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MILKING MACHINES:

III. As a Source of Bacteria in Milk.

IV. Methods of Maintaining in a Bacteria-free Condition.

G. L. A. RUEHLE, ROBERT S. BREED, and GEO. A. SMITH.

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BULLETIN No. 450.

MILKING MACHINES:

III. AS A SOURCE OF BACTERIA IN MILK.

IV. METHODS OF MAINTAINING IN A BACTERIA-FREE CONDITION.

G. L. A. RUEHLE, ROBERT S. BREED, AND GEO. A. SMITH.

SUMMARY.

1. In order to secure direct evidence as to the amount of bacterial contamination derived from milking machines, sterile water has been "milked" thru the machines under investigation from an artificial udder; and this rinse water has been analysed for its bacterial content. Thus all chance of outside contaminations from the body of the cow, the interior of the udder and other sources have been eliminated. The machine which has been studied the most intensively has been the Burrell-Lawrence-Kennedy, machines of this type having been in use at the Station since 1907. Some observations have been made upon Hinman and Empire milkers in use on dairy farms in the vicinity of Geneva.

2. Long continued observations have been made upon the bacterial quality of milk produced on dairy farms using each of these three types of machines.

3. The bacterial condition of the machines in use at the Station, and at some of the dairy farms, was found to be reasonably satisfactory and usually excellent. The condition of the machines on the majority of the dairy farms was found to be unsatisfactory; and in some cases very unsatisfactory, since in the latter case, in spite of the use of antiseptic solutions, the sterile water drawn thru the machines was found to contain millions of organisms per cubic centimeter.

4. The chief source of this heavy seeding with bacteria was found to be the teat-cups and rubber tubes; but the pails of the machines were also a prolific source of trouble in some cases.

5. The suction trap placed upon the recent types of B-L-K machines to prevent the return of contaminated condensation water from the vacuum piping into the pails was tested and found to be efficient for the purpose.

6. The stable air which enters the machines during milking was analysed and found to add relatively insignificant numbers of bacteria to the milk, not being responsible for increasing the bacterial content more than 34 per c.c. under the worst conditions tested. The cotton filters for which provision is made on B-L-K machines were found to remove more than two-thirds of the bacteria from the air and also a small amount of dust. Since the total amount of contamination from the air was insignificant, the improvement due to the filters was so slight as to be undetectable in ordinary analyses of machine-drawn milk.

7. Dropping the teat-cups to the floor in dirty bedding was found to cause relatively large amounts of dirt to appear in the pails of the machines; but the bacteriological analyses of the sterile water, milked thru the machines at the same time, did not reveal the excessively high counts that might be expected. These filthy and intolerable conditions failed to give counts in excess of 25,000 per c.c. Here, as in other cases in our studies of the amount of bacterial contamination derived from dust, the germ count has been found to be an unsatisfactory index of the amount of dirt and filth present.

8. It has been found possible to completely sterilize milking machines by means of steam applied to the metal parts; and by thoro cleanliness, combined with harmless antiseptics, applied to the rubber parts of the machines. The precautions necessary are, however, impracticable even for certified dairy farms, and quite impossible to maintain on the average dairy farm.

9. Satisfactory bacterial results have been secured where practicable and suitable methods were used for keeping the machines cleaned, provided the teat-cups and tubes were immersed in any of the following antiseptic solutions: (1) Brines containing at least 10 per ct. of salt; (2) solutions of chloride of lime; (3) a combination of brine and chloride of lime; (4) lime water; (5) cold running water; (6) "montanin" (not recommended). Chloride of lime and running water were ineffective if the temperature of the solution was as great as 60° F.

10. The solution recommended for general use is chloride of lime dissolved in a saturated brine. Antiseptic solutions were not found to be successful unless the teat-cups and tubes were maintained in a cleanly condition.

INTRODUCTION.

Since 1906, this Station has milked part of the herd with milking machines and has had some phase of the milking machine problem under investigation. Some results of the experimental work have already been published in two bulletins (Nos. 317 and 353). The present bulletin includes two further reports on this subject, constituting Part III and Part IV in the series. Besides the data published in this series, some other data bearing on the question of milking machines have been included in Bulletins 380 and 443.

In these bulletins it has been reported that machine milking is practicable, a conclusion which is supported by the fact that since 1906 when milking machines were first tried at the Station, there has been a gradual increase in the number of herds in the State which are milked by machine until in a recent agricultural census, which was 96 per ct. complete, over 4600 milking machines were found to be in use. Nevertheless, those who are familiar with the use of machines under practical conditions realize that there is still a tendency for owners to discard them after using them from one to three years. It is to be expected that this tendency will become less marked as the defects in the machines are corrected, and the methods of using and caring for them are better understood.

The present report upon the machines has been drawn up in two parts: (a) A discussion of data gathered in an attempt to estimate the relative importance of milking machines as a source of bacteria in fresh milk, and (b) the results of tests made to determine the best methods of keeping the machines in a sufficiently germ-free condition to make them practicable for use in producing high-grade milk.

The first part of the report belongs in the general series of investigations begun at this Station in 1906 on the relative importance of the various sources of bacteria found in fresh milk.[1] A recent bulletin in the series from the Illinois Station discusses the amount

[1] Harding, H. A., Wilson, J. K., and Smith, G. A. The modern milk pail. N. Y. Agr. Exp. Sta., Bul. 326. 1910.
Harding, H. A., Ruehle, G. L., Wilson, J. K., and Smith, G. A. The effect of certain dairy operations upon the germ content of milk. N. Y. Agr. Exp. Sta., Bul. 365. 1913.
Harding, H. A., and Wilson, J. K. A study of the udder flora of cows. N. Y. Agr. Exp. Sta., Tech. Bul. 27. 1913.
Ruehle, G. L. A., and Kulp, W. L. Germ content of stable air and its effect upon the germ content of milk. N. Y. Agr. Exp. Sta., Bul. 409. 1915.

of bacterial contamination from common dairy utensils other than milking machines.[2] The present bulletin is intended to continue and supplement this study of dairy utensils as well as to give the best methods of caring for machines.

