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BUREAU OF ANIMAL INDUSTRY.—BULLETIN No. 82.

A. D. MELVIN, CHIEF OF BUREAU.

UNGI IN CHEESE RIPENING:

CAMEMBERT AND ROQUEFORT.

BY

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LETTER OF TRANSMITTAL.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF ANIMAL INDUSTRY,
Washington, D. C., February 6, 1906.

SIR: I have the honor to transmit herewith the manuscript of an article entitled "Fungi in Cheese Ripening: Camembert and Roquefort," by Charles Thom, Ph. D., and to recommend its publication as Bulletin No. 82 of the series of this Bureau. This is the second paper dealing with the cooperative experiments in soft-cheese making undertaken by the Dairy Division of this Bureau in conjunction with the Storrs (Conn.) Agricultural Experiment Station, the first paper having been published as Bulletin No. 71 of this Bureau.

These experiments have been carried on at the Storrs Station under the general direction of Prof. L. A. Clinton, the station director, and under the personal supervision of Dr. H. W. Conn, the station bacteriologist, in accordance with the plan outlined in the introduction to Bulletin No. 71.

While there are many problems yet to be investigated with reference to the manufacture in this country of soft cheeses of the best European types, this article indicates that good headway is being made in that direction, and it is believed that the information here presented is of considerable scientific and economic value.

Respectfully,

A. D. MELVIN,
Chief of Bureau.

HON. JAMES WILSON,
Secretary of Agriculture.

CONTENTS.

ILLUSTRATIONS

FUNGI IN CHEESE RIPENING: CAMEMBERT AND ROQUEFORT.

INTRODUCTION.

It has been shown in a previous bulletin that certain fungi are the active agents indispensable to the ripening of Camembert cheese. The general results and the data upon which they rest are there discussed, but the more special mycological studies, involving several lines of work, remained to be brought out in greater detail. These fall naturally under two heads: (1) The physiological studies of the functions of particular species in the ripening processes of Camembert, Roquefort, and certain related types of cheese; (2) the classification and description of these and other forms occurring in dairy work. This paper includes only the work done under the first head. The description of the fungi occurring in dairy work is reserved for another paper.

Aside from such obligations as are mentioned in the discussion of special topics, the author wishes to acknowledge the assistance of Dr. B. B. Turner, Prof. W. A. Stocking, Mr. A. W. Bosworth, and Mr. T. W. Issajeff, members of the experiment station staff, in numerous cases where the work of each presupposes the results of the other, and especially to acknowledge the constant assistance of the supervisor of the investigation, Dr. H. W. Conn, with whom the cheese problems have been fully discussed at every stage.

CAMEMBERT CHEESE.

RÉSUMÉ OF PREVIOUS PAPER.

The biological conditions and the physical changes encountered in the production of a Camembert cheese from market milk may be restated from our former bulletin[1][a] as a basis for defining the special problems of the mycologist.

Milk as ordinarily received contains bacteria of many species and the germinating spores of numerous fungi from the stable and from the food of the cattle. When such milk is curdled for cheese making,

[a] The figure references are to bibliography at end of bulletin.

representatives of all of these species are inclosed in the mass of coagulum. Freshly made cheese from this curd, then, may contain any species of mold or bacterium found in the locality which is capable of living in milk or its products. The first step in the ripening of a Camembert cheese is the production of lactic acid. The lactic bacteria very soon increase their rate of multiplication so enormously as to become entirely dominant. The acid produced by these forms soon reaches a percentage sufficiently high to restrict the further growth of nearly every other species of bacteria, and even to eliminate the organisms themselves. In a time varying from a few hours to three or four days, according to the proportional numbers of these antagonistic species at the start, further bacterial growth seems to be entirely stopped. Bacterial development can not begin again until this acidity is reduced below the critical point for the species involved, and even then, since the acid is neutralized on the outside first, for most species it begins at the surface and works slowly inward. The uncertainties due to the presence of many species of bacteria in the milk are in this way avoided by the natural, simple, and almost universally successful process of souring.

The further ripening of a Camembert cheese is attended by a gradual reduction of this acidity until the ripe cheese is usually alkaline to litmus. At the same time the mold action in the mass of curd produces chemical changes which in from three to five weeks reduce the previously insoluble mass to a high percentage of solubility in water. In the later stages of this breaking down compounds are formed which give the characteristic odors and flavors to this type of cheese. Associated with these chemical changes there is a progressive physical change from the firm curd to a soft, buttery, or even semiliquid texture, characteristic of ripe cheese. The biological problems then were, in general, the determination of what organisms cause—

(1) The changes in the acidity of the curd.

(2) The breaking down of the casein, with the associated changes in the physical character of the cheese.

(3) The production of the flavors.

(4) The recognition and control of deleterious species.

CULTURE MEDIA AND METHODS.

The common dairy fungi grow readily upon any of the standard culture media. Among the media used have been peptone agar, whey gelatin, sugar gelatin with or without the addition of litmus, milk agar, gelatin and agar made with Raulin's fluid, potato agar, potato plugs, and sterilized milk and curd. Special studies have involved other preparations. The fact that these fungi grow readily upon all the common media has led to the selection of two preparations for constant use, and the careful study upon these of all species found. For this purpose the sugar gelatin, described by Conn[2] for the qualitative bacteriological analysis of milk, and potato agar have been used.

The sugar-gelatin formula produces an accurately titrated medium in which every effort is made to secure a uniform composition. Although absolute uniformity in chemical and physical properties is never obtained, the reaction of many species of fungi, when grown upon successive lots of gelatin made after this formula, have been so reliable as to commend its use for determining physiological characters. It seems clearly shown, therefore, that slight variations in the composition of the medium do not produce great differences in the species studied in this paper. In the discussion of the relation of a mold to this gelatin it must be borne in mind that the same results might not follow the use of any other formula.

The other medium, the potato agar, was selected because of its use in many mycological laboratories. In this medium uniform composition can hardly be claimed. The following process has been used in this work: The potatoes are carefully washed, pared, and sliced, then slowly heated for about two hours in approximately two volumes of water. At the close of the heating the water is allowed to boil. The whole is then filtered through cloth, and commonly through cotton also, water being added to make up the losses of evaporation and filtering. To this is added 1 per cent of shredded agar. It is then heated for from twenty to thirty minutes in the autoclave to 120° C. or higher, when it may at once be put into tubes for use, or, if cloudy, it may be very quickly filtered through absorbent cotton, after which it should be quite clear. The uncertainties in the composition of this medium result from the differences in the potato extract itself and from the fact that the difficulties in filtering this extract take out a varying amount, which is replaced with water. Titration shows that this medium is nearly neutral (4–6 acid on Fuller's scale) in cases tested to phenolphthalein; consequently it is used without neutralizing. Culture and study of the same species upon successive lots of this medium show that these differences in composition have little if any effect upon the morphology of the species studied.

Pétri-dish cultures have been used continually because they admit of direct study under the microscope. Slanted test tubes were found useful for stock cultures and for gross studies of physiological effects, but they are of little value for comparative work. It is useless to attempt to get a correct idea of the normal gross structure of these molds from fluid mounts. The extremely delicate hyphæ are so tangled in such preparations as to give but very little idea of their ordinary appearance, while the chains of conidia break up immediately when placed in any fluid. Such mounts are useful and necessary to get at details of cell structure and cell relations, but in comparative studies of species of such a genus as Penicillium their value is only that of a useful accessory. The primary source of comparative data must be direct study of the growing colony, undisturbed upon the culture medium, with the best lenses that admit of such use.

This method of study recognizes that morphology is the basis of fungus determination, but takes into consideration—

(1) That morphology must not only include the minutest details of cell structure and cell relations such as are undisturbed in fluid mounts, but also the appearance and character of the colony.

(2) That the morphology of the colony—i. e., the size of conidiophore and fructification, relation of these to substratum, appearance, and relations of aerial and submerged mycelium—is different upon various substrata, but has been found to be characteristic for each particular substratum.

(3) That a description of morphology to be of value must, therefore, specify the formula of the medium used and the conditions.

Dilution cultures have been necessary usually to obtain the colonies pure, but the direct transfer of large numbers of spores upon a platinum needle to the surface of gelatine or agar plates which have been allowed to cool has been found to give equally reliable results, and to have many advantages for the study of species once obtained in pure culture. This is often spoken of as inoculation of cold-poured plates. Litmus solution may be used with either gelatin or agar, and gives striking evidence of differences in species and the rate of their physiological action. Bacterial contamination has been usually restrained by the addition of from 2 to 4 drops of normal lactic acid to 8 or 10 c. c. of medium.

EFFECT OF A FUNGUS UPON A CULTURE MEDIUM.

In studying the relation of a fungus to a culture medium we find (1) that the fungus absorbs food from the surrounding medium; (2) that it may secrete or excrete substances into the medium which may transform its chemical composition and its appearance. The amount of food absorbed by the fungus is small, and for our purposes may be practically ignored, but the changes induced by indirect action—secretions from the mycelium—are great and far-reaching. To this latter group belong the changes in acidity, digestive effects, and flavors produced by fungi.

LITERATURE OF CHEESE FUNGI.

A review of the literature at the outset showed that no work on the fungous flora of the various types of soft cheese had been published in English. Epstein,[3] at Prague, studied the ripening of Camembert and Brie cheeses. He attributes the breaking down of the curd in French Brie to the action of *Penicillium album*, but denies the participation of molds in the ripening of Camembert. Johan-Olsen,[4] in Sweden, has published a brief review of the fungi related to the ripening of Gammelost, barely mentioning work done upon Camembert. Constantin and Ray,[5] in France, have described the appearance upon the cheese of the species of Penicillium involved in the ripening of the French Brie. Roger,[6] also in France, has attributed a single phase of Camembert cheese ripening to the activity of *Penicillium candidum*, for which he gives no description. Of these references, that of Epstein and that of Constantin and Ray describe the mold found upon the French Brie sufficiently clearly to aid in its recognition. A popular article, signed Margaret,[7] in the Creamery Journal of October, 1904, gives in entirely untechnical language a very satisfactory description of the appearance upon cheese of the penicillium concerned in the ripening of Camembert. The general insufficiency of the literature available made a first-hand study of the types of cheese found in American markets the only source from which definite information could be secured.

BIOLOGICAL ANALYSIS OF A CHEESE.

