Issued September 7, 1912.

U. S. DEPARTMENT OF AGRICULTURE,

BUREAU OF ANIMAL INDUSTRY.—BULLETIN 151.

A. D. MELVIN, CHIEF OF BUREAU.

STUDY OF THE GASES OF EMMENTAL CHEESE.

BY

WILLIAM MANSFIELD CLARK, PH. D.,

Chemist, Dairy Division.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Animal Industry,
Washington, D. C., April 23, 1912.

Sir: I have the honor to transmit, and to recommend for publication in the bulletin series of the bureau, the accompanying manuscript entitled "A Study of the Gases of Emmental Cheese," by Dr. William Mansfield Clark, chemist in the Dairy Division.

The so-called "eyes" in Swiss cheese are, as is well known, its most prominent characteristic, and its commercial value is largely dependent upon the proper size and spacing of these eyes. Furthermore, much depreciation in the value of this popular variety of cheese, in both the domestic and foreign kinds, is known to exist because of defects in eye formation. The experimental work herein described concerns the chemical contents of these eyes, and although considerable work has been done in Europe with the object of discovering the cause of eye formation, there has hitherto been no investigation made of the gases which are immediately concerned in the process. Dr. Clark's studies are therefore calculated to be of value to the scientific as well as the practical side of the industry.

Respectfully,

A. D. Melvin,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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INTRODUCTION.

The "eyes" of Swiss or Emmental cheese are its most striking characteristic. Their formation is a fascinating subject to the biological chemist, because of a supposed localization of reactions generating considerable quantities of gas, and because of the production of a plasticity among the colloids of the cheese, which makes possible the peculiar mold of the cavities.

To the cheese maker the formation of the "eyes" is a matter of great importance, since their size and proper spacing determine in large measure the commercial value of the cheese. In certain districts of Wisconsin visited by the writer the dealers rely almost entirely upon these features, and, shortly after the eyes have reached their proper development, relieve the maker of further care. The American makers of Swiss cheese are, therefore, unable to attend to their cheeses in that mellow old age upon which so much of the fine flavor of a true Emmental cheese depends. However much this quick marketing is to be deprecated, the fact remains that it raises the relative importance of the eye formation and adds significance to whatever knowledge can be gained concerning the process.

Some years ago Bächler,[1] [a] cited by Jensen,[11] estimated that 25 per cent of the cheeses made in Switzerland were considerably reduced in value because of imperfect eye formation. How far this enormous loss has been lessened in recent years as a result of scientifically controlled manufacture can not be said, but in this country, where large numbers of Swiss are still using the antiquated methods of their forefathers, Bächler's estimate is probably not too high. The wide difference in market price between domestic and imported Swiss cheese bears out this statement.

Considerable work has been done in Europe in the effort to uncover the cause of eye formation, and, through the labors particularly of

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[a] The reference figures relate to the list of references to literature at end of bulletin.

Von Freudenreich and Jensen, a well-founded theory has been proposed which will be discussed later. No one, however, has made a study of the gases which are themselves the immediate cause of the eye formation, and it was with the hope that such a study might furnish valuable data that the research herein described was undertaken. If nothing more is demonstrated than the composition of the gas in

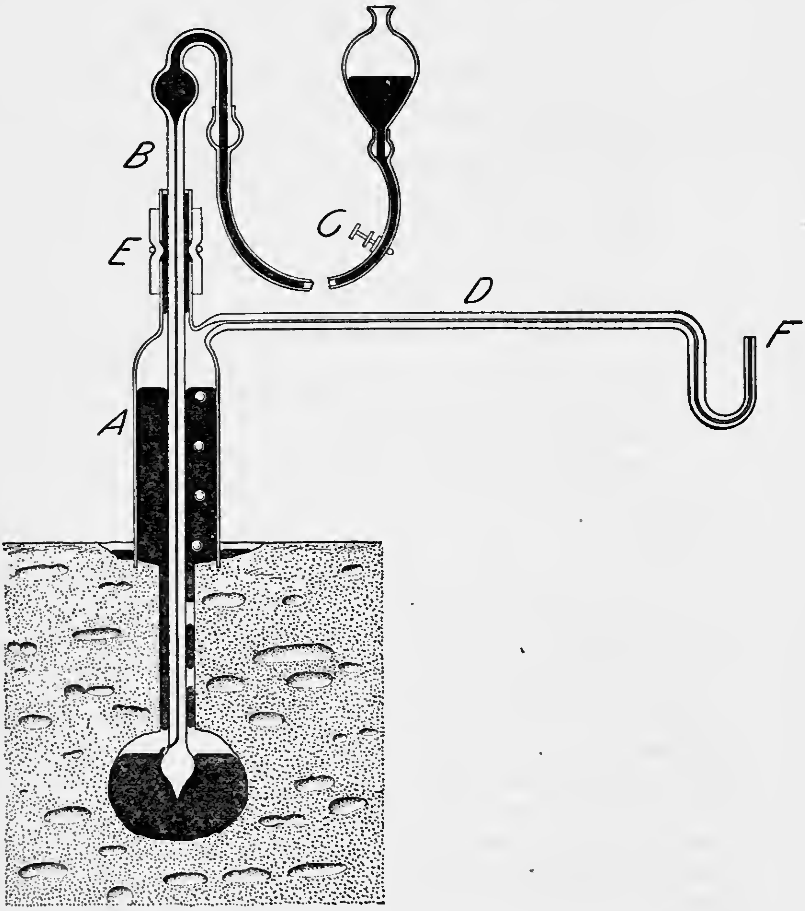

Fig. 1.—Apparatus for collecting gas from the eyes of Swiss or Emmental cheese.

the eyes, this alone justifies the work, for the extensive researches on the eye formation in Emmental cheese have led to but one conclusion that can be called positive, and that is that a final explanation will be reached only when every phase of the subject has been submitted to exact quantitative study.

DESCRIPTION OF APPARATUS AND METHODS OF COLLECTING THE GASES.

The collection of the gas in the eyes by cutting the cheese under a bell jar filled with water, as was done with Edam cheese by Boekhout and Ott de Vries,[2] is a simple and valuable method, but one which is hardly to be called accurate, owing to the high solubility of certain gases in water. In place of such a method an apparatus was devised for collecting the gas over mercury. This is shown in figure 1, the procedure being as follows:

METHOD I.

The glass cylinder A is forced a short distance into the body of the cheese until it is firmly held. It is then clamped in position. Around the outside the cheese is cut away sufficiently to leave a channel into which mercury moistened with mercuric chlorid solution is poured. This forms a seal preventing entrance of air. The head of the shaft B is now resting on the surface of the cheese. Through its capillary mercury is run into the cylinder, displacing the air until it finally runs out of the side arm D and up through the annular space between the shaft and the shoulder of the cylinder. The short length of thick rubber tubing at E is then very tightly bound with a rubber band, leaving mercury in the small cup above, and thus effectually closing this opening against the entrance of air. When the cylinder and side arm are thus completely filled with mercury, a receptacle filled with mercury is brought over the end of the side arm (in a mercury trough, of course) and serves to retain the collected gas until the time of the analysis. After these preparations the shaft is pushed down into the cheese. When it punctures an eye this can readily be felt. Since the head of the shaft is larger than the shank, there is left an annular space for the escape of the gas. This gas is displaced from the eye partially by the mercury of the cylinder, which finds its way to the lower level, but more largely by the mercury which runs in through the capillary in the shaft. The exit of this is prevented from becoming clogged with cheese by carefully blowing it out just behind the head, as shown in the diagram. When the gas is displaced from the eye it is displaced from the cylinder into the receiver by continuing to run in mercury through the shaft from the reservoir C. Between this reservoir and the shaft is placed a bulb which prevents the mercury from sweeping in bubbles of air.

