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THE ALCOHOL TEST IN RELATION TO MILK.

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INTRODUCTION.

The alcohol test as generally used consists in the mixing of equal volumes of alcohol and milk. Usually 2 cubic centimeters of 68 per cent alcohol are added to 2 cubic centimeters of milk and shaken gently in a test tube. The test is considered positive when a precipitate is formed, or in other terms, when a coagulum is produced. When a positive test is obtained with fresh milk from a single cow or small herd, it is generally believed that it indicates an abnormal milk, due to physiological or pathological conditions in the cow. A positive test with market milk is supposed to indicate that changes have been produced in the milk as a result of bacterial fermentations.

According to Fleischmann (11)[1] the first account of the alcohol test was published by Martinn in 1890 in the Deutsche (Berliner) Molkerei Zeitung. It is stated that Martinn used 68 per cent alcohol with equal parts of milk. Höft (13) in 1898 used the alcohol test to give an idea of the acidity of milk. He found that the higher the acidity the greater the amount of coagulation by alcohol. In the same year Petri and Maaszen (24) made use of the alcohol test to determine the quality of pasteurized milk, and Weber (31) in 1900 studied the alcohol test in relation to the so-called sterilized milk.

Since 1900 numerous investigators, mostly in Europe, have studied the alcohol test. Of those who have worked with this test Morres is probably its most ardent supporter. He strongly advocates the alcohol test in combination with the alizarin test, which he calls the alizarol test. This test will be described later. Morres and the other advocates of the alcohol test claim that it is of great value, since it affords a simple and quick means of determining the condition and keeping quality of milk.

In this country the alcohol test is used by only one large company which manufactures milk powder. Any milk which shows a precipi-

[1] See list of citations to literature at end of bulletin.

tate when mixed with equal volumes of 75 per cent alcohol is rejected by this company. We are not aware that any practical use of the test is made by any one else in America. In Europe the alcohol test is more generally used, but we are unable to state to what extent the test is employed at present, although Farrington and Woll (9) say that in European creameries and city milk depots the alcohol test is often applied to every can of milk received; milk that is sufficiently sour to be noticed by the taste will coagulate when mixed with an equal volume of 70 per cent alcohol.

The Berlin police regulation of 1902 (32) regarding the sale of milk and cream required that cow's milk coming from a distance must, at the time of delivery to the consumer, stand without coagulation the cooking or alcohol test (mixture of 70 per cent alcohol by volume with equal parts of milk). According to Devarda and Weich (6), only fresh milk, which shows no precipitate or only a very fine coagulation with the alcohol test, is accepted in the Vienna market.

OBJECT OF THIS WORK.

The principal object of this work was to determine the practical value of the alcohol test as a test for the quality of market milk. As incidental to our primary object, it was our purpose to determine some of the causes for the precipitation or coagulation of milk by alcohol.

METHOD OF MAKING THE ALCOHOL TEST.

In our work we have used the single alcohol test; that is to say, a mixture of equal volumes of alcohol and milk. A few investigators have used the double alcohol test, in which two parts by volume of alcohol are mixed with one part of milk. In general equal volumes of 68 per cent alcohol and milk are mixed for the test, but in our work 75 per cent, 68 per cent, and 44 per cent alcohol were used. Three tests were made on each sample of milk, 2 c. c. of alcohol being mixed with 2 c. c. of milk in a test tube. The milk was always at a temperature of from 15° to 20° C. After adding the milk to the alcohol the tube was shaken and examined for the appearance of a precipitate. The precipitate appears as flakes the size of which were recorded as follows: VS for very small, S for small, M for medium-sized, and L for large.

The different percentages of alcohol were obtained by diluting a high grade of absolute alcohol with distilled water. Reiss (27) has shown that alcohol should always be tested for acid before using in the alcohol test, as acetic acid sometimes found in the alcohol may make the milk sufficiently acid to cause a coagulation with alcohol. The acidity was determined by titrating 10 c. c. of milk with N/10 NaOH, and is expressed throughout this paper as per cent of normal acid.

Any special methods employed in this work will be discussed when mentioned in the text.

THE ALCOHOL TEST IN RELATION TO FRESH MILK FROM A SINGLE COW OR HERD.

While reviewing the literature on the alcohol test it became evident that the value of the test must be considered from two standpoints: First, its relation to fresh milk from a single cow or small herd, and, second, its relation to mixed market milk. Although our work on this subject deals principally with the relation of the alcohol test to mixed market milk, we feel justified, after a careful survey of the literature, first in briefly discussing the test in its relation to fresh milk from a single cow or herd.

In the consideration of fresh milk from a single, normal cow we must omit the changes in milk due to bacterial growth and the influences of the changes on the alcohol test. The changes as a result of bacterial activities are of greater importance in the relation of the alcohol test to the mixed market milk and will be discussed later.

It is evident from the results of other investigators and from our own tests on milk from a few cows that fresh, normal milk occasionally coagulates with 68 or 70 per cent alcohol when mixed in equal volumes. Henkel (12) found, after an examination of more than 1,600 samples of milk from a single cow, that 6 showed a coagulation with 68 or 70 per cent alcohol. This is a very low percentage of positive results and he concluded that, generally speaking, the milk of a single animal does not coagulate with 68 or 70 per cent alcohol. After an extensive study of the alcohol test Auzinger (2) concluded that the alcohol coagulation of fresh single milk is not so rare as Henkel had observed. Auzinger (2) also found great fluctuations in the alcohol test (70 per cent) with milk from single cows. Occasionally milk from the same cow gave a positive test in the morning and not in the evening, or vice versa. The test might be positive one day and not the next, but might reappear on the third day. Sometimes he found that the first and last milk from a single cow showed fluctuations in the alcohol test. Auzinger also found that milk from single quarters may coagulate with alcohol independently of the other quarters, although these cases were rare. He concludes that the alcohol test in normal milk from a single cow is independent of the acidity and when the test is positive it is caused by a change in the milk salts, especially the calcium, in their relation to the milk proteids. His opinion as to the reason for the occasional coagulation of fresh, normal milk is strengthened by one of his experiments, in which calcium phosphate was fed to a cow. It was found that the milk from this cow coagulated with a smaller volume of alcohol or with a lower percentage of alcohol than did the normal milk.

When fresh, normal milk from a single cow coagulates with 68 per cent alcohol it is evidently due to some slight change in the composition of the milk. What the exact changes are it is impossible at present to state.

When we speak of fresh, normal milk we mean fresh milk from a healthy cow in the middle of the period of lactation. Milk in the early period of lactation, that is, colostrum milk, or milk taken late in the lactation period—"old" milk, as it is sometimes called—usually coagulates with the alcohol test. Henkel (12), Metzger (17), and also Auzinger (2), found that the milk from a cow in the first of the lactation period, while apparently normal, may show a positive alcohol test at irregular intervals. Auzinger (2) believes that the high albumen and globulin content of colostrum milk and the calcium salts are responsible for the positive alcohol reaction.

EFFECT OF COLOSTRUM AND OF "OLD" MILK ON THE ALCOHOL TEST.

In Table 1 are shown the results of the alcohol tests which we have made on colostrum milk from two cows. Three tests were made, using 75, 68, and 44 per cent alcohol. The results show clearly that colostrum milk gives a positive alcohol test and that the stronger the alcohol the longer the test will be positive. It will be noticed that the milk from cow 16 gave a positive test with 68 per cent alcohol for 24 days, although the acidity was low after the fourth day. It is evident from these results and from those obtained by other investigators that the coagulation of milk in the first of the lactation period by alcohol is largely independent of acidity.

TABLE 1.—*Alcohol tests with colostrum milk.*

Cow No.	Days after calving.	Acidity.	Alcohol test.		
			75 per cent.	68 per cent.	44 per cent.
4	2	2.61	[1] +L	+L	+VS
	3	2.45	+L	+L	+VS
	4	2.25	+L	+M	—
	5	1.87	+L	+S	—
	6	1.80	+M	—	—
	8	1.50	+M	—	—
	9	1.70	+M	—	—
	10	1.55	—	—	—
	11	1.52	+S	—	—
	12	1.50	+M	—	—
	13	1.31	+S	—	—
	19	1.35	—	—	—
	21	2.10	—	—	—
	22	1.12	—	—	—
16	1	2.40	+L	+L	—
	2	2.20	+L	+S	—
	3	2.70	+M	+M	—
	4	2.26	+L	+S	—
	5	1.60	+M	+S	—
	6	1.84	+M	+S	—
	13	1.36	+L	+M	—
	15	1.65	+M	+S	—
	16	1.53	+M	+M	—
	19	1.57	+M	+S	—
	22	1.70	+M	+S	—
	23	1.60	+M	+S	—
	24	1.50	+M	+S	—
	25	1.45	+M	—	—

[1] In this and succeeding tables the initial letters denoting the degree of the positive (+) tests signify: L, large flakes; M, medium flakes; S, small flakes; and VS, very small flakes. Minus sign (—) signifies negative test.

In order to determine whether or not the alcohol test would be positive in a mixed colostrum and normal milk, one experiment was performed. Colostrum milk from two cows 24 hours after calving was mixed in various proportions with fresh, normal milk which gave a negative alcohol test. The results of this experiment, in Table 2, show that from 80 to 90 per cent of colostrum milk had to be mixed with normal milk in order to cause a positive test with 68 per cent alcohol. When 75 per cent alcohol was used the test was positive with as low as 25 per cent of colostrum milk from cow 5, but when colostrum milk from cow 16 was used, a mixture of 80 per cent was required to give a positive reaction with 75 per cent alcohol. It seems evident from these results that the mixing of colostrum and normal milk would not cause a positive alcohol test unless a very large percentage of the milk were colostrum milk.

TABLE 2.—*The alcohol test with a mixture of normal and colostrum milk.*

Colostrum milk from cow No.	Percentage of normal milk.	Percentage of colostrum milk.	Alcohol test. 75 per cent.	68 per cent.	44 per cent.
5	10	90	[1] +L	+ L	–
	20	80	+M	+VS	–
	25	75	+M	–	–
	50	50	+S	–	–
	75	25	+S	–	–
	90	10	–	–	–
16	10	90	+M	+ M	–
	20	80	+S	–	–
	25	75	–	–	–

[1] See footnote under Table 1.

Having discussed the relation of the alcohol test to colostrum milk, let us consider its relation to milk drawn at the last of the lactation period, or what is known as "old" milk. Several investigators have shown that "old" milk gives a positive alcohol test. It is well known that milk changes in composition toward the end of the lactation period, and it is undoubtedly these changes which cause the coagulation with alcohol. While no definite changes have been attributed to the positive alcohol reaction, it is believed by some to be due to the high content of solids (not fat). Henkel (12), however, found that this could not explain in all cases the coagulation by alcohol. Auzinger (2) believes that on account of the variation of solids (not fat) the alcohol test has no significance in milk from "old" milk cows.

SUMMARY OF CAUSES FOR POSITIVE TESTS IN MILK OF SINGLE COWS.

