

The phosphorus given above by titration is an average of from two to five results, done by the men at the works.

Standard phosphorus pig iron of Mr. Camp's.	Phosphorus by weighing yellow precipitate. Per cent.	Phosphorus by above method. Per cent.
1.....	0.109	0.108 \pm
2.....	0.109	0.109 \pm
3.....	0.109	0.109 \pm
4.....	0.109	0.109 \pm

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THE DETERMINATION OF AMMONIA IN MILK.

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THE object of this work was to find a method sufficiently delicate for the determination of such small quantities of ammonia as exist in fresh milk, and in which the danger of splitting off ammonia from organic matter during the determination should be reduced to a minimum. Such a method would not only be useful in the study of certain types of fermentation, but would also probably yield results of value from a sanitary standpoint. Changes which result in a breaking-down of proteid matter are more likely to render milk unwholesome than those which affect only the milk sugar, and, while it is possible that some better index of proteid decomposition may be found, the determination of ammonia naturally suggests itself in this connection.

The method which appears to have been commonly used for the determination of ammonia in milk or cheese is to boil either the aqueous infusion of the sample or the filtrate from the precipitation of proteids with magnesium oxide or barium or sodium carbonate under atmospheric pressure, the liberated ammonia being caught in standard acid. Under these conditions urea gradually breaks down with the formation of ammonia. The ammonia which could be thus split off from the amount of urea probably present would doubtless be negligible as compared with the amounts ordinarily found in the analysis of cheese, but might entirely vitiate the determination of ammonia in fresh milk. In

our earlier experiments we made several attempts to determine the free ammonia in milk on the principle used in water analysis, but found that even when 1 cc. of milk was diluted with 1000 cc. of ammonia-free water and distilled under atmospheric pressure with only 0.1 gram of sodium carbonate more ammonia was always found in the distillate than previously existed in the milk.

After a number of unsuccessful experiments with other methods, in which magnesium oxide, as well as sodium carbonate, were tried, we concluded that accurate results can only be expected when the greatest possible care is taken to prevent the cleavage of ammonia from organic compounds, and that this can best be done by lowering the temperature at which the ammonia is expelled from the alkaline liquid. Folin's method,¹ in which the ammonia is removed from the solution by a rapid current of air in the cold, offers distinct theoretical advantages, but is hardly adaptable to the conditions of the ordinary analytical laboratory. The Bous-singault-Shaffer method appeared to be equally efficient for the separation of ammonia from urea, and more promising for our present purpose. This method, developed by Shaffer² from the vacuum distillation process of Boussingault,³ has been applied by the former to urine with very satisfactory results. With the modifications which we have found necessary or desirable in adapting it to milk it is as follows:

THE BOUSSINGAULT-SHAFFER METHOD MODIFIED FOR MILK.

Fifty cc. each of the milk sample and of neutral methyl alcohol are brought into a 2-liter round-bottomed flask with 10 grams of sodium chloride⁴ and 0.5 gram of sodium carbonate.⁵

The flask is closed with a two-holed rubber stopper fitted with an inlet tube (to admit air at the end of the operation) and a Hopkins distilling head. By means of glass tubing the distilling head is connected with two absorption cylinders in series, and these in turn with a suction pump. On account of the tendency of the milk to froth during the distillation it is desirable to use a 2-liter flask when working with portions of 50 cc. If persistent

¹ *Ztschr. physiol. Chem.*, 1902-3, **37**, 161.

² *Am. J. Physiol.*, 1903, **8**, 330.

³ *Ann. chim. phys.*, 1850, **29**, 472; *J. prakt. Chem.*, 1850, **51**, 281.

⁴ The sodium chloride was not always used. See paragraph on influence of sodium chloride below.