In the samples of gas collected with this apparatus seldom was more than a trace of oxygen found. This in itself shows that the gas was obtained without contamination by air.

METHOD II.

For the collection of gas from "pinholes" the foregoing apparatus was of little use except in one instance to be mentioned later. To collect the gas from this form of hole, as well as the gas in the body of the cheese, the apparatus shown in figure 2 was used, as follows:

Samples of cheese taken with a trier were introduced into the glass cylinder A. The rubber stopper at B, attached to the mercury vacuum pump with or without the intermediate connection C, was forced in securely and protected from leakage by the mercury seal. Upon raising the leveling bulb D the cheese was flooded with mercury and the surrounding air was forced over into the pump until the mercury stood at the stopcock E. To prevent bubbles of air being trapped under the cheese the lower ends of the plugs were sharply beveled. Bubbles of air of course adhered to the rough surface of the cheese and its smaller exposed cavities. This error is inherent in the method, but was reduced by suddenly dropping the leveling bulb with the stopcock E closed, and then driving the air, which had expanded into the vacuum, past the open stopcock.

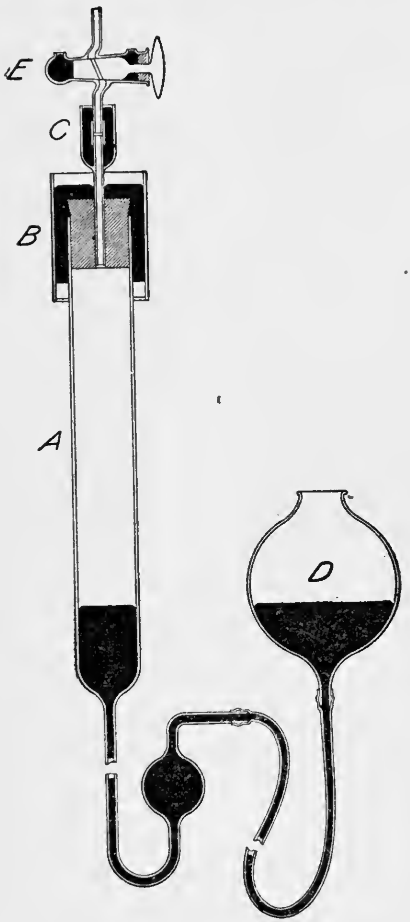

FIG. 2.—Apparatus for pumping gas from cheese.

The glass tube with its trap which connects A with the leveling bulb was made sufficiently long so that D might be lowered the barometric distance below A, and thus leave the cheese exposed to a fairly high vacuum even before the pumping commenced. After exhausting the pump up to E this cock was opened, and the gas pumped from the cheese and delivered into a receiver.

The mercury pump used in this as in other operations to be described later was Antropoff's modification of the Topler. A full description of the pump and its appurtenances will appear in the account of another investigation.

METHOD OF ANALYSIS.

The gas was analyzed with a special set of burettes and pipettes designed for the analysis of small quantities of gas produced by bacteria. A few of the first analyses were made with a burette specially designed for volumes as low as 0.5 c. c. In all the analyses the confining liquid was mercury, and use was made of a device for extremely accurate separation of gas from absorbent.

Thirty-three per cent potassium hydroxid solution, in quantities appropriate for the volume of gas analyzed, served as absorbent for carbon dioxid. Hydrogen sulphid, after preliminary qualitative tests, was assumed to be absent, although it is of course possible that, if present originally in the gas, it may have been taken up by the mercury. That any of this gas occurs in the eyes is, however, very improbable, for its odor was never detected. For hydrogen sulphid and mercaptans the nose is many times more sensitive than is the spectroscope for sodium,[6] and unless the other and milder odors of Swiss cheese exercise a surprisingly intense hindrance to the detection of hydrogen sulphid and mercaptans we may justly say that these vapors were absent. With Nessler's reagent very slight traces of ammonia were detected. For oxygen alkaline pyrogallol or long-continued contact with phosphorus was used. Combustible gases were estimated in several ways. Explosion with oxygen, in the presence of electrolytic gas when necessary, was used in several instances. For one case combustion with a platinum sponge was tried. For the small percentages of combustible gases found the method of Dennis and Hopkins [5] was found to be the most satisfactory. This consists, essentially, in leading the gas slowly into a measured volume of oxygen and there burning it slowly and quietly with a platinum wire heated by an electric current.

TABLE 1.—*Analyses of gas collected by puncturing apparatus from eyes of Swiss (Emmental) cheese—Method I.*

Designation of cheese.	Total volume of gas collected.	Contraction— Due to absorption with KOH.	Due to absorption for O_2.	Due to combustion.	Composition. CO_2.	O_2.	Hydrocarbons.	H_2.	N_2.	Description of cheese.
	C. c.	*C. c.*	*C. c.*	*C. c.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	
a	0.96	0.55	......	......	57.3	......	......	......	......	Imported, eyes normal.
b	2.73	2.29	0.00	......	83.9	0.0	......	......	......	Do.
c	1.66	1.11	.01	0.10	66.9	Trace.	(?)	4.00	29.1	Imported, eyes (?).
d	4.77	2.44	.02	.02	51.2	Trace.	0.0	Trace?	48.8	Imported, eyes normal.
e	1.25	1.00	.00	.00	80.0	0.0	0.0	0.0	20.0	Do.
f	3.44	2.23	.00	.60	64.8	0.0	0.0	11.6	23.6	Domestic, eyes thickly
	4.02	2.52	......	.45	62.7	......	0.0	7.5	29.8	crowded.
g	15.24	13.77	.00	.76	90.4	0.0	Trace?	3.3	6.3	Do.
h	7.56	6.14	.02	.43	81.2	Trace.	0.0	3.7	15.1	Imported, eyes thickly
	4.99	4.04	.03	.30	80.9	0.6	0.0	4.0	14.5	crowded.
i	14.47	12.91	.03	.00	89.2	0.2	0.0	0.0	10.6	Excellent imported, eyes
	9.99	8.95	.01	.09	89.5	Trace.	0.0	0.6	9.8	very regular.
j	5.42	3.04	.02	.06	56.0	Trace.	0.0?	Trace?	44.0	Imported, large hole.
39.61	4.96	2.35	.01	3.63	47.4	Trace.	0.0	48.8	3.8	Very gassy in press.

The analyses of the gas collected by Method I are given in Table 1, and of that collected by Method II in Table 2. All volumes are for 0° C. and 760 mm. When the gases were collected from a cheese procured at the market, a sufficiently large slice was purchased to prevent undue exposure of the eyes, and this was carried immediately the short distance to the laboratory, and the gas at once collected. In most cases the shaft punctured or grazed more than one eye, so that the analysis gives the true average for several eyes.

TABLE 2.—*Analyses of gas collected by pumping from Swiss (Emmental) cheese—Method II.*

No. of cheese.	Time pumping.	Total gas collected.	Weight of cheese evacuated.	Amount of gas per 100 grams of cheese.	Composition.				Description of cheese.
					CO_2.	O_2.	H_2.	N_2.	
	Hours.	*C. c.*	*Grams.*	*C. c.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	
3	20	2.36	....	....	76.3	1.7	0.0	22.0	Almost blind. Several small holes, either pinholes or inhibited eyes.
39–45	....	2.31	....	....	77.5	2.6	0.0	19.9	Do.
39-11-2	20	6.41	....	....	80.8	2.0	0.0	17.2	Do.
46-4-1	....	3.20	50	6.40	50.6	1.0	0.0	48.4	Do.
W2	20	13.60	53	25.7	84.5	2.2	0.0	13.3	Fine domestic cheese just beginning eye development.