It is apparent that fresh milk from a single cow may occasionally give a positive alcohol reaction with 68 or 70 per cent of alcohol. Colostrum milk gives a positive reaction, and the same is true usually of "old" milk—that is, milk from a cow in the last of its lactation period.

The causes for a positive alcohol test may be summarized by the opinion of Ernst (8) who states that a positive alcohol test of fresh milk from a single cow indicates a physiological or severe pathological condition of irritation of the milk glands. There is, however, a difference in the opinions of various investigators as to the reaction of the alcohol test to pathological conditions of the udder. Ruhm (28) noticed the alcohol test in milk from cows with infected udders. In some cases he found the test was positive during the infection and frequently a positive test was observed for three or four weeks later when the milk had a normal appearance and taste. He points out that in udder infection the milk may vary in many ways, and in consequence the alcohol test varies. Auzinger found that there was no relation between streptococci in infected udders and the alcohol test and that a positive test is produced through chemical changes in the secretions. Rullmann and Trommsdorff (29) also observed a positive alcohol reaction in milk from cows with infected udders, but according to these authors the alcohol test shows no definite relation to the leucocyte count. They point out that the variation in ash salts and high albumin content probably influences the alcohol test. Campbell (5) also believes that the alcohol test is of value in determining the diseased condition of the udder. Besides udder infection Auzinger (2) states that the general infections and infections of the vaginal canal may cause a positive alcohol test; also that milk from cows which have aborted may coagulate with alcohol. Metzger (17), however, after a study of the alcohol test with milk from sick cows concludes that the milk from them shows no relation between the acidity and alcohol test. According to this author fever had no influence on the acid and alcohol tests. There was no relation between tuberculosis of the animal and the alcohol test. When animals were very lean from disease the milk inclined toward coagulation with alcohol. Infectious inflammation of the vagina was without influence on the test. Infection of the uterus shows almost regularly with the alcohol test, but not without exception. Metzger also found that there was no relation between the alcohol test and various forms of indigestion. He points out that the chief value of the test lies in its use for the freshness of milk.

We have not had an opportunity to study the alcohol test in its relation to the milk from sick cows, but from a study of the literature on this subject we are inclined to believe that the alcohol test would be of but little value as a routine test of the milk from a single cow or from a small herd. If the alcohol test were used regularly to test fresh milk of single cows a positive reaction would indicate some change in the milk from normal. Subsequent examination of the cow might reveal some pathological condition, or there might be some physiological reason for a slight variation in the composition of the milk. If the test were performed on the milk from a few cows a positive reaction might be caused, as Auzinger (3) believes, by the mixing of milk which is changed by physiological or pathological conditions with milk from normal cows. If there were a large percentage of abnormal milk which gave an alcohol test with a coagulation with large flakes, the mixed milk might show a positive alcohol test in which the coagulation would be in the form of small flakes. When mixed milk from a large number of sources gives a positive alcohol test it must be interpreted in an entirely different manner, and this leads us to another phase of the subject.

THE ALCOHOL TEST IN RELATION TO MARKET MILK.

Since 1900 a considerable number of papers have appeared on the use of the alcohol test in its relation to market milk. According to Kirchner (15), Morres in 1905 showed that the alcohol test was of value for determining the keeping quality of milk and indicating its acidity. Reiss (26) in 1906 pointed out the practical value of the test, and Morres (18) again in 1909 showed the value of the alcohol test as a means of determining the keeping quality of milk. He added 2 c.c. of milk to 2 c.c. of 68 per cent (by volume) alcohol, and states that if the milk coagulates with alcohol then decomposition has already started and the extent is shown by the size of the flakes. If the precipitate is in fine flakes then the acidity corresponds to 4 degrees Soxhlet; however, the coagulation may not be due to an increase in acidity, but may be due to the action of rennet-forming bacteria. In later work Morres has combined the alcohol and alizarin tests. This will be discussed later. Morres considers that the coagulation of mixed market milk is due largely to the formation of acid or the action of rennet-forming bacteria or to a combination of both. Henkel (12) concludes from his work that the alcohol test does not afford a proper means for determining acidity, but that the value of the test lies in the fact that it gives a knowledge of the souring and other changes in the properties of milk or in variations from the normal properties which the acid test does not show. Other investigators believe that the alcohol test is of value only as a preliminary test. Fendler and Borkel (10) after a large number of tests to determine the relation of the acidity of milk to the alcohol test concluded that the double test with 70 per cent alcohol was not a proper criterion for the freshness of market milk, including infants' milk and superior grades of milk. They state that the double test using 50 per cent alcohol is suitable as a preliminary test for food inspectors, but the milk should be submitted to further tests. These authors also found that no consistent relation existed between the alcohol test and the acidity of milk. Rammstedt (25) also agrees with Fendler and Borkel, so far as he found, that no consistent relation existed between the alcohol test and the acidity of milk. He considers that the test gives preliminary knowledge of the hygienic quality of a milk.

It is evident from the literature that in a mixed market milk the acidity plays a part in connection with the alcohol test, so that in considering the factors which influence the test we may first take up the question of acidity.

THE INFLUENCE OF ACIDITY ON THE ALCOHOL TEST.

In our first experiments the acidity of milk was raised by the addition of N/10 lactic acid. The results of two experiments recorded in Table 3 show that a very slight increase in the acidity of milk may cause a positive alcohol test with 75 per cent and 68 per cent alcohol, but a considerably higher acidity is required to cause a positive test with 44 per cent alcohol.

These results show clearly that the alcohol test is sensitive to slight changes in acidity when these changes are produced by the addition of lactic acid. Since an increase in acidity will cause a positive alcohol test it is evident that the growth of acid-forming bacteria in milk will cause a positive test.

TABLE 3.—*Influence of acidity on the alcohol test.*

N/10 lactic acid added to 50 c. c. of milk.	Acidity.	Alcohol test.		
		75 per cent.	68 per cent.	44 per cent.
c. c.				
0	1.81	–	–	–
0.5	1.88	–	–	–
1.0	1.94	[1]+M	+M	–
3.0	2.21	+L	+L	–
3.5	2.38	+L	+L	–
4.0	2.47	+L	+L	+M
0	1.70	–	–	–
.5	1.76	+M	–	–
1.0	1.84	+M	+S	–
2.0	2.00	+L	+L	–
3.0	2.20	+L	+L	–
3.4	2.25	+L	+L	–
3.5	2.26	+L	+L	+few VS
4.0	2.31	+L	+L	+few L

[1] See footnote under Table 1.

In order to determine the relation between the number of acid-forming bacteria, the acidity, and the alcohol test, two experiments were performed, using a pure culture of a lactic-acid-producing organism. The culture was inoculated into sterile skim milk and incubated at 37° C. A bacterial count was made while the acidity and the alcohol test were determined at the same time. From the results shown in Table 4 it may be seen that in Experiment I the alcohol test was negative even after seven hours of incubation. At that time the acidity had increased from 1.98 to 2.14, and the bacteria from from 82,000 to 15,100,000 per cubic centimeter. It is interesting to note that an extensive multiplication of lactic-acid-forming bacteria may occur without causing a positive alcohol test. In the second experiment, also shown in Table 4, a heavier inoculation was used, and it will be seen that the milk at the beginning of the incubation period contained 480,000 bacteria per cubic centimeter. The 68 per cent alcohol test was not positive until the bacteria had increased to 31,400,000 per cubic centimeter.

These figures show that when a pure culture of lactic-acid-forming bacteria is grown in skim milk there must be a very great increase in order to produce acidity enough to cause a positive alcohol test. In these experiments there were no positive alcohol tests until the bacteria had increased from less than 500,000 to over 16,000,000 per cubic centimeter. From these results it is apparent that the growth of acid-forming bacteria in milk may, through the formation of acid, cause a positive alcohol test. However, when there is sufficient acid produced to cause a coagulation with 68 per cent alcohol the number of acid-forming bacteria would be very high.

TABLE 4.—*Influence on the alcohol test of acid produced by the growth of a pure culture of lactic-acid bacteria.*

Experiment No.	Age of culture in hours.	Bacteria per cubic centimeter.	Acidity.	Alcohol test.		
				75 per cent.	68 per cent.	44 per cent.
I	0	82,000	1.98	–	–	–
	2	113,000	....	–	–	–
	4	1,510,000	2.06	–	–	–
	5	4,300,000	2.08	–	–	–
	6	11,700,000	2.09	–	–	–
	7	15,100,000	2.14	–	–	–
II	0	480,000	1.94	–	–	–
	2	1,060,000	....	–	–	–
	4	7,500,000	2.08	–	–	–
	5	16,100,000	2.08	[1]+S	–	–
	6	31,400,000	2.30	+L	+M	–
	7	46,000,000	2.47	+L	+L	–

[1] See footnote under Table 1.

EFFECT OF PHOSPHATES.

We have so far discussed in a general way the effect of increasing the acidity of milk by the addition of lactic acid and by the generation of the acid in milk. Since the acidity of milk when titrated with phenolphthalein is due partly to acid phosphates, it will be of interest to show the effect on the alcohol test of the increase in acidity by acid phosphates. In Table 5 are shown the results of a few tests, using sodium and potassium acid phosphate. Various amounts of a 5 per cent solution of these salts were added to 50 c. c. of milk. It will be seen from the table that when the acidity was increased by sodium acid phosphate from 2.15 to 3.33 the alcohol test with 75 per cent alcohol was positive. At an acidity of 4.27 the milk coagulated with 68 per cent alcohol but the flakes were very small. In order to cause a coagulation with 68 per cent alcohol with medium-sized flakes it was necessary to increase the acidity to 6.16. When potassium acid phosphate was used the results were about the same.

These results show that it is possible by increasing the acidity of milk with acid phosphates to cause a coagulation with the alcohol test, but the acidity has to be increased to a high degree and there would never be enough acid phosphate in a mixed market milk for it to be entirely responsible for a positive alcohol test.

TABLE 5.—*Influence on the alcohol test of the addition of acid phosphates to milk.*

Sodium-acid phosphate.					Potassium-acid phosphate.				
Amount of 5 per cent solution of acid phosphate added to 50 c. c. of milk.	Acidity.	Alcohol test.			Amount of 5 per cent solution of acid phosphate added to 50 c. c. of milk.	Acidity.	Alcohol test.		
		75 per cent.	68 per cent.	44 per cent.			75 per cent.	68 per cent.	44 per cent.
C. c.					*C. c.*				
0	2.15	—	—	—	0	....	—	—	—
1	2.75	—	—	—	1	2.52	—	—	—
2	3.33	[1] +M	—	—	2	3.13	[1] +M	—	—
3	4.27	+M	+VS	—	3	4.00	+M	+VS	—
5	5.50	+M	+VS	—	5	5.20	+M	+VS	—
6	6.16	+M	+M	+VS	6	5.62	+M	+M	+VS

[1] See footnote under Table 1.

