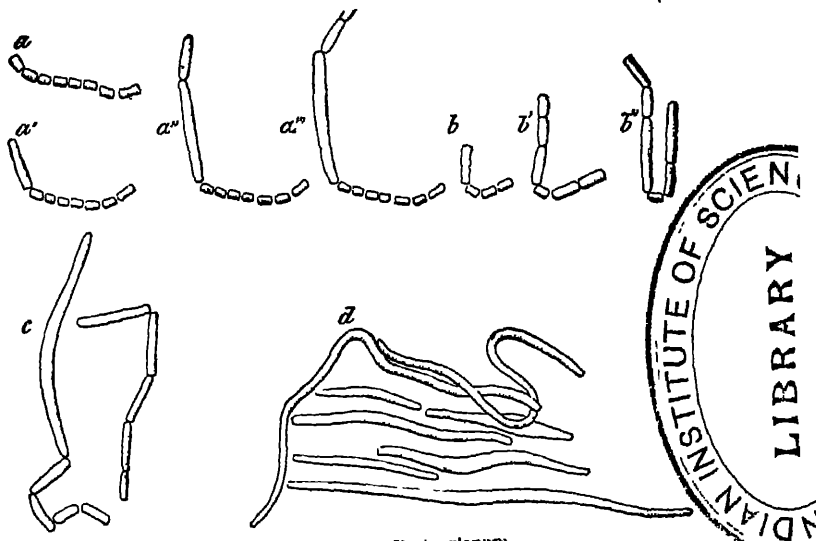
FIG 84.—Bacterium Pasteurianum

Morphological change from short rods to long threads. Culture on "doppel-bier" agar-agar in a Böttcher chamber at about 40.5° C   a. chain of eight short rods, a'–a''' the same after six, ten and twenty hours, b chain of five short rods, b'–b''. the same after five and nine hours, c and d after ten and twenty-one hours   Magn. 1000.   (After Hansen)
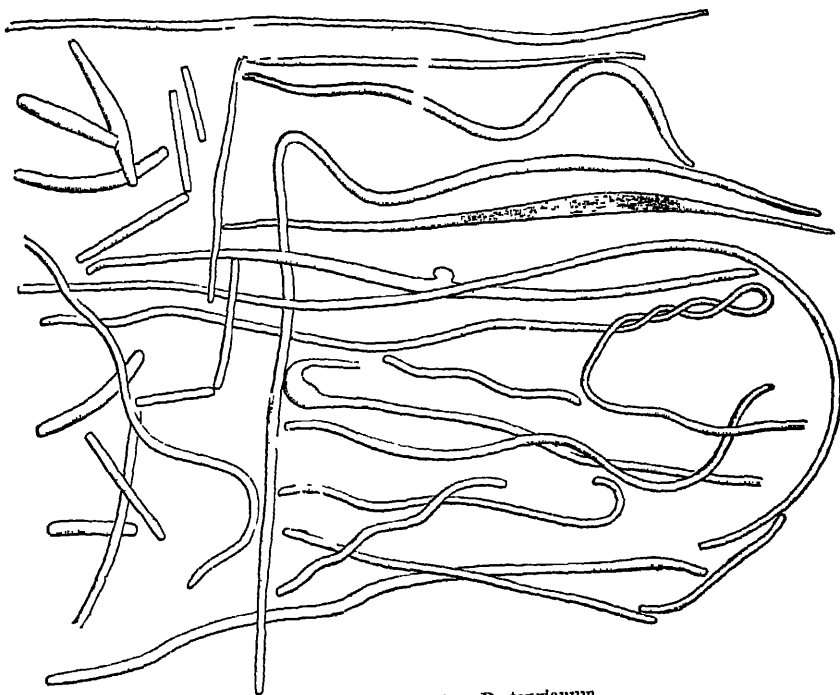
FIG 85.—Bacterium Pasteurianum
Long threads developed by twenty-four hours' cultivation at 40.5° C on "doppel-bier"
Magn 1000   (After Hansen.)

none but long rods are found, some of these being already disconnected, others still retaining the chain form. The latter also finally become dismembered, so that after a further four hours none but elongated cells, 40 μ and more in length are present. These now continue to grow, and in twenty-four hours from the commencement of the experiment long threads (Fig. 85), some of them measuring 200 μ in length, are found exclusively.

