THE ROLE OF THE LACTIC-ACID BACTERIA IN THE MANUFACTURE AND IN THE EARLY STAGES OF RIPENING OF CHEDDAR CHEESE.

H. A. HARDING.
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THE ROLE OF THE LACTIC-ACID BACTERIA IN THE MANUFACTURE AND IN THE EARLY STAGES OF RIPENING OF CHEDDAR CHEESE.

H. A. HARDING.

SUMMARY.

1. Lactic-acid bacteria are always present in factory milk.

2. These lactic-acid bacteria check the growth of other forms by breaking up the sugar into acid and finally make up more than ninety per cent of the total number of germs present in the milk and cheese.

3. The acid formed by these bacteria hastens the curdling action of the rennet.

4. This acid combines with paracasein to form at least two different compounds—paracasein monolactate and paracasein dilactate. The paracasein monolactate is normally formed in large quantities.

5. This formation of acid is necessary to the activity of the rennet pepsin.
INTRODUCTION.

As soon as American cheddar cheese and its method of manufacture were studied from a biological standpoint it was found that acid-forming bacteria thrive in the curd to such an extent as to make up more than 90 per ct. of the total flora of the cheese. It was natural to assume that the growth of this large number of bacteria, amounting to some millions in each gram of the curd, could not fail to have an influence upon the final result which we speak of as ripened cheese. Students of the problem have even gone so far as to hold that the lactic-acid bacteria are the principal, if not the only, cause of cheese ripening.

Scientific opinion is by no means unanimous as to the causes which bring about the ripening of cheese. Two other theories aside from the one just mentioned are supported by considerable evidence. According to certain students the ripening is brought about by a different class of bacteria characterized by the elaboration of enzymes capable of attacking and dissolving the coagulated casein. A third explanation assumes that the breaking down of the casein is largely due to the activity of enzymes secreted by the cow with the milk.

These wide differences of opinion concerning a problem which has been so long an object of study are the result of our inability to follow the changes going on within the cheese mass. While the problem is essentially one of applied biology, an exact knowledge of physiological chemistry, coupled with a proper appreciation of commercial quality is necessary for its solution. Since a sufficient knowledge of these three lines of human effort was not possessed by one man the study of the cause of the ripening of cheese was assigned by the Station Director to the cooperative activity of the Dairy Expert, the Chemist and the Bacteriologist. As a result of this cooperative effort a considerable advance has been made in our understanding of the problem of cheese ripening and results on a number of points have already
been published.\(^1\) In this bulletin, while the author accepts the responsibility for all conclusions drawn from the data, he takes pleasure in acknowledging the important activities of his colleagues in planning and executing the experiments.

Recognition should be given to the work of Mr. L. A. Rogers who, as Assistant Bacteriologist during a portion of the investigation, had charge of the routine bacteriological examinations. Since his transfer to the Department of Agriculture at Washington his part of the investigation has been carried on by his successor, Mr. J. F. Nicholson.

**ROLE OF LACTIC-ACID BACTERIA.**

**LACTIC-ACID BACTERIA ARE CONSTANTLY PRESENT IN FACTORY MILK.**

Everyone knows that fresh milk left in a warm room sours rapidly and nearly everyone knows that this souring is due to the formation of acid by bacteria. Since milk drawn with certain precautions does not undergo this rapid souring it is plain that these bacteria find their way into the milk after it is drawn.

Because the acid formed during this souring process is principally lactic these various acid-forming species were early spoken of collectively as the lactic-acid bacteria. Without knowing exactly why, it was found that the formation of acid by these germs was a necessary part of the manufacture of cheddar cheese and in order to insure the presence and activity of the most desirable kinds it has long been the custom to begin the process of manufacture by adding considerable quantities of so-called "starter."

This starter is simply milk containing rapidly multiplying acid-forming bacteria, and while the small amount of acid already formed is useful in quickening the action of the rennet the principal effect of a starter lies in the increased activity of the germs.

Thus, partly as the result of natural causes and partly because of the action of the maker, the milk from which cheese is made is normally well seeded with these lactic-acid bacteria. As these germs are present in the factory milk in such large numbers it is but natural that they should pass over into the cheese.

THE GROWTH OF LACTIC-ACID BACTERIA CHECKS THAT OF OTHER FORMS IN THE MILK.