⁵ If the milk is decidedly acid, determine the acidity and add 0.5 gram of sodium carbonate plus the amount required to neutralize the free acid present.

frothing occurs, it may be stopped by admitting a little air through the inlet tube. The absorption cylinders used were about 2.5 cm. in diameter and 20 cm. high, and together contained from 15 to 25 cc. of N/20 sulphuric acid, diluted with water to 40 cc. During the distillation these cylinders were surrounded by ice water. To guard against loss in case any liquid should be drawn out of these cylinders during the distillation, a clean suction bottle is interposed between the second cylinder and the pump. The general arrangement of apparatus was thus the same as that described and illustrated by Shaffer.¹

The apparatus being tightly connected, the suction is started and the flask is lowered into a water-bath having a temperature of 60° to 65°. In a minute or two the mixture begins to boil briskly and the temperature of the bath may then be allowed to fall several degrees, the boiling-point depending, of course, upon the efficiency of the suction. In our work the pressure was usually about 50 mm. and the boiling-point ranged from 56° to 62°. The mixture was usually distilled for fifteen minutes, the operation being discontinued by turning off the suction and at the same time admitting air through the inlet tube, both done carefully, so that the acid in the absorption cylinders shall neither be forced out into the suction bottle nor back into the distillation flask.

The distillation being finished the apparatus is disconnected, the volume of distillate noted and the whole contents of the two absorption cylinders, the suction bottle and the connecting tubing, are rinsed into a beaker, diluted to 250 cc. and the excess of acid determined by titration with N/20 sodium hydroxide. Three drops of 0.5 per cent. solution of congo red were used as indicator in all of these titrations.

After each determination the distilling flask and the pipette used in measuring the milk were carefully cleaned with a solution of potassium dichromate in strong sulphuric acid, thoroughly washed until every trace of acid or curd was removed and then rinsed once or twice with distilled water practically free from ammonia.

Correction for Methyl Alcohol.—The methyl alcohol which collects in the absorption cylinders was found to have a very appreciable influence upon the end-point in the final titration, re-

¹ *Loc. cit.*

quiring a correction which should be varied according to the amount present in each case. This is the reason for noting the volume of distillate collected. Since the standard acid used for the absorption was always diluted to 40 cc., the total volume of liquid in cylinders (and suction bottle) at the end of the distillation shows the amount of distillate which has been caught. As the result of many blank experiments it was found that when the volume of distillate was from 30 to 39 cc. the alcohol present affected the end point to an extent equivalent to 0.4 cc. of N/20 alkali. The corresponding correction for 40-49 cc. of distillate was 0.5 cc. and for 50-60 cc. of distillate, 0.6 cc. In our regular determinations the volume of distillate was always between 30 and 60 cc.—usually between 45 and 55 cc.

Influence of Sodium Chloride.—Obviously the saturation of the boiling mixture with sodium chloride as recommended by Shaffer will lessen the hydrolytic dissociation of the sodium carbonate and thus render less likely the cleavage of ammonia from organic matter. On the other hand, the purest available sodium chloride contained sufficient ammonia to require a correction of 0.34 mg. or as much as was found in 50 cc. of some fresh milks distilled without the addition of the salt. In view of the fact that many of our samples contained so little ammonia and that those first tested gave no indication that the use of salt was necessary, it was omitted in all of our earlier work. Later it was found that while the presence of salt made little if any difference in the distillation of fresh milk, most of the older samples yielded appreciably less ammonia when saturated with salt before distillation.

The following cases are typical.

A sample of bottled milk purchased from one of the largest dealers in the city on July 7, 1904, and examined the same day, yielded with salt 0.00033 per cent. ammonia; without salt 0.00049 per cent. The difference corresponds to only 0.1 cc. N/20 alkali and is therefore within the limits of experimental error. Four days later the same milk, which had been kept in a refrigerator except for a short time each day, yielded as the result of concordant duplicates, 0.0059 per cent. ammonia with salt and 0.0093 per cent. without, the salt in this case making a difference equivalent to 2.0 cc. of the N/20 solution.

Bottled milk from another dealer analyzed within one day after delivery, yielded 0.0004 per cent. without and 0.0002 per

cent. with salt, the difference being again within the limits of experimental error.

Portions of two or three bottles of "certified" milk from a high-class dairy, after having been kept in a refrigerator for two to four days, were mixed and allowed to stand in an open bottle in a warm room. Within a day the milk curdled without developing a sour odor but with some evolution of gas. The bottle was allowed to stand for about a week, the curd and cream rising until over half of the liquid was a nearly clear whey. The cream became rancid and the curd developed a slightly "cheesy" but not unpleasant odor. The whey was filtered into a sterilized flask and kept in a cool room for five weeks without developing any unpleasant odor. This sample yielded 0.0038 per cent. ammonia when distilled without and 0.0028 per cent. when distilled with salt. Although the amount of ammonia which had developed in this sample was remarkably small, the influence of the salt is unmistakable.