A fresh modification of form sets in as soon as these long threads are exposed to the original temperature of 34° C —they begin to bulge. These forms (Fig. 86
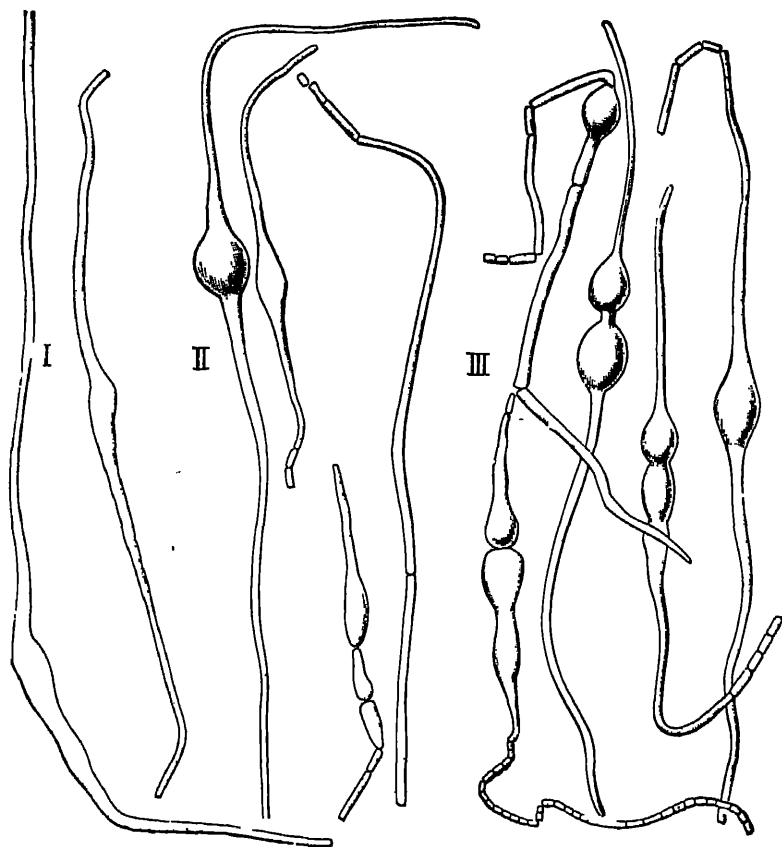
FIG 86 —Bacterium Pasteurianum.
Conversion of long threads into swollen (bulged) forms and chains   Culture in "doppel-bier" at 34°
I   condition after four hours, II after five hours, III after seven hours   Magn. 1000.   (After Hansen)

can be already noticed at the end of four hours, and their number rapidly increases from that time onward   At about the same time other portions of the threads begin to break up into fragments, the disruption beginning indifferently at either or both extremities, or in the middle of the thread, which is then modified into a chain of short rods, or one exhibiting both long rods, unaltered portions of threads, and bulged articulations, in short, great diversity prevail All intermediate stages of the last-named forms, between the very frequent spindle cells on the one hand, and pear-shaped rounded forms on the other, and

FIG 87 —Bacterium Pasteurianum.

met with. Globular cells, measuring up to 10 μ in diameter, are also by no means rare.

Finally, the threads become entirely dismembered into short rods (Fig. 87), even the bulged cells undergoing this conversion, and leaving only the thickest portion (Fig. 88) unchanged. This portion eventually, after the filamentous ends on either side have broken up into short rods, collapses in the surrounding liquid and dissolves. An examination made after the lapse of twenty-four hours then reveals only chains of short rods. We have thus induced a reversion to the original forms of cell, and have thereby learned the **morphological influence of temperature.** Of course, neither the composition of the nutrient medium nor the condition of the seed is a matter of indifference. Thus, for instance, if, instead of sowing the young cells presupposed in the foregoing demonstration, those already forty-eight hours old are employed, then the conversion into threads becomes very difficult. In the case of lager-beer the development proceeds somewhat differently to that occurring in the "doppel-bier" hitherto mentioned

FIG. 88.—Bacterium Pasteurianum.

Residue of swollen long threads after a sojourn of one to two days in "doppel-bier" at 34° C. In *a* the pear-shaped swelling has tapered out into two thin threads. In *b* the lower of these has divided into short rods In *c* the swelling has begun to disintegrate, a part of the plasmal cell contents escaping. In *d* the evacuation is complete, only the thick cell wall being left. *e,* spindle-shaped swollen form, with two long threads undergoing incipient subdivision. Magn 1000. (*After Hansen.*)

Bacterium aceti and *B Kutzingianum* behave very similarly under the circumstances now in question. A few small differences are, however, unmistakably evident. Thus, for instance, in harmony with the plumper form of the short rods of *B. Pasteurianum,* the breadth of its long threads is also greater, as will be evident on reference to Figs. 85 and 89, the long threads of *B. aceti* are thinner, but attain a greater length, viz , up to 500 μ. On the other hand, the long threads of *B. Kutzingianum* are considerably smaller than those of the other species. Finally, it should be stated that branching of the long threads occasionally occurs. A few of these comparatively rare forms are shown in Fig. 90. Pleomorphism seems to be a general property of the acetic acid bacteria, since it was also found by Hansen to prevail in four other species, including those discovered by Zeidler.

Like most of the other *Schizomycetes,* the acetic bacteria exhibit a preference for darkness. Their development—as M. GIUNTI (I.) discovered—is restricted (though not entirely prevented) by diffused daylight, as well as by direct exposure to the sun; but according to the discoveries made by G TOLOMEI (III.), this result is due to the chemically active light rays alone. TOLOMEI (IV) likewise found that the discharge of strong electric sparks at a short distance above the surface of the liquid also restricts development.

§ 212.—The Equation of Acetic Fermentation.