DISCUSSION OF THE ANALYSES.

If the values obtained in this study of the gases found in the eyes of Swiss cheese are compared with the values obtained by Boekhout and Ott de Vries [2] for the gases in Edam cheese, it is seen that the latter obtained much lower percentages of carbon dioxid and correspondingly higher percentages of nitrogen. The explanation becomes apparent when it is remembered that Boekhout and Ott de Vries collected the gas over water, while in this investigation it was collected over mercury. The two methods were compared in the case of cheese h, as follows:

Method.	CO_2.	O_2.	H_2.	N_2.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Collection over mercury	81.2	Trace.	3.7	15.1
Collection over mercury	80.9	0.6	4.0	14.5
Collection over water	34.8	1.9	1.9	61.4

This result is what might have been expected, namely, an absorption of much carbon dioxid and a little hydrogen by the water, and, in return, an increase in the amount of oxygen as well as an increase in percentage of nitrogen. Boekhout and Ott de Vries have themselves called attention to this, and claim only qualitative value for their results. The types of holes from which they isolated gas were small cracks corresponding to the Emmental "riszler," small round holes, and large cracks termed "knijpers."

Qualitatively the composition of the gases was the same, namely, carbon dioxid, hydrogen, nitrogen, and oxygen. Of these they eliminated oxygen as due to contamination. In the case of the "knijpers," or large cracks, 52 to 249 c. c. of gas were collected instead of 5 to 22 c. c. as in the case of the smaller holes. Assuming that the same volume of water was used, we would expect a truer value to be obtained for the analysis of the larger volumes, in which case the attention is struck by the large percentage of hydrogen. The significance of this will become apparent when the results on Emmental cheese have been assembled.

It is clear from the analyses of gas found in Emmental cheese that carbon dioxid and nitrogen are the chief constituents of the gas found in normal eyes. The oxygen in most cases is hardly more than would be expected to come from the minute bubbles or surface layers which adhere to the glass walls of the apparatus. To what gas the contraction after explosion with oxygen is to be ascribed is a difficult question to settle. In some cases, where the contraction was sufficiently large to justify further absorption with potassium hydroxid, the absence of any further contraction in volume justifies the conclusion that the combustible gas was chiefly hydrogen. In other cases the small contraction might have been due to any one of a number of gaseous combustions.

For further information it was decided to examine specimens of gas spectroscopically. The gas freed from carbon dioxid and possible oxygen was passed over phosphorus pentoxid into a dry, exhausted Plücker tube. The discharge of an induction coil was then passed between aluminum terminals, and the spectrum observed with a prism spectroscope. At the same time comparison was made with the spectrum of a similar tube containing pure hydrogen. Minute traces of hydrogen are to be expected when metal terminals are used, but, with the low resolving power of the spectroscope employed, the nitrogen spectrum so obscured the possibly present red line of hydrogen that it was not observed with specimens of pure nitrogen. A known sample of nitrogen containing about 0.05 per cent of hydrogen gave a brilliant hydrogen spectrum, whose intensity could be made more sharp at the expense of the nitrogen spectrum by suitable varying of the pressure.[15] The recognition of 0.05 per cent of hydrogen was therefore assured.

A small experimental cheese, which had begun an apparently normal eye formation and then ceased entirely, was pumped out by Method II and its gas submitted to spectroscopic examination. Slight evidences of hydrogen were observed. Samples of gas taken from cheeses which yielded 3 per cent of combustible gas gave very brilliant evidences of hydrogen.

In samples of gas taken from the normal eyes of two cheeses purchased on the market no hydrogen line was observed, nor was the hydrogen spectrum observed in the gases of a normal cheese evolved during the period of its maximum eye formation.

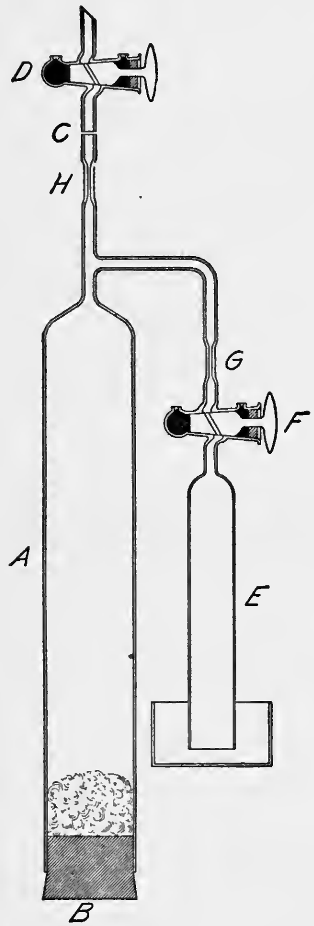

FIG. 3.—Apparatus for studying the absorption of oxygen by cheese.

These results, though not extensive, are sufficient to show that hydrogen plays no rôle in the formation of normal eyes, provided we assume that any hydrogen formed has not escaped collection by rapidly diffusing through the cheese. To make sure of this point the following experiments were conducted:

Two cheeses purchased in Wisconsin were found to be developing normal eyes. These eyes, though too thickly scattered for the modern market standard, would have been declared typical some years ago. When each cheese was apparently at the height of its eye formation, plugs were taken, and introduced into the tube A, figure 3, without that part illustrated at the side and lettered G, F, and E. To guard as far as possible against infection in transference the trier was flamed, and the tube was sterilized at 170° C., with cotton plugs at B and C. After introducing the plugs of cheese they were followed by the flamed cotton plug and then a rubber stopper dipped in hot rubber cement. The stopper was forced in and held in place till the cement[a] had cooled, when several layers of the same cement were added to the exterior. This made a thoroughly gas-tight seal. The capillary end was now attached to the mercury pump by means of securely tied rubber tubing completely covered with a mercury seal. Then the tube was exhausted.

Forty-six grams from one of the Wisconsin cheeses were exhausted for two hours, during which time it continued to give off small quantities of gas. The pressure was finally reduced to 2 mm. (measured on a McLeod gauge). The stopcock D was then closed, and the

[a] The cement was made by heating rosin several days with as much fine-grade rubber as it would dissolve. Dr. Nutting, of the Bureau of Standards, who kindly furnished the receipt, stated that he had used this cement in refined vacuum work with entire satisfaction.

tube allowed to remain in connection with the pump overnight. The next morning the pumping was resumed, and a pressure of 2 mm. again obtained. The gas which had collected overnight amounted to 7.23 c. c., N. T. P. Its analysis follows:

	C. c.
Original volume	7.23
Residue after absorption with KOH	.17
CO_2	7.05
Oxygen added up to	[a] 2.28
Volume after combustion with heated platinum spiral	2.26
Contraction	.02

The tube was then sealed off in a blowpipe at the constriction H and kept for six days at 25° C. To collect the gas from this sealed tube the following method was used. The capillary tip of the seal was scratched with a diamond, and then pushed up into the tube leading from the pump as at C, figure 3. Connection was made with a rubber tube securely tied and covered with a mercury seal. Having exhausted the pump up to the tip of the seal, the tube was turned slightly and sharply. The tip was broken at the scratch, and communication established between A and the pump.

The gas thus collected at the end of six days amounted to 10.12 c. c. The tube was allowed to stand connected with the pump overnight, after which an additional 2.75 c. c. of gas were collected.

These two volumes were united and analyzed 99.3 per cent carbon dioxid. The residue was hardly sufficient to justify further analysis. It was made, however, and a minute contraction observed, which was hardly more than the experimental errors of transference.