MILKING MACHINES: III. AS A SOURCE OF BACTERIA IN MILK.

PREVIOUS STUDIES.

A large number of papers discussing various aspects of the milking machine problem have already appeared. Some of these have dealt almost wholly with the effect of the machine on the amount of milk produced, or on the cost of operation of machines, and like problems. Others contain only general statements about the germ content of the machine-drawn milk, or about its keeping quality. Only those papers which give quantitative data on the germ content of machine-drawn milk are considered here.

Probably the first paper that discusses this problem is that of Harrison,[3] who in 1897 compared the germ content of hand-drawn and machine-drawn milk at the Experimental Farm at Guelph. In 235 analyses of machine-drawn milk the germ content as determined by a count of colonies on gelatin plates varied from 71,124 to 243,327 per c.c. with the greater number of the analyses showing counts between 100,000 and 200,000. In 94 analyses of hand-drawn milk from the same stable, the gelatin plate count varied between 645 and 63,422 per c.c., the numbers usually being between 1000 and 10,000. He concluded that milk drawn by the Thistle machine contained more bacteria than did hand-drawn milk.

In 1898, Backhaus and Cronheim[4] working at Königsberg, Prussia, reported a single analysis of milk drawn with a Thistle machine which showed a count of 1,187,000 colonies per c.c. They concluded that machine-drawn milk was low in sediment but high in germ content as compared with hand-drawn milk.

In 1902, Bordas and Raczkowski[5] in reporting bacteriological and acidity tests of milk from a dairy farm in France compared the milk as drawn by a mechanical milker with that drawn by hand. The number of colonies developed on lactose gelatin from milk drawn from the udder under aseptic precautions was 1700 per c.c. Milk drawn by hand and by machine when no aseptic precautions were used gave counts of 4,600 and 402,000 per c.c., respectively. When special precautions were taken, the hand-drawn milk had a count of 4,900 and the machine-drawn of 52,000 per c. c. The

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[2] Prucha, M. J., Weeter, H. M., and Chambers, W. H. Germ content of milk: II. As influenced by the utensils. Ill. Agr. Exp. Sta., Bul. 204. 1918.

[3] Harrison, F. C. Machine-drawn milk versus hand-drawn milk.— Some bacteriological considerations. Ontario Agr. Col. and Exp. Farm, Ann. Rept. 1897, p. 128–132. 1898. Also *Centralbl. f. Bakt.*, Abth. II, 5:183–189. 1899.

Harrison, F. C. The bacterial contamination of milk. *Rev. Gen. du Lait*, 2:457–463, 481–489, 510–519, 538–546. 1903.

[4] Backhaus and Cronheim, W. Ueber aseptische Milchgewinnung. *Ber. d. landw. Inst. Univ. Königsberg im Pr.*, 2:12–33. 1898.

[5] Bordas, F., and de Raczkowski. De la traite mécanique, dans l'industrie laitière. *Compt. rend. Acad. d. Sci. Paris*, 135:371–372. 1902.

acidity of the machine-drawn milk was noticeably higher after 24 and 36 hours than that of the hand-drawn milk. They conclude that machines could be kept in a satisfactory condition and that milk drawn by machine could not be contaminated with disease germs.

Later, Barthel[6] reporting as bacteriologist for the Aktiebolaget Separator in Sweden upon a new type of machine, in which the milk was drawn directly into the pail without passing thru rubber tubes, compared the germ content of milk drawn with a suction type of machine with that of hand-drawn milk. The two counts given for the machine-drawn milk are 19,540 and 88,400 per c.c., while those of the hand-drawn milk are 2520 and 1100 per c.c. When similar comparisons were made with the new type of machine, four tests of machine-drawn milk gave the following counts; 3400, 1000, 970, and 1070 per c c. The hand-drawn milk showed counts of 20,170, 6080, 5500, and 3900 per c.c. He concluded that the new type of machine had a great advantage over the older suction type of machine because of the lower germ content of the milk drawn with it.

Erf[7] recorded two comparisons between the germ content of hand-drawn and machine-drawn milk, the latter being drawn with a Burrell-Lawrence-Kennedy machine. In the first comparison, the hand-drawn milk gave a count of 3700 per c.c., while the machine-drawn milk gave 2200 per c.c. In the second comparison the counts were 1520 and 800 per c.c., respectively. He does not state what method of cleaning was used to produce these results. Tests were also made of the effectiveness of antiseptics in keeping the rubber tubes sweet, those used being a saturated solution of boracic acid, a one per ct. solution of formaldehyde and a solution of lime. Comparisons were made of the keeping quality of the milk drawn into the machines after treatment with these antiseptics, and the formaldehyde was found to be the most effective in keeping the tubes sweet; but because of its poisonous nature was not regarded as practicable. The lime water was found to be useful and the most practicable of the three.

Stocking,[8] in 1905, studied the same type of machine at two dairy farms making comparisons between hand-drawn and machine-drawn milk. At the first farm, eight tests showed the machine-drawn milk to have an average germ content of 2,790,100 per c.c. (maximum, 9,417,600, minimum, 105,000) while the hand-drawn milk had an average germ content of 768,382 per c.c. (maximum, 4,179,200, minimum 33,233) counts being made on agar plates. At the second farm, in three similar comparisons, the germ content averaged 172,958 per c.c. for the machine-drawn milk and 9,400 for the hand-drawn milk. These tests were made before special methods were used for cleaning the tubes. Later, similar comparisons were made when various antiseptics or antiseptic measures were used. These included (1) laying the tubes in brine, (2) boiling in a borax solution, (3) steaming the tubes, (4) scalding the tubes just before use and (5) boiling for three-quarters of an hour in water. The method of placing the tubes in brine was found to be the best when all things were considered. Using this method at one farm it was found to be easy to produce machine-drawn milk with a lower germ content than that of the hand-drawn milk; but at the other farm this was more difficult because of the lower germ content of the hand-drawn milk.