For a long time no clear perception was obtained of the mode of action of the "mother of vinegar." True, it was known that the acetic acid is formed from the alcohol present, and also that acidification does not occur when air is excluded,

but the reasons for these phenomena could not be given. The ABBÉ ROZIER (I.) concluded from his experiments that air is absorbed by the wine in process of turning sour, but LAVOISIER (I.) afterwards shewed that this is only true of one of the constituents of the atmosphere, viz., oxygen. He stated that "acetic fermentation is nothing more than the souring of wine, effected in the open air by absorption of oxygen." In 1821 Edmund Davy discovered platinum-black, a substance which, when moistened with spirits of wine, becomes white-hot, the formation of acetic acid being evidenced by the odour evolved. This observation was followed up by DÖBEREINER (I), who found that, in this reaction, the alcohol
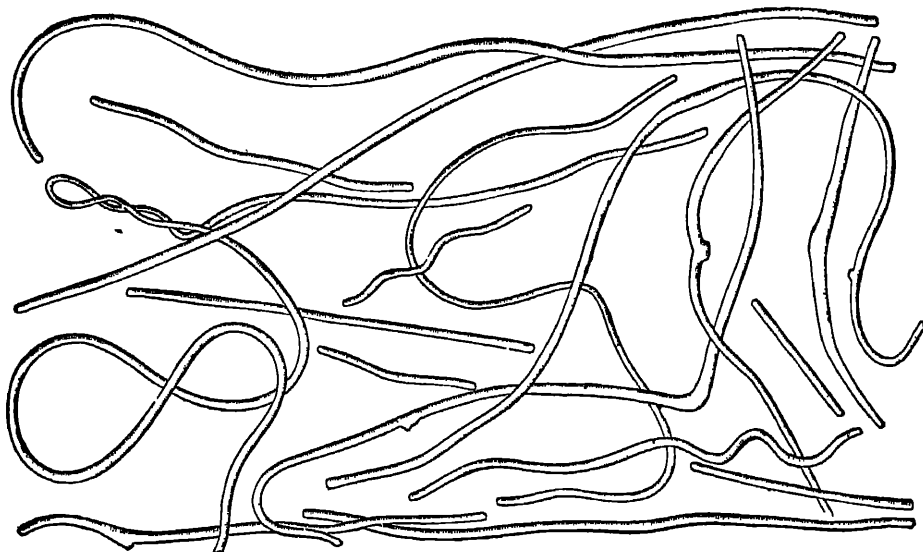
FIG 89 —Bacterium aceti

Long threads. Culture twenty-four hours old in "doppel-bier" at 40°–40.5° C   In several places the breadth of the threads is exaggerated   Magn. 1000   (After Hansen)

takes up oxygen—water and acetic acid, but no carbon dioxide, being formed By observing the volume of oxygen consumed by a weighed quantity of alcohol, he arrived at the following equation for this oxidation process:—

$$C_4H_6O_2 + 4O = C_4H_4O_4 + 2HO$$

which, translated from the symbolical language of equivalent formulæ to that of atomic formulæ, reads as follows

$$C_2H_6O + O_2 = C_2H_4O_2 + H_2O.$$
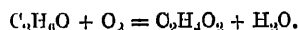
Hence, Döbereiner concluded that, for the production of acetic acid, only three substances are required alcohol, oxygen, and a body capable of absorbing and condensing the latter, and thus bringing it into more intimate contact with the first named, whereupon the reaction ensues.

The above experiment of Döbereiner's was taken by chemists as a starting-point in attempts at elucidating the phenomenon of acetic fermentation. The intermediary part played by the "mother of vinegar" in the souring of wine was obvious, since it was well known that without this "mother" no conversion

occurred. Nevertheless, more than one opinion was rife as to the mode of action of this mucinous skin.

Berzelius, in 1829, on the basis of his theory of catalytic action, ascribed the potency of this skin in acetic fermentation to the acetic acid " enclosed within its pores " Ten years later, and two years after the appearance of Kützing's work—which, being out of harmony with the spirit of the age, was consequently disregarded—Liebig published his theory of acetic fermentation, in which the " mother of vinegar " was classed along side platinum-black, their mode of action being defined as identical and of a purely chemical nature