In some cases where we increased the acidity of milk by adding lactic acid it was noticed that a very slight increase in acidity caused a positive alcohol test. At other times the acidity had to be increased to a considerable extent before the milk coagulated with alcohol. It occurred to us that the explanation for these differences might be that there were different amounts of dibasic phosphates present in milk and that the acid converted the dibasic phosphate into acid phosphate, which increased the acidity but did not cause a positive alcohol test. In order to test this theory one experiment was performed, the results of which are shown in Table 6. Two flasks of milk were used, each containing 50 c. c. of milk. One flask was left normal and 0.5 per cent dibasic sodium phosphate was added to the other. Various amounts of N/10 lactic acid were then added to each flask. As may be seen from the table, when 3 c. c. of N/10 lactic acid was added to the normal milk, the acidity was 2.37 and the alcohol test was positive with both 75 per cent and 68 per cent alcohol. The flakes were large and medium, respectively. The same amount of acid added to the milk with dibasic phosphate increased the acidity to 2.55 and yet the alcohol test was negative. When 7 c. c. of N/10 lactic acid was added to the normal milk, the acidity was increased to 3.00 and the milk coagulated with large flakes with all the different percentages of alcohol. When 7 c. c. of N/10 lactic acid was added to milk with dibasic phosphate the acidity was increased to 3.05 and only the 75 per cent alcohol test was positive, and the coagulation was in the form of small flakes. When 8 c. c. of acid was added to the milk with dibasic phosphate the acidity was 3.19 and the alcohol test with both 75 per cent and 68 per cent alcohol was positive. It was found by titration that 10 c. c. of a 0.5 solution of dibasic phosphate required 1.56 c. c. of N/10 lactic acid to convert the dibasic into the monobasic phosphate; therefore 50 c. c. of milk containing 0.5 per cent of dibasic sodium phosphate would require 7.8 c. c. of N/10 lactic acid to convert the dibasic into the monobasic phosphate. It will be seen from Table 6 that when from 7 to 8 c. c. of N/10 lactic acid was added to the milk with dibasic phosphate, the alcohol test became positive; that is, when the dibasic phosphate had been converted into monobasic phosphate then further increase in acidity caused a positive alcohol test.

As a very general explanation of this result it may be said that when acid is added to milk it converts the dibasic phosphate into the monobasic phosphate. It follows that the acid and also the monobasic phosphate probably affect the casein and thereby change it into a condition in which it is possible to precipitate the casein by alcohol and cause a positive test. This action on the dibasic phosphate probably explains in part the positive alcohol tests with different low acidities.

TABLE 6.—*Influence on the alcohol test of the addition of dibasic phosphate to milk.*

N/10 lactic acid added to 50 c. c. of milk.	Normal milk.				Normal milk + 0.5 per cent dibasic sodium phosphate.			
	Acidity.	Alcohol test.			Acidity.	Alcohol test.		
		75 per cent.	68 per cent.	44 per cent.		75 per cent.	68 per cent.	44 per cent.
c. c.								
0	1.85	–	–	–	2.03	–	–	–
1	2.01	[1] +S	–	–	2.13	–	–	–
3	2.37	+L	+M	–	2.55	–	–	–
5	2.63	+L	+L	+S	2.81	–	–	–
6	2.80	+L	+L	+M	2.92	–	–	–
7	3.00	+L	+L	+L	3.05	+S	–	–
8	....	....	....	....	3.19	+L	+M	–
9	....	....	....	....	3.43	+L	+L	–
11	....	....	....	....	3.97	+L	+L	+L

[1] See footnote under Table 1.

RESULT OF MIXING SOUR AND NORMAL MILK.

Since a positive alcohol test may be produced by increasing the acidity, several investigators have pointed out that a mixture of sour and normal milks will give a positive test. The amount of sour milk which can be added to fresh milk without causing a positive alcohol test will, of course, depend upon the acidity of the sour milk. In one experiment, the results of which are shown in Table 7, various percentages of sour, raw, and pasteurized milk were added to fresh milk. The addition of 1 per cent of sour milk caused a positive test with 75 per cent alcohol, 2.5 per cent caused a positive test with 68 per cent alcohol, and the addition of 10 per cent of sour milk was necessary to cause a positive test with 44 per cent alcohol.

It must be taken into consideration in this experiment that the sour milk had a high acidity. If the acidity had been low a much higher per cent could undoubtedly have been added to the fresh milk without increasing the acidity sufficiently to cause a positive alcohol test.

TABLE 7.—*The alcohol test with a mixture of normal and sour milk.*

Addition of sour raw milk. Acidity 10.23.					Addition of sour pasteurized milk. Acidity 9.87.				
Per cent of sour milk added.	Acidity.	Alcohol test.			Per cent of sour milk added.	Acidity.	Alcohol test.		
		75 per cent.	68 per cent.	44 per cent.			75 per cent.	68 per cent.	44 per cent.
0.0	1.84	–	–	–	....	....	–	–	–
1.0	1.93	[1] +S	–	–	1.0	1.88	+S	–	–
2.5	2.06	+M	+M	–	2.5	2.00	+M	+M	–
5.0	2.30	+L	+L	–	5.0	2.29	+L	+L	–
10.0	2.70	+L	+L	+M	10.0	2.68	+L	+L	+VS

[1] See footnote under Table 1.

In connection with the relation of acidity to the alcohol test the question arises as to whether or not the acidity of a sour milk can be neutralized so that the alcohol test will be negative. Some investigators have shown that the neutralization of the acidity does not cause a positive test to become negative, although the size of the flakes in the coagulation is somewhat reduced. We have tried one experiment in which various amounts of normal lactic acid were added to fresh milk, after which the acidity was reduced to the original acidity by the addition of sodium hydrate. From the results which are shown in Table 8 it will be seen that when the acidity was increased to 4.3, then neutralized to 1.90, the 68 per cent alcohol test was positive. The positive alcohol tests with 68 per cent alcohol could be made negative at acidities below 4.30 by reducing to about the original acidity of the normal milk.

TABLE 8.—*Effect on the alcohol test produced by neutralizing the acidity of milk.*

Amount of normal lactic acid added to 50 c. c. of milk.	Acidity.	Alcohol test.			Acidity after neutralizing.	Alcohol test.		
		75 per cent.	68 per cent.	44 per cent.		75 per cent.	68 per cent.	44 per cent.
c. c.								
0.0	1.84	–	–	–	....	....	....	....
0.1	2.08	[1] +M	+M	–	1.80	–	–	–
0.3	2.42	+M	+M	–	1.81	–	–	–
0.5	2.99	+L	+M	+VS	1.64	–	–	–
0.8	3.54	+L	+L	+M	1.60	+VS	–	–
1.0	3.94	+L	+L	+L	1.60	+S	–	–
1.5	4.30	+L	+L	+L	1.90	+L	+M	–

[1] See footnote under Table 1.

EFFECT OF HEAT COMBINED WITH ACIDITY.

As a matter of general interest we may mention the effect of heating milk which gave a positive alcohol test. Auzinger (2) found that a milk which gave a positive test at 15° C. sometimes did not give the test when heated for 30 minutes at 60° C. Then again he found that the test might remain positive in milk heated to boiling. In Table 9 is shown the result of an experiment showing the effect of heat on the alcohol test with milk of two different acidities. No effect of heat was found on the sample of milk with an acidity of 2.30, but there was a marked effect when the acidity was lower.

We have no explanation to offer for this negative result of the test when the acidity is low. This action of heat might be of importance when the alcohol test is applied to pasteurized milk.

TABLE 9.—*Effect of heat on the alcohol test which is positive on account of acid action.*

Milk heated to—	Alcohol test.			
	Acidity, 2.		Acidity, 2.30.	
	75 per cent.	68 per cent.	75 per cent.	68 per cent.
°*C.*				
Not heated...	[1] +M	+S	+L	+L
40............	+M	+S	+L	+L
60............	+M	+S	+L	+L
70............	+M	+VS	+L	+L
80............	+S	–	+L	+L
90............	–	–	+L	+L
100...........	–	–	+L	+L

[1] See footnote under Table 1.

INFLUENCE OF THE ACTION OF RENNET.

The relation of the alcohol test to the acidity of milk shows that acidity is one factor which may cause a positive alcohol reaction, but from the work of other investigators it is evident that it is not the sole cause. Morres throughout his papers points out that the alcohol test may be caused by an acid fermentation or by a rennet fermentation or by a mixture of both fermentations.

In order to determine the effect of rennet action in relation to the test, we first tried the effect of prepared rennet. Four flasks of fresh milk were used and to each a different percentage of rennet was added. The milk in each flask was tested by the alcohol test at intervals of one hour. It will be seen from Table 10 that four different percentages of rennet were used, ranging from 0.00005 per cent to 0.0015 per cent. The acidity of the milk increased during the four hours from 1.64 to 1.70; therefore the influence of acidity can be neglected, since it is only a slight change.

The results show that the action of rennet in milk may produce changes which cause a positive alcohol test and that two main factors are of importance, namely, the amount of rennet and the length of time the rennet has to act. Undoubtedly a third factor must be taken into consideration; that is, the temperature at which the milk is held. In our experiments the milk was held at room temperature. These results confirm those obtained by other investigators and indicate that the action of the rennet-forming bacteria might cause a positive alcohol reaction.

TABLE 10.—*Influence of rennet on the alcohol test.*

Hours.	Rennet added.	Alcohol test.		
		75 per cent.	68 per cent.	44 per cent.
	Per cent.			
0	.00005	–	–	–
	.00025	–	–	–
	.0005	–	–	–
	.0015	–	–	–
1	.00005	–	–	–
	.00025	–	–	–
	.0005	–	–	–
	.0015	[1] +M	+M	–
2	.00005	–	–	–
	.00025	–	–	–
	.0005	+M	+M	–
	.0015	+L	+L	+M
3	.00005	–	–	–
	.00025	+M	+M	–
	.0005	+M	+M	–
	.0015	+L	+L	+M
4	.00005	–	–	–
	.00025	+L	+L	L
	.0005	+L	+L	+VS
	.0015	(2)	(2)	(2)

[1] See footnote under Table 1.
[2] Milk curdled by rennet.

In order to show the effect of rennet of bacterial origin, the action of a pure culture of a rennet-forming organism was studied. Two flasks of sterile skim milk were inoculated with different amounts of a pure culture of a rennet-forming organism. These flasks were incubated at 37° C., and the bacterial increase was determined at definite intervals, together with the alcohol test. The results are shown in Table 11. From a study of the table it is evident that rennet-forming bacteria will cause a positive alcohol test, but there must be a large bacterial increase to produce rennet enough to cause a positive test.

The acidity was also increased during the incubation, but we believe this acidity played a minor part in causing the positive alcohol test.