In the biological analysis of a market cheese it is carefully unwrapped to avoid contamination as far as possible. Series of dilution cultures on neutral and acid media are made at once from each part of its surface which shows any variation in appearance. In this way all the surface molds and bacteria are secured in one set of plates. Afterwards this surface is examined in detail, usually with a lens, the appearance of the different areas being noted, and direct transfers from each area made to cold agar or gelatin plates. The cheese is then cut with a sterilized scalpel and cultures are made from various portions of the interior. Usually the transfers were made from the center and from the area just inside the rind. Any part showing special appearances is reserved for a separate series of cultures.

Most of the brands of Camembert cheese found in our markets, as well as some sent by Roger, have been examined in this way. For comparison, similar studies have been made from several specimens of Roquefort cheese bought in different markets, and from individual specimens of Gorgonzola and Stilton. Single studies for molds have been made from Limburger, Port du Salut, Brinse, and from several brands of prepared cheese found in the market. From these cultures all species of bacteria found have been isolated and handed over to the bacteriologists. Each variety of mold occurring upon these cheeses has been isolated and studied. It has been possible in this way to show that a comparatively small number of species characteristically occur upon soft cheese. Although this list may be greatly extended by including forms which are occasionally found, it is rather surprising that a restricted group of species occurs with much regularity in studies of cheese from so widely different countries.

To study the origin and distribution of these molds several laboratories and cheese factories have been visited and cultures taken. Correspondents in distant States have kindly sent cultures of molds occurring in their work. Among those who have sent material are Dr. C. E. Marshall, Agricultural College, Mich.; Mr. E. G. Hastings, Madison, Wis.; Prof. F. C. Harrison, Guelph, Ontario; Dr. H. A. Harding, Geneva, N. Y., and Prof. P. H. Rolfs, Miami, Fla. Thus, in addition to a large number of cultures from the dairy laboratories of the stations at Storrs and at Middletown, we have accumulated from various sources a considerable number of species representing the characteristic molds occurring in dairy work, as well as many forms collected in the field and from laboratories not associated with dairy investigation.

THE FLORA OF CAMEMBERT CHEESE.

Although a considerable variety of molds appeared in cultures from Camembert cheeses, a list of possibly twenty species would include those which were often found. Among these there are perhaps six species of Penicillium, two or three of Aspergillus, *Oidium lactis*, *Cladosporium herbarum*, one or two of Mucor, one or more of Fusarium, *Monilia candida*, and two species perhaps related to it, with the incidental occurrence of *Acrostalagmus cinnabarinus*, a Cephalosporium, various species of Alternaria, and Stysanus. Besides these, yeasts in large numbers and considerable variety are found in many cases.

The comparison of the results of culture with comparative studies of the surfaces of different brands of cheese showed that a single species of Penicillium was present upon every Camembert cheese examined. In partially ripened cheeses this mold often covered the larger part of the surface. We shall call this the "Camembert Penicillium" or the "Camembert mold." This species develops a large and characteristic growth of aerial mycelium in addition to a densely felted mass of threads which penetrate the surface of the cheese for 1 or 2 mm. and largely constitute the rind. In all except a few very old cheeses which were almost covered with red slime of bacterial origin it was readily seen to be the dominant species upon the surface.

Similarly, cultural data showed *Oidium* (*Oöspora*) *lactis* to be abundant upon every brand of Camembert. This mold is practically indistinguishable upon the surface by its characters, except under very favorable conditions, and at best its recognition, even with a hand lens, is not often certain. Mycelium of this fungus develops only in very moist substrata, and is usually entirely submerged. Only part of its chains of conidia even rise above the surface. In old and very ripe cheese, when the rind is covered with yeasts and bacteria, it is often difficult under the microscope to find the spores of Oidium. In such cases, unless one is familiar with the peculiar smell associated with its action, he must depend entirely upon the culture for evidence of its presence.

No other species of mold has been found upon every cheese examined, although no market cheese has failed to show contamination with at least one or two of the other fungi listed above. In other words, comparative biological examination of imported Camembert cheeses established the fact that these two species of mold were present upon them all, however abundantly they might be contaminated with other forms. The examination of hundreds of cheeses in the city markets has shown the presence of the same two molds upon all the brands of Camembert offered for sale. Such analyses clearly established the presence of these molds upon the ripe cheese, but gave no information either as to whether they were necessary or what function, if any, they might have. Experiments were therefore devised to test the relationship of these molds to the ripening processes outlined above. The constant occurrence of other molds upon the cheese brings up the question, How and to what extent do the latter affect the ripening process? The experiments, therefore, have been made to include as many species as possible. Where detailed chemical analyses had to be made the work has necessarily been restricted to a few forms.

For this purpose, in addition to the Camembert Penicillium and *Oidium lactis*, the Penicillium found in Roquefort cheese ("der Edelpilz" of German authors) has been generally used. For convenience it is called the "Roquefort Penicillium" or "Roquefort mold." One of the Mucors, probably *Mucor* or *Chlamydomucor racemosus*, is so commonly found that it has often been included. A pure white mold closely related to the Camembert Penicillium has given some interesting contrasts. When reference is made to any of the numerous undetermined green species of Penicillium, they will be indicated by the letter or number under which they appear in the record book of cultures, and under which the origin and subsequent cultural history of all species studied has been kept.

OUTLINE OF THE WORK.

These studies involve two classes of data, first, those experiments requiring quantitative analyses, which have been conducted in cooperation with Mr. A. W. Bosworth, chemist to this investigation, the results of which series of analyses will appear in his report; second, experiments which show the physiological characters of the fungi by physical changes in the appearance, texture, or color of the medium used, or by the production of flavors.

The results may be anticipated here by noting that these two classes of data did not prove mutually interdependent, but that analysis may show in general the right stage of chemical changes called for in a ripe cheese without the necessary texture and flavor; and, conversely, the practically necessary texture and flavor may be obtained in a cheese differing considerably in its chemical characteristics from the standard market article. In our practical experiments we sought first for proper appearance, texture, and flavor of the cheeses; then, without disturbing these, endeavored so to control the processes of ripening as to satisfy the standard of chemical composition established from the study of market cheeses.

RELATION OF MOLDS TO ACIDITY.

The development of lactic acid has been shown to be of primary importance in the control of deleterious bacteria. In our previous paper it has also been seen that after doing its work this acidity gradually disappears in the ripening process. The disappearance of the acid has been attributed by Roger,[6] by Epstein,[8] and by Mazé[9] to the activity of molds, and interpreted as preparing the way for the action of peptonizing bacteria. This view of the relation of molds to cheese ripening has been widely quoted as their only function in the process.

The acid exerts practically no selective action upon any of the molds studied. Stoll has recently shown that species of Penicillium grow readily in media containing a much higher percentage of acid than ever occurs in cheese work. The use of acid in fungous cultures to restrain bacteria is practically universal, but the action of the different species of mold upon the acid is very different. This is strikingly shown by the introduction of a solution of litmus into the culture media used. Litmus gelatin or litmus agar may be a deep blue if used at 15 acid on Fuller's scale, as is usual for bacterial studies, or a clear bright red if 2 to 4 drops of normal lactic or other acid are added to 10 c. c. of medium. No mold cultivated in this work has failed to show some definite relation to acidity indicated by litmus reaction. Some fungi, as soon as they develop visible colonies, begin to change red (acid) media to blue (alkaline), and consistently maintain this character. Many others, when grown in blue gelatin (designating by blue gelatin 15 points acid to phenolphthalein = 10 points alkaline to litmus on Fuller's scale), begin by changing the blue to red. This change may vary from the faintest tinge of red in only that part of the medium directly in contact with the threads of the young colony to deep red over large areas. *Oidium lactis* and Roquefort Penicillium produce at times a very slight pink, which barely traces the outer limits of the young colonies before the blue reaction begins to appear. At other times the red, if appearing at all, has been so evanescent as to be overlooked. It has been suggested that this slight appearance of acidity might be due to the excretion of carbon dioxide in respiration, which, although continuous, is afterwards masked by many times larger changes in other substances.

The Camembert Penicillium, and several of the very common green species of Penicillium, when grown upon blue gelatin, at first turn all the substratum in contact with the growing colonies to a bright red. Some species produce areas of red beyond the limits of the mycelium. These effects are most clearly seen by examining the colony from the under side. Later a spot of blue appears in the center of the colony below and gradually extends outward until commonly the entire mass of culture medium has become blue. This often involves a change of reaction in agar or gelatin 2 to 3 cm. beyond the colony. It is thus clear that there must be either the secretion or the excretion by the mycelium into the medium of a substance capable of changing this reaction or the absorption from the medium of some substance, thus changing its reaction. The exact nature of this change has not been determined. Increase in the percentage of acidity or of alkalinity retards the change of reaction. In certain experiments phenolphthalein was introduced into red litmus media and several species of Penicillium and *Oidium lactis* were grown upon it. With the Camembert Penicillium the entire mass of agar became blue in a few days, and remained so for nearly three weeks. Then the characteristic pink color for the alkaline reaction of phenolphthalein appeared on the under side of the colony. This was tested by opening the colony with a platinum needle and introducing a very small drop of normal acid, when the pink area was changed first to blue and then to red. As the acid diffused outward from the center the wave of blue traveled outward, being replaced constantly by red until all trace of the phenolphthalein reaction was gone. The other species used did not give this reaction. There are forms including some species of Penicillium, *Aspergillus niger*, *Monilia fructigena*, and others, which produce the acid reaction in litmus media without any change to blue. Several species of Penicillium rapidly produce the purplish color which is characteristic of the turning point of litmus at which their further development occurs. Apparently these bring acid or alkaline media to that point without further change. It would appear, then, that the relations of these molds to acidity, as indicated by the litmus reaction, is reasonably uniform. To determine whether the litmus reaction would be reliable upon a medium closely allied to cheese, test tubes of separated milk were prepared, blue litmus added, and the tubes sterilized. Eleven species of Penicillium were inoculated into these tubes and observations made every day. Of the eleven species, four, including the Camembert Penicillium, produced a layer of red milk for a few millimeters below the colonies, which later was changed back to blue. The other species either intensified the blue or produced no change.