Stocking and Mason[9] reported additional studies with the Burrell-Lawrence-Kennedy machine at the Storrs Experiment Station dairy. Comparisons were made of the germ content of machine-drawn and hand-drawn milk of two sets of cows which were frequently alternated. In ten comparisons when no special methods

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[6] Barthel, Chr. La traite méchanique au point de vue bactériologique. IIe. Cong. Internat. de Laiterie à Paris, Repts. Prélim., Sect. II, Sous-Sect. 7A, 3e Question, pp. 4. 1905.

[7] Erf, Oscar. Milking machines. Kan. Agr. Exp. Sta., Bul. 140. 1906.

[8] Stocking, W. A., Jr. Bacteriological studies of a milking machine. U. S. Dept. Agr., Bur. An. Ind., Bul. 92, Pt. 2, p. 33-55. 1907.

[9] Stocking, W. A., Jr., and Mason, C. J. Milking machines. Pt. 1. Effect upon quality of milk. Storrs Agr. Exp. Sta., Bul. 47, p. 105-129. 1907.

of sterilizing the tubes were used, the results averaged 15,524 per c.c. (maximum 35,300, minimum, 1660) in machine-drawn milk and 3144 per c. c. (maximum, 8890, minimum, 200) in hand-drawn milk. Tests were also made of the efficiency of the following as methods of keeping the tubes free from germs: (1) streaming steam, (2) lime water, (3) solution of borax (1 to 15), (4) 2½ and 3½ per ct. formalin, (5) 10 per ct. brine, and (6) "gold dust" solution (1 to 300). With the exception of the tests with the formalin, the results secured were not as good as those obtained at the same time with hand milking. In the course of this investigation air-relief filters were added to the machines, in which cotton was used as a means of filtering bacteria out of the air which entered the machine during the milking process. The original purpose of the first filters, which were suggested by Mr. Loomis Burrell, was to prevent contamination of the milk thru moisture entering the vacuum pipes and its return when the vacuum was broken at the end of milking. The work of Stocking and Mason with the air-relief filters led them to believe that the cotton was a very effective agent in reducing the bacterial contamination of the milk.

Hastings and Hoffman [10] compared the germ content of hand-drawn and machine-drawn milk, using separate sets of cows for each. The hand-milking was done in 12-inch open pails, and the rubber parts of the machines were kept in lime water between milkings. The analyses of 150 samples of machine-drawn and of 136 samples of hand-drawn milk gave results which slightly favored machine milking. Twenty-two per ct. of the machine-drawn samples gave agar plate counts under 1000 per c.c., 55.3 per ct. were between 1000 and 5000, while 22.7 per ct. were over 5000 (maximum count, 174,000). Of the hand-drawn samples, 19.8 per ct. gave counts less than 1000 per c.c., 44.1 per ct. gave counts between 1000 and 5000, while 36.1 per ct. gave counts higher than 5000 (maximum count, 892,000).

Edwards [11] studied the Burrell-Lawrence-Kennedy machine at Guelph comparing the germ content of machine-drawn and hand-drawn milk. In seven tests on machine-drawn milk, the counts varied between 203,000 and 1,208,000 per c.c. while the germ content of six hand-drawn samples varied between 3200 and 68,900 per c.c. No special precautions other than ordinary methods of cleaning were used to keep the machines free from bacteria. In nine tests where the rubber parts were boiled once per week, the germ content of the machine-drawn milk was greatly reduced on the date of boiling; but quickly rose to high numbers again before the next treatment. In two tests when extreme precautions, such as boiling the tubes, steaming and thoro cleaning, were used the counts were reduced to 1407 and 1776 per c.c. The author concluded that where the tubes were cleaned and boiled every day under strict sanitary precautions the germ content of machine-drawn and hand-drawn milk was approximately the same, but that milk drawn with a B-L-K machine may contain many more bacteria than hand-drawn milk.

The germ content of machine-drawn and hand-drawn milk has also been compared by Miss Meek.[12] At the beginning of her tests, the rubber parts of the machines were rinsed successively in cold, tepid and hot water and then hung up until used. Later the pails were steamed and the rubber parts laid in brine between milkings. In several dozen samples of milk from individual cows drawn by machine, she found an average germ content of 47,870 per c.c. while an approximately equal number of hand-drawn samples taken at the same time averaged 21,115 per c.c. A similar number of composite samples taken from the mixed milk of four or five cows averaged 83,143 per c.c. for machine-drawn and 26,895 for hand-drawn milk. At the beginning the hand-drawn milk clearly contained fewer germs than the machine-drawn milk but as the work progressed the machine-drawn milk came to have a better quality.

[10] Hastings, E. G., and Hoffman, C. Bacterial content of machine-drawn and hand-drawn milk. Wis. Agr. Exp. Sta., 24th Ann. Rept. 1906–7, p. 214–223. 1907. Also Wis. Agr. Exp. Sta., Res. Bul. 3. 1909. Also *Centlbl. Bakt.*, Abth. II, 22:222–231. 1909.

[11] Edwards, S. F. Bacteria and the Burrell-Lawrence-Kennedy milking machine. Ontario Dept. Agr., Bul. 159, p. 20–24. 1907.

[12] Meek, Elizabeth B. Bacterial efficiency of the milking machine. Penn. Agr. Exp. Sta., Ann. Rept. 1907–8, p. 146–159. 1908.