TABLE 11.—*Influence on the alcohol test of rennet produced in milk by the growth of a pure culture of a rennet-forming organism.*

Experiment No.	Age of culture.	Bacteria per cubic centimeter.	Acidity.	Alcohol test.		
				75 per cent.	68 per cent.	44 per cent.
	Hours.					
I	0	34,000	1.98	–	–	–
	2	62,000	....	–	–	–
	4	4,700,000	2.02	–	–	–
	5	9,000,000	2.06	[1] +L	–	–
	6	21,000,000	2.10	+L	+L	–
	7	31,000,000	2.11	+L	+L	+L
II	0	147,000	1.94	–	–	–
	2	200,000	....	–	–	–
	4	15,000,000	2.10	+L	+L	+S

[1] See footnote under Table 1.

DIFFERENTIATION BETWEEN ACIDITY AND RENNET ACTION.

The fact that reducing the acidity did not cause a negative alcohol test, as mentioned above, led us to believe that it might be possible to differentiate between a positive alcohol test caused by acidity and one caused by rennet action. In order to determine whether this was true two flasks of sterile skim milk were prepared. One was inoculated with a pure culture of a lactic-acid-forming organism and the other with equal amounts of a pure culture of lactic-acid bacteria and rennet-forming bacteria. The two flasks were then incubated at 37° C. As may be seen from Table 12, the milk containing the lactic-acid bacteria had an acidity of 2.23 after 3 hours' incubation and the test was positive with both 75 per cent and 68 per cent alcohol. When the acidity was reduced to 1.49 all the alcohol tests were negative. The milk containing a mixed culture of lactic-acid bacteria and rennet-forming bacteria after 3 hours' incubation had an acidity of 2.32 and the alcohol test was positive with 75 per cent and 68 per cent alcohol. In both cases the coagulation was in the form of large flakes. When the acidity was reduced to 1.70 the alcohol test remained positive, although the size of the flakes was reduced. This milk after 5½ hours' incubation had an acidity of 4.38 and the milk coagulated with large flakes with each percentage of alcohol. When the acidity was reduced to 1.49 the alcohol test remained positive, the only change being with the 44 per cent alcohol, in which case the size of the flakes was reduced. This experiment was repeated, as will be seen from Table 12, and the results confirmed those of the first experiment. These results indicate that it may be possible to differentiate between an acid and an acid-and-rennet fermentation in milk, provided the acidity is not high.

TABLE 12.—*Differentiation between an acid and a mixed acid-and-rennet fermentation by neutralizing the acidity and using the alcohol test.*

Experiment No.	Pure culture of—	After incubation at 37° C. for 3 hours.				After incubation at 37° C. for 5½ hours.			
		Acidity.	Alcohol test.			Acidity.	Alcohol test.		
			75 per cent.	68 per cent.	44 per cent.		75 per cent.	68 per cent.	44 per cent.
I.	Lactic-acid bacteria.	2.23	[1]+L	+L	–	3.21	+L	+L	+L
		Neutralized to 1.66.	–	–	–	Neutralized to 1.49.	–	–	–
	Mixture of rennet-forming and lactic-acid bacteria.	2.32	+L	+L	–	4.38	+L	+L	+L
		Neutralized to 1.70.	+S	+VS	–	Neutralized to 1.49.	+L	+L	+S
II.	Lactic-acid bacteria.	....	....	....	....	[2] 2.70	+L	+M	–
	Mixture of rennet-forming and lactic-acid bacteria.	[3] 2.00	+L	+M	–	[2] 3.42	+L	+L	+L
		Neutralized to 1.80.	–	–	–	Neutralized to 1.60.	+L	+M	+VS

NOTE.—Acidity of normal milk in experiment I, 1.75; in experiment II, 1.78.

[1] See footnote under Table 1.
[2] After 4½ hours incubation at 37° C.
[3] Acidity after adding pure cultures to milk and before incubation.

EFFECT OF HEAT COMBINED WITH RENNET ACTION.

Earlier in this paper we have shown the effect of heat on the alcohol test with milk of high and low acidity, and as a matter of general interest the effect of heat on the alcohol test produced by rennet action may now be considered. The results of two experiments shown in Table 13 explain themselves clearly. Sufficient rennet was added to two samples of milk to cause a positive alcohol test with 75 and 68 per cent alcohol. The milk was then heated, and it was found that at 90° C. the milk no longer gave a positive alcohol test. Both experiments showed the same results.

TABLE 13.—*Effect of heat on the alcohol test with milk in which the positive test is due to rennet action.*

Milk heated to—	Alcohol test.			
	75 per cent.		68 per cent.	
	Experiment I.	Experiment II.	Experiment I.	Experiment II.
°*C.*				
Not heated...	[1]+L	+L	+L	+L
40............	+L	+L	+L	+L
60............	+L	+L	+L	+L
70............	+L	+L	+L	+L
80............	+S	+VS	+S	+VS
90............	—	—	—	—

NOTE.—Acidity of milk in experiment I, 1.82; in experiment II, 1.84.

[1] See footnote under Table 1.

The results which we have shown on the effect of rennet action in relation to the alcohol test confirm the work of other investigators, and it is evident that the rennet-forming group of bacteria in milk can play an important part in the production of a positive alcohol test.

INFLUENCE ON THE ALCOHOL TEST OF CARBON DIOXID IN MILK.

There are probably numerous minor factors which influence the alcohol test with market milk. While the two principal factors are probably acidity and the effect of rennet action, it is believed by some investigators that carbon dioxid plays a more or less important part. Auzinger (2) found that milk one hour old which gave a positive alcohol test gave a negative test after it had been held for 18 hours. He believes that carbon dioxid might be partly responsible for such a change.

We have passed carbon-dioxid gas into milk many times and have always been able to cause a positive alcohol test. In one experiment carbon-dioxid gas was passed into milk until the acidity was 2.36 (titration in cold milk with phenolphthalein as an indicator), and a positive alcohol test was obtained with 75 per cent and 68 per cent alcohol. As is shown in Table 14, this milk was heated at different temperatures up to 100° C. With the increase in temperature the acidity was reduced, due probably to the expelling of the CO_2. Barillé (4) has shown that carbon dioxid forms a very unstable compound, which he calls carbono phosphate of calcium and is easily broken up by heat. When the temperature reached 70° C., the alcohol test with 68 per cent alcohol was negative and the acidity had been reduced from 2.36 to 2.05. At 90° C. the acidity was 1.91 and the alcohol test was negative with 75 per cent alcohol.

There can be no doubt as to the fact that carbon dioxid may cause a positive alcohol test, provided there is a large enough amount in the milk. In order to determine how much carbon dioxid was required to cause a positive test with 68 per cent alcohol, the gas was passed into a flask of fresh milk until a positive alcohol test was produced. The amount of CO_2 in this milk and in the original milk was then determined.[1] It was found that the normal milk contained 0.76 per cent of CO_2 by volume at 32° C., and the milk through which the gas had been passed contained 13.05 per cent of CO_2 by volume. In this experiment it was necessary to increase the CO_2 content to 13.05 per cent by volume in order to cause a positive alcohol reaction with 68 per cent alcohol. According to Kastle and Roberts (14) carbon dioxid is present in milk to the extent of 3 to 4 per cent by volume and partly escapes into the air when the milk is drawn. This being the case, it is evident that there is not enough carbon-dioxid gas in normal milk to cause of its own accord a positive alcohol test with 68 per cent alcohol. Of course, the presence of CO_2 may assist other factors to cause a positive alcohol test and in the case of bacterial fermentation where the gas is produced it might play a small part, but we believe that when 68 per cent alcohol is used in the test the influence of CO_2 in mixed market milk would be very small, if it has any effect.

TABLE 14.—*Effect of heat on alcohol test with milk-made acid to phenolphthalein with carbon dioxid.*

Milk heated to—	Acidity.	Alcohol test.	
		75 per cent.	68 per cent.
° *C.*			
Not heated	2.36	[1] +L	+L
40	2.32	+L	+L
50	2.30	+L	+M
60	2.19	+L	+VS
70	2.05	+M	−
80	2.05	+VS	−
90	1.91	−	−
100	1.92	−	−
Original milk	1.90	−	−

[1] See footnote under Table 1.

THE RELATION OF THE ALCOHOL TEST TO THE BACTERIA IN MIXED MARKET MILK.

Having discussed the effect of acidity and the effect of rennet action on the alcohol test, let us consider the relation of the test to the bacteria in market milk. Since an increased acidity and also rennet action may cause a positive test, it is natural to suppose that there may be some definite relation between the alcohol test and the number of bacteria in milk, as the increase in the acidity and the rennet in milk is the result of bacterial growth.

It is claimed by some authorities that the alcohol test is of great value for determining the freshness of milk, and as this is a question

[1] We are indebted to Dr. Clark, of the Dairy Division laboratory, for this analysis.

of great importance we have examined a number of samples of market milk which had been held for a number of days.

In Table 15 are shown the results of the examination of four samples of market milk. One was raw milk and the others were pasteurized. Each bottle of milk was obtained from a different dairy and was held in a refrigerator at a temperature of about 9° C. The acidity and alcohol test were determined daily and bacterial counts were made on the first day and again when the 68 per cent alcohol gave a positive test.

It is evident from the results obtained that the alcohol test (68 per cent) does not show the freshness of milk. The samples were held from 8 to 13 days at 9° C. before the alcohol test became positive, and during that time the bacteria had increased to more than 100,000,000 per cubic centimeter.

TABLE 15.—*Effect on the alcohol test of holding milk at 9° C.*

Days held.	Raw milk.					Pasteurized milk A.				
	Acidity.	Alcohol test. 75 per cent.	68 per cent.	44 per cent.	Bacteria per cubic centimeter.	Acidity.	Alcohol test. 75 per cent.	68 per cent.	44 per cent.	Bacteria per cubic centimeter.
0	1.72	[1]+V.S.	—	—	8,200		—	—	—	37,000
1		+V. S.	—	—			—	—	—	
2		+V. S.	—	—			—	—	—	
3		+V. S.	—	—			—	—	—	
4										
5		+V. S.	—	—			—	—	—	
6		—	—	—			—	—	—	
7		—	—	—			—	—	—	
8	1.88	+V. S.	—	—		1.78	—	—	—	
9	1.95	—	—	—		1.88	+S.	—	—	
10	2.02	—	—	—		1.93	+S.	—	—	
11										
12	2.08	+ M.	—	—		2.30	+L.	+M.	—	242,000,000
13	2.43	+ L.	+M.	—		2.48	+L.	+L.	—	
14	2.72	+ L.	+ L.	—	146,000,000	2.75	+L.	+L.	+L.	626,000,000
Days held.	Pasteurized milk B.					Pasteurized milk C.				
	Acidity.	Alcohol test. 75 per cent.	68 per cent.	44 per cent.	Bacteria per cubic centimeter.	Acidity.	Alcohol test. 75 per cent.	68 per cent.	44 per cent.	Bacteria per cubic centimeter.
0	1.74	+M.	—	—	182,000		—	—	—	133,000
1		+ L.	—	—			—	—	—	
2		+M.	—	—		1.75	—	—	—	
3		+M.	—	—			—	—	—	
4										
5		+M.	—	—			—	—	—	
6	1.88	+M.	—	—			—	—	—	
7		+M.	—	—			—	—	—	
8	2.00	+M.	+M.	—	145,000,000	2.12	+M.	+M.	—	221,000,000
9	2.10	+M.	+M.	—		2.34	+ L.	+ L.	—	
10	2.38	+ L.	+ L.	—		2.77	+ L.	+ L.	+M.	650,000,000
11										
12	2.45	+ L.	+ L.	+M.	700,000,000					
13										
14										

[1] See footnote under Table 1.