The suggestion has been made that neutralization of acid is due to the production of ammonia. A series of cultures were made in cooperation with Mr. A. W. Bosworth to test the production of ammonia compounds by mold action. The species used were the Roquefort Penicillium, the Camembert Penicillium, *Penicillium* sp. (record No. 310), *Oidium lactis*, *Oidium* sp. (record B), and *Aspergillus niger*. These were grown upon potato ager, to which litmus and lactic acid were added. The Aspergillus culture remained bright red; all the others became deep blue. Upon analysis the *Aspergillus niger* was found to have produced the largest amount of ammonia. Study of the figures showed that the ammonia alone was not sufficient to neutralize the acid used in any case. It is clear, then, that the lactic acid must have been neutralized by some other basic products of digestion rather than by ammonia. If the acid were absorbed and dissociated after absorption the area of blue would be restricted to the neighborhood of the hyphæ, or the diffusion of the acid for considerable distances would produce purple tones instead of sharply marked areas of red and blue. The data seem to indicate that chemical decomposition or neutralization of acid must be the action of some product excreted by the fungus, probably an enzyme.

It has thus been shown by many experiments that the Camembert Penicillium and *Oidium lactis* are two of many species capable of reducing the acidity of the media upon which they grow. Many other species of the same genus produce this effect more quickly than the Camembert Penicillium and some act at about the same rate. The reduction of the acidity of the cheese may clearly be attributed to these molds; but the study of the relations of many other molds to acid indicates that any of a large number of species might be equally or more useful for the accomplishment of this step in cheese ripening. If, therefore, these particular molds are essential to Camembert cheese ripening, their special function must be sought in other steps of the process.

THE BREAKING DOWN OF CASEIN.

The changes in firm sour curd which result in the production of the soft, buttery, or semiliquid texture of the Camembert cheese present some very complex problems. These may be grouped as (1) the purely chemical questions, which involve qualitative and quantitative analyses of the material at every stage; (2) The biological and physical questions, which deal with the agents and conditions which produce these results and with the gross appearances of the final products, whose descriptions do not depend upon detailed chemical analysis.

(1) The chemist describes the general course and extent of these processes[1] as a change in which the insoluble or but slightly soluble compounds of casein found in sour curd are rendered almost completely soluble in water. The details of the process and the data will appear later in the report of the chemist.

(2) To determine what relation the molds might have to this change involved a great many cultures on different media. In some experiments the number of species used was large and the results acquired in that way a comparative value, but in the more complicated trials the work was limited to those mentioned above.

It is practically impossible to produce a normal cheese in such a way as to avoid contamination with bacteria or molds. It is difficult, therefore, to study directly upon cheese the relations of organisms to the steps of cheese ripening. Even were this possible, the complexity of the changes encountered would make the interpretation of the phenomena difficult. The activities of these molds have, therefore, been studied in pure culture upon a series of media which would give information as to steps of the process. While these cultural studies were proceeding, many cheeses were made and inoculated with the Camembert and Roquefort Penicillia. The measure of success obtained from cheese inoculated with the Camembert Penicillium gave good, practical ground for its continued study. These detail studies may be discussed best separately.

LIQUEFACTION OF GELATIN.

The liquefaction of gelatin media has been much used as an index of digestive activity. All species obtained have been grown upon neutral and acid sugar gelatin and the effects noted carefully.

The difference in action between the molds important in this investigation are striking. The Mucor produces a slow but rather complete liquefaction; *Oidium lactis* will gradually soften the gelatin so that the center of the colony is liquefied; a pigment-producing Penicillium (recorded simply as O) will liquefy all the gelatin in contact with it so quickly that it becomes in a week a floating colony in a watery pool twice its own diameter. Several other species of Penicillium have the same effect. The Roquefort Penicillium softens gelatin somewhat, but never produces a watery liquefaction. The Camembert Penicillium often produces a slight liquefaction under the center of the colony, but never extends that liquid area to half the total size of the colony. This seems to indicate that the Penicillium O and its allies would produce a rapid digestion, that the Mucor would be somewhat slower, that the Camembert mold might have some digestive effect and the Roquefort mold very little, if any, value. The test of the ability to liquefy the gelatin used gives, therefore, only indefinite or negative results as to any advantageous relation of these particular species to cheese ripening.

Comparative study of numerous cultures of many species of fungi upon gelatin gives, however, some very interesting suggestions. In many species which liquefy litmus gelatin rapidly, the area of liquefaction is surrounded by a blue (alkaline) band. For example, in one experiment with Penicillium 392 at its most active period of growth a colony 15 mm. in diameter was surrounded by a liquefied area 4 to 8 mm. wide. This area was in turn surrounded by a band of intense blue shading gradually in a width of perhaps 10 mm. into unchanged red litmus gelatin. The medium which had been liquefied was almost colorless.

Several suggestions may be drawn from many such observations. The change in acidity of the medium, as has been noted above, may be effected at a distance of 2 to 3 cm. from the colony. This change of litmus reaction advances faster than the area of liquefaction of the gelatin. The breadth of the area of liquefaction shows that the action of the fungus is not a digestion by contact, but the secretion into the medium of diffusible agents, that is, enzymes. In most of these species liquefaction occurs only in areas having alkaline reaction. No general relation between acidity and digestion is established. The substantial uniformity of the results of repeated cultures of the same species of fungi upon gelatin made after the formula used established its usefulness as a test of the ability of an organism to perform this particular digestion. It will be shown later that the ability to liquefy this variety of gelatin is not to be regarded as a general test of the ability of a species to produce active proteolytic enzymes.

RAULIN'S FLUID.

To test the ability of these species to grow in a medium entirely lacking in proteid, Raulin's fluid was used as given by Smith and Swingle,[10] but modified by leaving out the potassium silicate and zinc sulphate. Sterilized flasks of this solution were inoculated with Mucor, *Oidium lactis*, Camembert Penicillium, and Roquefort Penicillium. All four grew. The *Oidium lactis* and Mucor did not appear to develop in an entirely normal way. Both species of Penicillium grew richly and fruited normally. The culture of the Camembert mold, after growing several weeks, was examined chemically and digestive experiments conducted by Mr. Bosworth demonstrated the presence of a proteolytic enzyme. In this way it was shown that this fungus could not only construct proteid from inorganic compounds of nitrogen, but would produce proteolytic enzymes in such a solution. Enzyme studies were not made for the other species used in this experiment.

CASEIN.

For a medium at the opposite extreme, the chemists prepared pure casein. This was weighed into 2-gram lots, moistened, sterilized in the autoclave, and inoculated with five species of mold. All grew and fruited luxuriantly. This experiment showed only that the species used were able to break down casein and to grow normally upon the products of this digestion without the addition of other nutrients.

STERILE MILK AND CURD.

Sterilized milk and sterilized curd offer a substratum related to cheese. Sterilized milk in quantities varying from 40 c. c. to 150 c. c. in test tubes and Erlenmeyer flasks has often been used. Nearly all species of Penicillium grow luxuriantly, forming a felted mass of mycelium often 2 to 4 mm. in thickness upon the surface of the milk. With the absorption of the milk in such cultures of the Camembert and Roquefort species the mass of mycelium buckles and bends, tubercles of mycelium arise on the under side of the mass and grow downward, keeping the mold in connection with the fluid. In this way a culture may continue to grow for several months until it forms tough, irregular masses of felted hyphæ, filling the test tube for an inch or more downward from the original surface of the milk. The milk below the colony soon becomes transparent, giving reactions for digestion, with a residue of curd at the bottom, which in the course of time may be almost completely dissolved. With the *Oidium lactis*, on the contrary, the colonies largely sink below the surface, so that the milk may be quite well filled with mycelium upon which chains of spores are only produced in quantity at or just below the surface. Similar experiments with 100 grams of sterilized curd in flasks, inoculated with the Camembert and Roquefort molds, have shown that either species is able to change the chemical composition until the derivatives of casein are amost completely water soluble. Such cultures were plated to show their freedom from contamination by bacteria before analysis. The resulting products give the standard reactions for digestion. These experiments show that either of these molds is capable of producing digestive changes comparable in their completeness, rapidity, and general nature to those shown by analysis to have occurred in the ripening of Camembert cheeses.

DOES THE MYCELIUM PENETRATE THE CHEESE?

It must be noted carefully that this action of the Camembert mold goes on without the complete penetration of the substratum by the mycelium of the mold. That this is true is readily seen in milk cultures, where the limits of the development of the mycelium are sharp and clear. The same fact has been demonstrated for cheese by hundreds of sections and careful cultural studies many times repeated. The mycelium forms a dense mat upon the surface of the fluid or the mass of curd, or the newly made cheese. It follows the irregularities of the surface and is not found to enter well-packed curd to any extent. It is very difficult to prove that hyphæ of this mold actually appear in curd of uniform texture below 1 or 2 mm. When found deeper, careful search usually shows a cracking of the surface, so that the mycelium may follow the opening already made. In no case of many hundreds of cheeses studied and experiments performed has the mold been found to fruit in cavities not opening broadly upon the surface. This is in marked contrast to the habit of the Penicillium instrumental in the ripening of Roquefort cheese, which penetrates the channels of the substratum and fruits in every cavity large enough to accommodate a conidiophore. The Roquefort mold will make every cavity in a cracker or piece of bread green with spores, while the Camembert mold will fruit upon the surface of the bread or cracker with only vegetative mycelium inside the bread.

Definite experiments to prove that this digestive power on the part of the Penicillium is due to the secretion of one or more enzymes have given characteristic reactions for digestion many times. Without here discussing these chemical reactions, it has been shown that the chemical action of the fungus is carried on at distances from the mycelium which preclude direct action. The enzyme must therefore be secreted and diffuse outward from the mycelium into the substratum. This explains why the Camembert cheese begins to ripen just under the surface and the process progresses inward from all sides until the cheese is entirely ripe. Before this process is complete the center is simply sour curd. A good illustration of this action is seen in cheeses which are ripened without turning. In such cases the development of mold and enzyme on the lower surface is prevented, and as a consequence ripening is delayed on that surface.

CAMEMBERT PENICILLIUM UPON CHEESE.

Many cheeses have been made and inoculated with this mold in conjunction with pure cultures of lactic starter. Little difficulty is found in this, since, if an abundance of spores are put upon the cheese when made, this mold seems capable of taking and maintaining the lead of all others. A cheese made in this way and ripened for from three to four weeks will finally be rendered creamy, or, under some conditions, waxy throughout, in color white within, in flavor almost neutral, having no particular character—good or bad—and hence, to one fond of Camembert cheese, tasteless and insipid. The important features of this ripening process are, then, the completeness of its action and the entire absence of any objectionable character in its flavor. Biological analysis has shown that the center of such a ripened cheese may be practically a pure culture of lactic organisms. The texture is, therefore, obtainable by the use of the Penicillium alone.

COMPARATIVE STUDIES OF FUNGOUS DIGESTION.