Conn & Esten\(^2\) recently published the results of a careful study of the bacteria present in fresh milk and the rate at which the various kinds develop as the milk becomes older. Up to the time the milk is sour practically all the species of bacteria present in it continue to multiply, although as the latter stage is approached the germs other than the lactic-acid group increase more and more slowly. Very few lactic-acid bacteria are found in the fresh milk but they increase rapidly and in 12 to 18 hours at 20° C. (68° F.) they usually outnumber all those of the other kinds. At the higher temperature to which the milk is commonly exposed during the warmer portions of the summer the growth of all the bacteria is accelerated, and consequently at such times the lactic-acid bacteria more quickly make their presence felt. At the time of souring the acid bacteria commonly make up more than 95 per ct. of the total number.

While this problem has never before been so carefully studied from the biological standpoint the fact that an abundant growth of lactic-acid bacteria in milk checks the activity of other forms has long been known and utilized in cheese-making. It is a matter of common experience that the effect upon the cheese of various objectionable fermentations in the milk can be modified and often removed by the addition of liberal amounts of a vigorous lactic-acid starter in the cheese vat.

THIS CHECKING IS DUE TO THE CHANGE OF SUGAR INTO ACID.

The ability to form acid, as the name implies, is the point of similarity upon which the classification of the lactic-acid group is based. Shirokich\(^3\) has shown that when representatives of this group were grown in milk the nitrogenous portion was not attacked to a measurable extent. Chodat & Hoffman-Bang\(^4\) analyzed milk cultures of five lactic-acid organisms and found that at the end of five weeks the milk sugar had decreased 1 to 1.25 per ct. A considerable portion of this sugar was converted into acid. While the larger part of this acid was believed to be lactic they determined the presence of small quantities of formic, valeric and acetic acids.

The destruction of this amount of sugar does not measure the limit of the ability of the germs to attack the sugar but rather shows the limit of the ability of the compounds present to dispose of the acid as formed. In similar milk cultures to which chalk had been added to combine with the excess of acid as formed, all of the 5.77 per ct. of sugar had disappeared in four of the five flasks at the end of five weeks.

The majority of organisms aside from this group prefer a neutral or even faintly alkaline reaction, while the lactic-acid organisms grow best in a slightly acid reaction. As soon, therefore, as they break up an appreciable quantity of sugar into acid they place their competitors in the milk at a disadvantage and this disadvantage increases with the increased formation of acid.

THE EFFECT OF THIS ACID UPON THE CURDLING ACTION OF THE RENNENET.

The direct relation between the extent of acid formation and the rate of rennet action is well known. This fact is taken advantage of in practical cheese-making by the addition of partially soured starters to sweet milk in order to quicken the rate of coagulation. The effect of these starters is twofold. They have an immediate effect due to the acid which has already been formed and a later effect due to the increased rate at which the sugar of the milk is changed to acid.

\(^3\) Shirokich. *Ann. Past.*, 12:400. (1898.)

The effect of acid formation upon the action of the rennet may be directly observed by adding measured amounts of acid to milk and noting the decrease in the time required to curdle portions of the same with rennet.

In the following experiment 12½ litres (25 pounds) of milk was used. The time required for the action of the rennet according to the Monrad rennet test was determined at the beginning of the experiment and after the addition of successive portions of acid, 100 cc. (3½ oz.) of the milk being used in each rennet test. Before each test, after the first, 2.5 cc. (1-12 oz.) of chemically pure lactic acid diluted with 100 cc. of water was added to the milk.

**Table I.—Effect of Acid Upon the Curdling Action of the Rennet.**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>225</td>
<td>103</td>
</tr>
<tr>
<td>2.5</td>
<td>2.5</td>
<td>122</td>
<td>44</td>
</tr>
<tr>
<td>2.5</td>
<td>5.0</td>
<td>78</td>
<td>44</td>
</tr>
<tr>
<td>2.5</td>
<td>7.5</td>
<td>63</td>
<td>16</td>
</tr>
<tr>
<td>2.5</td>
<td>10.0</td>
<td>47</td>
<td>15</td>
</tr>
<tr>
<td>2.5</td>
<td>12.5</td>
<td>43</td>
<td>4</td>
</tr>
</tbody>
</table>

From this it is clearly seen that the addition of acid quickens the rate of curdling by rennet since the addition of 12.5 cc. of acid (about 0.1 per ct.) shortened the time of curdling from 3 minutes 45 seconds to 43 seconds. In other words the addition of 0.1 per ct. of acid to fairly sweet milk changed it as much as is allowable for milk intended for cheese-making.