Owing to the endeavours of Pasteur, the theory promulgated by Kützing was experimentally shown to be correct, and the true import of the vinegar-mother once more recognised It would, however, be going too far to also credit the French physiologist with having recognised acetic fermentation as a purely physiological process ; for—remarkable as it may now appear to us—Pasteur, with his followers stopped half-way and defined the vinegar fungus as " acting after the manner of spongy platinum." He characterised the skin-like zooglœa of the fission fungus in question as " vegetations endowed with the remarkable peculiarity of retaining the oxygen of the air and condensing it after the manner of spongy platinum, by inducing the combustion of alcohol and acetic acid." With VON KNIERIEM and AD. MAYER (I share the credit of having convincingly proved, in 1873, that the oxidation of alcohol by means of platinum-black cannot be classed along with the fermentation set up by the "mother of vinegar." Platinum - black oxidises both concentrated and dilute alcohol whereas, according to the experience
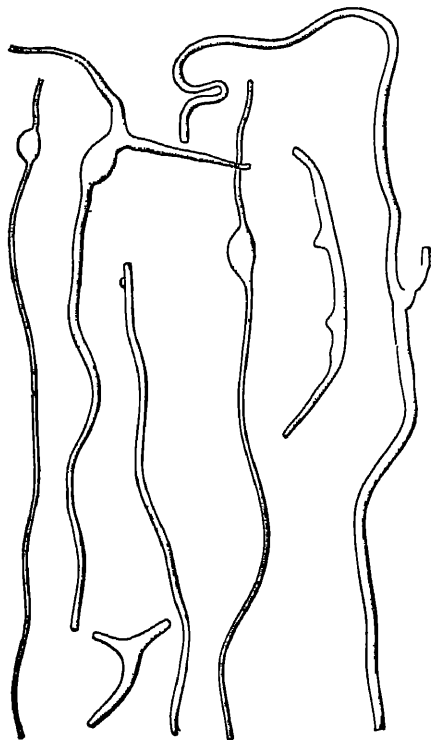
FIG 90 —Bacterium aceti

Filamentous cells of unusual form from cultures (several days old) on wort and on " doppel-bier " at 39°-41° C. Magn. 1000. (*After Hansen.*)

of vinegar-makers, acetic fermentation cannot proceed in presence of more than 14 per cent. of alcohol Moreover, with regard to temperature, highly important differences—touching the very existence of [the question—are observed. Thus, whereas acetic fermentation proceeds most satisfactorily at about 35° C. and is arrested altogether at 40° C., the energy of the oxidation effected by platinum-black (starting at 35° C.) increases as the temperature rises, and may become so violent that the alcohol ignites explosively and burns away to water and carbon dioxide. Hence the composition of the (by no means uniform) oxidation products thus formed differs greatly from those obtained from acetic fermentation.

This latter process, whose purely physiological nature was placed beyond doubt by these investigations, was examined more minutely by A. J. BROWN (III in 1886. Meanwhile, Hansen's discovery of the existence of at least two speci

of acetic acid bacteria considerably enlarged the field of research, since thenceforward "acetic fermentation" could no longer be spoken of without coupling with it the name of the organism by which it was caused. The species forming the subject of Brown's researches was obtained by him from sour (acetic) beer, and was called *Bacterium aceti*, though not identical with Hansen's species bearing the same name. Pasteur's discovery that the "acetic acid bacteria" first convert alcohol into acetic acid, and then burn the latter to carbon dioxide and water, was also made by Brown in connection with his *B. aceti*, but he did not institute any closer examination (more particularly in connection with the ratio of transformation) on this point, so that this theoretically and practically most important question has still to be investigated.

Methyl alcohol, isobutyl alcohol, and amyl alcohol are not attacked by Brown's *B. aceti*, but normal propyl alcohol is oxidised to propionic acid. If the nutrient medium (yeast-water) contains dextrose but no alcohol, then gluconic acid is formed, a fact already established by BOUTROUX (I.) in 1880, in connection with another species of bacterium (of questionable purity). Saccharose, lactose, and starch remain unaltered, but mannite is converted into lævulose, which then remains unchanged. Dulcite is unaffected, whilst glycol is converted into glycollic acid. The behaviour of *Bacterium xylinum* is approximately the same as in the organism just described. The extensive mucinous envelopes, consisting of cellulose, are produced when the nutrient solution contains dextrose, lævulose, or mannite ; whilst, on the other hand, cane-sugar and starch are useless for this purpose.

We are indebted to G. BERTRAND (I.) for a beautiful experiment with a fission fungus, not accurately identified, but presumably very closely allied to *Bacterium xylinum*. Mountain-ash berries, *i.e.* the fruit of *Sorbus aucuparia*, *S. intermedia*, and *S. latifolia*, contain, in addition to glucose, an alcohol isomeric with mannite, viz., Sorbitol ($C_6H_{14}O_6$). If now the juice of these berries be subjected to alcoholic fermentation (which sets in rapidly and spontaneously), the glucose is decomposed, but not the sorbitol, this latter only being attacked when the above-mentioned fission fungus obtains access into the fermented liquid, which it does through the mediation of a small red fly (*Drosophila funebris*, Fabricius, *D. cellaris*, Macquart), known to all fermentation technicists as the "vinegar-fly." This insect haunts places where alcoholic juices (especially fermented fruit-juices) are being stored and converted into vinegar, and there loads itself with acetic acid bacteria, which it then transfers to other localities. The bacterium introduced by these flies into the fermented juice of the mountain-ash berry oxidises the hexavalent alcohol sorbitol to the ketose sorbin (also known as sorbinose or sorbose), according to the equation—

$$C_6H_{14}O_6 + O = C_6H_{12}O_6 + H_2O.$$
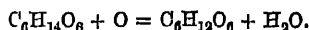
This affords a convenient method for the production of sorbose.