Comparative tests of digestive action have been made for a number of molds. The Roquefort Penicillium has been used in parallel cultures with the Camembert Penicillium in many determinations. It has shown equal or greater ability to digest milk and curd. A typical example of several series consisted of the cultivation of 11 species of Penicillium upon sterilized milk in large test tubes. Observation of results after seven days showed digestion by 7 of these species. In 5 of them the amount of action exceeded that of the Camembert Penicillium, and some of them appeared to digest milk at least twice as rapidly as did that species in the first week.

In another series milk agar was made by dissolving 1 to 2 per cent of the agar in water at 130° C. and pouring together equal quantities of the hot agar and hot sterilized milk. If poured into Petri dishes at once this medium was smooth and clear, but if acidified or sterilized after mixing, flakes of precipitate appeared. The flaky precipitate in the acidified cultures was found very useful as an indication of digestion. In cultures upon the surface of such plates where digestive action was strong the flakes would entirely disappear. Twenty-three species of mold were tested upon milk agar in this way. Of these, 8 produced a distinctly stronger digestion than the Camembert Penicillium; 5 produced digestion approximately equaling that species, and 10 produced less digestion. These cultures were mostly made in duplicate, and both results in all but two cases agreed fully. *Oidium lactis* produced comparatively little effect upon this medium.

TABLE I.—*Reaction of certain species of molds.*

Species.	Litmus.	Liquefaction of gelatin.	Rate of digestion of curd.	Rate of digestion of milk.	5° to 10° C.
Camembert P.	Red, then blue	Partial	Medium	Medium	Grow, slow fruiting.
Roquefort P.	Blue	Softening	Rapid	Rapid	Characteristic growth.
Oidium	Blue	Incomplete	Slow	Slow	Characteristic.
Mucor 12	Blue	Incomplete	Slow		Poor growth.
Mucor 191	Blue	Incomplete	Slow		
O	Blue	Rapid	Rapid	Rapid	
300	Blue	Partial	Medium to rapid.	Rapid	Retarded.
132	Red, then slowly blue.	Slight	Medium		
310	Red, then blue	Slight	Slow to medium.	Slight	Slow growth.
68	Red, then blue	Partial	Slow	Medium	Slow fruiting.
Monilia candida 198.	Blue	Rapid	Rapid	Rapid	
P. brevicaule	Blue	Rapid	Rapid	Rapid	
392	Blue	Rapid	Slight	Rather slow	
240	Blue	Rapid	Rapid		
Aspergillus niger.	Red	Rapid		Slow	
135	Blue	Rapid		Rapid	
136	Red to purple blue.	Partial softening.	Medium		Characteristic.

Two species of Penicillia, 68 and 310, found closely associated upon cheese with the Camembert Penicillium, produced little digestion. The Roquefort Penicillium and several other molds often found upon Camembert cheese appeared to act much more rapidly than the Camembert mold itself.

All of these series of cultures under different conditions have many times shown the same results and prove that the ability to digest curd is common to many species of fungi. The species we have been led to call the Camembert Penicillium possesses this character in common with numerous other molds, many of which act more rapidly than this one.

After the ability of several molds to digest curd is established, the relation of any particular mold to cheese ripening must be determined by the character of the products of that digestion and the flavors associated with it. No pure culture upon a medium previously sterilized by heat has given a taste resembling that of Camembert cheese. Cheese made and kept in an atmosphere of chloroform, which prevented mold and bacterial development, refused to ripen. Numerous cheeses made and not inoculated with molds have uniformly failed to develop the texture and flavor of Camembert cheese, although such cheeses have usually become covered with molds of various species. The type of cheese made and sold in this country as Isigny and Brie, and sometimes labeled Camembert, which always shows *Oidium lactis* associated with bacteria, differs entirely in appearance, texture, odor, and flavor from Camembert; yet *Oidium lactis* is capable of neutralizing the acid of the cheese much more rapidly than the Camembert Penicillium. Nevertheless the center of such a cheese remains acid for a longer time than is required to ripen a Camembert cheese, while the texture of Camembert is not produced. The necessity for the presence of another agent in this ripening is clearly established.

More than 2,000 cheeses have been made and ripened at this station with the Camembert mold under varying conditions. Hundreds of these cheeses have shown repeatedly that cheese so made will assume in ripening the texture of the best imported article. The Camembert Penicillium, therefore, is seen to be able to neutralize the acid of the freshly made cheese and to produce the texture desired, but not the flavor. It remains to determine whether other molds may not be equally useful in this process. For comparison cheeses have been made and inoculated with the Roquefort Penicillium with undetermined species of Penicillium appearing on the record as O, 300, 310, 68, 132. Of these species one, 310, when cultivated upon every medium used except the cheese duplicated the reactions of the Camembert mold completely. Its morphology is scarcely distinguishable. It differs only in that it remains pure white during its entire cycle of development, while the Camembert species turns gray-green in age. The close relationship apparent, together with a promising test, led to its use upon over 100 cheeses. The breaking down resulting from its action was widely different. These cheeses were drier, waxy, with a mealy crumbling layer just under the rind. The physical character of the results and the flavor produced were so different that the cheeses were entirely worthless. This mold was originally isolated from a market Camembert cheese, where it was found mixed with others.

The presence of the Roquefort Penicillium may be seen by the spots of green it produces and may be detected by a sharp, bitter, perhaps astringent, taste. The texture of the cheese produced is different, and the flavor when it is present in any large amount is so strong as to be very objectionable to many. When present in small amounts upon a cheese it gives a certain sharpness or piquancy to it, such as has been found often in certain brands of imported cheese, and is sought for by some buyers.

The species marked O and 300 secrete a bright yellow pigment into the cheese, which colors every area with which it comes in contact. A cheese was inoculated with No. 300 and examined when 8 weeks old. It had produced no trace of the texture of Camembert. The center of the cheese remained practically sour curd, while the portion for perhaps one-fourth of an inch under the colony was decomposed.

The species marked 68 has been obtained from cheese from widely different sources. In cultures upon milk and milk agar it produced little change. A cheese inoculated with it remained largely sour curd for two months. The species marked 132 is a very common green form, appearing in dairy and other cultures. It has given no satisfactory results when grown upon cheese. In this way related species found in cheese work have been tested in their effects upon cheese and shown not to produce digestion comparable in physical character to that demanded in a Camembert cheese and constantly obtained by the use of the Camembert Penicillium. There seems to be no further question that this species of Penicillium, among all the molds so far studied, is the only agent capable of producing the characteristic texture of the best type of Camembert cheese, with no objectionable flavors or colors.

FLAVORS.

All attempts to produce the flavor of Camembert cheese in pure cultures upon milk and curd with particular organisms have failed. Here again we have had to depend upon the use of cheeses so that direct, positive proofs have not been possible. The value of the indirect or circumstantial evidence offered must depend upon the completeness with which all factors have been considered. It has been previously shown that a cheese may be ripened to the texture of the best Camembert by the action of lactic bacteria and the Camembert Penicillium, but that it will lack flavor. A series of difficulties are met here. The typical flavor does not begin to appear until ripening is well along. This would indicate that the flavor-producing agent or agents must act upon already partially ripened cheese to produce the particular end products which give this flavor. But coincident with this change the acidity of the curd has become so far reduced that bacterial development may now occur on the surface at least, and as a matter of observation few cheeses begin to show flavor until cultures from their surface show swarms of bacteria of various species. It has not been practically possible to change these conditions sufficiently to make cheeses bearing only pure cultures upon the surface. The problem becomes, then, one of comparative study and the elimination of the unnecessary factors one by one, rather than the direct production of the flavor sought in a single conclusive experiment.

Some organism or organisms must be sought for to produce the flavor. The appearance of the flavor of the imported article in certain experimental cheeses at this stage of the investigation led to their immediate study. This showed that *Oidium lactis* was abundant upon these cheeses and emphasized the fact that it had always appeared in cultures from market cheeses. Oidium had been excluded from many experiments in cheese making because it had been found to be associated with odors that seemed undesirable, as well as because of the conclusion of Epstein from his researches, that the presence of Oidium is uniformly deleterious. The inoculation with spores of Oidium of a half-ripened cheese entirely lacking flavor produced the flavor distinctly in a single week, but since bacterial action seemed always associated with this, further evidence was necessary. Roger and Epstein have attributed the ripening of Camembert to the action of certain bacteria without distinguishing that the production of the texture of the cheese is accomplished by a different agent from the production of flavor. In their descriptions ripened Camembert is always referred to as slightly reddish in color, and the appearance of this color is regarded as an indication of the progress of ripening. In cheeses selected and forwarded by M. Roger this red color was very prominent and the red layer was found to consist of myriads of bacteria of a few species. Cultures from these cheeses showed that *Oidium lactis* was also present in abundance. Numerous tests have been made with the bacteria found associated with the various brands of Camembert cheese hitherto without producing the flavor in any case independently of the molds. The comparative study of many cheeses from the market and from our own cellars seems to show that cheeses may have the typical Camembert flavor without the development of any specific surface growth of bacteria. The character of the bacterial growth upon the surface appears, therefore, to be incidental or accidental, though its presence may be necessary to exclude air, as maintained by Mazé[9] in a recent paper.

Cheeses of good flavor have been produced here and also purchased in the market, which indicate that particular surface appearances are not essential to the typical flavor. Similarly the introduction into new cheeses of species of bacteria found in cultures from the interior of good cheeses has produced either no effect whatever or disagreeable flavors. Thus far, therefore, no species of bacterium has been found capable of producing the Camembert flavor. Although the flavor question is manifestly still unsettled, we may offer the following summary of the data at hand upon relation of molds to flavor in Camembert cheese:

(1) *Oidium lactis* has been found in every brand of Camembert cheese studied.

(2) It has never been found upon a ripened Camembert cheese which lacked the flavor.

(3) The flavor has never been found in a cheese without the Oidium.

(4) Every other species with which the flavor seemed obtainable has been eliminated from one or more experiments without loss of flavor.

(5) Bacteria or other molds do in many cases modify the flavor of Camembert cheese, but do not seem to be able to produce it independently of the mold. There thus arise characteristic secondary flavors which are associated with the output of certain factories and which command special markets. These varieties are usually more highly flavored than what we have regarded as typical.

The essential relation of the Camembert Penicillium and *Oidium lactis* to the production of Camembert cheese is, therefore, well established. Several mycological questions remain: What are the optimum conditions of temperature and moisture for the use of these molds in cheese ripening? What are the most practicable means of cultivating material for inoculation? How can the proper inoculation with these molds be most effectually secured? What other fungi occur as contaminating species and how can they be controlled?