EFFECT OF HOLDING THE MILK AT DIFFERENT TEMPERATURES.

The temperature at which the milk is held would, of course, affect the length of time before the alcohol test becomes positive. To show the effect of temperature we held samples of raw and pasteurized milk at 9° C. and also at room temperature, and examined them in the same manner as in the preceding experiment. The results are shown in Table 16. In order to have about the same bacterial content in the milk held at the different temperatures, a quart of milk was thoroughly mixed and placed in sterilized pint bottles.

The results show clearly that the temperature at which milk is held has a marked influence on the time when the alcohol test will be positive. Also, as shown by sample of raw milk C, the bacterial content of the milk is an important factor. In all the samples it will be noticed that the bacterial counts show an exceedingly high number when the 68 per cent alcohol test was positive.

TABLE 16.—*Comparison of the alcohol tests with milk held at 9° C. and at 24° C.*

Temperature at which held.	Days held.	Raw milk A.					Pasteurized milk B.				
		Acidity.	Alcohol test.			Bacteria per cubic centimeter.	Acidity.	Alcohol test.			Bacteria per cubic centimeter.
			75 per cent.	68 per cent.	44 per cent.			75 per cent.	68 per cent.	44 per cent.	
9° C. . .	0	1.85	—	—	—	1,600	1.65	—	—	—	139,000
	1	1.85	—	—	—	......	1.60	—	—	—	......
	2	1.90	—	—	—	......	1.60	—	—	—	......
	3	1.80	—	—	—	......	1.70	—	—	—	......
	4	1.79	—	—	—	......	1.71	—	—	—	......
	5	......	......	......	......	......	......	......	......	......	......
	6	1.85	—	—	—	......	1.60	—	—	—	......
	7	1.90	—	—	—	......	1.80	—	—	—	......
	8	1.80	—	—	—	......	1.65	—	—	—	......
	9	......	—	—	—	......	......	—	—	—	......
	10	1.80	—	—	—	......	1.72	—	—	—	......
	11	......	—	—	—	......	2.00	[1]+S	—	—	197,000,000
	12	......	......	......	......	......	......	......	......	......	......
	13	2.70	+L	+M	—	400,000,000	2.3	+L	+M	—	960,000,000
	14	......	—	—	—	......	......	......	......	......	......
	17	4.1	+L	+L	+L	440,000,000	4.3	+L	+L	+L	......
Room temperature[12]	0	1.85	—	—	—	2,900	1.65	—	—	—	146,000
	1 (9 a.m.)	1.90	—	—	—	......	1.85	+S	—	—	32,600,000
	1 (3 p.m.)	3.1	+L	+L	+L	534,000,000	3.70	+L	+L	+L	694,000,000

[1] See footnote under Table 1. [2] About 24° C.

TABLE 16.—*Comparison of the alcohol tests with milk held at 9° C. and at 24° C.*—Continued.

Temperature at which held.	Days held.	Raw milk C.				
		Acidity.	Alcohol test. 75 per cent.	Alcohol test. 68 per cent.	Alcohol test. 44 per cent.	Bacteria per cubic centimeter.
9° C.	0	1.85	–	–	–	7,870,000
	1	1.80	–	–	–	
	2	1.95	[1] +S	–	–	93,000,000
	3	2.35	+M	+S	–	130,000,000
	4	2.36	+L	+L	–	188,000,000
	5					
	6	2.88	+L	+L	+M	430,000,000
	7					
	8					
	9					
	10					
	11					
	12					
	13					
	14					
	17					
Room temperature [2]	0	1.85	–	–	–	7,610,000
	1 (9 a.m.)	4.90				Curdled.

[1] See footnote under Table 1. [2] About 24° C.

At various times different investigators have used the alcohol test on market milk. Aurnhammer (1) in an examination of 250 samples of market milk during July and August of 1907 found the 68 per cent alcohol test positive in 82 samples. In a study of market milk in Philadelphia, Campbell (5) found that 37 of 100 samples of milk gave a positive test with 68 per cent alcohol. Of these 37 samples 17 contained less than and 20 more than 1,000,000 bacteria per cubic centimeter. It was found by Nurenberg and Lythgoe (23) during an examination of 2,600 samples of market milk that only 63 gave a positive test with 68 per cent alcohol.

We made alcohol tests on 236 samples of Washington market milk during the period from March 20 to June 4, 1914. These samples and their bacterial counts were supplied by the Health Department, District of Columbia.[1] Of the 236 samples we found that 37 gave an alcohol test with 75 per cent alcohol, 20 with 68 per cent alcohol, and 5 with 44 per cent alcohol. The samples which gave a positive test are tabulated in Table 17 with their acidity and bacterial counts. There were 177 samples of raw milk and 59 samples of pasteurized milk in the 236 samples examined. As may be seen from the table, 35 of the raw-milk samples gave a positive test with 75 per cent alcohol and only 2 of the 59 samples of pasteurized milk.

[1] We take this occasion to express our thanks to Dr. Kinyoun and Dr. Dieter, of the Health Department, for the samples of market milk and their bacterial counts which they so kindly furnished us.

TABLE 17.—*Raw and pasteurized market milk which gave positive alcohol tests.*

Milk.	Sample number.	Acidity.	Bacteria per cubic centimeter.	Alcohol tests.		
				75 per cent.	68 per cent.	44 per cent.
Raw	1	2.01	2,100	[1] +M	+VS	–
	2	2.30	7,000	+L	+M	–
	3	2.00	12,000	+M	–	–
	4	1.60	14,000	+M	+S	–
	5	1.97	18,000	+M	–	–
	6	1.92	24,000	+M	+S	+VS
	7	1.91	24,000	+S	–	–
	8	1.70	29,000	+S	–	–
	9	1.62	51,000	+M	+S	–
	10	2.30	67,000	+L	+M	–
	11	1.79	121,000	+M	+S	+VS
	12	1.75	156,000	+S	–	–
	13	2.00	200,000	+M	–	–
	14	1.94	350,000	+M	–	–
	15	1.90	442,000	+S	–	–
	16	2.06	464,000	+L	+M	–
	17	1.95	1,200,000	+M	+VS	–
	18	1.76	1,300,000	+VS	–	–
	19	1.75	1,500,000	+S	–	–
	20	1.80	1,600,000	+M	+S	–
	21	1.70	2,100,000	+M	–	–
	22	2.03	2,120,000	+S	–	–
	23	1.90	2,200,000	+S	–	–
	24	1.95	2,300,000	+M	+S	–
	25	2.45	2,600,000	+L	+M	+VS
	26	1.95	4,100,000	+M	+M	–
	27	1.93	4,700,000	+VS	–	–
	28	2.15	7,200,000	+M	+S	–
	29	2.19	8,600,000	+M	+S	–
	30	3.65	10,200,000	+L	+L	+L
	31	1.90	10,500,000	+M	–	–
	32	2.05	20,200,000	+M	–	–
	33	1.95	20,400,000	+M	+M	–
	34	2.55	20,600,000	+L	+L	+L
	35	2.10	21,200,000	+L	+M	–
Pasteurized	1	1.68	2,000	+S	–	–
	2	1.90	8,000	+M	+S	–

[1] See footnote under Table 1.

RESULTS OF TESTS WITH SAMPLES OF KNOWN BACTERIAL CONTENT.

When we consider the alcohol test in relation to the number of bacteria in milk, a short survey of the results is sufficient to show that there is no definite relation. Of the 35 samples of raw milk which showed a positive test with 75 per cent alcohol, 16, or 45.7 per cent, contained less than 500,000 bacteria per cubic centimeter. Of the 19 samples positive with 68 per cent alcohol, 8, or 42.1 per cent, contained less, and 11, or 57.9 per cent, more than 500,000 bacteria per cubic centimeter. Of the 5 samples positive with 44 per cent alcohol, 2 samples, or 40 per cent, contained less, and 3, or 60 per cent, more than 500,000 bacteria per cubic centimeter. The number of bacteria in samples which gave a positive alcohol test ranged from 2,100 to 21,200,000 per cubic centimeter.

The samples of pasteurized milk which showed a positive alcohol test had a very low bacterial count.

In order further to show that the alcohol test has no definite relation to the bacterial count, there are tabulated in Tables 18 and 19 the samples of raw and pasteurized milk which gave negative alcohol tests, together with their acidity and bacterial counts. We wish to call particular attention to the bacterial counts of 142 samples of raw milk which ranged from 2,000 to 19,600,000 bacteria per cubic centimeter. Of these 142 samples none gave a positive alcohol test, yet 86, or 60.6 per cent, contained less than and 39.4 per cent more than 500,000 bacteria per cubic centimeter.

The bacterial counts of the samples of pasteurized milk which gave a negative alcohol test ranged from 1,200 to 3,600,000 per cubic centimeter, as may be seen from Table 19.

From our results we believe that there is no definite relation between the alcohol test and the bacterial count, except in special cases where the bacteria have developed to a point where there is sufficient acid produced or where rennet-forming bacteria have acted sufficiently to influence the test.