With regard to the fermentative capacity of *B. aceti* Hansen, and *B. Pasteurianum*, the author, in 1895, published comparative investigations, showing that a sowing of the first-named species on pale lager-beer is able to develop and exert a powerful acidifying effect at $4°$–$4.5°$ C ; whereas *B. Pasteurianum* is unable to do this, or to reproduce itself at all, even at $4.5°$–$5°$ C.

§ 213.—Pure Culture Ferments in the Manufacture of Vinegar.

Searching investigations into the chemical activity of the different species of acetic acid bacteria would be not only opportune in the interests of science, but also highly important to the practice of the vinegar industry. In this business

I   U

the employment of selected pure culture ferments is not yet regarded as a fundamental rule, everything being still left to the mercy of chance.

As every reader will be aware, there are two different methods of making vinegar. In one of them wine forms the raw material, this method being known as the Orleans process, from having long been extensively carried on in that locality. There (as elsewhere) the work is still performed in the same manner as it was centuries ago, as follows —A number of oaken casks, each of a capacity of some 55 gallons, are arranged in rows in a chamber maintained at a constant temperature of 18° to 22° C   In the upper part of the front end (head) of each cask a circular aperture (a few cm. in width) is provided, through which the cask can be filled or emptied, and which is generally kept closed, whilst near it is a very small hole (vent) always left open for the admission of air.   In normal work each cask is about half full.   Before setting a new cask in work it is scalded out several times with steam or hot water, in order to extract the sap from the wood, and is then "soured" by impregnating it with good, boiling hot vinegar.   About 1 hl (22 galls.) of good clear vinegar and 2 l. (o 44 gall. of wine are then placed in the cask, another 3 l. of wine being added at the end of eight days, 4 to 5 l. more after the lapse of another week, and so on until the cask contains about 180–200 l. (40–44 galls.)   Then, for the first time, vinegar is drawn from the cask, and in such quantity that about 22 galls are left behind in the vessel.   From that time the cask ("mother") is in continuou use, 10 litres (2.2 galls.) of vinegar being withdrawn every week and replaced by an equal quantity of wine.   The "mother" casks may remain in work during six or eight years without interruption, but at the end of this period they wil contain such a considerable accumulation of deposited yeast, tartar and mothe of vinegar, as to necessitate their being emptied and cleansed.   A skin, known as vinegar-flowers or mother of vinegar, and composed of acetic acid bacteria develops on the surface of the liquid, and the manner and luxuriance of it growth enables the operator to judge the progress of the fermentation.   How ever, at the outset the growth proceeds very slowly, since the wine employe mostly contains but very few of these bacteria.   Consequently an opportunit is afforded for the development of rapid-growing injurious organisms, chiefl certain budding fungi, which consume the acetic acid.   The aerobic "vinega eels" also make their appearance   To obviate this source of loss, PASTEUR (XV. in 1862, proposed that, instead of waiting until the acetic acid bacteria in th wine had increased sufficiently to form a protective skin of "vinegar-flowers the necessary ferment should be cultivated in small vessels, the skin thu obtained being then carefully transferred in pieces of sufficient size, by the a of a wooden spatula, on to the surface of the wine to be soured, which w: placed in shallow open vats.   This process was adopted by Breton-Lorion Orleans, in particular, and would be suitable for general application if th presence of not more than one species of acetic ferment could be thereby ensure This, however, is not the case, and it is purely a matter of chance whether th skin prepared beforehand by cultivation is composed of beneficial or injurio organisms.   According to circumstances, there may be present several ve different species with divergent properties, faculties, conditions of vitality a metabolic products.   By reason of this uncertainty alone, the Pasteur method liable to produce very irregular results, and may, on occasion, actually give ri to losses; and, as a matter of fact, it is just on this account that the method h been abandoned both in France and Germany, where it was introduced E. WURM (I).   Up to the present it does not appear that any one has attempt to work with really pure cultures. ✓

In the second method actually employed for making vinegar, spirit is us instead of wine.   This method has been evolved from that originally prescrib

by Hermann Boerhave, and has attained its present condition (since 1823) mainly through the instrumentality of Karl Schüzenbach. The French term it the " German method," but in Germany it is generally known as the " quick vinegar method " (*Schnellessig-Fabrikation*). A detailed description cannot be given here, but the gist of the process consists in slowly running the "goods" (*i e.* spirit diluted with vinegar) to be turned into vinegar over shavings or strips of beech-wood contained in a closed vat (the vinegar-generator), so that the liquid presents a large surface to the air, which is admitted through special ventilating holes below and makes its escape at the top. That the fermentative activity of micro-organisms also comes into play in this method can no longer be doubted since the searching investigations of Pasteur, which were confirmed (on repetition) by Mayer and Knieriem. Pasteur showed that no acidification takes place if the alcohol be allowed to trickle over shavings destitute of fungi. He assumed that the organism taking part in the quick vinegar process is the same as that forming the superficial skin in the Orleans method, the fungus being supposed to settle on the shavings in the vinegar-generator and convert the slowly running vinegar goods into acetic acid. Up to the present no precise investigations on the bacteria acting in this branch of industry have been made public. This highly necessitous industry has, more perhaps than any other, to struggle against a variety of difficulties; the actual losses of alcohol are enormous, and no one is able to offer any reliable explanation of their cause. The introduction and intelligent use of suitable pure culture ferments would be a great boon. How much still remains to be done and ascertained in this instance can be estimated by a comparative glance at the conduct of fermentation in the operation of brewing. Not least among the advantages to be derived from such a method of working—which we may hope will soon be elaborated—would be the possibility (not afforded by the present method) of combating the "vinegar eels." With regard to these objectionable parasites, it may be mentioned that detailed morphological and physiological information concerning *Anguillula aceti* will be found both in Czernat's monograph (excerpts from which are contained in Borgmann's trans-lation of Pasteur's *Études sur le Vinaigre*) and in a treatise by G. LINDNER (I.), which latter work chiefly deals with the pathogenic potency of these worms. As SADEBECK (I.) has found, these parasites are occasionally themselves infested and killed by a fungoid parasite belonging to the group of *Oomycetes* (mentioned in the second volume), and known as *Pythium Anguillulœ aceti*.