This should help to emphasize the importance of delivering milk intended for cheese-making before the bacteria in it form any considerable quantities of acid.

It is also interesting to observe how much more noticeable is the effect from the first addition of acid. Here the time of curdling was shortened 1 minute 43 seconds, while the addition of an equal amount of acid at the close of the experiment shortened the time of curdling but 4 seconds. This is quite in accord with factory experience where the addition of sour milk has an immediate effect upon the rate of rennet action although the acid added amounts to only a very insignificant proportion.
THIS ACID COMBINES WITH THE CASEIN IN A DEFINITE WAY.

In the experiment by Chodat & Hoffman-Bang already referred to, which was a modification of an earlier experiment by von Freudrenreich, it was shown that, in the presence of chalk to combine with the acid, these germs were able to break down all the sugar. That a similar fixation of acid takes place during the manufacture and ripening of cheese is rendered probable by the rapidity with which the sugar is broken down during the process.

Sugar determinations made by Hart showed that in one instance shortly after the curd was cut the whey contained 4.75 per ct. of sugar while the whey obtained at the time the curd was ready for the press contained but 1.83 per ct. The destruction of the sugar which is left in the curd continues steadily and the sugar disappears from the cheese after a few days at ordinary curing temperatures. In spite of this destruction of sugar with its accompanying formation of acid the presence of free lactic acid in cheese has never been satisfactorily demonstrated.

In connection with this work upon cheese ripening Van Slyke and Hart have studied two compounds of acid with casein (or paracasein) and have described their chemical properties in Bulletin No. 214 of this Station.

The more important of these two compounds from the standpoint of our present knowledge of cheese ripening is an unsaturated combination of paracasein and acid called paracasein monolactate. This compound can be formed from paracasein in large quantities in the presence of dilute acid and is a compound constantly present during the process of cheese-making and ripening. This compound is insoluble in water but is soluble in dilute solutions of common salt (NaCl). Prepared in a fairly pure condition this compound draws out in fine threads when applied to a hot iron. It seems that this well known "hot iron" test of the progress of acid formation has thus received a satisfactory explanation.

A second salt of paracasein and acid called paracasein dilactate is formed from the monolactate by treating the latter with more acid. This compound is insoluble both in water and in dilute salt solutions and as yet little is known as to either its chemical properties or the part which it plays in cheese-ripening.

**PARACASEIN MONOLACTATE IS FORMED IN CHEESE CURD BY LACTIC-ACID BACTERIA.**

In order to determine whether paracasein monolactate could be formed in cheese through the activity of lactic acid organisms the following experiment was carried out.

Fresh milk was curdled by rennet in the presence of ether to prevent acid formation and after the moisture had been satisfactorily expelled from the curd the whey was drawn and the curd washed in three changes of warm water to remove the major part of the sugar and the water soluble compounds. The resulting mass contained 0.3 per ct. of sugar and about 4 per ct. of salt-soluble compounds.

Two series of flasks were prepared, each flask containing 50 cc. of water and 25 grams of curd ground with sand (922 mgs. nitrogen). These flasks were sterilized in steam for 10 minutes at 120° C. After cooling, Series I received an inoculation from a pure culture of an acid-forming bacillus agreeing closely with the description of *Bacillus lactis aerogenes*. Series II received an inoculation from the same organism and in addition 0.5 grams of sterile lactose in each flask. A similar sterile flask received neither sugar nor organism but 0.5 cc. of chemically pure lactic acid. Two flasks of each series were taken at each analysis. Bacteriological examinations of the flasks at the time they were taken for analysis indicated that they were free from contamination. The amount of nitrogen insoluble in water but soluble in a 5 per ct. solution of sodium chloride at the end of various intervals expressed in percentage of the total nitrogen is given in the following table.
### TABLE II.—PARACASEIN MONOLACTATE FORMED IN CHEESE CURD BY BACTERIA AND BY ACID.