Forty-five grams of the second Wisconsin cheese submitted to the same procedure as described above gave the following data:

	C. c.
Gas collected on first standing overnight	11.48
Residue after absorption with KOH	.22
CO_2	11.26
Residue after absorption with phosphorus	.22
Oxygen added up to	[a] 2.41
Volume after combustion with heated platinum spiral	2.39

Tube sealed off and incubated six days at 25° C.

	C. c.
Gas collected after 6 days	9.29
Residue after absorption with KOH	.16
CO_2	9.13
Oxygen added up to	[a] 4.91
Volume after combustion with hot platinum spiral	4.85
Gas collected after again standing overnight	5.33
Residue after absorption with KOH	Trace.

[a] This comparatively large volume was made necessary because of the disadvantageous form of the Dennis-Hopkins pipette used.

In the above analyses the contraction due to combustion was so small that further analyses to determine the products of combustion were impracticable. Nor was it necessary, for, even if the contraction were due to but one gas, for example hydrogen, the amount was such that this gas may be said to be without significance in the formation of eyes. Doubtless the contraction was in reality due to volatile organic bodies. The above experiments show that when all the gas from an actively gas-producing region is collected no significant amount of hydrogen is found, and thereby the contention is refuted that, in the analysis of gas in the eyes, hydrogen escaped detection because of its rapid diffusion out through the cheese.

Pains were taken in these studies to make a strenuous hunt for hydrogen for the following reason: In Emmental cheese there is what Duclaux has termed the "initial fermentation" during which the sugar inclosed in the curd undergoes bacterial decomposition. Several of the earlier workers on this cheese thought it was the gaseous fermentation of this sugar which caused the development of eyes. If so, one would expect to find the gas composed of a large percentage of hydrogen, since hydrogen is a characteristic product in the fermentation of sugars by bacteria. This deduction is of course not rigid, but, from our present knowledge of the gaseous fermentation of sugars by bacteria, it is highly probable.

Jensen [11] in 1898 pointed out clearly that the gaseous fermentation of sugar must not be looked upon as in any way directly connected with the production of normal eyes in Emmental cheese. He found no trace of sugar in a cheese five days old, although the normal eye formation had not yet begun. This confirms the analyses made by various authors. Jensen cited Klenze [13] as stating that the sugar disappears in 48 hours. But, while the sugar disappears rapidly, normal eyes seldom begin to develop before the eighth day, and reach the height of their development long after every trace of sugar has disappeared.

These facts alone demonstrate that the eye formation does not depend upon the presence of sugar. Additional reason for so believing is found in the results herein, in so far as the absence of hydrogen in the gas indicates an absence of gaseous sugar fermentation.

But it also follows from this reasoning that when a gaseous fermentation occurs while sugar is still present in the cheese, hydrogen is to be expected. Such a fermentation frequently occurs while the cheese is in press. Fortunately a cheese was obtained (No. 39–61) which was known to have given marked signs of gas while under press. From this cheese gas was collected by the previously described Method I, with the following analysis:

Total volume of gas collected	Cubic centimeters	4.96
Residue after absorption with KOH	do	2.61
CO_2	do	2.35
Residue after absorption with phosphorus	do	2.60

Oxygen added up to	Cubic centimeters..	6.14
Volume after combustion with platinum spiral	do....	2.51
Contraction	do....	3.63
Residue after absorption with KOH	do....	2.51
Hydrogen	per cent..	48.80

Upon attempting to make a second puncture the mercury broke through into the hole previously made. The cheese was then opened, and found to be so spongy that the walls separating the individual cells were very thin—too thin to withstand the weight of mercury.

To obtain a second sample of gas for confirmatory analysis recourse was had to Method II of collecting gas, previously described. A high percentage of hydrogen was again found.

In the further study of this case 52 grams of the cheese were introduced into the vacuum tube described on page 14 and evacuated to 1 mm. pressure. There collected overnight 7.84 c. c. of gas.

Analysis:

	C. c.
Total volume	7.84
Residue after absorption with KOH	.28
Residue after absorption with phosphorus	.27
Oxygen added up to	1.08
Volume after combustion with platinum spiral	.99
Contraction	.09

The tube was then sealed off and kept nine days at 25° C. Upon opening it and pumping out the gas by the method previously described 7.49 c. c. of gas were collected. The residue after absorption with potassium hydroxid was only 0.07 c. c.

It is therefore apparent that the production of hydrogen, which was very active while the cheese was in press, had soon ceased, presumably with the disappearance of the sugar.

The occasional occurrence of hydrogen in small percentages, as shown in the table, generally accompanied eyes which in the writer's judgment were not typically normal. They were either crowded and distorted or associated with numerous pinholes. It is not, perhaps, incorrect to say that in all probability there had occurred in these cases a slight initial gaseous fermentation of the sugar, with the production of hydrogen which lingered to contaminate the gas of the normal fermentation.

An extremely interesting observation was made in the case of cheese i. (See Table 1, p. 11.) This was an excellent imported cheese with large and perfectly rounded eyes, well spaced in a body of fine texture and flavor. In the first analysis of the gas from these eyes no trace of a combustible gas was found. The second analysis gave 0.6 per cent of hydrogen. Upon exposing the eyes punctured it was observed that a slight crack extended to within a centimeter of one of the eyes punctured on the second collection. This crack was found to lead directly to a hole some 2 cm. in diameter, the irregular and apparently corroded walls of which proclaimed it distinctly abnormal.

It is of interest to note that in the case of cheese j, gas was obtained from a hole the size of one's fist, and that this contained practically no hydrogen. The appearance of this hole was that of a strictly normal eye except in size.

It was hoped that the gas of a typical "blow hole" could be obtained. For this purpose a cheese containing such a hole was purchased in Wisconsin. When it arrived at the laboratory it was found that the cheesemaker had punctured it.

From the results obtained it is clear that there are at least two distinct types of gas formation.[a] The one is highly detrimental, and is accompanied with hydrogen; the other is that demanded in a good Emmental cheese. One is dependent upon the presence of sugar; the other occurs in the absence of sugar.

The presence of hydrogen in considerable quantities in the gas isolated from Edam cheese by Boekhout and Ott de Vries is very suggestive of a gaseous fermentation of sugar, and to this Jensen [11] has ascribed the formation of gas holes in Edam cheese.

At this point it may be well to call attention to a source of error overlooked by various investigators in their attempts to establish the cause of any particular gas formation in cheese. Frequent examples are to be found in which gas production by bacteria in milk is interpreted to mean that these bacteria can produce gas in cheese. Although this may frequently be true, it must nevertheless be remembered that the two media differ not only in chemical constitution but also vary greatly in physical chemical condition.

Baumann,[3] for instance, attributed the formation of eyes in hard cheeses to *Bacillus diatrypeticus casei*. From an experiment in which this bacillus produced in milk gas containing 63 per cent of carbon dioxid and the remainder almost entirely hydrogen, Baumann concluded that the gas of normal as well as faulty eyes is carbon dioxid and hydrogen. The error of attributing the reactions of a bacillus when cultivated in milk, which contains sugar, to cheese, which after the initial fermentation contains no sugar, is so evident, and the error in stating that the gas of normal eyes contains hydrogen, without having first analyzed this gas, is so evident, that Baumann's conclusions might be left unnoticed at this late date were they not typical of several found in the more recent literature.

ABSORPTION OF OXYGEN.