TABLE 18.—*Acidity and bacterial count of samples of raw market milk which gave negative alcohol tests with 75 per cent, 68 per cent, and 44 per cent alcohol.*

Sample No.	Acidity.	Bacteria per cubic centimeter.	Sample No.	Acidity.	Bacteria per cubic centimeter.	Sample No.	Acidity.	Bacteria per cubic centimeter.
1	1.08	2,000	49	1.82	82,000	97	1.95	812,000
2	2.10	5,000	50	1.75	86,000	98	1.85	840,000
3	1.85	6,000	51	1.08	92,000	99	1.80	860,000
4	1.75	7,000	52	1.90	93,000	100	1.60	880,000
5	1.82	8,000	53	1.85	93,000	101	1.62	906,000
6	2.05	8,000	54	1.60	105,000	102	1.69	910,000
7	2.05	8,500	55	1.70	109,000	103	1.90	910,000
8	1.83	11,000	56	1.93	115,000	104	1.90	910,000
9	1.92	13,000	57	2.00	118,000	105	1.80	920,000
10	1.97	13,000	58	1.87	120,000	106	1.75	1,040,000
11	1.75	14,000	59	2.06	120,000	107	1.75	1,100,000
12	2.00	14,000	60	1.75	130,000	108	1.75	1,170,000
13	2.15	16,000	61	1.75	132,000	109	1.65	1,200,000
14	2.10	18,000	62	2.00	147,000	110	1.70	1,200,000
15	1.75	21,000	63	1.85	149,000	111	1.90	1,210,000
16	1.95	21,000	64	1.90	150,000	112	1.70	1,400,000
17	1.62	22,000	65	1.90	157,000	113	1.85	1,400,000
18	2.15	22,000	66	1.48	160,000	114	1.85	1,460,000
19	1.80	25,000	67	2.00	164,000	115	1.80	1,600,000
20	1.52	26,000	68	1.85	172,000	116	2.08	1,600,000
21	1.80	26,000	69	1.80	206,000	117	1.70	1,630,000
22	1.95	26,000	70	2.00	210,000	118	1.98	1,710,000
23	1.89	27,000	71	2.03	212,000	119	2.20	1,800,000
24	1.65	29,000	72	1.90	214,000	120	2.10	2,120,000
25	2.00	32,000	73	1.85	216,000	121	1.74	2,210,000
26	1.60	33,000	74	2.00	220,000	122	1.75	2,260,000
27	1.84	33,000	75	1.82	238,000	123	1.85	2,340,000
28	1.65	34,000	76	1.83	238,000	124	1.90	2,580,000
29	1.76	34,000	77	2.00	242,000	125	1.85	2,710,000
30	2.10	35,000	78	1.70	266,000	126	1.71	2,840,000
31	1.90	36,000	79	2.02	268,000	127	1.70	2,900,000
32	1.97	36,000	80	1.90	270,000	128	1.80	2,920,000
33	1.74	37,000	81	1.80	278,000	129	1.70	3,300,000
34	1.75	37,000	82	1.80	310,000	130	2.07	3,800,000
35	1.55	38,000	83	1.80	350,000	131	1.85	4,300,000
36	1.80	38,000	84	1.75	360,000	132	1.96	4,800,000
37	2.00	39,000	85	....	422,000	133	2.15	5,100,000
38	1.75	42,000	86	2.10	451,000	134	2.05	5,300,000
39	1.81	42,000	87	1.80	506,000	135	1.80	5,700,000
40	1.70	43,000	88	2.05	510,000	136	1.80	6,400,000
41	1.80	46,000	89	1.90	560,000	137	1.85	6,900,000
42	1.78	51,000	90	1.75	610,000	138	1.90	6,900,000
43	1.90	54,000	91	2.10	620,000	139	1.80	8,800,000
44	1.86	56,000	92	1.88	624,000	140	1.90	12,600,000
45	1.60	63,000	93	1.70	640,000	141	1.85	12,700,000
46	1.75	69,000	94	1.74	740,000	142	2.15	19,600,000
47	1.90	74,000	95	1.75	740,000			
48	1.71	79,000	96	2.00	800,000			

TABLE 19.—*Acidity and bacterial count of samples of pasteurized market milk which gave negative alcohol tests with 75 per cent, 68 per cent, and 44 per cent alcohol.*

Sample No.	Acidity.	Bacteria per cubic centimeter.	Sample No.	Acidity.	Bacteria per cubic centimeter.	Sample No.	Acidity.	Bacteria per cubic centimeter.
1	1.85	1,200	20	1.75	15,000	39	1.85	104,000
2	1.77	1,200	21	1.70	16,000	40	2.05	110,000
3	1.66	1,900	22	1.65	16,000	41	1.65	114,000
4	1.75	3,000	23	2.05	16,000	42	1.76	120,000
5	1.66	4,000	24	1.69	17,500	43	1.80	133,000
6	1.80	5,000	25	1.78	21,000	44	1.75	194,000
7	1.85	7,000	26	1.66	21,000	46	1.73	264,000
8	1.85	7,600	27	1.80	24,000	47	1.70	284,000
9	1.80	8,000	28	1.83	32,000	48	1.90	340,000
10	1.80	9,000	29	1.67	37,000	49	1.76	446,000
11	1.71	9,000	30	1.96	41,000	50	1.85	720,000
12	1.75	11,000	31	1.75	52,000	51	1.65	740,000
13	1.85	11,000	32	1.90	59,000	52	1.75	940,000
14	1.70	11,000	33	1.85	62,000	53	1.74	1,280,000
15	1.65	12,000	34	1.85	64,000	54	1.60	1,660,000
16	1.85	13,000	35	1.70	65,000	55	1.97	2,460,000
17	1.75	14,000	36	1.75	68,000	56	1.60	3,100,000
18	1.75	15,000	37	1.80	71,000	57	2.00	3,600,000
19	1.80	15,000	38	1.70	74,000			

In the early stages of the growth of acid-forming bacteria in milk, when the numbers are low, there is a period in which a rapid increase in numbers takes place without any increase in acidity which can be detected by ordinary chemical methods, or it may occur with only a slight increase in acidity; consequently if the alcohol test were made during that period there would be a high bacterial count and yet not high acidity enough to cause a positive alcohol test. The same is true of the action of the rennet-forming bacteria in their growth and action, as we have shown earlier in this paper when dealing with the relation of acidity, and also the effect of rennet on the alcohol test. Besides these facts there are other groups of bacteria which may develop in milk and yet have no influence on the alcohol test, as, for example, the alkali-forming group of bacteria. We have tried cultures of this group of organisms and found that they did not produce a positive alcohol test. There are other groups of bacteria in the flora of milk, such as the inert group, which also would probably develop without influencing the alcohol test in any way. When we consider all these facts it is not strange that there is no definite relation between the bacterial flora of milk and the bacterial count.

When the 68 per cent alcohol test is positive with a sample of market milk, it is evidence that there is some change in the milk from normal. In some cases it may be due to an increased acidity and in consequence a change in the casein of the milk, due to bacterial action. In other cases it may be due to a pure rennet fermentation or there may be a combination of an acid-and-rennet fermentation. In such cases the bacterial count would undoubtedly be high. However, there still remains to be explained the reason for a positive alcohol test in samples of market milk with a low bacterial count and low acidity.

We can not see that the alcohol test is of any particular value in the control of a market milk supply except as a means of evidence that milk from a particular source is abnormal in some way and should be examined by other tests. It might be of value at a receiving station as a means of detecting sour milk, but the test would be expensive compared with the use of alkaline tablets for the rapid determination of acidity as described by Farrington and Woll (9).

THE TITRATION METHOD OF APPLYING THE ALCOHOL TEST.

From the simple alcohol test in which a definite volume of a definite-percentage alcohol is added to an equal volume of milk there has developed a method in which a definite volume of milk is titrated with certain percentages of alcohol until a coagulation of the milk is produced.

Löhnis (16) has found this titration method to be of value as a test for the quality of market milk. He found that there was quite a definite relation between the titration with 80 per cent alcohol and the bacterial content of market milk. He titrated 2 c. c. of milk in a beaker against a black background with 90, 80, and 70 per cent alcohols, the titration being made at a temperature of from 15° to 20° C. The first appearance of flakes was considered the end point.

We have used this method in the titration of 116 samples of market milk furnished with bacterial counts by Dr. Kinyoun and Dr. Dieter, of the Health Department of the District of Columbia. In our titrations of 92 samples of raw and 24 samples of pasteurized milk we have not found any definite relation between the titration with 90 per cent and 80 per cent alcohols and the bacterial count. In Table 20 is shown the acidity, bacterial counts, and alcohol titration of 92 samples of raw milk, and in Table 21 the results of an examination of 24 samples of pasteurized milk. The bacterial counts of the raw milk ranged from 2,100 to 20,600,000 per cubic centimeter, and the pasteurized milk from 1,200 to 3,100,000 bacteria per cubic centimeter. Consequently we were able to titrate samples having a great variation in their bacterial content. If a study is made of the bacterial counts and the alcohol titrations shown in Tables 20 and 21 it will be seen that there is no definite relation between them. In order to bring this point out more clearly the titrations of samples containing more than 500,000 and less than 500,000 bacteria per cubic centimeter have been averaged, as shown in Table 22. The average titration with 90 per cent alcohol of 46 samples of raw milk containing more than 500,000 bacteria per cubic centimeter was 1.95 c. c., while the average titration of 46 samples containing less than 500,000 per cubic centimeter was 2.39 c. c. The average titration of 46 samples with 80 per cent alcohol was 4.61 c. c. when the bacterial count was more than 500,000 per cubic centimeter and 5.61 c. c. when the counts were less than 500,000 per cubic centimeter. The average titrations of the pasteurized milk samples showed even smaller differences. The small differences in the average titration of samples with a high and a low bacterial count show that there is little, if any, relation between the alcohol titration and the bacterial count. This is shown even more strikingly in Table 23, where the range in titrations among samples grouped according to bacterial counts is recorded. With these extreme ranges among samples of milk with high and low bacterial contents it would be almost impossible to interpret an alcohol titration in terms of bacteria.

TABLE 20.—*Alcohol titrations of raw market milk.*

Sample No.	Acidity.	Bacteria per cubic centimeter.	Alcohol titration.		Sample No.	Acidity.	Bacteria per cubic centimeter.	Alcohol titration.	
			90 per cent.	80 per cent.				90 per cent.	80 per cent.
			c. c.	*c. c.*				*c. c.*	*c. c.*
1	2.01	2,100	1.03	1.86	47	1.80	506,000	1.50	1.80
2	2.30	7,000	.51	.93	48	1.75	610,000	2.93	5.25
3	1.75	7,000	2.17	5.30	49	2.10	620,000	1.80	5.10
4	1.82	8,000	2.76	4.54	50	1.88	624,000	2.91	9.20
5	2.05	8,500	2.00	5.30	51	1.74	740,000	2.96	6.80
6	1.75	14,000	2.21	4.67	52	1.75	812,000	1.91	6.76
7	2.15	16,000	2.80	7.60	53	1.80	860,000	1.62	3.00
8	1.95	21,000	2.70	7.43	54	1.62	906,000	1.68	3.30
9	1.91	24,000	1.59	3.08	55	1.69	910,000	2.08	6.13
10	1.80	25,000	3.29	11.73	56	1.80	920,000	1.85	4.50
11	1.52	26,000	3.12	7.28	57	1.75	1,040,000	1.92	4.37
12	1.65	29,000	2.68	10.07	58	1.75	1,100,000	2.36	4.00
13	2.00	32,000	3.64	9.59	59	1.75	1,170,000	2.20	6.04
14	1.60	33,000	2.56	5.55	60	1.65	1,200,000	1.90	5.76
15	1.65	34,000	2.48	5.51	61	1.76	1,300,000	2.72	6.59
16	2.10	35,000	1.24	2.71	62	1.70	1,400,000	1.63	3.74
17	1.75	37,000	2.26	4.40	63	1.85	1,400,000	2.36	4.42
18	1.80	38,000	2.71	6.50	64	1.85	1,460,000	3.04	9.61
19	1.55	38,000	3.06	6.92	65	1.80	1,600,000	1.23	5.40
20	1.75	42,000	3.00	4.28	66	2.08	1,600,000	2.51	5.94
21	1.80	46,000	2.78	9.68	67	1.70	1,630,000	2.40	6.00
22	2.30	67,000	.50	1.1	68	2.20	1,800,000	1.30	2.37
23	1.75	69,000	2.58	5.98	69	1.70	2,100,000	1.16	1.60
24	1.90	74,000	3.03	6.33	70	2.03	2,120,000	1.16	2.34
25	1.71	79,000	3.50	6.91	71	1.74	2,210,000	2.10	3.96
26	1.85	93,000	1.57	4.36	72	1.75	2,260,000	1.81	4.12
27	1.70	109,000	3.04	5.20	73	1.85	2,340,000	2.42	4.78
28	1.87	120,000	2.46	5.80	74	2.45	2,600,000	.78	.97
29	1.75	130,000	1.60	3.20	75	1.71	2,840,000	2.95	3.97
30	1.75	132,000	2.72	10.45	76	1.70	2,900,000	2.12	6.36
31	2.00	147,000	1.72	3.80	77	1.80	2,920,000	3.00	6.85
32	1.85	149,000	3.54	8.17	78	1.70	3,300,000	2.80	6.30
33	1.80	160,000	1.48	2.49	79	2.00	3,600,000	1.90	5.40
34	1.80	206,000	3.16	5.52	80	1.85	4,300,000	2.05	5.00
35	2.00	210,000	2.68	7.00	81	1.93	4,700,000	1.40	3.90
36	2.03	212,000	2.35	5.70	82	1.96	4,800,000	1.46	2.70
37	1.90	214,000	1.68	4.52	83	2.05	5,300,000	1.60	2.68
38	1.82	238,000	2.75	7.00	84	1.80	5,700,000	2.94	7.25
39	2.00	242,000	1.24	2.90	85	1.85	6,900,000	1.01	2.41
40	1.76	266,000	1.95	3.94	86	2.19	8,600,000	.92	1.86
41	2.02	268,000	2.57	5.20	87	1.80	8,800,000	2.96	10.83
42	1.90	270,000	2.74	4.45	88	1.90	12,600,000	1.83	5.10
43	1.80	278,000	3.22	8.70	89	1.85	12,700,000	1.40	2.43
44	1.80	310,000	2.34	4.58	90	2.15	19,600,000	1.56	3.10
45	1.80	350,600	2.68	4.67	91	1.95	20,400,000	.86	1.40
46	1.75	360,000	2.35	5.20	92	2.55	20,600,000	.52	.60