Rinsings from the can of the machine, and from the cups and hose were examined bacteriologically but the method of taking these samples is not described in detail. The first of these samples, taken before special precautions were used in keeping the tubes clean, gave counts in the hundreds of thousands or millions per c.c. of rinse water. Later when the tubes were washed more carefully and laid in brine between milkings, these numbers were reduced to a few thousand and finally to less than 100 per c.c.

Haecker and Little [13] give three comparisons between the germ content of machine-drawn and hand-drawn milk taken in the early part of their work before any special precautions were used in cleaning the machines. These averaged 3310 per c.c. for the hand-drawn and 37,090 per c.c. for the machine-drawn milk. A few comparisons were made later when (1) the tubes were taken apart and scrubbed each day, (2) the tubes were placed in lime water, (3) the tubes, were boiled for twenty minutes each day before being placed in lime water, or (4) were boiled five minutes each day and placed in lime water. In general they report lime water ineffective unless accompanied by boiling and emphasize the necessity of the most painstaking cleaning of the machines if milk of low germ content is to be secured from them.

A report on 26 tests of machine-drawn milk is given by Brainerd,[14] part of these being made before special precautions were taken, and part after lime water was used for keeping the tubes germ-free. Three tests, made when lime water was not used, gave counts of 32,000, 35,000 and 1,250,000 per c.c. In 23 tests where lime water was used the counts varied from 34,000 to 100,000 per c.c., with an average of 60,000 per c.c. The author concludes that when lime water is used, the germ content of the machine-drawn milk compares favorably with that of hand-drawn milk.

Orr,[15] who made an investigation of the sanitary quality of milk for several counties in England, examined the milk from two dairy farms which were using milking machines of different types. In both, however, the milk was drawn thru rubber tubing. Two tests made on different occasions showed the milk from the first farm to have a germ content of 172,500 and 494,000 per c.c. Similar tests from the second farm showed counts of 1,392,000 and 986,000 per c.c. The last two counts were higher than any obtained from hand-drawn samples gathered in the course of a rather extensive survey of the contamination of a local milk supply. The author concludes that the machine was alone responsible for the high counts obtained. They were cleaned by drawing a strong soda water solution thru them, this being followed by tepid water.

Soon after this, the first report upon milking machines by Harding, Wilson and one of us (S) [16] was issued from this Station. This bulletin dealt primarily with the effect of the method of handling on the germ content of the milk, two types of machines having been studied, the Globe and the Burrell-Lawrence-Kennedy. When the Globe machine was carefully washed but no further precautions taken, the germ content of 20 samples of milk was found to average 692,542 per c.c. An equal number of hand-drawn samples had a germ content of 16,643 per c.c. The cows from which the samples were taken were milked alternately by hand and machine. The analyses of 33 samples of machine-drawn milk indicated that there was a slight reduction in the germ content of the milk in three successive milkings with the same machine. When the B-L-K machine was put into use, the effect of keeping the rubber tubes and teat-cups in a 10 per ct. brine was tested. Eleven samples taken when brine was used had an average germ content of 17,086, while a twelfth sample gave a count

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[13] Haecker, A. L., and Little, E. M. Milking machines. Neb. Agr. Exp. Sta., Bul. 108. 1908.

[14] Brainerd, W. K. The production of clean and sanitary milk. Va. Agr. Exp. Sta., Bul. 185. 1909.

[15] Orr, Thomas. Report on an investigation as to the contamination of milk. pp. 113, 18 figs., Beverly, England. 1908.

[16] Harding, H. A., Wilson, J. K., and Smith, G. A. Milking machines: Effect of the method of handling on the germ content of the milk. N. Y. Agr. Exp. Sta., Bul. 317. 1909.

of 176,620 per c.c. Thirty-six samples when brine was not used gave an average count of 188,580 per c.c. (maximum, 708,000, minimum, 9,500 per c.c.). Seven other series of tests showed the effect of excluding all air bubbles from the tubes while in the brine and of the effect of filtering the air entering the machine. Four other series of tests were made of minor points, the whole of the analyses leading the authors to the conclusion that both the brine solution and the cotton air filters were important aids in securing milk with a low germ content. In the later types of B-L-K machines used, the germ content of the milk was almost always less than 10,000 per c.c. when the proper precautions were used. However, dropping the teat-cups on the floor during the milking process or any gross carelessness in handling the machine caused surprising rises in the germ content of the milk and occasionally very high counts were obtained where no definite cause could be found.

Gorini [17] has studied the germ content of machine-drawn milk on a dairy farm in northern Italy where the Lawrence-Kennedy-Gillies machine was in use and was able to get milk of a lower germ content from the machine milkers than from hand milking. In one case, the hand-drawn milk had a germ content of 12,600 while the machine-drawn milk had a count of 3400 per c.c. The machine-drawn milk had a markedly better keeping quality than did the hand-drawn milk. He did not use brine or other antiseptics in this case but he carefully cleaned the machines, which were new when the tests were made.

Hoffman-Bang [18] studied machines of the same type in a Danish dairy, both from the standpoint of the germ content of the milk and of the effect of the machine milking upon the milk flow. By thoroly scrubbing the tubes and immersing them in a hot salt solution, he obtained results varying between 2000 and 231,000 per c.c. with an average of 27,000. The milk from the same cows drawn on alternate days by hand showed a germ content between 3000 and 34,000 per c.c. with an average of 11,000. Other antiseptics and antiseptic measures tried with varying success were (1) lime water, (2) a 15 per ct. salt solution, (3) live steam and (4) boiling the tubes for 15 to 20 minutes. He concluded that even with a sterile machine large numbers of germs were washed from the outside of the teats and udders of the cows and advised washing the teats and udders with warm water before milking.