In all the analyses no appreciable amount of oxygen was found. The presence of large percentages of nitrogen with this absence of oxygen raises the question, Does air diffuse into the cheese with absorption of oxygen? Evidence of an active absorption of oxygen was

[a] This does not preclude there being a number of distinct fermentations or reactions of either type.

accidentally obtained. In attempting to study the gases produced in sealed tubes a faulty form of tube was first used, which evidently leaked. On attempting to exhaust, the lowest pressure which could be obtained was 3.6 mm. It was soon ascertained that there was no leak in the pump, but a leak in the tube was suspected. The tube was left connected with the pump (connecting stopcock closed) over night. The next morning 37.20 c. c. of gas was pumped out. The first portion of 19.15 c. c. gave 4.57 c. c. of carbon dioxid and 2.21 c. c. of oxygen. The residue was lost but was considered to be nitrogen. The second portion was then pumped out, and of the 18.05 c. c. thus collected there were 4.85 c. c. of carbon dioxid, 1.45 c. c. of oxygen, and the residue entirely nitrogen. The total oxygen amounted to 3.66 c. c., which, had it come by leakage, would have indicated an entrance of 13.7 c. c. of nitrogen. There was actually found 24.12 c. c. of nitrogen. This leaves 10.42 c. c. of nitrogen to be accounted for. The carbon dioxid amounted to only 9.42 c. c. and, since the ratio $\frac{10.42}{9.42}$ is much larger than that obtained in other similar pumpings where no leak occurred, it was suspected that oxygen had been absorbed.

To definitely determine this the apparatus shown in figure 3 was used. With plugs of cotton at B, C, and in the bend above G, the tube was sterilized at 170° C. Then 28.5 grams from one of the Wisconsin cheeses were carefully taken with trier and spatula flamed to prevent contamination as far as possible, and the plugs introduced into A and sealed in as previously described. Mercury was drawn up into the tube E until it had just passed the stopcock F. After attachment had been made to the pump the whole was evacuated 5 hours and finally at a pressure of 1.2 mm. the capillary at H was sealed off in a blowpipe flame. There was introduced into E 7.47 c. c. N. T. P. of oxygen from a tank. At the same time a sample of the same gas was taken for analysis, and found to contain 98.1 per cent of oxygen. Upon opening the cock F atmospheric pressure forced the gas over into the tube A. The mercury behind this gas was allowed to rise until it had entered the capillary G. As close to this mercury as was possible G was then fused off with a blowpipe. There was left of the 7.47 c. c. introduced only a small bubble in the capillary, and this at reduced pressure. After 6 days at 25° C. the tube was opened by the usual method and the gas was pumped out and analyzed, with the following result:

	c. c.
Total volume of gas collected	11.90
Carbon dioxid	10.96
Oxygen	.53
Residue, all nitrogen	.41

From the percentage composition of the 7.47 c. c. of gas added at the beginning of the experiment it is known that 7.33 c. c. of oxygen was added. At the end of the experiment there remained only 0.53 c. c. of oxygen. There must, therefore, have been 6.80 c. c. of oxygen absorbed, or 0.239 c. c. per gram of cheese.

Such an active absorption of oxygen lends itself to the argument that the nitrogen of the eyes found its way there by the diffusion in of air. But, before such an argument can be considered valid, the following points must be determined: First, to what extent is cheese permeable to gases in general and nitrogen in particular? Second, how much of the nitrogen present is due to nitrogen dissolved in the cheese at the time of manufacture? Third, what evidences are there to show that the nitrogen does not arise in situ from bacterial or chemical reactions?

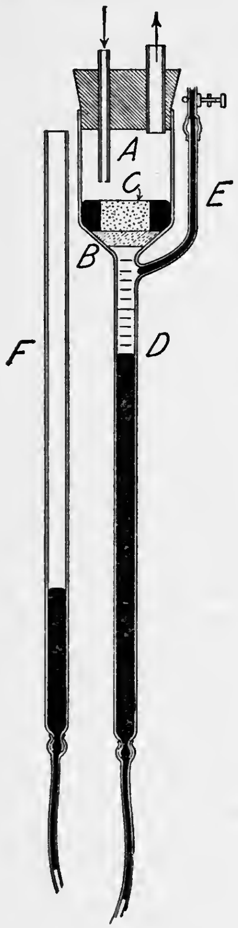

FIG. 4.—Device for ascertaining permeability of cheese to gases.

THE PERMEABILITY OF CHEESE TO GASES.

After various unsuccessful efforts to make an impermeable adhesive that would stick to cheese, and so enable a slice to be sealed into a diffusion apparatus, the following device was made (fig. 4):

At B a membrane of plaster of Paris was formed whose strength was reenforced by a perforated brass plate not shown in the diagram. This membrane was desiccated until its permeability was high, that of transfusion.[10]

Most of the air was forced out of D through the membrane and E by raising the mercury. A carefully taken disk of cheese was then placed on the plaster of Paris bed. It was gently held there while it was completely covered with mercury. Then, by lowering F, the space in D was left under greatly reduced pressure. This caused such a difference in pressure between the upper and lower surfaces of the cheese that the disk was held firmly against the plaster bed, and the surrounding mercury was unable to float it. Preliminary experiments showed that no mercury crept between the disk and the plaster, and that the plaster did not become clogged with mercury or cheese. After partial vacuum had been produced in D a few moments elapsed before the gas retained in the plaster came to equilibrium. When this was reached the mercury was carefully withdrawn from the top of the disk of cheese until the surface was exposed. The mercury left at the side prevented entrance of gas there, so that the only path between the chambers A and D by which gas could enter D was through the cheese.

The disks of cheese used were about 1 cm. in diameter and 2 to 2.5 mm. thick. They were taken from sound portions of freshly cut cheese by means of a cork borer, and carefully sectioned with a sharp razor. Every precaution was used in cutting and handling to prevent distortion and breaking of the texture. In one case the exposed surface was that of an eye. The gas whose diffusion it was desired to study was flooded into the chamber A. With both air and carbon dioxid there was apparently no diffusion during an hour, even though the pressure in D was reduced as much as possible. Longer experiments were not practicable, because a continuous watch had to be kept to see that no bubble of air entered through the rubber connecting tube between D and F and altered the volume in D. With a trap to prevent such a source of error the same impermeability for air was observed during an experiment lasting several days.

This result was so remarkable that it was tested further in the following manner: Instead of the parts E, D, F (fig. 4), a glass tube led from B to a mercury pump. With the cheese slab C covered with mercury the pump was operated till the lowest vacuum which could be obtained was reached. By reason of the gas being given off by the cheese, this was of course not so high a vacuum as the pump can produce. When the vacuum was considered sufficient the pump was allowed to rest in order to discover leakage, and, if there were none, to allow the residual gas to distribute itself so that a reliable reading on the McLeod gauge could be made. Then the mercury was carefully withdrawn from the top of the cheese, leaving its upper surface exposed. Entrance of gas could now be detected by the McLeod gauge. An experiment is given in detail below:

[Disk of cheese 7 mm. diameter, 2.5 mm. thick, taken 10.50 a. m., Dec. 19, 1911, 15 mm. from the nearest rind.]

	Time of reading.	Pressure.
	a. m.	*Mm.*
Apparatus exhausted, and, with cheese covered with mercury, pump pressure at	11.14	0.075
Pump resting	11.25	.140
Do	11.30	.150
Increase in pressure assumed to be due to gas evolved from cheese.		
After 7 minutes pumping	11.37	.070
Cheese exposed to air	11.40	.070
	p. m.	
Do	12.15	.150
Do	1.15	.270
Do	1.50	.320
Do	2.15	.350
After 15 minutes pumping	2.30	.025
Cheese exposed to CO_2	3.10	.060
Do	3.30	.090
After 5 minutes pumping	3.35	.025
Cheese exposed to H_2	4.10	.050
Do	4.30	.065
After 15 minutes pumping	4.45	.010
	a. m.	
Cheese left overnight exposed to air	9.30	.430

When the disk of cheese and the mercury were removed air entered rapidly, showing that the plaster had not become plugged. Furthermore, there was no evidence of mercury having crept between the cheese and the plaster. It is not claimed that all the above listed readings on the McLeod are very accurate, since the readings were sometimes made before equilibrium was obtained. All that was desired was the order of magnitude. Since the variation in temperature during the experiment was only between the extremes 17° C. and 19° C. and since the volume of the pump, gauge, and diffusion apparatus was found to be 159 c. c., we may calculate from pressures the approximate amount of gas which had apparently diffused through the cheese. This amounted to about 0.09 c. c. during the first 5 hours and 0.09 c. c. during the last 17 hours.