A sample of milk of fair quality one day old was contaminated during handling with a few drops of old decomposed milk, and then kept at room temperature for about five months. Considerable gas was formed, and the acidity rose until 10 cc. neutralized 35.5 cc. of N/10 alkali, using phenolphthalein as indicator. The sample developed an odor suggesting sour kumyss and a very disagreeable bitter taste. It yielded 0.0175 per cent. ammonia with, and 0.0233 per cent. without salt.

Another portion of the same lot of milk was kept uncontaminated and was mixed with about 1 part formaldehyde to 1,000 parts of milk. This milk did not curdle until about four weeks old and only a moderate amount of gas was formed after curdling. The odor resembled that of sweet buttermilk. Though the formaldehyde prevented souring for several weeks, the acidity at the end of five months was three-fourths as high as in the preceding sample, 10 cc. neutralizing 27.3 cc. of N/10 alkali. This sample when five months old, yielded 0.021 per cent. ammonia, whether distilled with or without salt.

On the whole, then, it appears that with fresh milk it makes little if any difference whether or not the solution is saturated with salt before boiling with sodium carbonate. In other words, fresh milk contains little if any material which can be decomposed so as to liberate ammonia when treated with sodium carbonate under the conditions obtaining in the vacuum distillation method while milk which has been kept without a preservative and allowed to become stale does contain some such easily decomposable constituent, so that it is necessary to diminish the dissociation of the sodium carbonate if the true amount of free ammonia is

to be found by this method. The excess of ammonia found in the absence, over that found in the presence, of salt may conveniently be referred to as "cleavage ammonia," since it undoubtedly results from the cleavage of nitrogenous organic compounds.

So far as examined, all samples which had been kept for two days or more without a preservative contained appreciable amounts of "cleavage ammonia," the quantity being, in general, roughly proportional to the degree of decomposition which had taken place in the milk. Whether the absence of "cleavage ammonia" in the sample preserved by formaldehyde is to be attributed to the further change of the unstable decomposition products into simpler and more stable forms, or to the influence of the preservative in checking the growth of those organisms to which the formation of the unstable compounds is due, cannot be stated with certainty until further observations have been made. The latter explanation appears, however, the more probable.

Evidently in the examination of samples of unknown history the use of salt to diminish the hydrolytic dissociation of the sodium carbonate should never be omitted if the true amount of free ammonia is desired. In many cases, however, it is believed that the amount of "cleavage ammonia" will throw important light upon the nature of the fermentation which has taken place. Moreover, since both "free" and "cleavage" ammonia increase as the milk becomes stale it is not improbable that the sum of the two—*i. e.*, the amount of ammonia yielded by distillation without salt—may be more useful than the true amount of free ammonia as a practical measure of proteid decomposition.

Check Experiments.—As a check upon the accuracy of the method above described a number of determinations were made with solutions containing known amounts of pure ammonium chloride. The following results were obtained:

Expt. No.	Ammonia taken. Gram.	Ammonia recovered. Gram.	Difference. Ammonia. Gram.	Equivalent to N/20 solution. cc.
1	0.0005 in water	0.00043	0.00007	0.08
2	0.0020 " "	0.00200
3	0.0040 " "	0.00393	0.00007	0.08
4	0.0060 " "	0.00597	0.00003	0.04
5	0.0080 " "	0.00809	0.00009	0.10
6	0.0120 " "	0.01195	0.00005	0.06
7	0.0020 in milk	0.00187	0.00013	0.15
8	0.0060 " "	0.00595	0.00005	0.06

While Shaffer used the method for the determination of much larger amounts of ammonia in urine, we prefer not to distil more than 10 or 12 mg. of ammonia in a determination. This corresponds to over 0.02 per cent. of ammonia, which would rarely be found except in very highly fermented samples. In such cases we prefer to use a smaller quantity of the sample diluted with water to 50 cc. The above check experiments, therefore, cover the range of all ordinary work, and allowing for the unavoidable errors of manipulation, it will be seen that the recovery of ammonia was quantitative in each case.

Since some of the samples used were preserved by means of formaldehyde (usually 1 part to 1000 of milk) check determinations were also made to prove that the addition of formaldehyde in this proportion did not interfere with the distillation and determination of ammonia by this method.

RICHARDS-WOODMAN METHOD FOR "ALKALINITY."