CHAPTER XXXVIII.

THE OXYDASES.

§ 214.—The Browning of Wines.

In addition to the purely chemical absorption or fixation of oxygen (e.g. in th conversion of SO_2 into SO_3), on the one hand, and the oxidation effected directl by the vital activity of micro-organisms on the other, there is a third method of transferring this gas, viz., by the action of enzymes, to which the name oxydase. proposed for them by Weigert, may be generally applied. How many kinds of oxydases exist is a matter for future research to determine. At the presen time the subject is merely in an incipient stage, though the commencemen made is a highly promising one, and has already led to the explanation of several phenomena which only a short time back were regarded as extreme puzzling.

One of these is the so-called "browning" of wines, known in France as "la casse," "le cassage," or "cassure" (Ger. Rahn-Werden or Braunwerden. This occurs chiefly in white wines and was for a long time classed along with the malady known as loss of colour in (red) wines. In France attention was fir directed to its distinct character by Arm. Gautier (I.) in 1878, but in German this was known at an earlier date. The most important characteristic of "v cassé" is the rapid change of colour undergone by the wine as soon as it is poure out of the cask or bottle into an open glass, the colour of the upper laye (exposed to the air) of the hitherto pale liquid becoming darker, and finally (the course of a few hours) becoming brown. This coloration also gradually pr gresses in the deeper layers, and, at the same time, the flavour becomes unpleasa (air taste). Turbidity then ensues, but disappears in proportion as a fine da brown pulverulent sediment settles down. The liquid is now (three to fo hours after the commencement of the experiment) again bright, though dark in shade than when newly drawn from the cask. The taste has also improv again, without, however, being equal to what it was at first.

In view of the fact (indubitably proved by Nessler's experiments) th this malady only occurs when air is admitted, it was regarded as an oxi tion process, without any more precise acceptable explanation being forthcomir After Gautier had presumed, and A. Bouffard (I.) in 1894 had denied, t probability of bacterial activity in this phenomenon, it was shown by Gouirand (I.) that we have here to do with the action of an enzyme which pla the part of a carrier of oxygen. He isolated the same (though not in a pu state) from browned (white and red) wines, and produced therewith the sai malady in previously sterilised sound wines. This enzyme must probably be garded as acting by the absorption of atmospheric oxygen, which it then gives again, not only to the colouring-matter in the wine, but also to the tannin, a thus converting them into insoluble dark-coloured compounds. It is to be hop that, ere long, this matter will have been made clear by further investigation

It is found by experience that the wines obtained in wet autumns fr rotten grapes, as also those affected with "sweet-rot" (Edelfaule; pourrit noble), and such as are poor in acid, are subject to this malady with comparat

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frequency. Nessler made searching investigations into the means of combating this complaint in practical viticulture. The most important result obtained was the discovery that the browning of wine can be prevented by thoroughly fumigating the casks with 1-2 grams of sulphur per hectolitre—22 galls. or 3.53 cubic feet—of cask-room before use, the malady being found, in Nessler's experiments, not to ensue when the wine contained a minimum quantity of 0.003 *per mil.* of SO_2. According to the researches of Gouirand, this enzyme is destroyed by a temperature of 80° C, 60° C. being apparently insufficiently reliable for this purpose. MÜLLER-THURGAU (VI.) made the discovery, important in cellar management, that the tendency of wine to turn brown could be prevented by Pasteurisation, *i.e* keeping it for some time at a temperature of 60°-62° C., which it will endure without acquiring the so-called "boiled" taste.