<table>
<thead>
<tr>
<th>Date</th>
<th>Lactic-acid bacteria.</th>
<th>Sterile.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No lactose.</td>
<td>.5 gm. lactose.</td>
</tr>
<tr>
<td>Feb. 21—Initial</td>
<td>3.90</td>
<td>3.90</td>
</tr>
<tr>
<td></td>
<td>4.34</td>
<td>4.34</td>
</tr>
<tr>
<td>March 24</td>
<td>3.52</td>
<td>40.65</td>
</tr>
<tr>
<td></td>
<td>4.07</td>
<td>28.46</td>
</tr>
<tr>
<td>May 22</td>
<td>2.71</td>
<td>20.87</td>
</tr>
<tr>
<td></td>
<td>2.98</td>
<td>17.89</td>
</tr>
<tr>
<td>Aug. 15</td>
<td>2.98</td>
<td>8.94</td>
</tr>
<tr>
<td></td>
<td>2.71</td>
<td>8.94</td>
</tr>
</tbody>
</table>

It is seen from this table that in the presence of cheese curd containing but a trace of sugar this organism was not able to form measurable quantities of salt-soluble material.

On the other hand the addition of lactose to similar flasks resulted in the formation of considerable amounts of the salt-soluble compound.

That the formation of this monolactate is really due to the transformation of sugar into acid is rendered probable by the action of the corresponding amount of lactic acid upon the sterile curd.

**THE AMOUNT OF PARACASEIN MONOLACTATE IN MILK AND CHEESE.**

While it is supposed that compounds of acid and casein are formed in the milk their measurement in this medium is beset with difficulties. However, the presence and amount of monosalt in cheese curd can be determined in a fairly satisfactory manner.

In connection with the determination of sugar at different stages in the cheese-making process previously mentioned the amount of paracasein monolactate in the curd was estimated. Shortly after the cutting of the curd, when the milk sugar in the whey amounted to 4.75 per ct., there was but 5 per ct. of this salt-soluble compound in the curd; while at the time the curd was ready for the press, when the sugar in the whey had fallen to 1.83 per ct., the salt-soluble material in the curd had risen to 31.7 per ct. In normal cheddar cheese from one-half to three-fourths of
the nitrogen is found in the form of paracasein monolactate during the first week after the cheese is made.

From the table on page 173 it is seen that in the breaking down of \( \frac{1}{2} \) gram of lactose about one-third of the nitrogen in the 25 grams of curd was changed into the monosalt. This indicates that the breaking down, by bacteria, of sugar amounting to more than two per ct. of the fresh curd is necessary in order to account for the formation of the usual amount of monosalt in cheese.

**RESULT OF TOO GREAT FORMATION OF ACID.**

Flasks containing 50 cc. of water and 25 grams of the same cheese curd used in the preceding experiment were sterilized in steam at 120° C. Each of a series of these flasks received 1 gram of sterile lactose and was inoculated with the same culture of lactic-acid bacteria which had been used in the preceding experiment. Additional sterile flasks received 1 cc., 1.5 cc., and 2 cc. of chemically pure lactic acid but were not inoculated with bacteria. Bacteriological examinations made in connection with each analysis showed that the lactose series contained but the single species of bacteria with which it had been inoculated while the sterile flasks to which acid had been added remained sterile. The amount of nitrogen insoluble in water but soluble in a 5 per cent. solution of sodium chloride at the end of various intervals expressed in per cents of the total nitrogen is given in the following table.

**Table III.**—Paracasein Monolactate Formed in Cheese Curd by Bacteria and by Acid.

<table>
<thead>
<tr>
<th>Date,</th>
<th>Lactic-acid bacteria</th>
<th>Sterile flasks and chemically pure lactic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 gm. lactose.</td>
<td>1 cc.</td>
</tr>
<tr>
<td>February 21—Initial</td>
<td>3.90</td>
<td>3.90</td>
</tr>
<tr>
<td>March 10</td>
<td>4.34</td>
<td>4.34</td>
</tr>
<tr>
<td>March 24</td>
<td>23.31</td>
<td>20.60</td>
</tr>
<tr>
<td>May 22</td>
<td>9.76</td>
<td>7.32</td>
</tr>
<tr>
<td>August 15</td>
<td>3.52</td>
<td>3.25</td>
</tr>
<tr>
<td></td>
<td>3.25</td>
<td>3.52</td>
</tr>
</tbody>
</table>
From the above results it would seem that 1 cc. or more of lactic acid to 25 grams of curd (or 4 per ct.) was too great an amount for the existence of paracasein monolactate.

In the flasks containing the bacteria and 1 gram of lactose we are able to follow in some detail the results of the gradual formation of a large amount of acid. On March 10 a considerable portion of the sugar in these flasks was yet untouched and about \( \frac{3}{4} \) of the nitrogen was present as the monosalt. Two weeks later the sugar had nearly disappeared and less than 1-10 of the nitrogen remained in the salt-soluble form. By May 22 all the sugar had disappeared and the amount of monolactate had fallen to a very low figure where it remained August 15.