For the determination of the products of alkaline fermentation—"ammonia, or some substance which yields ammonia on distillation"—Richards and Woodman have published¹ the following method:

"Alkalinity.—Measure into a 750 cc. round-bottomed flask 25 cc. of the milk. Add 350 cc. of ammonia-free water and 0.5 gram of sodium carbonate and distil over about 200 cc. into a flask containing about 20 cc. of dilute sulphuric acid (1:40). Neutralize the distillate with sodium carbonate and redistil it, receiving the distillate into 15 cc. of N/10 hydrochloric acid. Titrate the excess of acid with N/10 sodium hydroxide."

It was thought that a comparison of the "alkalinity" figures obtained by this method with the "ammonia" figures obtained by vacuum distillation would be of interest, since each is designed to serve as a measure of the decomposition of proteid matter in the sanitary examination of milk.

In carrying out the Richards-Woodman process we adhered as closely as possible to the prescribed conditions and were careful to have the volume and alkalinity the same when the first distillate was redistilled, as in the original distillation. The result thus obtained, calculated as percentage of ammonia, is designated "Alkalinity figure A."

¹ "Air, Water and Food," New York, 1900, p. 152.

The liquid remaining in the flask at the end of the first distillation was restored to the original volume by adding 200 cc. of ammonia-free water and was again distilled until 200 cc. had passed over, this distillate also being caught in standard acid. Usually the volume was again restored and a third portion of 200 cc. distilled, but in most cases there was little difference in the amounts of ammonia found in the second and third distillates. Since the alkalinity of the second distillate is the result of decomposition which was doubtless also going on during the first distillation, the difference between the two might be interpreted as a measure of pre-existent ammonia or very closely allied substances. The alkalinity of the first distillate minus that of the second is designated "Alkalinity figure AA."

Determinations were also made in which the first distillate was caught directly in standard acid and titrated. The result thus obtained is given as "Alkalinity figure B." This result, corrected by subtracting the alkalinity of the second distillate, as just described, is designated "Alkalinity figure BB."

Naturally the alkalinity figure obtained by the Richards-Woodman method was, in each case, higher than the "ammonia" content as found by vacuum distillation. The table shows, however, that corrected alkalinity figures obtained by subtracting the alkalinity of the second distillate from that of the first are also almost always higher than the "ammonia" figures.

The redistillation recommended by Richards and Woodman does not seem to have a very marked influence, since the results obtained by distilling directly into standard acid are sometimes higher, sometimes lower and sometimes the same as those obtained by redistillation as in the published method.¹ The differences appear to be due mainly to the rate at which the distillation is carried on. If a sample froths badly, so that longer boiling is required to obtain the 200 cc. of distillate, the yield of ammonia is higher.

COMPARISON OF RESULTS.

Comparing the alkalinity figure obtained by the Richards-Woodman process with the ammonia obtained by vacuum distillation without the addition of salt, it will be seen that in fresh milk the former figure is several times as high as the latter. As the

¹ This point was tested in seventeen cases, the average of all the results being 0.0262 per cent. for "Alkalinity figure A" and 0.0250 per cent. for "Alkalinity figure B."

COMPARISON OF "AMMONIA" AND "ALKALINITY" FIGURES.

Sample.	Boussingault-Shaffer method ¹ ammonia. Per cent.	Alkalinity figure A as ammonia. Per cent.	Alkalinity figure AA as ammonia. Per cent.	Alkalinity figure B as ammonia. Per cent.	Alkalinity figure BB as ammonia. Per cent.
Fresh milk "A".....	0.0000	0.0079	0.0050	0.0066	0.0026
Fresh milk "A," pasteurized and kept five weeks at room temperature.....	0.0086	0.0191	0.0165	0.0175	0.0149
Fresh milk "B".....	0.0007	0.0083	0.0050	0.0069	0.0036
Fresh milk "B," kept untreated four weeks in re- frigerator.....	0.0069	0.0158	0.0135	0.0149	0.0124
Fresh milk "B," kept untreated seven weeks in re- frigerator.....	0.0206	0.0300	0.0265	0.0318	0.0280
Milk + 0.1 per cent. formaldehyde, kept six weeks at room temperature.....	0.0013	0.0119	0.0089	0.0129	0.0099
Milk + 0.1 per cent. formaldehyde, kept twenty weeks at room temperature.....	0.0210	0.0277	0.0247	0.0260	0.0234
Contaminated milk + 0.1 per cent. formalde- hyde, kept at room temperature for three weeks.....	0.0012	0.0063	0.0036
Contaminated milk + 0.1 per cent. formalde- hyde, kept at room temperature for six weeks	0.0074	0.0096	0.0076	0.0092	0.0069
Contaminated milk, kept without preservative at room temperature six weeks.....	0.0098	0.0248	0.0235	0.0248	0.0235
Contaminated milk II + 0.1 per cent. formalde- hyde, kept at room temperature one week....	0.0063	0.0109	0.0069	0.0115	0.0085
Contaminated milk II + 0.1 per cent. formalde- hyde, kept at room temperature five weeks...	0.0124	0.0191	0.0168	0.0182	0.0152
Mouldy, decomposed milk.....	0.1122	0.1254	0.1139	0.1238	0.1122