The acquisition of a more accurate characterisation of the enzyme, and the consequent possibility of distinguishing it from other oxydases, is desirable, this being a necessary preliminary to the elucidation of its origin. Possibly the enzyme is not formed anterior to fermentation, but, on the other hand, its presence in the grapes themselves and in the must is not absolutely precluded. V. MARTINAND (I.) has actually found oxidising enzymes in wine-must on many occasions. The elucidation of the conditions under which browning may be caused in wines is a subject requiring further investigation, the question whether the presence of special metabolic products is essential, or whether the oxydase here concerned differs from those observed by Martinand, being still unsolved. Moreover, it appears from the discoveries of this observer that, in the maturing of wine, the alterations of flavour occurring—and which may be accelerated by the influence of oxidising agents (ozone, the electric current)—are, under natural conditions, brought about by the agency of oxydases which still require closer identification. The same applies to the darkening of the colour of wine during storage. According to G. TOLOMEI (V.), oxydases are also produced by the wine-yeasts *Saccharomyces apiculatus* and *Sacch. ellipsoideus.*

§ 215.—The Rapid Discoloration of Fresh Vegetable Juices

is in many cases attributable to the action of oxydases. Technical interest in the discoveries made on this point is chiefly centred in the researches of G. BERTRAND (II) on Japanese lacquer, that lustrous and extremely durable varnish employed in Eastern Asia for coating wooden furniture and similar articles. By making incisions in the bark of the indigenous *Rhus vernicifera*—a tree of the family *Anacardiaceæ* and closely allied to the European garden-tree *Rhus cotinus* (Venice sumach)—a juice is obtained which, on admixture with the oil from *Bignonia tomentosa* and (for red lacquer) vermilion, yields the lacquer in question. This juice resembles a thick pale cream and will keep unchanged for a long time if stored in closed bottles, but quickly turns brown when air is admitted, becoming covered in a few minutes with a tough black skin, and finally hardening—this being, in fact, the property for which it is so highly prized. That a process of oxidation is here in question cannot be doubted. The constituent thus converted has been isolated by Bertrand under the name of laccol, and recognised as a compound allied to the polyatomic phenols, and capable of producing extremely violent reddening and inflammation of the skin if applied in even very minute quantities. The juice also contains an oxydase, named by Bertrand laccase, by the known oxygen-carrying powers of which the laccol is rapidly converted into a hard, black oxy-compound, insoluble in water, alcohol, &c. This product is not obtained in the absence of the enzyme, only a resinous soluble grease, that remains sticky for a long time, being obtained under

such circumstances. In addition to laccol, other polyatomic phenols (pyrogallol, hydroquinone, &c.) and their acid derivatives (e.g. gallic acid and tannin) are quickly oxidised by laccase in presence of air. According to the further discoveries of BERTRAND (III.), the polyphenols containing at least two groups of OH or NH_2 (either in the ortho- or in the para-position) are also easily and readily oxidised by this oxydase.

This interesting discovery gave an impulse to the elucidation of several other phenomena interesting both to the food-stuff chemist and the agriculturist. It is well known that the freshly broken or cut surfaces of raw apples rapidly become discoloured on exposure to the air, at first turning reddish and then becoming brown. This is the cause of the ugly colour of expressed apple-must. Housewives skilled in cookery are aware that this alteration of colour does not ensue if the cellular structure of this fruit is preserved unbroken until after the apple has been boiled. L. LINDET (I.) in 1893 explained this discoloration as resulting from the action of an enzyme, to which he subsequently gave the name of laccase—without, however, implying the identity of this with the oxydase of the lac-tree. The name of malase would probably be more suitable for this apple enzyme. In the case of apple-juice also, oxygen is carried by the enzyme to the tannin, and thus dark-coloured oxy-compounds are produced, which are precipitated on the cell walls as a fast, permanent dye. The spotting of sound apples under the rind, the so-called brown spotting, is explainable in the same manner. So long as the structure of the cell remains perfectly intact, the atmospheric oxygen cannot obtain access to the enzyme (in the plasma) or to the tannin. As soon, however, as by mechanical action (e.g. the dropping of the apple from the tree, pressure in packing or transit, &c.) any of the cells become ruptured, then an opportunity is afforded the oxygen to act on the now exposed constituents of the plasma. If the rind of the fruit remains uninjured, the air gains admission to the interior merely through the intracellular spaces alone, and, in such event, will produce only a faint reaction and slight discoloration Whether, as assumed by Lindet, the enzyme and tannin are contained in separate cells (i.e. distinct from each other), is a question still requiring more accurate research on the part of the botanist to decide.

The darkening of beet-juice, or the rapid discoloration of the fresh slices of beet in the sugar-works evidenced even when cutting-tools devoid of iron are employed, is equally attributable to the action of an oxydase present in the sugar-beet. This was discovered by G. BERTRAND (IV.), and received the name of Tyrosinase, because it carries atmospheric oxygen to the tyrosine—well known to be abundantly present in the cells of the sugar-beet—and thus produces the discoloration in question. On the other hand, laccase has no effect on the said amido-compound. Apart from this property, tyrosinase is also characterised by its greater susceptibility both to heat and chemical influences. It occurs in other plants, e.g. the bulbs of the dahlia (Dahlia variabilis) According to the researches of G. BERTRAND (V.), oxidising enzymes are also found in other plants, e.g. in the carrot; the tubers of the potato (which, as is well known, rapidly become discoloured when cut in an uncooked state); in the pear, quince and chestnut; in the sprouts of asparagus, clover, lucerne, and rye-grass; in the leaves of the potato, sugar-beet, &c. For detecting this class of enzymes Bertrand recommends the employment of guaiacum tincture, which produces therewith a blue coloration when dabbed or poured on to the cut surface or juice under examination. To isolate these enzymes the plant juice is mixed with alcohol, the resulting precipitate being dissolved in a little water and filtered On pouring the filtrate into five volumes of alcohol, a precipitate consisting of the desired enzyme will be formed.