From these two experiments it seems fair to conclude that in forming the amount of monolactate ordinarily present in cheese the bacteria use up an amount of sugar equal to more than 2 per ct. and less than 4 per ct. of the cheese mass.

THE PRACTICAL OBJECTION TO TOO MUCH ACID.

The formation of acid is an unavoidable and apparently a necessary step in our present method of making cheddar cheese. However, the amount of acid which is really needed is small and is very easily exceeded to the detriment of the product. The exact extent to which the development of acid is desirable varies considerably, depending upon the temperature of curing and the market for which the cheese is intended, but in general the formation of acid is carried to the point where there is a decided mellowing of the curd. Whenever the formation of acid is carried much beyond this point the curd rapidly becomes plastic and refuses to part with the whey still contained within it.

Put to press in this condition the sugar contained in the excess of whey is broken down to acid, changing the curd from the plastic condition into a tough, resistant mass with a distinct acid odor. With this change in consistency the whey is set free and runs out upon the shelves. The ripening processes of such cheese are commonly retarded and the resulting flavor is bad.

LACTIC-ACID BACTERIA ARE NUMEROUS AND ACTIVE IN CHEESE.

It is but natural that very many of the lactic-acid bacteria which are present in the factory milk should pass over into the
cheese curd. Here the presence of the acid reaction resulting from the breaking up of sugar gives them an advantage over their competitors and this advantage is increased by the continued breaking down of the sugar contained in the curd.

The earliest attempt at determining the number of bacteria of different kinds present in ripening cheese by Adametz\(^6\) showed that in both emmenthaler and backstein the lactic acid group included by far the larger part of the individuals. The work of von Freudenreich\(^7\) on emmenthaler cheese has emphasized the fact that during the ripening period of this cheese there are few but lactic bacteria present. Russell & Weinziirl\(^8\) showed that while a considerable number of liquefying bacteria was present in the milk at the time the rennet was added this group suffered a marked decline during the curing, while the lactic-acid germs flourished, especially during the first few weeks of the life of a cheddar cheese.

In a recent article Babcock, Russell, Vivian & Hastings\(^9\) have demonstrated in detail that the ability of lactic-acid bacteria to displace the other forms in cheese depends primarily upon the action of these germs upon the milk sugar. When the milk sugar was largely removed from the fresh curd by repeatedly washing it with warm water the liquefying bacteria were abundant in the resulting cheese and this cheese differed markedly from the normal in its physical and chemical properties. In a duplicate portion of this washed curd in which the sugar was artificially replaced, this increase in the number of liquefying germs was prevented and this cheese did not differ widely from the normal in its flora or in its physical or chemical properties. The most evident difference between the normal cheese and that made from washed curd with the subsequent addition of sugar lay in the failure of the latter to reproduce exactly the normal cheese flavor.

When considering the flora of cheese, interest is commonly so centered upon this striking increase in the lactic forms that the

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presence of other organisms is usually overlooked. It should not be forgotten that while the number of liquefiers rarely amounts to more than one per ct. of the total during the early history of the cheddar cheese, even under these circumstances their number is considerable. It is also not unreasonable to suppose that an enzyme formed by this class of organisms will continue to act in the cheese even after the disappearance of the living cells. The long-continued presence of this small number of liquefying organisms in the case of a 28-pound cheddar cheese is well illustrated by the following determinations made by Nicholson.

**Table IV.—Bacteria Per Gram in a Ripening Cheddar Cheese.**

<table>
<thead>
<tr>
<th>Age in days</th>
<th>2.</th>
<th>4.</th>
<th>6.</th>
<th>21.</th>
<th>30.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number</td>
<td>13,582,000</td>
<td>18,990,000</td>
<td>17,387,000</td>
<td>12,846,000</td>
<td>19,500,000</td>
</tr>
<tr>
<td>Liquefiers</td>
<td>1,200</td>
<td>*920</td>
<td>*840</td>
<td>4,100</td>
<td>13,250</td>
</tr>
</tbody>
</table>

* Spores determined by heating to 65° C.

<table>
<thead>
<tr>
<th>Age in days</th>
<th>49.</th>
<th>57.</th>
<th>62.</th>
<th>68.</th>
<th>79.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number</td>
<td>3,730,000</td>
<td>3,285,000</td>
<td>60,300</td>
<td>67,460</td>
<td>24,500</td>
</tr>
<tr>
<td>Liquefiers</td>
<td>9,500</td>
<td>2,000</td>
<td>2,000</td>
<td>400</td>
<td>0</td>
</tr>
</tbody>
</table>

At the end of 62 days this cheese was pronounced ripened and of fine quality from the commercial standpoint.