TABLE 21.—*Alcohol titrations of pasteurized market milk.*

Sample No.	Acidity.	Bacteria per cubic centimeter.	Alcohol titration. 90 per cent.	Alcohol titration. 80 per cent.	Sample No.	Acidity.	Bacteria per cubic centimeter.	Alcohol titration. 90 per cent.	Alcohol titration. 80 per cent.
			c. c.	*c. c.*				*c. c.*	*c. c.*
1	1.77	1,200	2.20	5.74	13	1.70	65,000	2.88	5.04
2	1.70	11,000	2.84	6.76	14	1.75	68,000	2.40	5.50
3	1.65	12,000	3.08	8.80	15	1.80	71,000	2.50	4.49
4	1.85	13,000	1.56	4.43	16	1.85	104,000	2.43	3.96
5	1.75	14,000	1.65	3.10	17	1.70	120,000	2.10	5.96
6	1.75	15,000	2.22	3.42	18	1.75	194,000	3.05	6.01
7	1.70	16,000	2.76	4.10	19	1.73	264,000	2.24	3.00
8	1.78	21,000	2.33	5.91	20	1.70	284,000	2.86	5.48
9	1.80	24,000	1.69	6.10	21	1.76	446,000	1.82	5.34
10	1.90	59,000	2.57	3.68	22	1.60	1,600,000	2.10	4.78
11	1.85	62,000	2.23	5.47	23	1.97	2,460,000	2.08	3.66
12	1.60	63,000	2.53	3.24	24	1.60	3,100,000	3.53	7.07

TABLE 22.—*Average alcohol titrations of samples of raw and pasteurized market milk in tables 20 and 21.*

Milk.	Number of samples.	Bacteria per cubic centimeter.	Average alcohol titration. 90 per cent.	Average alcohol titration. 80 per cent.
			c. c.	*c. c.*
Raw..........	46	More than 500,000.	1.95	4.61
	46	Less than 500,000..	2.39	5.61
Pasteurized..	3	More than 500,000..	2.57	5.17
	21	Less than 500,000..	2.28	5.02

TABLE 23.—*Range in alcohol titrations of market milk shown in detail in tables 20 and 21.*

Bacteria per cubic centimeter.	Alcohol titration. 90 per cent. Lowest.	Alcohol titration. 90 per cent. Highest.	Alcohol titration. 80 per cent. Lowest.	Alcohol titration. 80 per cent. Highest.
	c. c.	*c. c.*	*c. c.*	*c. c.*
26 samples with less than 100,000......	0.51	3.64	0.93	11.73
30 samples with from 100,000 to 1,000,000	1.24	3.54	1.80	10.45
36 samples with over 1,000,000..........	.52	3.04	.60	10.83

For the sake of clearness we have plotted in figure 1 the bacterial counts and the 90 per cent alcohol titration. In this figure the titrations of 116 samples of milk were plotted as ordinates and the logarithms of the bacterial counts as abscissæ. The numbers 3, 4, 5, 6, 7, and 8 represent the mantissa of the logarithms of the bacterial counts. Consequently from 3 to 4 was plotted the logarithm of samples with a bacterial count of from 1,000 to 9,999, from 4 to 5 counts from 10,000 to 99,999, and so on, as may be seen from the figure. By this method of plotting it is possible to plot bacterial counts ranging from low to high numbers, which would otherwise be impossible in a limited space. A glance at the plot which shows the 90 per cent alcohol titration and the bacterial count of 116 samples indicates clearly that there is no definite relation between them. In figure 2 we have plotted in the same way the 80 per cent alcohol

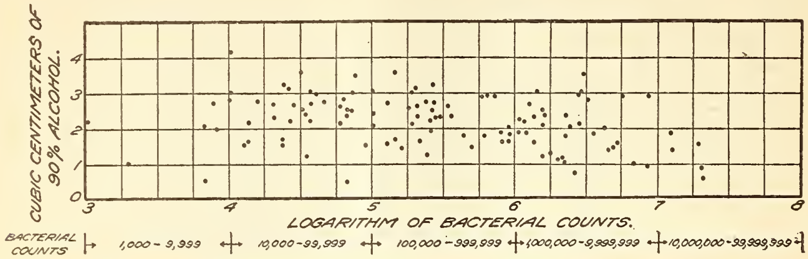

FIG. 1.—Relation o falcohol titration to the bacterial count of milk. Titrations of 116 samples of raw and pasteurized market milk with 90 per cent alcohol.

titration and the bacterial counts. It may be seen that among the 116 samples plotted there is a wide range in titration of samples with low and high bacterial counts. Some samples with a low count show a low titration and others a high titration. Among samples with a high count some show a low and others a high titration.

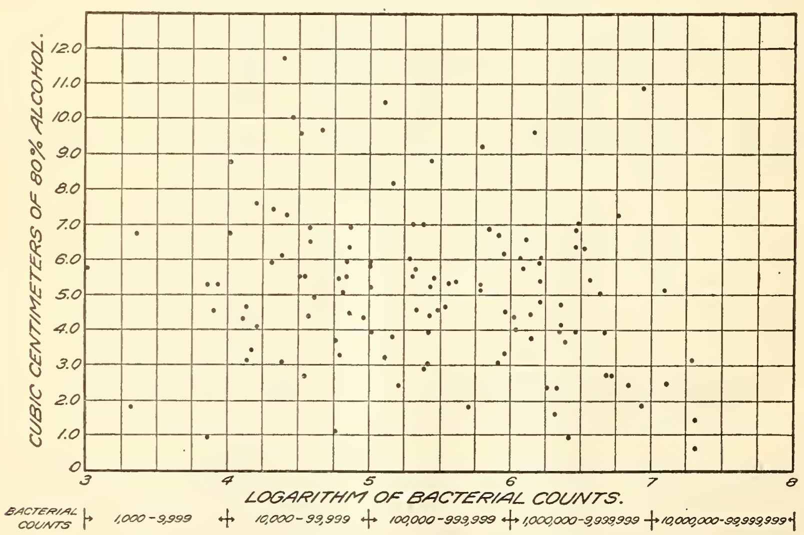

FIG. 2.—Relation of alcohol titration to the bacterial count of milk. Titrations of 116 samples of raw and pasteurized market milk with 80 per cent alcohol.

Our results indicate that there is no definite relation between alcohol titration and acidity unless the acidity is more than about 2.20. This is shown in figure 3, where 116 samples are plotted according to their acidity and titration with 90 per cent alcohol, and also in figure 4, where the 80 per cent titrations and acidities are plotted. The plots show that there is a wide range in the alcohol titration at all acidities until they reach about 2.20, after which the alcohol titration becomes lower in general as the acidities increase. This fact holds true for the small number of samples at these high

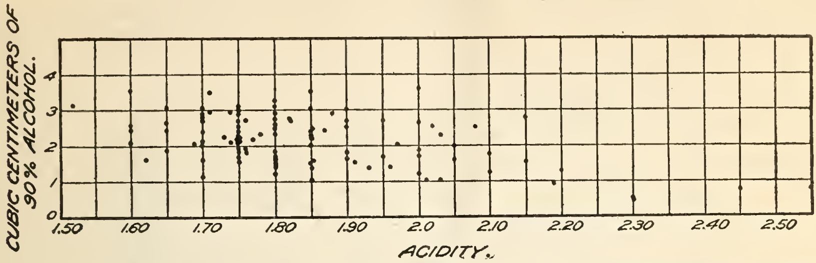

FIG. 3.—Relation of alcohol titration to the acidity of milk. Titrations of 116 samples of raw and pasteurized market milk with 90 per cent alcohol.

acidities and would probably have been brought out more clearly if we had had a larger number of samples with acidity above 2.20.

If we were dealing with pure cultures of organisms which influence the alcohol test the titration with alcohol might be of value in giving an idea of the bacterial numbers, as is shown in Table 24, from the

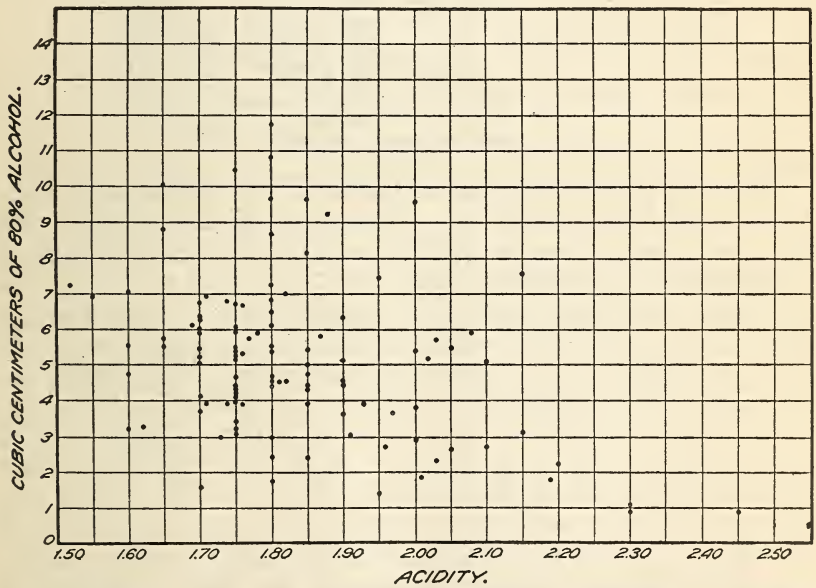

FIG. 4.—Relation of alcohol titration to acidity of milk. Titrations of 116 samples of raw and pasteurized market milk with 80 per cent alcohol.

results of experiments in which we used pure cultures of lactic-acid and rennet-forming bacteria. In milk, however, we have a varied bacterial flora to contend with and we can not see from our results that the alcohol titration method is of much greater value than the simple alcohol test.