¹ In these determinations the milk was not saturated with salt before heating with sodium carbonate.

milk ages both figures increase and in general at about the same rate, so that while the absolute difference is not greatly changed, the relative difference becomes much less. Relatively, therefore, the ammonia figure increases much more rapidly as the milk becomes stale than does the alkalinity figure.

Since the actual amount of nitrogen involved in the discrepancy between the two figures is in no case as great as the amount which is said to be present in the form of urea, the following experiment was made: A solution of pure urea was prepared of such strength as to contain 0.018 per cent. of nitrogen, this being the amount of "urea-nitrogen" found in cows' milk by Camerer and Soldner.¹ This solution was tested as in the Richards-Woodman process, with the modification described above. Twenty-five cc. were mixed with 350 cc. of water and 0.5 gram sodium carbonate and distilled, catching the distillate in standard acid until 200 cc. were obtained. The solution was then restored to its original volume by adding 200 cc. of water and another portion of 200 cc. distilled off. This was repeated until five successive distillates had been obtained. On titrating these distillates it was found that in each case 0.5 mg. of ammonia, or 0.002 per cent. of ammonia, calculated on the original solution, had distilled over. This is about the amount usually found in the second and third distillations in the examination of fresh milk by the Richards-Woodman process. This experiment indicates, therefore, that the discrepancy, due to urea, is practically eliminated by the method of correction used in obtaining the figures AA and BB above, and that in most cases the greater part of the discrepancy between the "alkalinity" and "ammonia" figures must be due to some other form of organic matter.

In order to study the influence of proteids, two samples, one fresh, the other seven weeks old, were treated with tannin to remove proteids and the filtrates distilled by the "ammonia" and "alkalinity" methods and by the modifications of the latter method above described.

So far as can be judged from these experiments, it appears that in fresh milk the discrepancy between the "ammonia" and "alkalinity" figures is due to the liberation of ammonia in the alkalinity determination, (1) from urea or some substance which behaves like it, (2) from proteid matter; for when the latter was

¹ *Ztschr. Biol.*, 1898, **36**, 277-313.

removed by tannin and the influence of the former was eliminated by subtracting the ammonia obtained by redistillation, as already described, the difference between the two figures disappeared.

With the old and partially decomposed milk the alkalinity figure was higher than the results of the vacuum distillation even after both these corrections had been applied. In this case, therefore, there seems to have been present some compound other than urea and not precipitated by tannin, which yields ammonia when boiled with very dilute sodium carbonate at ordinary, but not at reduced, pressure. In this case the removal of proteids lowered the amount of ammonia found by vacuum distillation, showing that the latter split off some ammonia from the partially decomposed, though apparently none from the fresh, proteids. It has already been stated that our later experiments showed a greater yield of ammonia from old samples when distilled without, than when distilled with, the addition of sodium chloride to lower the dissociation of the sodium carbonate.

As yet sufficient experiments have not been made to determine whether the cleavage of ammonia from decomposing proteid matter can be entirely prevented by the addition of salt, under the conditions of the vacuum distillation method as here described.

The determination of ammonia by vacuum distillation is, in our hands, a more delicate process than the alkalinity determination, and even if it does not always show the amount of ammonia which actually exists free in the sample, it has the advantage over the alkalinity figure of showing much greater proportional differences between pure and contaminated samples, or between the same sample when fresh and when stale or beginning to decompose.

COMPARISON OF ACIDITY AND AMMONIA.