These results are quite in accord with the determinations which we have made upon a considerable number of cheddar cheeses. During the early period of its life history when the cheese is rapidly passing through the ripening changes the flora consists very largely of lactic-acid organisms with a small proportion of liquefying bacteria.

**Effect of Acid Upon the Digestive Action of the Rennet.**

It has been known for many years that the use of larger quantities of rennet would quicken the rate of ripening in cheese and cheese-makers use this knowledge in hastening the ripening
of their product. However it was not until 1900\textsuperscript{10} that experimental proof was brought forth to show that this quickening of the ripening process was due to the digestive action of a peptic enzyme in the rennet.

The part taken by the rennet enzyme in the ripening of cheese has been discussed at length in Bulletin No. 233 of this Station and the reader is referred to that bulletin for a detailed treatment of the subject. In the present connection it will be sufficient to point out that under normal conditions the change of sugar into acid is necessary in order that the peptic enzyme of the rennet may play its important part in the breaking down of the cheese casein. As illustrative of this point there is given below a summary of the chemical changes observed in two of a series of cheeses made for the purpose of studying this relation.

In order to measure the digestive action of the rennet it was necessary to remove the other factors which might assist in the breaking down of the casein. It has been shown in Bulletin No. 233 that a momentary heating to 85° C. will destroy the enzymes in the milk itself and the milk used in making these cheeses was heated above 95° C. for this purpose. In order to prevent bacterial action 2 to 3 per ct. of chloroform by volume was added to the milk shortly after the above heating.

The milk was made into cheese in the usual manner except that calcium chloride was added to the milk to assist the curdling action of the rennet. In cheese 6.17IV a total of .2 per ct. lactic acid was added in small diluted portions at intervals during the process of manufacture to simulate the formation of the same by bacteria under normal conditions. In 6.17V this acid was omitted.

\textsuperscript{10} Babcock, Russell & Vivian. Ann. Rept. Wis. Station, 17:102. (1900.)
Also Cent. f. Bakt., 11 Abt., 6:817. (1900.)
TABLE V.—Effect of Acid Compounds Upon the Digestive Action of the Rennet.

<table>
<thead>
<tr>
<th>Age in months</th>
<th>Percentage of nitrogen of total nitrogen in cheese.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6.17 IV (acid)</td>
</tr>
<tr>
<td></td>
<td>Salt-soluble</td>
</tr>
<tr>
<td>Initial</td>
<td>26.62</td>
</tr>
<tr>
<td>3</td>
<td>28.52</td>
</tr>
<tr>
<td>6</td>
<td>19.06</td>
</tr>
<tr>
<td>9</td>
<td>19.94</td>
</tr>
<tr>
<td>12</td>
<td>11.97</td>
</tr>
</tbody>
</table>

From the above results it is clearly seen that in the presence of the salt-soluble compound the rennet enzyme was able to change considerable quantities of nitrogen into a water-soluble form while under similar conditions except for the action of the acid the rennet enzyme did very little work. From this we conclude that the activity of the lactic-acid bacteria in changing sugar into acid is a necessary preliminary to the digestive action of the rennet in cheese under normal conditions.

CONCLUSION.

In the preceding pages the attempt has been made to follow the process of cheese-making and the first steps of cheese-ripening and to trace in outline the part played by the acid-forming bacteria. It has been shown that all of the observed changes up to the point where the digestion by the rennet leaves off are either the direct result of the action of these bacteria or are largely influenced by bacterial action.

A discussion of the early stages of cheese-ripening would be incomplete unless consideration was given to the part played by the enzymes of the milk itself. However, the effect of these enzymes is not confined to the first stages of the ripening, and as the subject will be later treated in a separate bulletin it will not be further discussed at this time.

Under the conditions which exist in normal cheddar cheese the action of the rennet enzymes probably extends but little beyond
the formation of peptones, while a ripened cheese is characterized by the presence of large quantities of the simpler nitrogenous compounds. Since the flavors, which lend greatest value to cheese, are probably associated with the formation of these simpler compounds, this unexplained portion of cheese-ripening is of the greatest practical interest and the influence of bacteria upon this formation as well as the part taken in the same by the enzymes of the milk are at present the subject of investigation.