Williams, Golding and Mackintosh [19] made bacteriological studies of eleven different makes of machines in a competitive trial arranged by the Royal Agricultural Society in England in 1913; but no machines were allowed to enter the competition where antiseptics of any sort were used in keeping them clean. Three of the machines that entered the trials were of the pressure type in which no rubber tubing is used, and eight were of the suction type. The bacteriological counts obtained from the three pressure-type machines averaged 675, 4603 and 5161 per c.c., respectively, while the averages from the eight suction-type machines varied from 1579 to 41,419 per c.c. Ten samples were analysed in each case. A series of twelve hand-drawn samples averaged 2666 per c.c. while the average of eleven machine-drawn samples taken at the same time was 6727 per c.c. So far as known the only one of the eleven machines now being sold in New York State is the Omega, the machine which was regarded as the best one submitted in the trials.

Miss Wing [20] studied the Burrell-Lawrence-Kennedy machine. Seventy-one tests of machine-drawn milk from seven cows when the rubber parts of the machine were immersed in a 15 per ct. brine gave counts on agar plates which varied between 150

[17] Gorini, C. Studi sulla mungitura meccanica, specialmente sotto il rispetto igienico batteriologico. Boll. Uffic. de Ministero di Agricultura, Indust. e Commer., Ann. 8 (1909), Vol. 2, Ser. C, No. 1, pp. 23–28. 1909. Abs. in *Milch Zeit.*, 39:183–185. 1910.

[18] Hoffman-Bang, N. O. Forsög med Molkemaskiner. Kgl. Veterinaerog Landbohöjskoles Labor. for Landökonomiske Forsög, 6Sde Peretning, p. 3–79. 1910.

[19] Report on the trials of milking machines, 1913. Royal Agr. Soc. England (London), pp. 23, 2 figs., 1914. Also paper presented at the Australia meeting British Assoc. Adv. Sci. 1914. Sect. M, Ref. 210.

[20] Wing, Lois W. Milking machines: Their sterilization and their efficiency in producing clean milk. Cornell Agr. Exp. Sta., Circ. 18. 1913.

and 12,300 colonies per c.c. Still lower counts were obtained in 21 tests when chloride of lime was added to the brine used in sterilizing the rubber parts, the individual tests varying between 150 and 3125 per c.c. A later test on six cows where 34 samples were analysed gave results varying between 350 and 4650 per c.c. (average 2342 per c.c.). A series of 24 comparisons between hand-drawn and machine-drawn milk in which the machine tubes were not cleaned after milking but placed immediately in the brine and chloride of lime solution gave average counts of 513 per c.c. for the hand-drawn and 3068 per c.c. for the machine-drawn milk. Other antiseptics tested to a limited extent but discarded for various reasons were (1) brine and hydrogen peroxide, (2) brine and alcohol, (3) brine and permanganate of potash, (4) brine and calcium peroxide, (5) copper sulphate, (6) vinegar, (7) acetic acid, (8) formaldehyde. She concluded that brine alone did not completely sterilize the tubes, but that chloride of lime and brine did practically do so.

Larsen, White and Fuller [21] compared the germ content of hand-drawn and machine-drawn milk, using the Hazelwood machine. In a series of 38 comparisons the average germ content of the hand-drawn milk was 10,739 per c.c., while that of the machine-drawn milk was 509,285 per c.c. No special methods of cleaning the machines were used. When the tubes were placed in brine between milkings the germ content of the machine-drawn milk averaged 252,166 per c.c. for 38 samples. When $2\frac{1}{2}$ per ct. formalin was used the average was 72,058 per c.c. for 17 samples. When a saturated brine solution with 5 per ct. calcium chloride added was used, the average count was 134,070 per c.c. for 17 samples. The use of cotton filters reduced this to an average of 57,708 per c.c. for 12 samples. The investigators concluded that machine-drawn milk is higher in germ content than hand-drawn milk; but that soaking the tubes in brine and 5 per ct. calcium chloride and using air filters would greatly reduce the contamination from the machine. Later Larsen,[22] without giving additional data, states that lime water is the best germicide to use.

Hooper and Nutter [23] report tests with the Sharples milking machine, lime water being used as a solution in which to keep the milking tubes. In 21 tests made at the Kentucky Station dairy the average germ content was 3657 per c.c. In addition they give the results of the examination of the milk supplied from the dairy and sold in the City of Lexington. This milk, which was drawn largely by machine, was found to have during a one year period, an average germ content of 10,620 per c.c. when tested at the Experiment Station, or 8487 per c.c. when tested by the Board of Health of the City of Lexington. Results are also given for 178 samples taken from 57 cows milked by Sharples machines in a neighboring commercial dairy. The average germ content of these samples was 3389 per c.c.

A limited study was also made of the value of strong brine and a 5 per ct. soda solution as antiseptics in which to keep the rubber tubes; but both were discarded when it was found that the solutions themselves were not sterile. They concluded that with care milk with a low germ content could be produced on any dairy farm.

Güssow [24] made forty comparisons between hand-drawn and machine-drawn milk using a Sharples machine. No special precautions were taken in sterilizing the rubber parts of the machines. The average germ content for the hand-drawn milk was 4551 per c.c. while that for the machine-drawn milk was 70,646 per c.c.

Harrison, Savage and Sadler [25] report upon a few tests of milk as delivered in Montreal which came from farms where machines were in use. The average germ

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[21] Larsen, C., White, Wm., and Fuller, J. W. Preliminary report on the milking machine. So. Dak. Agr. Exp. Sta., Bul. 144. 1913.

[22] Larsen, C. Important factors affecting machine milking. So. Dak. Agr. Exp. Sta., Bul. 166. 1916.

[23] Hooper, J. J., and Nutter, J. W. Experiments with the Sharples mechanical milker. Kentucky Agr. Exp. Sta., Bul. 186. 1914.

[24] Güssow, H. T. Milk bacteriological investigations. Append. Rept. Minist. Agr., Exper. Farms Rept. for 1912-3, p. 478–480. Ottawa, 1914.