TEMPERATURE.

Since the higher temperatures of the ripening cellar lead more rapidly to the development of bacteria, it is necessary to determine the lowest temperature which will permit mold growth and also enzyme action. The different species respond quite differently to temperature. In one experiment eight species were inoculated into slanted tubes of gelatin and put in a refrigerator where the temperature varied from 5° to 10° C. Of these the Camembert Penicillium and two nearly related species, Nos. 68 and 310, grew, but fruited very slowly, showing an inhibiting effect. The Roquefort Penicillium grew and fruited normally, as also did *Oidium lactis*. The species of Mucor used developed very slowly and fruited only slightly. Two of the very common green species of Penicillium grew richly. *Oidium lactis* grows abundantly in the Brie and Isigny cellars visited. In these the temperature was 50° to 55° F. (11° to 12° C.). Numerous experiments in the ripening cellar show that the Camembert Penicillium does not grow its best in a room cooler than 60° F. (15° C.), and that to obtain rapid development the room should be slightly warmer. Until this mold is well established, therefore, it is distinctly an advantage to grow it at a temperature of 65° to 70° F. Repeated experiments have shown that lowering the temperature to 52° to 55° F. checks the rate of ripening very materially. A difference of less than 10 degrees between two rooms will often make as much as two weeks' difference in the ripening period of cheeses from the same lot in the two rooms. A temperature as low as 54° to 55° F., as given in an article in the Creamery Journal previously referred to, appears to prolong the ripening period without contributing any compensating advantages. A half-ripened cheese was cut, the progress of the softening of the curd was noted, and the cheese put in a refrigerator, where it was held for four weeks at 48° F. It was then found to be completely ripened and perhaps a little old in one place, but the changes noted at the end of this period would have been produced within a single week at 60° F. The cold-storage possibilities suggested by this experiment will be further studied.

Some experiments were made to show the resistance of spores to heat. The spores of the Camembert and Roquefort Penicillia were inoculated into gelatin and placed in an incubator. Heating for an hour and fifteen minutes at 56° C. killed all spores of the Camembert species. Only a few spores of this mold grew after one hour at the same temperature, while some spores of the Roquefort Penicillium grew after two and one-half hours.

HUMIDITY.

The use of very moist cellars and caves in the ripening of this class of cheeses is practically universal. The richest development of mold is seen in rooms where the atmosphere is saturated or nearly so. This appears to be exceptionally true for species like the Camembert Penicillium, which is peculiarly a milk fungus, and in which there is a large development of thin-walled aerial mycelium. So dependent is the Camembert mold upon abundance of moisture that it has been found difficult to secure a rich growth upon the surface of a cheese which has been drained for two or three days before inoculation. Contrary to directions commonly given for ripening these cheeses, which call for a particular degree of humidity, cheeses have been ripened successfully in our cellars at the saturation point, as well as at various degrees of humidity below that. A good illustration of a mold which has adapted itself to changes of moisture is found in mold No. 198 Upon a fresh cheese in a moist room this mold forms a circular, ringlike colony of floccose hyphæ standing often 8 mm. high upon the surface of the cheese. In a drier situation, or when the cheese is nearly ripe and the rind becomes harder and dried, the same mold produces conidiophores which barely rise above the substratum, so that the surface of the cheese is covered by a white, powdery layer which is practically pure spores. The Mucors are so sensitive to moisture that they scarcely develop upon the cheese, except sometimes during the first few days, when the surfaces are very wet. They appear to be unable to withstand the rate at which surface evaporation proceeds in the ripening cellars.

INOCULATING MATERIAL.

The problem of propagation of the Camembert Penicillium for inoculation purposes presents some difficulties. This species bears spores only upon the surface of the culture medium used, in contrast to the Roquefort species, which, when grown upon bread, develops spores in every air space, as well as on the surface. To produce spores in quantity, therefore, material must be capable of sterilization and must present the largest possible amount of free surface in proportion to the space occupied. For the preparation of such material, quart fruit jars have been used. Various styles of crackers have been tried. Most of these were not successful. The most suitable appears to be the hard, dry "water cracker." The jar is filled with crackers and dry sterilized at 140° to 160° C. for an hour or more, better twice on successive days. The spores may be added directly, or first inoculated into about 100 c. c. of sterile water (acidified with 1.5 per cent of lactic acid usually) and this poured into the jar and shaken until all the crackers are wet. Various types of "milk cracker" soften to a pasty mass in this moistening process. The best water crackers are not very satisfactory, because the mycelium tends to transform bread or cracker into a soft, gummy mass. The crackers become matted together until they present much less actual surface than might be expected. The substitutes tried have been excelsior, hay, and sheets of cardboard wetted with milk or whey. Although some of these have advantages, they were on the whole less satisfactory than the water crackers. So far, therefore, on account of the very different habit of our mold, no material has been found so easily prepared and so satisfactory as the "Schimmelbrot" of the Roquefort cheese makers.

From the point of view of the use of pure cultures the *Oidium lactis* is even more troublesome. This mold produces a large proportion, and in some strains all of its spores as chains below the surface of the substratum. For pure-culture work Petri-dish cultures have been the only satisfactory vessels used. Its exceedingly rapid development, however, makes possible the propagation of a culture from day to day from the draining boards upon which the cheese is made. These become heavily coated with a slimy mass of mycelium and spores upon standing overnight. Direct transfers from them have been used with apparently no serious trouble from contamination. In fact, so capable is the Oidium of self-propagation in dairy work that Epstein declares it to be present in all dairy work. Although Roger in his published statement does not mention it at all, it was found abundantly upon the cheese forwarded by him to this station. We have succeeded by careful work in making many cheeses entirely free from Oidium, but with the ordinary treatment of dairy utensils it appears constantly in factory practice. It is practically possible to rely to a considerable extent upon the ability of the Oidium to propagate itself, as has hitherto been done in the factories.

INOCULATION WITH PENICILLIUM.

With the Penicillium, however, numerous experiments indicate that there is much advantage in early and effective inoculation from cultures of known purity. Whether such inoculation must be always made from specially grown laboratory cultures is questionable. In factory practice, the making room and the ripening cellar are usually adjacent. If precautions are taken always to have on hand some cheeses bearing pure cultures (and the cheese maker must know his mold so well that there will be no question about it), one or two such cheeses will furnish enough inoculation material for much newly made product. This would be indicated by the rough calculation that from the abundance of the chains of fruit and the size of the spores (0.005 mm. in diameter) probably about enough spores are produced to cover evenly the surface upon which they grow—perhaps 25,000,000 to the square inch. Very successful inoculation in 75 pounds of milk has commonly been secured by tapping a Petri-dish culture over the vat, or by breaking a piece of cracker about an inch square or less and stirring it into the milk.

The most economical and successful method of inoculation so far devised has been the use of a sprinkling jar or can. For this purpose holes 1 mm. or less in diameter in the jar lid are demanded. A small amount of water is put into the jar, a piece of cracker or cheese covered with mold is broken into the water, the top is then screwed on, and the jar thoroughly shaken. The water is then sprinkled upon the newly made cheese at the time of first turning, so that both sides of each cheese receive a few drops of water. Excellent results have been obtained in this way with the smallest amount of inoculating material and the least requirement of labor and skill. Such a jar should be emptied and washed immediately after using. The mixture is made fresh each time. Milk may be used instead of water, as was first suggested and tried by Doctor Conn; but the water has been found the more easily managed. The practical method for factory use will probably vary with the conditions and skill of the maker.

VITALITY OF SPORES.

Studies have been made upon the vitality of the spores of the species used. This varies greatly in different species. In some of the most common forms spores have been reputed to remain viable for several years. Recent studies by Wehmer showed that five species of Penicillium used in his experiment were entirely dead in laboratory cultures at the end of two and one-half years. Cultures of the Camembert Penicillium grown upon potato in test tubes plugged with cotton have refused entirely to germinate at the age of one year. Other cultures have seemed entirely dead inside of six months. In fact, the spores of this mold are very thin walled and die very rapidly when stored. Under such conditions they lose turgidity and become crenulated or indented. Spores of *Monilia candida* and several others have grown after more than a year in laboratory cultures, but their germination was much retarded. *Oidium lactis* seems to be very easily killed by drying, as would be expected from a species with such thin-walled spores. The Roquefort Penicillium under some conditions is more resistant, but loses vitality quite rapidly. It is certain, therefore, that to give the best results material for inoculation should be fresh and vigorous. Under ordinary circumstances it would not be desirable to use material more than a few weeks old.

CONTAMINATIONS.

The number of molds found upon market Camembert cheese shows the need of care in guarding against contamination of cultures. Extraneous molds may come from the milk or from the utensils used or from the clothes and hands of the workmen. Although the milk is the primary source of most infections, practical experiments have shown that if the proper molds are put upon the cheese at the time of making the troubles arising in this way may be minimized. In fact, sufficient contamination from this source directly to ruin a cheese is very uncommon.

The very habit in some countries of washing or rinsing cheese-making utensils in whey will account readily for the universal presence of *Oidium lactis* and perhaps for many of the bacterial infections that result in loss. But the source of the most trouble in a cheese cellar is found to be the cheese maker himself. The cheeses are commonly exposed upon curing boards and turned and examined in the hands. In this way spores from molds or bacteria occurring accidentally as single colonies upon single cheeses are distributed by thousands to hundreds of cheeses. The product of a factory may almost be identified in the markets by the contaminations upon the surface of its cheeses. Certain brands of the cheese always bear *Monilia candida* and commonly one or two other Monilias. A species of Fusarium is distinctive of another brand, with *Acrostalagmus cinnabarinus* occasionally present. After numerous experiences with all sorts of contamination this trouble has been practically eliminated from our experimental work by putting the fresh cheeses, as soon as they are drained, salted, and comparatively dry upon the surface, into boxes which are slightly larger than the cheeses, leaving air space and room for mold to develop normally. In this way fingering is done away with, the cheese is turned by turning the box, and examined by removing the lid without touching the surface, so that a colony of mold appearing upon one cheese is no longer distributed throughout the cellar.

It is therefore possible to produce cheeses practically free from molds other than those inoculated upon their surface. Although such boxing upon a large scale may be practically undesirable on account of expense, it remains certain that it may be useful in eliminating certain troubles without so large a loss as would come from discarding all infected cheeses, many of which would ripen satisfactorily but for the danger of spreading obnoxious fungi over great numbers of cheeses.