Allowing nothing for possible small leaks, which were difficult to avoid in the delicate manipulations required, the observed volume of gas indicates a very remarkable impermeability. Practically the same result was obtained with a disk of Cheddar cheese and other samples of Swiss cheese.

The question at once arises, How to explain the evolution of carbon dioxid which there is every reason to suppose does diffuse from cheese? Van Slyke and Hart [17] found that a normal Cheddar cheese evolved during 32 weeks 15.099 grams of carbon dioxid. Since they took care to exclude surface growths of molds, it seems highly improbable that this amount of carbon dioxid could have come to any great extent from the surface layers alone. It must have diffused from the interior of the cheese into the surrounding bell jar.

The following explanation will doubtless be found reasonable: Becquerel [4] found that when the tegument of peas was mounted at the end of a barometer tube, and a partial vacuum of 5 to 10 mm. obtained upon the one side, with atmospheric pressure on the other, the tegument was impermeable to gas when dry, although permeable when moist. In so far as the tegument of peas and a disk of cheese are both colloidal they may be compared. In the present experiments the disks of cheese dried considerably both from exposure to gases of low vapor content on the one side and the moisture free vacuum on the other. By analogy with Becquerel's experiments one would expect to find the dry cheese more or less impermeable. Reference to the experiment detailed on page 21 will indeed show that the permeability decreased as the time of the experiment increased, or, in other terms, as the cheese became drier. Furthermore, in an experiment in which the exposed surface of the cheese was kept exposed to carbon dioxid, which was saturated with vapor, 1.04 c. c. of gas was found to have passed through in 5 hours; ten times as much as in the experiment with drying cheese.

It therefore seems probable that the permeability of cheese to gases is due to the diffusion of dissolved gases, and that as the free solvent becomes more and more attenuated the gas is more and more unable to find its way through the gel.

Since in Emmental cheese a more or less dry rind is produced, it seems probable that little air can diffuse into the cheese. And from the fact that in the manufacture of Cheddar a less dry rind as well as a more open texture is produced, it seems probable that escape of carbon dioxid more easily occurs in this type than in the Swiss type of cheese.

It must be remembered, however, that the above experiments only cover a very limited time, and that, even were the permeability as low as the experiments seem to show, there is still the possibility that nitrogen may make its way slowly through the gel during the long period of ripening. Possibly more extensive investigation would reveal that the larger percentages of nitrogen found in the eyes of some cheeses are proportional to the age of the cheeses. Nevertheless this penetration can only take place slowly.

The fact that penetration of air is so slow, together with the avidity with which oxygen is absorbed, only tends to emphasize the completeness of the anaerobiosis in the interior of the cheese, a condition which Troili-Peterson[16] found necessary for the best development of the propionic bacteria.

These experiments on the permeability of cheese to gases make it evident that in pumping the gases from plugs of cheese we should expect the gas to be slowly evolved. Such was, indeed, found to be the case. The reason for this was not fully appreciated at the time the pumpings were made, and it is very doubtful if all the occluded gas was completely exhausted even after 20 hours exposure to high vacuum. Reference to the experiment with plugs of cheese kept 6 days in vacuo (p. 15) reveals the interesting fact that the amount of gas evolved per gram of cheese was dependent more upon the state of the vacuum than upon time. This is illustrated in the following statement, in which the figures represent cubic centimeters of gas evolved per gram of cheese per hour:

	1	2
First 18 hours	0.0087	0.0042
Succeeding 6 days	.0015	.0014
Last 18 hours	.0033	.0066

During the middle period, of course, the tubes were sealed, and the evolved gas increased the pressure. Evidently, then, the higher the vacuum to which the sample was subjected the more rapidly was the gas evolved, indicating that a considerable proportion of the gas was dissolved or occluded gas rather than that formed during the time of the experiment.

It may also be true that there is loose combination of carbon dioxid with inorganic salts, or with calcium and amino bodies, as in the carbo-amino reaction, and that the stability of these compounds is a function of the imposed pressure.

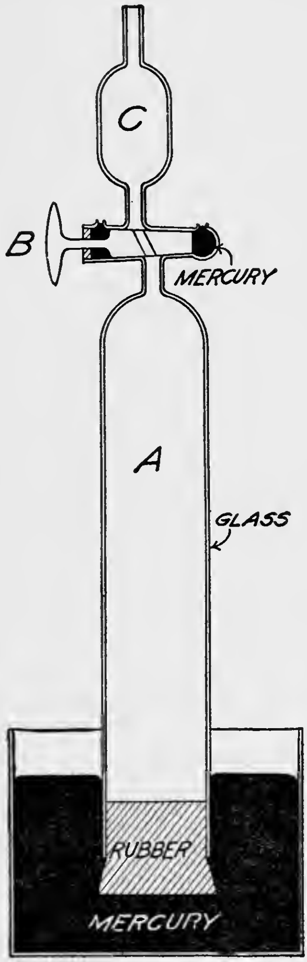

FIG. 5.—Apparatus for determining amount of nitrogen in curd.

NITROGEN DISSOLVED IN CURD.

Let us now consider how much of the nitrogen found in the eyes is attributable to nitrogen occluded in the original curd.

One would expect the curd to be well aerated by the vigorous stirring it gets during the process of manufacture. Marshall [14] has shown that aerated milk contains considerable quantities of nitrogen, but, unfortunately for the purposes desired, his data are only expressed in percentage composition and not very definitely in cubic centimeters of gas per cubic centimeter of milk.

A rough approximation of the amount of nitrogen occluded in the curd was obtained in the following way: A liter of milk was treated as in the process of making Swiss cheese. When the curd had reached the stage when it was suitable to hoop, the greater part of the whey was decanted, and then the residual whey and curd were poured carefully into the glass cylinder A, figure 5 (inverted). As the curd settled, the overlying whey was drawn off and more of the mixture poured in. This was repeated until the tube was filled with curd grains completely surrounded by whey. The rubber stopper was then forced in. The tube was next inverted to the position shown in the figure, and the mercury seal stopcock B was opened to relieve the pressure. The rubber stopper was then forced farther in, and the whey displaced by it escaped into C. By covering the stoppered end of the tube with rubber-rosin cement and keeping it under mercury, it was made perfectly gas tight. The cock B was then closed, and, after the surplus whey in C had been drained out, the apparatus was connected to the vacuum pump in the usual way.

When the pump and chamber C were completely exhausted, the cock B was opened. It was found that the gas expanding in A drove the whey almost completely up through the interstices of the curd and into C.

An interesting point was observed. Comparatively little of the gas came from the whey, while the major portion came from the curd particles. Since a separation of whey and curd was accomplished, it could not have been true that the gas evolved from the curd particles originated in the whey, using curd particles as nuclei for the formation of bubbles. Furthermore, there was comparatively little frothing of the whey in C, most of the gas collected having bubbled through C from A. Examination of curd particles will show why this is so; for they have adhering to them minute bubbles, apparently froth taken up during the stirring. It is quite evident that the column of whey in C through which the gas had to make its way prevented a very complete exhaustion. Since the pumping was continued several hours and the tube then allowed to stand overnight before the final pumping, this error was reduced to some extent. If occasion arises to repeat these experiments this error will be avoided.