[25] Harrison, F. C., Savage, A., and Sadler, W. The milk supply of Montreal. 67 pp., 1914.

content for seven such samples was 778,428 per c c. (maximum, 2,900,000, minimum, 100,000). A single comparison between hand-drawn and machine-drawn milk was given which was stated to be typical of many. The count of the machine-drawn milk as made from agar plates was 700,000 per c.c. while the hand-drawn milk had a count of 36,000 per c.c. They concluded that under practical farm conditions, machine-drawn milk had more bacteria in it than ordinary hand-drawn milk.

Moak,[26] who has charge of the inspection of the certified milk sent to Brooklyn, N. Y., reports upon the results obtained on ordinary and certified farms which use the Burrell-Lawrence-Kennedy machine. Chloride of lime and brine, or montanin (see page 174) were used as antiseptics for keeping the rubber tubes free from bacteria. At first the type of machine used was similar to the one used by Harding, Wilson and one of us (S) and also by Miss Wing. In this type of machine cotton filters are used at the place where the machines are connected with the vacuum piping system. Moak found that the filters did not stop the entrance of condensation water into the pail. An analysis of the water having shown that it contained large numbers of bacteria, a trap was devised by the manufacturers to prevent the entrance of this contaminated water into the milk. Four tests made on a dairy farm showed the following counts, where the older type of machine was used; 278,000, 275,000, and 288,000 and 214,000 per c.c. Under identical conditions where the new type of machine was used the counts were 4280 and 2960 per c.c. In 182 tests from a certified farm where the new type of machine was used, the counts averaged 2962 per c.c. for milk as delivered in Brooklyn. He also gives counts from machine-drawn milk of 11 individual cows which varied between 640 and 2520 per c.c. Another series of 16 counts from individual cows gave an average count of 880 per c.c., with two counts as low as 40 per c.c. After using a 2 per ct. montanin solution for 49 days, rinsings of the tubes with sterile water showed them to be free from bacteria. A series of 16 tests of the milk from two certified farms as delivered in the city showed an average germ content under these conditions of 5360 per c.c. He concludes that certified milk may be produced on farms where milking machines are used but does not urge their use for this purpose.

Burri and Hohl,[27] working at Liebefeld, Switzerland, have compared the germ content of hand-drawn milk with that of milk drawn with the Omega milker. When no special precautions were taken to render the machine free from bacteria, the germ content of the milk was ordinarily between 100,000 and 500,000 per c.c., the average of 54 milkings being 204,574 per c.c. When the same set of cows was milked by hand, the average of 48 tests showed 6377 per c.c. (maximum 34,000, minimum, 500). Tests of rinse water showed that the excess contamination came from the interior of the machine. When steam was used to sterilize all of the machine except the four rubber-lined teat-cups, the germ content of the milk was as low as from the hand-milked cows (about 6000 per c.c.). This was further reduced to about 1,000 per c.c. by careful washing of the udder and teats of the cows. Similar low counts were obtained when the machines were taken apart, cleaned in hot soda water, and dried.

Ruediger,[28] in examining the germ content of milk received on a city market, has found that the milk from six farms where machine milking was practiced usually gave counts on agar plates in excess of 1,000,000 per cc., only one count (and that 785,000) being less than this number. The milk received from 6 farms in the same district where hand milking was practiced gave counts varying from 52,500 to 1,795,000 per c.c. When the machines were scalded just before use, the germ counts

[26] Moak, Harris. Recent experiments with the milking machine in the production of certified milk. Proc. Cert. Milk Pro. Assoc. of America, 7th (1914) Ann. Conv., p. 28–38. 1915.

[27] Burri, R., and Hohl, Joh. Einfluss des Melkens mit der Melkmaschine "Omega" auf die bakteriologische Beschaffenheit der Milch. *Landw. Jahrb. d. Schweiz*, Jahrg. 30, p. 240–255. 1916.

[28] Ruediger, G. F. The milking machine a source of bacterial contamination of milk. *Jour. Inf. Dis.*, 19:652–654. 1916.

were found to vary between 2430 and 130,000 per c.c. He concluded that the proprietary product "Bacilli-Kil" and chloride of lime solutions are unsatisfactory for preventing bacterial growth in the tubes and that when the machines are not carefully cleaned, and scalded just before use they are liable to be badly contaminated.

From the foregoing review, it appears that there is practically unanimous agreement that the suction type of milking machines (the most successful type from the mechanical standpoint) must be cared for by special methods of cleaning if they are to be maintained in a sufficiently germ-free condition to yield milk containing as few germs as that obtained by careful hand milking. Under practical farm conditions where dairymen have used methods of cleaning the machines comparable to those used in cleaning simple dairy utensils such as pails, strainers and the like, investigators have found that the machine-drawn milk frequently has a germ content of millions per c.c. Similar, tho not as high counts, have been obtained in practically all cases even where steam was available for sterilizing the pails and metal parts of the machines. Even on high grade farms such as those of agricultural colleges and experiment stations, the counts from machine-drawn milk have in almost all cases been higher than those from hand-drawn milk from the same stables. Where bacteriological tests have been made and special methods of caring for the machines developed, several investigators report as good or better results from machine-drawn as from hand-drawn milk. Probably the most extensive results of this sort are those of Hastings and Hoffman,[29] Hooper and Nutter,[30] and Moak.[31]

The majority of the workers agree that it is the difficulty of preventing the growth of germs in the teat-cups and rubber tubes which is chiefly responsible for the high germ content of machine-drawn milk. A few have regarded the air that enters the suction type of machines at the end of the milking process when the vacuum is relieved as a significant source of contamination; and the same workers have also held that, in machines of the B-L-K type where air continuously enters the interior of the tubing and teat-cups, a significant number of germs enters with it. As indicated in the foregoing review, Stocking and Mason[32] were the first to test the

[29] See footnote 10.
[30] See footnote 23.
[31] See footnote 26.
[32] See footnote 9.