ROQUEFORT CHEESE.

The well-known Roquefort cheese is another highly flavored cheese in which mold has long been known to play a part. In manufacture this cheese approaches the hard type, but the ripened cheese bears a closer relation to the soft cheeses. Many complete descriptions give the details of its making and curing. These need not be repeated here. Roquefort is by description a goat's or sheep's milk cheese, made in France principally, though cheese of nearly the same quality is said to be made in other parts of Europe from mixed cow's and sheep's milk or from cow's milk alone.

The great popularity of Roquefort cheese makes information as to the biology of its ripening processes very desirable. To this end numerous specimens of Roquefort have been purchased and analyzed. The results of this work have been very much simpler than the studies of Camembert. The ordinary Roquefort cheese before it is sent to the market is carefully cleaned and covered with tin foil. Its surface would, therefore, tell very little. When cut it is seen to be traversed by channels or holes made by the prickle machine (Stechmaschine) and by cracks. Every air space is lined with green Penicillium, so that the cut surface is said to be marbled with green. The texture of the cheese is reasonably uniform, with every indication that ripening is simultaneous throughout the cheese or at least approximately so. Its texture is rather crumbling than waxy, with a tendency to dissolve readily in the mouth. The taste is a characteristic sharp flavor, in which a rather high salt content is noticeable. Its odor is strong, cheesy rather than offensive in any way, except as pronounced putrefactive odors are sometimes developed in the rind. Cultures from the surface often show various species of fungi. There is no regularity about the surface, however, while uniformity of texture and appearance is universal on the inside. Cultures from the interior show a remarkable uniformity. In many cheeses examined a pure culture of a single species of Penicillium has been found. The extremely rare appearance of any other mold in the cultures has been remarkab e. Similarly the bacterial content is usually limited to typical lactic forms. Sufficient analyses have been made to establish clearly that a first-class Roquefort cheese should conta n only lact c bacteria and the Roquefort Penicillium. This Penicillium is often referred to by writers as *P. glaucum* and regarded as the common green species, but as it has very characteristic morphological and physiological characters it seems best to designate it as the Roquefort Penicillium, even though it quite often occurs upon other substrata.

The cultures which have been conducted in connection with the study of Camembert cheese have shown that the Roquefort Penicillium is capable of digesting curd very completely. Here, as in Camembert cheese, chemical analyses have shown that the derivatives of casein become almost completely water soluble. Further pure-culture experiments upon sterile curd have shown that this mold during the process of digestion produces bitter flavors during the first few weeks, but that its continued action changes these to typical flavors of the Roquefort cheese. Here, then, we have a definite, positive result. It is thus shown that the Roquefort Penicillium, acting with the lactic bacteria, is capable of ripening Roquefort cheese without the introduction of other enzyme-producing or flavor-producing organisms. The investigations of the chemical nature of these changes have barely been touched upon at this time. In a recent experiment a cheese of the Roquefort type was made of cow's milk inoculated with the Roquefort Penicillium and kept in a room at a temperature of about 60° F. At the end of five weeks this cheese was found to have acquired both the texture and the flavor of genuine Roquefort. There seems to be no doubt that it will be possible to develop methods of making and ripening that will produce the Roquefort type of cheese successfully in the United States. Details of making and handling will then be offered.

CHEESES RELATED TO ROQUEFORT.

Single studies have been made from the Italian Gorgonzola, English Stilton, and Hungarian Brinse (Brindze or Brimse). Gorgonzola and Stilton are made from cow's milk. Brinse is described as made from sheep's milk, mixed sometimes with goat's milk. These three varieties of cheese are found marbled with green Penicillia in pure cultures, which are unquestionably one or more strains of the Roquefort Penicillium. In the Gorgonzola and Stilton cheeses examined lactic species were the only bacteria found. Comparison of the flavors in these cheeses shows that the differences lie in the qualities of the materials used in the making and the handling of the cheeses rather than in the qualities attributable to ripening organisms. It is peculiarly interesting to find the same species of mold in the interior of ripened cheese in four countries so widely separated, where no efforts at the use of pure cultures are known to be made. Experiments show that in every locality so far studied there are many green species of Penicillium. It is evident, then, that the food material or the conditions, or both, presented by these types of cheese must exert a selective influence upon the molds, which results in the dominance of the one species so universally found. This species has been introduced into experimental cheeses at this station.

AMERICAN BRIE AND ISIGNY.

Cheeses of the type referred to in our previous bulletin as the American Brie have been studied for comparison. This was a collective term suggested to cover cheese sold under various labels as Brie, Isigny, Wiener, Miniature, and others, designated commonly by the retailer simply as Brie. The name "Brie" seems to be applied in the French dairy literature to a cheese which differs from the Camembert in the process of making, but ripened by the same fungi and approximately in the same way as Camembert. The domestic product so far as examined is quite different, with the exception of the output of one factory, which is conducted by imported cheese makers. The cheese met in the eastern markets under these names shows no trace of the Camembert Penicillium. Numerous brands have been examined in the market and many hundreds of cheeses have been seen in the cellars of two of the largest cheese companies. *Oidium lactis* is universally present upon these cheeses, but its presence goes practically unnoticed by the makers, since it produces neither color nor aerial mycelium. All noticeable molds are washed or scraped from the surface of the cheese. The washing produces exactly the best conditions for the growth of bacteria and Oidium. This treatment results in a cheese without a very definite fungous rind and with a strong flavor and smell.

Cultures from this type of cheese indicate that there is an associative action between the *Oidium lactis* and various species of bacteria. Several species of Penicillium occur as contaminations in these cellars and sometimes are found upon the cheeses in the market. Every effort is made to eliminate mold action other than that of *Oidium lactis*, which usually passes unrecognized. Cheeses of this type usually bear rich growths of yeasts, giving a characteristic greasy feeling to the surface. Exactly what parts these various organisms play in the production of Brie is as yet undetermined.

Single studies have shown that *Oidium lactis* is the dominant mold upon the surface of some brands of Limburger, brick, and Port du Salut. There is, then, good reason to believe that this fungus is associated with nearly every type of highly flavored, ripened soft cheese met in the American market.

MOLDS REFERRED TO IN THIS PAPER.

The Camembert and Roquefort molds belong to the hyphomycete genus Penicillium, which has been characterized by one author—

Hyphæ broadly effused, creeping; conidiophore branched at the apex in an irregularly verticillate manner, producing brush or broom-like forms; conidia in chains, hyaline or bright colored, spherical or elliptical.

This genus of fungi contains a large number of very poorly described forms which are everywhere abundant as the "green" or "blue" mold of the household, the dairy, and the granary. They form patches upon and just under the surface of the materials upon which they grow. The patches are composed of delicate threads of mold, which are matted together, forming more or less cottony surfaces, never rising more than a small fraction of an inch above the substratum. At first these areas are always white, but in most species the ripening of a crop of spores is indicated by the change to a color which is usually some shade of green, though this may later give place to a brown. In a few species other colors appear. These spores (conidia), or propagating bodies, are minute thin-walled cells averaging possibly one five-thousandth of an inch in diameter, and so light that they float freely in the air. A breath upon the surface of such a colony carries away thousands of them, when if held in a proper position they may commonly be seen to rise in a cloud. If the colony be held to the nose and inhaled they give the sensation commonly called the "smell of mold." They are, then, exceedingly light; they are produced in immense numbers; they are capable of growing in almost every conceivable situation, upon anything which is not definitely and strongly poisonous. Some of these spores are short lived, others cling tenaciously to their power to germinate. Of the species, probably a dozen common ones may be expected in any locality, perhaps more. Our studies have shown that they affect very differently the substances upon which they grow. It is, then, clearly necessary that by thorough study of their characters and habits we know the forms we are to use, and just as important that we know how to get rid and stay rid, if it be possible, of those we do not want. The discussion of the whole group will be reserved for another paper. Here we may describe in simple terms the two cheese fungi we find important, but it may as well be acknowledged at the outset that, with the possible exception of the Camembert species, safe recognition of species without technical knowledge and cultural study is out of the question.

THE CAMEMBERT MOLD (PENICILLIUM CAMEMBERTI).

The spores of the Camembert mold grow rather slowly in comparison with the other molds of the group. They first swell to nearly double size, and then produce fine threads or hyphæ at from one to three points on their surface. Upon a cheese or in laboratory culture the subsequent growth of these threads forms a colony large enough to be visible to the naked eye, in ordinary room temperature, in about two days. Usually in four or five days the colony will have become loosely white, cottony, about one-half inch or less in diameter, and perhaps standing one-twentieth of an inch above the surrounding surface. At or about this stage the center of this colony begins to turn a shade of greenish gray, which is characteristic of this species, though one or two other forms produce colors closely resembling this shade, and difficult to distinguish from it except to one very familiar with the colors in question. This is due to the presence of ripe spores. Upon the cheese in the cellar this color often does not appear in less than a week or even ten days. Microscopic examination shows that the submerged threads of mycelium of such a colony do not go deeper into the solid media than one-sixteenth of an inch, and that the superficial portion of the mycelium spreads as fast, or nearly so, as the part beneath the surface of the substratum. This fungus grows and fruits for about two weeks—in some cases this may be prolonged to three weeks—and at the end of that period no further growth is to be expected from the primary colonies, nor, if the medium is undisturbed, is there a secondary growth from the germination of the spores produced by the first colony. In case the rind of the cheese is broken so that a fresh surface is presented, the spores will develop new colonies upon such areas. A colony, then, produces a single crop of spores and dies, under ordinary circumstances, and in undisturbed cultures there is usually no second growth from the spores or from the old mycelium, although the contrary has been claimed for this fungus by a recent writer (Mazé [9]). A cheese inoculated with this mold will become

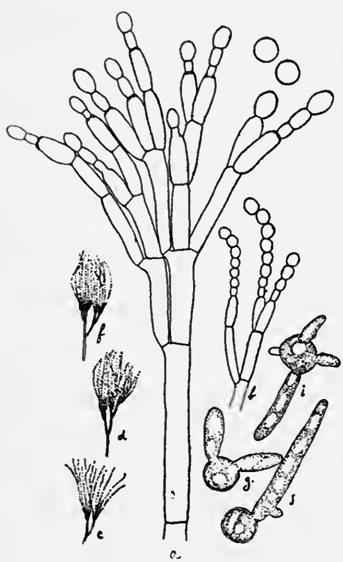

FIG. 1.—Camembert Penicillium (*P. camemberti*). *a*, conidiophore showing a common type of branching and the production of basidia and conidia, highly magnified; *b*, a common form showing much less branching; *c*, *d*, *f*, diagrams of large fructifications (× 80); *g*, *i*, *j*, germinating conidia.

covered with pure white cottony mycelium in about a week. The color will then begin to show the gray-green shade characteristic of the species, which spreads, until at the end of the second week the entire surface, if left undisturbed, will be colored.