By the method described, 1.35 c. c. of gas was collected in one instance and 0.86 c. c. in another. Of this, there was 0.58 c. c. of nitrogen in one case and 0.39 c. c. in the other; average, 0.5 c. c. The curd was roughly estimated to represent 20 grams of cheese. Consequently there would be approximately 2.5 c. c. of nitrogen per 100 grams of cheese. How this nitrogen would partition itself between the body of the cheese and an eye is a question whose solution would be mere guesswork without further data.

While the 2.5 c. c. per 100 grams of cheese is a mere approximation, and a figure which would vary not only with the extent to which the curd is stirred, but also with the form of the curd particles and their ability to absorb foam, nevertheless it is sufficiently accurate to show that a large part of the free nitrogen found in cheese comes from occluded air.

DOES NITROGEN ORIGINATE IN SITU?

The question of whether any of the nitrogen found in the eyes is set free in situ is a difficult one to answer, and one which can not be definitely answered without further research. From the following considerations, however, it is highly probable that it is not produced during the course of that reaction which furnishes the gas to distend the eyes. In those experiments in which samples from a cheese at the period of its maximum eye formation were held in vacuo, the nitrogen in the evolved gas steadily and rapidly declined in percentage, finally reaching almost nothing. This indicates that the nitrogen collected was simply that dissolved in the cheese, and as this was removed there was no evolution of free nitrogen to take its place, such as occurred in the case of the carbon dioxid.

RELATION BETWEEN CARBON DIOXID AND VOLATILE ACIDS.

The results of the whole investigation show clearly that the only gas which plays an important rôle in the formation of normal eyes is carbon dioxid. This is in entire harmony with the assumption which has heretofore been accepted as a fact by various authors.

It remains to be seen whether there is any quantitative relation between the amount of carbon dioxid evolved and that called for by the process to which Von Freudenreich and Jensen ascribe the formation of eyes.

A study of the volatile fatty acids of Emmental cheese by Jensen [12] disclosed the fact that they are chiefly propionic and acetic, and that often the ratio of these approximates 2:1.

Fitz [7] had previously shown that certain bacteria are capable of producing this ratio of propionic and acetic acids from lactic acid, and he ascribed to their action the equation:

$$\underset{\text{lactic}}{3C_3H_6O_3} = \underset{\text{propionic}}{2C_3H_6O_2} + \underset{\text{acetic}}{C_2H_4O_2} + CO_2 + H_2O$$

Subsequently Von Freudenreich and Jensen [8] isolated from Emmental cheese an organism which did ferment lactates according to the above equation of Fitz, and whose introduction into cheese was followed by an eye formation of which it was thought to be the cause.

The conclusion seems evident that here is an organism to whose action may be attributed the formation of normal eyes.

The evidence is undoubtedly the clearest that has yet been presented. There are, however, one or two points which will bear inspection before the theory can be accepted as a full explanation.

According to the equation of Fitz three molecules of volatile fatty acids are accompanied by the liberation of one molecule of carbon dioxid. Consequently it can be shown that a titer of 1 c. c. of tenth-normal alkali for these volatile fatty acids should indicate the liberation of 0.74 c. c. of carbon dioxid (N. T. P.). If, then, it is found that the volatile acids from 100 grams of cheese neutralize 100 c. c. of tenth-normal alkali, and it is assumed that these acids are acetic and propionic in the ratio in which they occur in Fitz's equation, we would have liberated 74 c. c. of carbon dioxid per 100 grams of cheese.

This amount of gas is considerably more than is required to fill the eyes, but the question remains how much is to be found in the body of the cheese itself.

Reference to the experiments described on page 15 shows that at an age of 55 days 37.2 c. c. of carbon dioxid per 100 grams of cheese were collected after the cheese has been held in vacuo one week. At the time of the experiment it was thought that this gas was produced during that week. After the study which shows how impermeable cheese is, this view had to be modified, for, even after considerable pumping, an appreciable quantity of gas must have remained and appeared as "evolved" gas at the end of the week. In order to make a better estimation of the dissolved gas, plugs of this same cheese (at an age of 4 months) were sliced into thin disks to facilitate the removal of dissolved gas, and introduced into a tube. They were sealed in with the usual rubber stopper and rubber-rosin cement, and the tube joined to the mercury pump. After evacuating the pump the connecting cock was opened and the disks of cheese evacuated. The air surrounding them in the tube was of course pumped out too. The total gas thus collected after 5 hours continuous pumping contained 17.05 c. c. of carbon dioxid. The weight of cheese was 42 grams. Hence, there were collected 40.6 c. c. of carbon dioxid per 100 grams of cheese (19 hours later 0.9 c. c. of carbon dioxid was collected, or 2.1 c. c. per 100 grams of cheese).

A duplicate determination gave 46.2 c. c. of carbon dioxid per 100 grams of cheese (with an additional 1.03 c. c. per 100 grams after 19 hours). The average for the first 5 hours' pumping was 43.4 c. c. of carbon dioxid per 100 grams of cheese, and this we may fairly consider the quantity occluded at the time the plugs were taken. At the same age (4 months) the volatile fatty acids corresponded to 40.9 c. c. of tenth-normal alkali per 100 grams of cheese.

Similarly, duplicate determinations of dissolved carbon dioxid and volatile acids in an excellent imported cheese (No. *i*) gave the following data: Carbon dioxid per 100 grams, 67.8 c. c. and 54.8 c. c., average 61.3 c. c. Total volatile fatty acids in cubic centimeters of tenth-normal alkali per 100 grams 95.1 and 97.7, average 96.4.

Assuming that all the volatile fatty acids were produced in strict accordance with the equation of Fitz, the amount of these acids in the first cheese indicates that there had been liberated 30.6 c. c. of carbon dioxid against 43.4 c. c. found occluded; and in the second cheese the liberation of 71.3 c. c. of carbon dioxid against 61.3 c. c. found occluded. There is a somewhat striking apparent relationship in this data, and the averages, 51.0 c. c. calculated, against 52.3 c. c. found, are in such close agreement that they are tempting. A little consideration will show, however, that this agreement may be only accidental. At the time these analyses were made each of the cheeses had probably reached a state of little activity. The volatile acids represent almost entirely the total amount produced in the interior from which the samples for analyses were taken; while, if we are to accept the results on Cheddar cheese by Van Slyke and Hart as at all applicable to Emmental, it is certain that a considerable quantity of carbon dioxid must have escaped in the months since manufacture. Furthermore, although the actual volume of the eyes represents but a small portion of the gas in a given volume of cheese, the normal volume of this gas in the eyes leaps into considerable significance when it is remembered that it must have been under considerable pressure. That it is under pressure was made evident in some cases by its vigorous escape when using the puncturing apparatus for its collection.

Unfortunately long delay in obtaining apparatus suitable for a study of the gas escaping from cheese, as was done by Van Slyke and Hart for Cheddar, have made it impossible to present any data on this point. As before mentioned, the data on carbon dioxid evolved from plugs of cheeses taken at the height of their gaseous fermentation and kept in vacuo a week is complicated by the fact that there was probably a slow yielding of dissolved gas from the solid plugs as well as the normal production of gas. Two other experiments, however, indicate to what extent carbon dioxid was being formed during this period of maximum fermentation.

Portions of cheese W 2 from regions without eyes were carefully selected and sealed up in a tube as described on page 14. The eye membranes were carefully removed from a large number of eyes and similarly treated. The tubes were simultaneously exhausted with a Boltwood pump for several hours. Since in these cases the cheese was in a more finely divided state, it is reasonable to assume that predissolved gas was pretty thoroughly removed. After exhaustion, the tubes were sealed off in a blowpipe flame and held at 25° C. for seven days. At the end of this period the gas was collected:

34 grams eye membranes gave 14.95 c. c. of gas, 99.3 per cent of carbon dioxid, or 44 c. c. per 100 grams.