While the time available for the present investigation was almost entirely occupied with the study of methods for determining ammonia or alkalinity, several determinations of acidity were made in connection with this work and two experiments were carried out with the purpose of comparing the development of acidity with the formation of ammonia in milk kept under ordinary conditions. These results may be shown by tabulation as follows:

	Acidity. Degrees.	Ammonia by vacuum distillation without salt. Per cent.	Lactic acid : ammonia : : 100:
<i>First Experiment</i> (milk kept at about 10° C.).			
Bottled milk July 7, 1904, as purchased	1.4	0.0005	0.4
Same sample one day after purchased..	1.6	0.0009	0.6
Same sample three days after purchased	9.4	0.0093	1.1
<i>Second Experiment</i> (milk kept at about 10° C.).			
Fresh milk, July 12, 1904, as purchased	1.7	0.0003	0.2
Same sample one day after purchased..	1.7	0.0005	0.3
Same sample two days after purchased	5.2	0.0005	0.1
Same sample six days after purchased..	10.2	0.0063	0.7
Same sample eight days after purchased	10.3	0.0078	0.8
<i>Miscellaneous Samples</i> (kept at room temperature).			
Fresh bottled milk June 15, 1904, as purchased.....	1—2 ¹	0.0005	0.3—0.6 ¹
Same sample after two days at room temperature.....	9.8	0.0112	1.3
Milk, five months old, ² kept in cool, dark room unopened.....	29.2	0.0257	1.0
Duplicate ² with formaldehyde 1:15,000 ³	29.2	0.0224	0.9
Duplicate ² with 3 per cent. chloroform	2.5	0.0033	1.4
Milk, ⁴ two months old, with formalde- hyde 1:1000.....	2.5	0.0009	0.4
Skim milk, eighteen months old, form- aldehyde 1:1000 ³ added when sample was two days old.....	4.9	0.0112	2.5
Milk with formaldehyde 1:1000, twenty weeks old, frequently opened.....	27.3	0.0205	0.8
Contaminated milk (see above), twenty weeks old, frequently opened.....	35.2	0.0233	0.7
Milk ⁴ + formaldehyde 1:1000; three years old ³	15.9	0.0284	2.0
Milk ⁴ + formaldehyde 1:1000; four years old ³	12.5	0.0386	3.4

Without attempting any detailed discussion of these results, it may be pointed out that they show great variations in the relative amounts of acid and ammonia depending upon the nature of the milk and the conditions under which it has been kept. So far as they go they confirm the view that the acidity and the am-

¹ In this case the determination of acidity was accidentally omitted. The limits given are estimated from the acidity determinations made on other bottles of milk from the same source.

² These samples of milk were put into clean sterile bottles within two hours after milking and remained unopened until they were examined five months later.

³ When examined at the ages stated the formaldehyde had disappeared.

⁴ These samples were treated with formaldehyde within two hours after milking, were opened for analysis within the next few days and after that remained closed at room temperature in diffused daylight.

monia content serve as measures of distinct types of fermentation, and that one may increase rapidly while the other increases slowly or not at all. A further study of some of these points has been undertaken in this laboratory.

SUMMARY.

The Boussingault-Shaffer method in which the sample is mixed with methyl alcohol, made alkaline with sodium carbonate and distilled under diminished pressure, has been slightly modified and found to be sufficiently delicate for the determination of ammonia in milk where the amount is often less than 0.001 per cent.

With an alkalinity corresponding to 0.5 per cent. sodium carbonate in the mixture of milk and alcohol, a cleavage of ammonia from organic matter appeared to be brought about by this process in the case of stale, but not of fresh, milk.

If the boiling mixture is saturated with sodium chloride to reduce the dissociation of the sodium carbonate, this cleavage is largely, if not entirely, prevented. The results thus obtained are believed to represent very closely the true amount of ammonia in the samples, while the amount of "cleavage ammonia," yielded by a sample when submitted to this process without the addition of salt, appears to be of value as an indication of the condition of the proteid matter.

Methods in which the milk is made alkaline and boiled under atmospheric pressure to expel the ammonia gave high results even when the dilution of the alkali was very great. Attempts to correct the results by subtracting the amount of ammonia given off in a second period of distillation were not satisfactory.

Preliminary experiments indicate that while milk ordinarily tends to develop both acidity and ammonia on standing, there is no necessary connection between the two, since either may increase rapidly while the other increases slowly, if at all.