Persistent search has failed to find a single colony in America whose presence can be attributed to anything but Camembert cheese imported from Europe. The mold may then be regarded as a typical dairy form which is not well adapted to cosmopolitan conditions and to the struggle for existence on all sorts of media. In fact, in the course of laboratory practice involving thousands of cultures, even in the laboratories of this station, this mold rarely appears as a contamination, although it has been cultivated in quantity and used in the inoculation of large numbers of cheeses in the same building with the bacteriological laboratory. Moreover, the spores are easily killed by heat and retain their vitality for only a few weeks in ordinary cultures allowed to dry in the air at room temperature.

TECHNICAL CHARACTERIZATION OF THE CAMEMBERT MOLD.[a]

The following technical characterization of *Penicillium camemberti* (fig. 1) may be offered, based upon studies made upon the sugar gelatin and potato agar described in this paper:

Colonies effused, white, slowly changing to gray-green (glaucous); surface of colony floccose, of loosely felted hyphæ about 5 μ in diameter; reverse of colony yellowish white; conidiophores 300 to 800 μ in length, 3 to 4 μ in diameter, septate, cells thin-walled, often collapsing in age, arising as branches of aerial hyphæ; fructification sometimes 175 μ in length, but usually much less, consisting commonly of one main branch and one lateral sparingly branched to produce rather few basidia, which bear long, loosely divergent chains of conidia. Basidia 8 to 11 by 2.4 to 3 μ; conidia at first cylindrical, then elliptical, and finally globose when ripe, smooth, bluish-green by transmitted light, thin-walled and commonly guttulate, 4.5 to 5.5 μ in diameter, swelling in germination to 8 to 10 μ. Germ-tubes one to several. Cells of mycelium about 5 by 20 to 40 μ; liquefies sugar gelatin only under the center of the colony. Changes blue litmus to red strongly at first, then after four to six days begins to turn the red back to blue at the center and continues outward concentrically until all has become blue. Growing and fruiting period about two weeks. Fruits only upon exposed surfaces of the substrata—never produces spores in cavities not very broadly open. Habitat, cheese.

[a] *Penicillium camemberti* (nomen novum). This species is unquestionably the one referred to by Mazé in his recent papers as *P. album* Epstein. Professor Mazé was kind enough to show me the cultures. But the name *P. album* was already used by Preuss some fifty years earlier for a species of Penicillium, hence by the rules of nomenclature should not be used again for a species whose identity with *P. album* Preuss is not claimed by Epstein. Upon this ground Lindau, in Rabenhorst's Kryptogamenflora, has changed the name of Epstein's fungus to *P. epsteini* Lindau, and extracted from the article written by Epstein a brief and totally insufficient diagnosis. A careful study of the physiological data given by Epstein shows that they differ from the data so far found for this species so materially as to lead to the probability that he was studying another form entirely. I therefore give *P. album* Epstein in the list of possible synonymy only, because the name is accepted by Mazé for what I know to be this species.

THE ROQUEFORT MOLD (PENICILLIUM ROQUEFORTI).

The spores of the Roquefort mold grow very rapidly, often producing new mycelium and ripe spores within thirty-six hours. The colonies are white at the very first, but begin to become green at the center within two days in a rapidly growing colony. Such a colony may become a half inch in diameter in the first two days. The mycelium is mostly submerged, but very close to the surface, and grows rapidly outward from the starting point in a radial manner, which is rendered prominent by certain of the threads lying just under the surface for the most part, but making loops into the air by rising just above the substratum for a little way, then reentering the medium again. This gives a grayish, almost cobwebby (arachnoid), appearance to the margin of the young colony. The rate of growth is not uniform in the circumference of such a colony, which makes the border of a colony uneven instead of regularly circular, as most species appear. The superficial portion of the Roquefort mold is almost entirely composed of the fruiting hyphæ or conidiophores, the vast majority of which arise as branches of submerged hyphæ and consequently stand separately as short, unbranched threads of approximately equal length, which gives the surface a velvety appearance. They are usually 0.2 or 0.3 mm. or less in length, say one seventy-fifth of an inch. Such a colony spreads indefinitely in the substratum, so that the center will be composed of ripe fruit, while the margin is still actively growing. In laboratory culture, however, the development is so rapid that the entire surface is covered within the first few days; then growth ceases. The mycelium here, as in the Camembert mold, produces but a single crop of spores, then dies. These spores are a bright green at first, but in a short time become a dirty-brown color in dry culture. The spores of this fungus are much more resistant than those of the Camembert mold both to heat and to natural exposures. They will retain their viability for months in old cultures under the ordinary conditions of exposure in the laboratory. Upon a cheese this mold produces a bright green area which extends rapidly. Its action can be detected in a few days by the bitter taste of the curd near to the mycelium. A similar taste is, however, produced at least in some measure by other green forms, so that it is not diagnostic except as between this and the Camembert species. A colony upon the surface of a cheese becomes brown in two or three weeks, but colonies growing in the cavities which are so characteristic of the center of this type of cheese retain their bright green color for long periods.

This mold is not limited to dairy products, but is widely distributed. It has been sent to the laboratory from the most distant correspondents. It has been found in silage, and in laboratory cultures from many substances. It has been found to be the green mold of Stilton, Gorgonzola, and Brinse, as well as in certain types of prepared cheese purchased in the market. Once in a laboratory it stays and seems to get into everything. In other words, this is one of the cosmopolitan and omnivorous species of the genus. One character seems to differentiate this mold from most of the others—that is, its power of growing into and fruiting normally within narrow cavities, such as appear in cheese. It appears that this character exerts a sort of automatic (perhaps we may call it a truly "natural") selection which eliminates all other species from the ripening processes of Roquefort and related types of cheese.

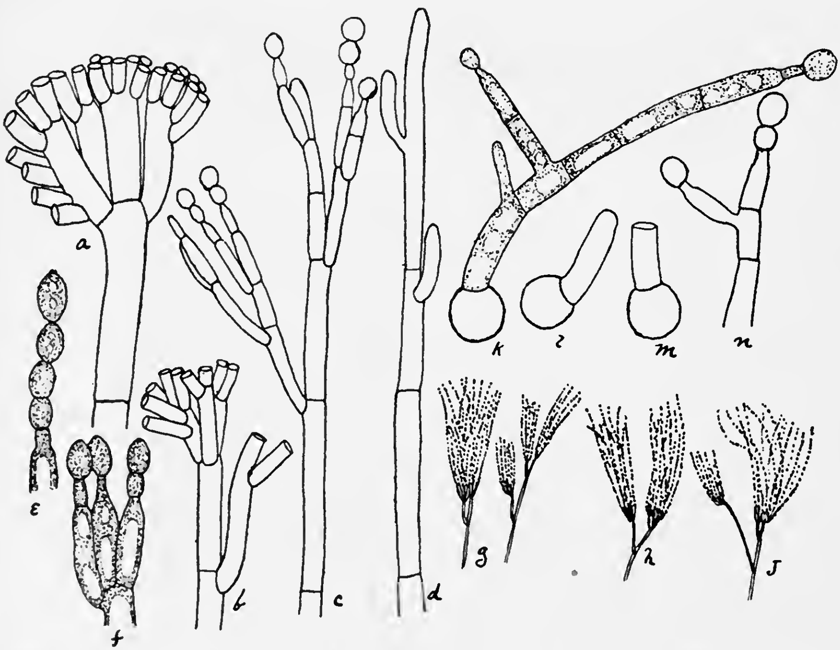

FIG. 2.—Roquefort Penicillium (*P. roqueforti*). *a*, part of conidiophore and of bas of fructification, highly magnified, showing the production of basidia on the sides as well as at the apex of the basidiophore; *b*, *c*, other types of branching; *d*, young conidiophore just branching; *e*, *f*, basidia and the formation of conidia, highly magnified; *g*, *h*, *j*, diagrams of types of fructification as seen under low power (× 80); *k*, *l*, *m*, *n*, germination of conidia and new conidia produced directly on the first hyphæ.

TECHNICAL CHARACTERIZATION OF THE ROQUEFORT MOLD.[a]

A technical characterization is offered of *Penicillium roqueforti* (fig. 2), as follows:

Colonies quickly turning green, becoming a dirty brown in age, velvety strict, indeterminately spreading by large main radiating, branching hyphæ, giving a somewhat uneven or

[a] *Penicillium roqueforti* (nomen novum). In offering a new specific name for this well-known fungus, the author is perfectly aware that the mold is often referred to in the literature as *P. glaucum*. A careful study of the literature fails to disclose a single description which indicates that this is identical with the plant described as *P. glaucum*. As a preliminary step, therefore, to the proper determination of the green species of Penicillium which have hitherto been collectively referred to as *P. glaucum*, this very distinct and easily recognized form is named from its universal occurrence *P. roqueforti*.

indefinite margin, which gets a white, fibrous, almost spider-web appearance from its alternation of submerged parts of hyphæ with short prostrate aerial loops; reverse of colony yellowish white. Conidiophores arising separately and in acropetal succession from the growing parts of submerged hyphæ (comparatively few from aerial parts, but some), 200 to 300 μ septate. Fructification 90 to 120 μ or at times 160 μ by 30 to 60 μ at broadest place, usually appearing double by the divergence of the lowest branch; branchlets (basidiophores) irregularly verticillate, bearing crowded verticils of appressed basidia 9 to 11 μ by 2.5 μ with long divergent chains of conidia. Conidia bluish green, cylindrical to globose, smooth, rather firm-walled, 4 to 5 μ in diameter, germinating by a straight tube. Colonies do not liquefy sugar gelatin, though they soften it somewhat. The fungus changes litmus from red to blue very rapidly and strongly, almost from the beginning of growth. Fruiting period short, but one crop of spores upon the mycelium. Cosmopolitan and omnivorous, or nearly so. Characteristic of Roquefort and related types of cheese.

OIDIUM LACTIS.