36 grams from regions without eyes gave 10.06 c. c. of gas, 98.2 per cent of carbon dioxid, or 28 c. c. per 100 grams.

From this one pair of experiments it is not advisable to claim confidently that the eye surfaces always produce the much larger quantity of carbon dioxid, although this is plainly evident in the above case. The significant fact is that such a large quantity was produced by each region in the period of only one week. Of course it may be claimed that although the division of the cheese was done in a dust-free room and with sterile instruments, and the cheese introduced into sterile tubes, yet the long manipulation admitted a heavy reinoculation by bacteria, and that these produced a renewed evolution of carbon dioxid. Such an argument can not be completely refuted, but the probability of a heavy enough infection is small. The most likely source of carbon dioxid producing infection was by molds, but these could not have grown in the complete anaerobic condition in which the cheese quickly found itself.

The following experiment serves to confirm the last. Into a sterilized combustion tube were quickly slipped plugs of cheese taken with sterile instruments. Each end of the tube was guarded with cotton plugs. Carbon dioxid free air was then passed through, and the carbon dioxid evolved from the cheese absorbed in the customary train with all due precautions for exact estimation of carbon dioxid. In the case of this experiment we would expect a higher amount of carbon dioxid, since there would be collected not only the carbon dioxid produced, but a large portion of the predissolved carbon dioxid. Such was found to be the case.

One hundred and five grams in plugs taken from cheese W 1 when at the height of its fermentation gave:

First 24 hours, 81.6 c. c. of carbon dioxid.
Second 24 hours, 66.7 c. c. of carbon dioxid.
Third 24 hours, 44.5 c. c. of carbon dioxid.
Fourth 24 hours, 63.7 c. c. of carbon dioxid.

The increase on the fourth day was thought to be due possibly to growth of molds with which Van Slyke and Hart found difficulty in their work on Cheddar cheese. The experiment was therefore discontinued, although no growth was visible.

A final word must be urged against the too liberal use of the equation of Fitz. As a terse representation of the probable relation of the end products the equation has a legitimate use. As a comprehensive portraiture it is colored with presumption. The literature of fermentation is littered with equations, two or three members of which are known to stand in certain quantitative relationships, while the other members are given values which fit. This stoichiometrical adjusting is particularly true of the gaseous products. One has only to review the literature on the gas production of *B. coli* to assure himself of the fact.

While Von Freudenreich and Jensen's use of the Fitz equation has been interpreted quite rigidly in the preceding pages, this was done simply as a test. From this basis alone one can not reasonably jump to a final conclusion; but it must be remembered that the liberal use made of the Fitz equation was generous to the theory of Von Freudenreich and Jensen in that all the volatile fatty acids, as determined by Jensen's method, were assumed to have been produced in accordance with this equation.

From a comprehensive view of the matter it appears to be quite evident that the theory of Von Freudenreich and Jensen is not capable of accounting for all the carbon dioxid produced. Indeed, it is not necessary nor expected that it should, but we have reached a point where it has become advisable to distinguish between a primary and a secondary cause of eye formation, and to at least define clearly what we mean when we attribute to any organism or to any reaction the function of forming eyes.

Suppose that the propionic bacteria are active, but that they are never sufficiently localized to concentrate carbon dioxid rapidly enough at one point to produce an eye. In this case the gas would be more or less evenly produced throughout the body of the cheese. Now this state of more or less complete saturation of the body with carbon dioxid is exactly the condition necessary for the most advantageous eye formation by any other reaction which may follow, else the gas evolved at a point would be largely absorbed and its inflating energy dissipated. Of course it can be said that this saturation proceeds from the point where the eye is formed, and that the delay observed before an eye commences to grow represents the time necessary to effect this saturation.

This, however, is merely presenting the other horn of a dilemma from which escape is possible only when the localization of the propionic bacteria is conclusively demonstrated. Gorini [9] has contended that the localization of colonies may often be of as great importance as their isolation; and it is interesting to note that he found no correlation between the colonies which stained on his sections of Grana cheese and the gas bubbles.

If, then, we distinguish between a "saturating" gas production and an "inflating" gas production, we will have at least defined a possibility which must be squarely met, and a hypothesis which may lead to a differentiation between a primary and a secondary cause of eye growth.

The favorable results obtained with cheese inoculated with propionic bacteria indicate that they may play an important rôle. But is this rôle primary or secondary? Is it a strictly localized action or is it simply the provision of that saturation without which some primary and strictly localized reaction would be without avail? The same question arises in the case of the glycerin-fermenting bacteria to which Troili-Petersson [16] has ascribed an important rôle in the holing of Swedish cheeses. In fact experimental cheesemaking of the past, though not so thoroughly controlled as in the experiments of Troili-Petersson and those of Von Freudenreich and Jensen, bear evidence that any one of a number of gas-producing bacteria may provide the saturation, not to mention those reactions which Van Slyke and Hart [17] have proposed as contributing to the carbon dioxid in Cheddar cheese. On the other hand, any one of these may be the primary "inflator" and the other the secondary "saturator."

In this connection it may be of interest to note a peculiar phenomenon met with in some experimental cheeses. A number of these made with "artificial" rennet by Mr. Doane were reported in their early stages to have begun a normal eye formation. Seldom, however, did this beginning develop into a normal holing. These cheeses were of small size, and, since it is known that small-sized cheeses for some reason not yet clearly defined seldom develop large eyes, the failure in these cases may on general principles be vaguely attributed to size. However that may be, it was found upon pumping out the dissolved gas that the amount was low. The three cheeses examined were 39–45, 39–11–2, and 46–4–1. (See Table 2, p. 12.)

It is well known from the work of Jensen and others that the bacteria found in "natural" rennet are often distinct from those found in "artificial" rennet. Since the cheeses under discussion were made with the latter, is it not possible that the reaction which started the eye formation was rendered inadequate because the gas-producing propionic bacteria, which might have saturated the cheese with carbon dioxid, were absent? That the observed holes were truly the beginnings of normal eyes, and were not a pinhole formation resulting from an initial gaseous fermentation of sugar, is evinced by the fact that hydrogen was absent.

Exhaustive research alone can unravel this tangle; but it is hoped that the present investigation has provided both a clearer definition of the problem and a sound basis of fact.

SUMMARY.

1. The gases of normal "eyes" in Emmental cheese are exclusively carbon dioxid and nitrogen, and of these only the carbon dioxid is of significance.

2. The nitrogen accompanying the carbon dioxid in normal eyes is that of air originally occluded in the curd at the time of manufacture.

3. There sometimes occurs during the initial fermentation an evolution of gas characterized by the presence of hydrogen. This is believed to be due to the gaseous fermentation of sugar.

4. The hydrogen from such an initial fermentation may sometimes linger to contaminate the gas of normal eyes.

5. The two fermentations are distinct and are characterized by their gaseous products. The one is detrimental, the other that demanded of a good Emmental cheese.

6. High oxygen-absorbing power combined with low permeability of the cheese to air render the interior thoroughly anaerobic, and consequently favorable to the growth of anaerobic bacteria.

7. A comparison between the amount of carbon dioxid evolved and the total volatile fatty acids shows that the activity of the propionic bacteria of Von Freudenreich and Jensen is not sufficient to account for all the carbon dioxid found.

8. It was found that cheese is capable of retaining a very large amount of carbon dioxid.

9. The possibility is suggested that there are two phases in the formation of normal eyes, a saturation of the body with carbon dioxid, and an inflation of eyes; and the bearing of this hypothesis on the production of gas by a specific cause is discussed.

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