The so-called rusting or tarnishing of many of the agarics, i.e. the rapid

discoloration of freshly broken or cut surfaces in the body of the fungus, is well known. The bluing of two of these, which he styled *Boletus luridus* and *Agaricus sanguineus* (?), was explained by CH. SCHÖNBEIN (I.) in 1856, by stating that these fungi contain a resinous substance soluble in alcohol (the above-mentioned reaction with guaiacum tincture will be remembered !), and becoming converted into a blue oxy-compound when brought into contact with ozone. The formation of this latter from the oxygen of the air is accomplished by the activity of another substance, also present in the fungi, and destructible by heat. This active substance was subsequently (in 1895) proved by E. BOURQUELOT and G. BERTRAND (I.) to be an oxydase, and was detected by them in 59 out of 107 species examined · *e.g.* in 18 species of the genera *Russula* and *Lactarius*, 10 species of the genus *Boletus*, 2 species of the genus *Amanita*, &c. According to the researches of BOURQUELOT and BERTRAND (II.), the enzyme giving rise to the bluing of *Boletus cyanescens* is similar to laccase ; but another oxydase, causing the freshly fractured surfaces of *Russula nigricans* to first turn red and finally become black, is certainly different.

G. TOLOMEI (VI.) discovered in ripe olives an oxydase which he called olease. In many parts of Italy it is customary to allow the olives, before putting them through the press, to undergo a spontaneous decomposition, which is chiefly effected by this olease, but has not yet been sufficiently investigated. This enzyme also passes into the oil prepared at temperatures below 70° C., and presumably continues to convey oxygen gradually thereto as well, oleic acid, acetic acid, sebacic acid, &c., being formed.

Another phenomenon not yet accurately known (but possibly also attributable to enzymatic activity) will now be considered, since otherwise no convenient opportunity would offer, and that is

§ 216.—The Bittering of Wine.

This malady makes its appearance in many districts, such as the French Jura (Burgundy wine), the Ahr valley (Rheinland), Vöslau near Vienna, and Sicily (Vino del Faro di Messina), with comparative frequency, and almost exclusively affects red wines. The commencement of the disease is evidenced by a reduction in the acid content, the wine becoming apparently sweeter again (French cellar-masters say " *le vin doucine* ") By degrees the liquid turns paler, and is finally decolorised completely, the colouring-matter being deposited as an insoluble sediment or covering the walls of the bottle as with a skin. Concurrently, the wine develops a strange odour and a bitter after-taste, which finally becomes so strong as to render the liquid undrinkable. This malady first makes itself apparent in the second or third year of storage, and oftentimes not before the wine is bottled for maturing.

Respecting the cause of this incurable disease of wine, nothing reliable can as yet be stated. PASTEUR (XVI.) attributed it to the activity of a rod-shaped fission fungus, without, however, being able to throw any further light on the matter. The bacteria found in large numbers in bitter wine are for the most part covered with flakes and fragments of the precipitated brownish red colouring-matter, and hence very often assume remarkable shapes. They may be freed from these incrustations by the addition of a droplet of a solvent mixture of alcohol and tartaric acid to the preparation. R. ADERHOLD (I.) unsuccessfully attempted to prepare pure cultures of the organism suspected of causing this malady, but PERRONCITO and MAGGIORA (I.) were able to artificially induce the complaint in sound wines by inoculating them with a bouillon culture of microbes discovered in bitter wine ; the infection, however, succeeding only in such samples as contained less than 8.5 per cent. of alcohol. The attempts at

noculation made by E. Kramer with a bittered white wine from the province of
Kustenland (Austria) did not prove satisfactory. At present, uncertainty pre-
vails not only with regard to the organism causing this complaint and the
external conditions influencing its development, but also as to the nature of the
bitter principle itself. The opinion expressed by Mulder, that citric ether is in
question, was refuted by O. NEUBAUER (I), who proved that this (still uninvesti-
gated) bitter principle is a compound that is not volatilised by boiling the wine.
From experiments made by J. BERSCH (I.), it is permissible to conclude that the
tannin present is decomposed and consumed by the organisms here in question.
This observation would suffice to explain the fact mentioned at the commence-
ment of this paragraph, that bittering is almost exclusively confined to red wines,
these containing, as is well known, a somewhat large amount of tannin absorbed
from the skins and kernels of the grape during the primary fermentation.

It may be useful to casually mention, in conclusion, that the bittering of
alcoholic beverages, beer in particular, may also be occasioned by higher fungi
(yeasts). Fuller particulars will be found in a subsequent chapter in the second
volume, dealing with *Saccharomyces Pastorianus,* and to which the reader is
hereby referred.

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