The mold (fig. 3) variously known as *Oidium*, or *Oöspera, lactis* is another cosmopolitan organism. This fungus differs widely from the species previously described. Inoculated into any suitable medium it grows with enormous rapidity. A single spore (or oidium) may give rise to several centimeters of mycelium and hundreds of spores in twenty-four hours. It prefers very moist situations, since almost the entire mycelium is developed below the surface of the substratum. It is therefore passed unnoticed many times or produces changes which are attributed by the observer to bacteria. Description, therefore, must depend upon microscopic characters. The study of the border of the young colony shows numerous vegetative hyphæ radiating outward. Each of these is found to divide dichotomously (fig. 3, *a*, *b*), so that the border is a crowded series of forking branches. In the older parts of the mycelium a branch may be produced at each end of every cell, or several at each end, and these branch indefinitely. The fruiting branches are mostly produced as outgrowths from the distal ends of the cells. These extend upward into the air or remain entirely submerged in many cases. From the ends of these outgrowths one to several rows of oblong or cylindrical cells begin to be pinched off. If extending above the surface this gives rise to chains of delicate shimmering cells appearing as a powdery covering upon the surface, which can be seen with a good lens to be arranged in chains. In some strains of Oidium all of these chains (and some of the chains in all strains) of spores remain submerged and germinate at once, so that they give rise to unintelligible mats of hyphæ. Oidium produces a very slight acid reaction to litmus at first, then a strong and continued alkaline reaction. It liquefies sugar gelatin under the colonies, but does not extend the area of liquefaction beyond the edge of the colony. Oidium always and everywhere tested has produced a strong and very characteristic odor. Once familiar with this odor the worker may recognize its presence by its spores or oidia, which are hyaline, smooth, cylindrical, 3.5 to 5 μ by 6 to 30 μ, varying with the conditions and the substratum and perhaps at times exceeding these limits. These swell variously and germinate in many ways, so that no germination characters are definite. Upon some media this mold may be induced to produce a large growth of aerial mycelium, but the limits here defined will include the variations to be found upon the usual culture media.

Oidium lactis is described as universally present on milk and its products. Epstein even suggests that experiments upon milk and cheese can not be freed from its presence without sterilizing. The

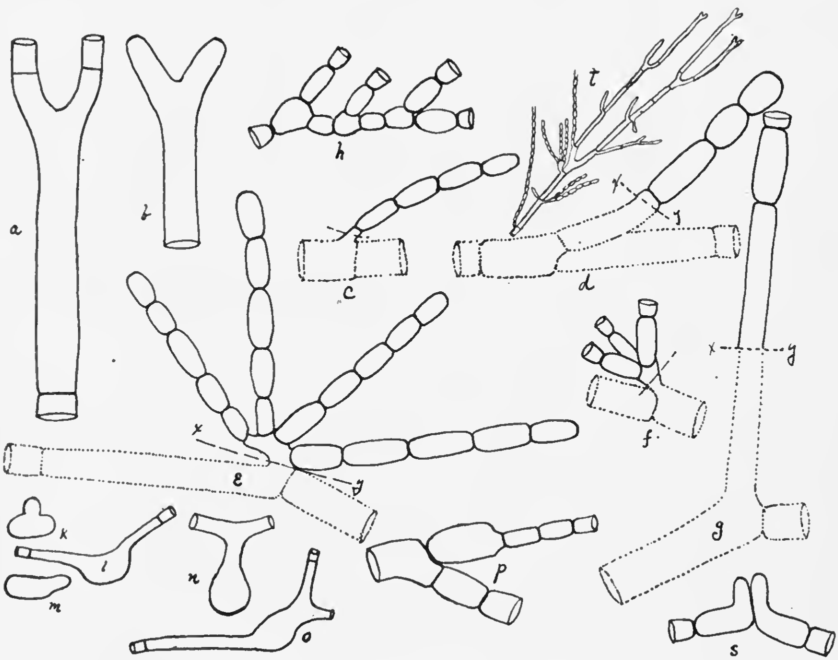

FIG. 3.—*Oidium lactis*. *a, b*, dichotomous branching of growing hyphæ; *c, d, g*, simple chains of oidia breaking through substratum at dotted line *x–y*, dotted portions submerged; *e, f*, chains of oidia from a branching outgrowth of a submerged cell; *h*, branching chain of oidia; *k, l, m, n, o, p, s*, types of germination of oidia under varying conditions; *t*, diagram of a portion of a colony showing habit of *Oidium lactis* as seen in culture media.

same or almost indistinguishable forms are found upon decaying vegetables and fruits, which may give reason for the statement that the odor produced by Oidium is that of rotten cabbage. There seems to be good reason for saying that all these forms are but varieties or strains of the same species. Comparison of several of them shows that under uniform conditions the morphology of all these forms is very nearly the same. This is largely true also of their physiological effects. This mold has been much studied and numerous papers discuss its nature and physiological effects as well as its relationships.

It will be sufficient to describe here the fungus and to give figures to assist in its recognition. Its relations to the problems of cheese ripening have already been indicated.

SUMMARY.

CAMEMBERT CHEESE.

The acidity of the curd resulting from the action of lactic organisms reduces where it does not entirely eliminate the growth of objectionable bacteria.

Many species of dairy fungi exert in the course of their development the power of changing this reaction to alkaline. The Camembert Penicillium and *Oidium lactis* possess this power, but not in greater degree than many other species.

Many species of fungi possess the ability to change curd to a greater or less extent.

The breaking down of curd by fungi is due in the cases studied to the production of enzymes.

The texture, appearance, and flavor of curd acted upon by such fungi are different for different species.

The Camembert Penicillium (*P. camemberti*) is the only species so far studied with which the particular appearance and texture sought in the ripened Camembert can be produced from curd soured by lactic bacteria without producing any objectionable flavor.

Oidium lactis is always found upon Camembert cheese and so closely associated with the presence of the flavor as to indicate its agency in flavor production, though only circumstantial proof of such function has been possible thus far. The participation of bacteria in flavor production is not excluded by these results.

Other species of fungi have been shown to produce variations in this flavor such as have been often found in certain market cheeses. In this way it is possible to look for the cause of differences in flavor in contamination of the cultures upon the cheeses. This points toward the use of pure cultures for inoculation, with the addition of special organisms if certain variations from what we have regarded as typical flavor are found to be of value in the market rather than dependence upon accidental occurrence of the desired species in the factory.

ROQUEFORT CHEESE.

In the ripening of Roquefort cheese the only organisms found necessary are lactic bacteria and the Roquefort species of Penicillium.

The Roquefort Penicillium has been shown to possess the power to reduce the acidity, to digest the curd, and to produce the typical flavor.

OTHER VARIETIES OF CHEESE.

The Roquefort species of Penicillium is found in the imported Stilton, Gorgonzola, and Brinse, as well as in Roquefort cheese.

Oidium lactis alone of the forms studied has been found upon the various brands of Limburger, Brie (American type), Isigny, and related cheeses found in the market. Other species incidentally occur, but not uniformly, and such occurrence is avoided as far as possible by the makers.

BIBLIOGRAPHY.

(1) Conn, Herbert William; Thom, Charles; Bosworth, A. W.; Stocking, W. A., Jr., and Issajeff, T. W. The Camembert type of soft cheese in the United States. Bull. No. 71, U. S. Department of Agriculture, Bureau of Animal Industry. Washington, 1905. Also published as Bull. No. 35 of the Storrs Agricultural Experiment Station, Storrs, Conn., Apr., 1905.

(2) Conn, Herbert William. Bacteria in milk and its products. Illus. 306. pp. Philadelphia, Blakiston's Sons & Co., 1903. See p. 268.

(3) Epstein, Stanislaus. Untersuchungen über die Reifung von Weichkäsen. Arch. f. Hyg., Bd. 43, Hft. 1, pp. 1–20; Bd. 45, Hft. 4, pp. 354–376. Munich and Leipzig, 1902.

(4) Johan-Olsen, Olav. Die bei der Käsereifung wirksamen Pilze. Cent. f. Bakt., Abt. 2. Bd. 4, No. 5, pp. 162–169. Jena, March 5, 1898.

(5) Constantin, J., and Ray, J. Sur les champignons du fromage de Brie. Compt. rend. Soc. de biol., Paris, sér. 10, t. 5, No. 16, pp. 504–507. Paris, May 13, 1898.

(6) Roger, Georges. [Article in] Revue hebdomadaire, v. 7, p. 334. Paris.

(7) Margaret, pseudonym. The practice of cheesemaking at home and abroad. The Creamery Journal, v. 1, No. 11, pp. 313–315. London, July 20, 1905.

(8) Epstein, Stanislaus. See Citation 3, above, p. 373.

(9) Mazé, P. Les microbes dans l'industrie fromagère. Ann. de l'Inst. Past., ann. 19, No. 6, pp. 378–403, June 25; No. 8, pp. 481–493, August 25. Paris, 1905.

(10) Smith, Erwin F., and Swingle, Dean B. The dry rot of potatoes, due to *Fusarium oxysporum*. Bull. No. 55, U. S. Department of Agriculture, Bureau of Plant Industry. Washington, February 16, 1904.

Lang, M., and Freudenreich, Eduard von. Über *Oidium lactis*. Landwirthschaftl. Jahrbuch der Schweiz, Bd. 7, pp. 229–237. Bern, 1893.

Marpmann, G. Beiträge zur Käseflora. Ztschr. f. angewandte Mikroskopie, Bd. 2, Hft. 3, pp. 68–79. Berlin, June, 1896.

Teichert, Kurt. Beiträge zur Biologie einiger in Molkereiproduction vorkommenden Schimmelpilzen. Milch-Zeitung, v. 32, No. 50, pp. 786–787. Bremen, December 12, 1903.

Thom, Charles. Some suggestions from the study of dairy fungi. Jrn. of Mycology, v. 2, No. 77, pp. 117–124. Columbus, Ohio, May, 1905.

O

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Albuquerque, N. Mex.—Dr. Louis Metsker, room 22 N. T. Armijo Building.
Denver, Colo.—Dr. Lowell Clarke, room 320 Quincy Building.
Fargo, N. Dak.—Dr. R. H. Treacy.
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Philadelphia, Pa.—Dr. C. A. Schaufler, 134 South Second street.
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Orono, Me.—Dr. F. L. Russell.
Port Huron, Mich.—Dr. David Cumming, 912 Lapeer avenue.
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San Antonio, Tex.—Dr. Joseph W. Parker.
San Diego, Cal.—Dr. Robert Darling, care Charles S. Hardy.

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