use of cotton filters as a means of reducing this contamination. At the same time they noted that condensation water in the vacuum pipes ran back into the milk pails when the vacuum was broken at the end of milking; but the improvement noted from using the cotton filters was ascribed to the effect of the cotton acting as a filter in removing germs from the air. These filters on the B-L-K machines were enlarged and improved in the course of the work done at this Station by Harding, Wilson and one of us (S)[33]; Larsen, White and Fuller[34] also report that cotton filters are effective in lowering the germ content of the milk drawn by the Hazelwood machine. Later Moak[35] has shown that the condensation water from the vacuum pipes is badly contaminated and the manufacturers of the machines have found that the cotton filter used at the connection with the vacuum pipes was not effective in preventing the return of this water into the milk. These studies resulted in the use of a trap for collecting the condensation water and the placing of a cotton filter at another place on the head of the machine thru which the air entered when the vacuum in the pail is relieved at the end of the milking process. The lessening of the germ content of the milk which followed the use of these devices led Moak to regard them as important aids in securing milk of low germ content.

Many workers have reported trouble caused by the accidental dropping of the teat-cups of the vacuum type of machine during milking. These, acting as suction cleaners, quickly draw noticeable quantities of dirt into the machines. Altho no direct evidence has been presented to show the relative importance of this source of contamination, those who have spoken of it have regarded it as important.

The authors of three reports have called attention to the possibility that bacteria are drawn into the teat-cups from the outer surfaces of the udder or are washed from the outer surfaces of the teats by the milk. Harrison[36] mentions this possibility but presents no data. Hoffman-Bang,[37] and Burri and Hohl[38] present some data and conclude that these are significant sources of bacteria.

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[33] See footnote 16.
[34] See footnote 21.
[35] See footnote 26.
[36] See footnote 3.
[37] See footnote 18.
[38] See footnote 27.

The metal parts of the machine such as the pail and cover may add large numbers of bacteria to the milk, in the same way that milk pails, cans and other metal dairy utensils add bacteria to the milk if they are not properly cleaned. This is not a problem peculiar to milking machines, but is identical with the general utensil problem which has been discussed by Prucha, Weeter and Chambers.[39]

PRESENT STUDIES.

EFFECT OF MILKING MACHINES ON THE GERM CONTENT OF THE MILK DRAWN.

Observations have been made upon the total amount of bacterial contamination found in machine-drawn milk both as drawn in the Station stable and under ordinary commercial dairy conditions. The relative importance of stable air, dropping the teat-cups on the floor, and the condensation water in the vacuum piping as sources of bacteria have also been studied in detail. Since the present studies concern only those contaminations derived from the machines themselves, no attempt has been made to measure the importance of the exterior of the teats and udder as sources of bacteria in machine-drawn milk.

In earlier studies, the majority of the investigators have used the amount of contamination of hand-drawn milk as a standard for comparison with machine-drawn milk. However, this is an unsatisfactory standard as the amount of contamination of hand-drawn milk is highly variable. A much better standard is a numerical one based partly upon the experience of practical farmers in the production of certified milk, and also partly upon such numerical measures of the amount of contamination of milk as have been secured in special investigations. Thus Harding and Wilson[40] have shown that normal healthy udders, free in large measure from streptococcic infections, give milk with an average germ content of approximately 500 per c.c., Reuhle and Kulp[41] have shown that the dust in the air is a still less important source of bacteria, rarely being responsible for increasing the germ content of the milk more than 10 to 100 per c.c. Prucha, Weeter and Chambers,[42] on

[39] See footnote 2.
[40] See footnote 1, third reference.
[41] See footnote 1, fourth reference.
[42] See footnote 2.

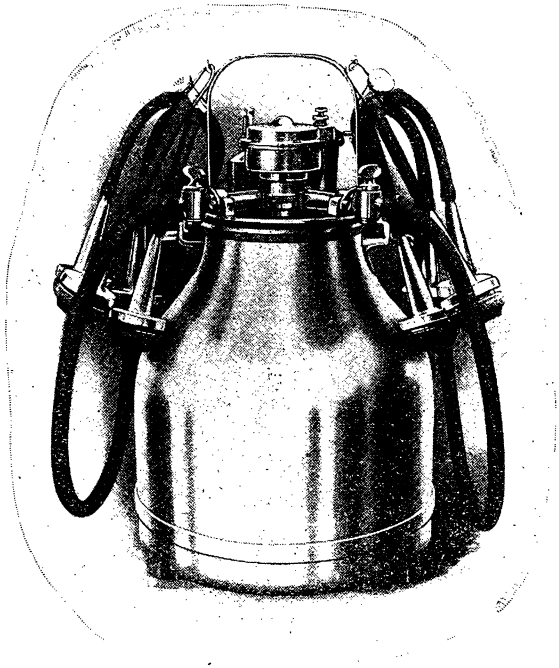

FIG. 1.—THE BURRELL-LAWRENCE-KENNEDY MILKER.

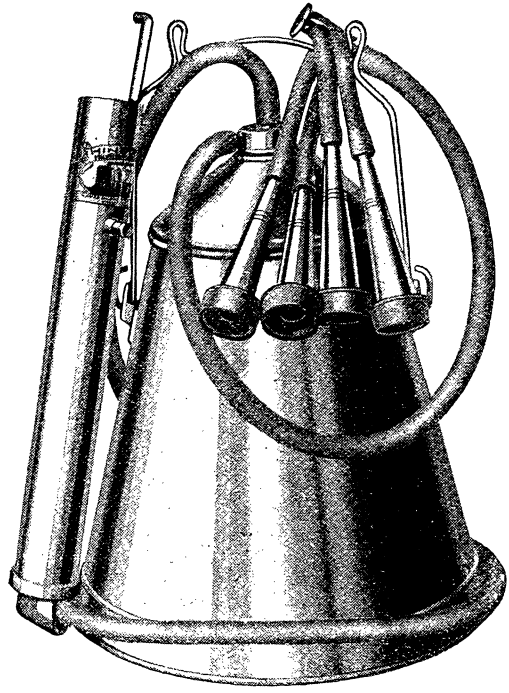

FIG. 2.— HINMAN MILKER WITH PUMP (AT LEFT).