TABLE 24.—*Alcohol titrations of milk inoculated with pure cultures of bacteria.*

Experiment.	Culture.	Age of milk culture.	Acidity.	Bacteria per cubic centimeter.	Alcohol titration.	
					90 per cent to 2 c.c. of milk.	80 per cent to 2 c.c. of milk.
1.....	Lactic-acid bacteria..........	*Hours.*			*c. c.*	*c. c.*
		0	1.95	64,000	3.08	8.88
		2	1.95	....	2.86	9.13
		3	1.90	131,000	3.14	9.42
		4	1.95	120,000	2.60	9.52
		5	1.95	361,000	3.05	9.40
		6	2.05	736,000	3.05	8.85
		6½	2.07	1,660,000	1.85	7.08
	Rennet-forming bacteria.......	0	1.95	....	3.12	9.02
		2	1.90	1,600	3.03	9.52
		3	1.95	70,000	2.50	9.48
		4	2.03	230,000	2.44	9.83
		5	2.04	2,850,000	3.20	9.90
		6	2.04	9,300,000	2.80	8.12
		6½	2.07	11,100,000	1.87	6.18
2.....	Lactic-acid bacteria..........	0	2.00	25,000	3.33	12.55
		2	2.00	184,000	3.58	13.82
		3	2.08	475,000	3.52	11.18
		4	2.04	1,710,000	3.73	9.85
		5	2.18	4,900,000	2.00	7.32
		6	2.26	8,400,000	2.00	5.50
		7	2.33	22,500,000	1.06	1.79
	Rennet-forming bacteria.......	0	2.00	6,100	3.32	13.95
		2	2.00	51,500	3.40	13.36
		3	2.00	234,000	3.98	10.52
		4	2.00	1,325,000	3.57	9.35
		5	2.00	1,300,000	1.56	8.98
		6	2.04	13,000,000	1.25	2.93
		7	2.20	21,800,000	0.83	1.21

THE ALIZAROL TEST.

When the alcohol has alizarin added to it to act as an indicator for the acidity the alcohol test is known as the alizarol test. This name was given to the test by Morres (21). The use of alizarin as an indicator for the acidity of milk has been known for a long time, but Morres (19) was probably the first to combine the alcohol and alizarin test. He pointed out that the alcohol test was of more value than the litmus test and that the combination with alizarin was better than the combination of litmus and alcohol. Morres (20) used a 68 per cent alcohol with 1.2 grams of fresh alizarin paste, or 0.4 gram of dry alizarin to 1,000 c. c. of alcohol. Two cubic centimeters of this alizarin-alcohol solution are mixed with 2 c. c. of milk, the same as in the alcohol test. This author found that from the coagulation by alcohol and the color of the alizarin it was possible to obtain a picture of the condition of the milk. According to Morres (20) the alizarol test shows the following conditions:

1. Lilac-red color. (Milk titrated 7° acid.)
   - (*a*) With no coagulation. The milk should keep sweet more than 6 hours.
   - (*b*) With fine flaky coagulation. The beginning of rennet production is shown here.
   - (*c*) With heavy flocculent coagulation. This indicates advanced rennet formation.

2. Pale-red color. (Milk titrated 8° acid.)
   (*a*) With no coagulation or only very fine coagulation. This shows the beginning of lactic-acid fermentation.
   (*b*) With flaky coagulation. Acid and rennet fermentation is indicated.
   (*c*) With coagulation with very thick flakes. A mixed fermentation is indicated with advanced rennet and the beginning of acid fermentation.
3. Brownish-red color. (Milk titrated 9° acid.)
   (*a*) With coagulation with fine flakes. Well-advanced pure acid fermentation is indicated.
   (*b*) With coagulation with thick flakes. A mixed fermentation with advanced rennet and strong acid fermentation is indicated.
   (*c*) With coagulation with very thick flakes. A very advanced rennet production and little less important acid fermentation is indicated.
4. Reddish-brown color. (Milk titrates 10° acid.)
   (*a*) With flaky coagulation. Advanced pure acid fermentation is indicated.
   (*b*) With thick flaky coagulation. Advanced acid fermentation and the beginning of rennet production is indicated.
   (*c*) With very thick flaky coagulation. A proportional mixed fermentation which is well advanced is indicated.
5. Brown color. (Milk titrates 11° acid.)
   (*a*) With thick flaky coagulation. Pure acid fermentation is indicated. Milk is sour; to be detected by smell.
   (*b*) With very thick flaky coagulation. Some rennet production and well advanced acid fermentation is indicated.
6. Yellowish-brown color. (Milk titrates 12° acid.)
   (*a*) With very thick flaky coagulation. Acid fermentation is indicated. Milk tastes acid.
7. Brownish-yellow color. (Milk titrates 14° acid.)
   (*a*) With very thick flaky coagulation. Sour taste is distinctly noticeable.
8. Yellow color. (Milk titrates 20° acid.)
   (*a*) With very thick flaky coagulation. Pure acid fermentation is indicated. Milk smells and tastes strongly acid and is near the normal coagulation point.
9. Violet color. (Milk titrates 7° acid.)
   No fermentation is indicated, but the milk is abnormal.

It can not be disputed that a simple test which will picture conditions in milk, as claimed by Morres, would be of considerable value. But will the alizarol test indicate all that Morres claims? Devarda and Weich (6) in 1913, after working with this test, decided that it had no value over the alcohol test. In a later paper Devarda (7) draws conclusions as follows:

1. For market control the alcohol test is satisfactory for the determination of the quality of milk.
2. The assertion of Morres that the alizarol test can show a pure rennet and mixed fermentation is without scientific or practical significance.
3. In a pure lactic fermentation the alizarol test stands close to the acidity in its color relation, but for the determination of the keeping quality of milk it is of slight significance.
4. The diagnostic value of the alizarol test is limited to an empirical test for milk, principally as to its suitability for cheese making which was already employed by Eugling in 1882.

Thöni (30), in a study of the milk supply of Berne, found that 12 of 85 samples examined were more or less abnormal, according to the alizarol test. Among the other 73 samples of milk, which according to the alizarol test were normal, there were samples which had a high bacterial content and which were abnormal according to the leucocyte and other tests. From his results Thöni believes that the alizarol test is not sufficiently delicate for use in market-milk investigations. However, he believes the test is of value as a quick means for detecting udder infection in animals.

It is evident that there is a diversity of opinion as to the value of the alizarol test, and our experiments have not been extensive enough for us to form a definite opinion in regard to it.

We have tried the test on a number of samples of milk and have not been able to obtain all the color changes which are described by Morres. When the acidity was slightly above normal we found a change from lilac red to pale red and brownish red. In one sample of milk we increased the acidity by the addition of lactic acid and obtained the colors named below.

Amount of N/10 lactic added to 50 c. c. of milk.	Acidity.	Color of alizarol test.
Normal milk	1.85	Lilac red.
2 c. c.	2.10	Pale red.
4 c. c.	2.42	Brownish red.
6 c. c.	2.73	Do.
8 c. c.	3.00	Do.
1.5 c. c. normal acid	5.15	Do.

From our results we believe that alizarin will show slight changes in the acidity when the acidity is low, but that the indicator did not seem to be very sensitive to high acidities in milk. Morres (22), in a paper in 1913, also states that alizarin is of greatest value in indicating the first changes in acidity and that the color change is so gradual at acidities over 16° that the test is of no particular value.

In regard to the value of the alizarol test we believe that wherever the alcohol test can be considered of value, the addition of an indicator, such as alizarin, may increase the value of the alcohol test by possibly giving additional information as to acidity.

On account of the complexity of the bacterial fermentations in market milk we do not believe that the alizarol test gives any very valuable information as to the conditions existing in the milk.

CONCLUSIONS.

In conclusion, we wish to point out again that the alcohol test must be considered from two standpoints: First, in its relation to the milk from a single cow or small herd, and, second, in its relation to mixed market milk.

As to the relation of the alcohol test to milk from a single cow, it seems evident from the work of other investigators, which is confirmed to some extent by our results, that a positive 68 per cent alcohol test indicates some change in the milk from its normal condition. In our opinion the value of the alcohol test with milk from a single cow or small herd lies in the fact that it would show that the milk was abnormal, and in consequence a careful examination should be made of the herd.

When the relation of the alcohol test to mixed market milk is discussed, we must consider it on an entirely different basis. In this case the test with 68 per cent alcohol may be positive as a result of changes produced in milk through bacterial action. The results of our work confirm some of the results of other investigators and show that the alcohol test may be positive as a result of the growth in milk of lactic-acid and rennet-forming bacteria. When the growth of these bacteria has reached a point where the acid or rennet is produced in sufficient quantities to affect the casein, a coagulation is produced when equal volumes of 68 per cent alcohol and milk are mixed. Our results, however, do not show that there is any definite relation between the alcohol test and the number of bacteria in milk. During an examination of 177 samples of raw milk we found that 20 samples gave a positive test with 68 per cent alcohol. Of these 20 samples 8, or 42.1 per cent, contained less than 500,000, and 11, or 57.9 per cent, more than 500,000 bacteria per cubic centimeter. It was also found that 39.4 per cent of 142 samples of milk which gave no positive alcohol tests contained over 500,000 bacteria per cubic centimeter. That there is no definite relation is probably explained by the fact that bacteria may increase in large numbers before there is much acid or rennet produced. Consequently, if an alcohol test were made during that period there would be a high bacterial content and yet not enough change produced in the milk by acid or rennet to cause a positive test. Besides this point it must be remembered that in market milk there is a bacterial flora representing many different species, many of which may increase without influencing the alcohol test.

As stated before, generally speaking, when the bacterial fermentations have advanced to a point where chemical changes are produced, the alcohol test will be positive as a result of lactic or rennet fermentations, or a mixture of both. In such cases the alizarol test may be of more value than the plain alcohol test, so far as it may give additional information as to the kind of fermentation. From our results it seems evident that the acid-and-rennet fermentations may be differentiated by means of neutralization of the acidity by sodium hydrate.

The alcohol titration method according to our tests seems to offer no particular advantages over the alcohol test. In a study of 116 samples we were not able to find any definite relation between the alcohol titration and the bacterial count.

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