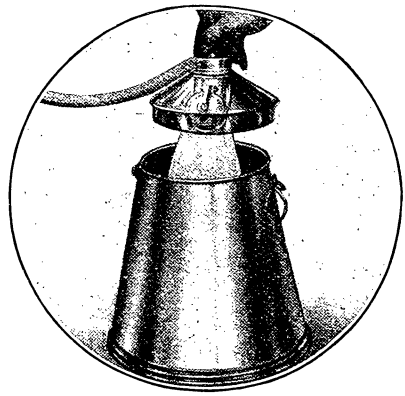

FIG. 3.— HINMAN MILKER.
Milk entering pail.

the other hand, have shown that the amount of contamination of milk from ordinary utensils is highly variable and is dependent upon the methods used in cleaning and caring for them. With proper care of utensils and reasonably good dairy conditions the germ content of fresh milk from cows with uninfected udders ought not to exceed 10,000 per c.c.

Machines used.— The machines studied were the Burrell-Lawrence-Kennedy (Fig. 1), the Hinman (Figs. 2 and 3) and the Empire (Fig. 4). The B-L-K machine was used at the Station and on two commercial dairy farms. The Hinman and the Empire milkers were each used

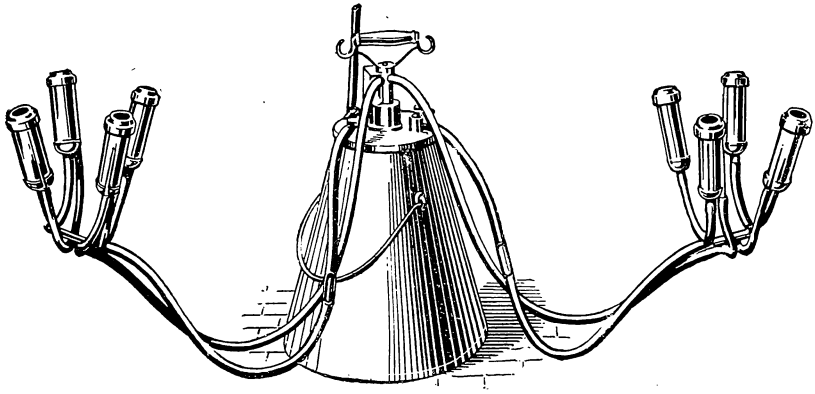

FIG. 4.— EMPIRE MILKER.

on four dairy farms. All of the dairy farms were located in the vicinity of Geneva and all of them supplied milk for the City of Geneva.

The Empire machine is one which uses both suction and air pressure on the teats, having double-lined teat-cups for this purpose. The other two machines use simple, conical, all-metal teat-cups with rubber mouthpieces. The main differences in principle between the Hinman machine and the other two machines lies in the fact that (a) the Hinman machine maintains no vacuum in the pail; but has a small vacuum chamber in the cover of the pail (Fig. 3); and (b) that the vacuum is secured by simple individual pumps (Fig. 2) for each milking unit. Both the B-L-K and the Empire machines maintain a 15-inch vacuum in the pail of the machine.

It should be stated that this selection of machines was a chance one; and in no way indicates our judgment as to the desirability of these three types.

Technique used.— In attempting to determine the relative importance of each of several possible sources of bacterial contamination of machine-drawn milk, it was necessary to devise some method by which each source could be studied separately. This was accomplished by using sterile water in an artificial udder (Fig. 5), and drawing it into the machine in a manner which simulates the true milking process very closely. Five liters of water have been used in each case and the time consumed in drawing this into the pail has usually varied between three and four minutes, a period of time which compares closely with that ordinarily consumed in drawing the principal part of the milk yield of an ordinary cow.

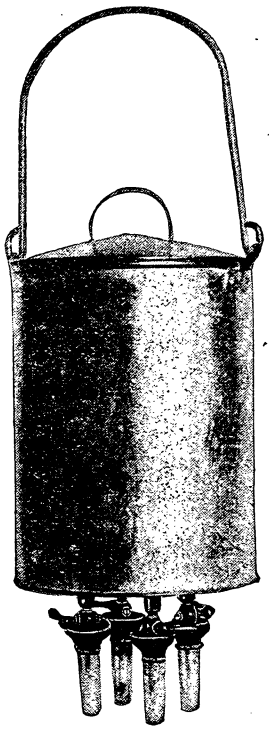

FIG. 5.—ARTIFICIAL UDDER USED IN EXPERIMENTAL WORK.

In attempting to study the effect of each of several possible sources of bacteria about the machine itself, such as the dust in the air, the condensation water from the vacuum pipes, the rubber tubes and the like, it was necessary to develop still other methods of attacking the problem.

Thus in an attempt to determine whether the bacteria got into the milk (1) during its passage thru the teat-cups and rubber connecting hose, (2) from the head of the machine or, (3) from the pail of the machine, means were devised for collecting samples of water drawn from the "udder" at two points in its passage into the pail. The apparatus used is shown in Fig. 6. "A" was a device for collecting samples from the teat-cups and hose. It consisted of a 45 per c.c. sterile glass cylinder which was fitted with glass tubes so that a portion of the water which passed thru the connecting hose would be retained in the cylinder at the end of the milking process. In operation, however, it was found that this

cylinder was almost completely emptied at each pulsation so that the water retained was not representative of the entire "milking." "B" was a small sterile specimen jar of 200 per c.c. capacity which was hung by means of a bent wire from the partition of the pail so that it received a portion of the water as it sprayed into the pail at each pulsation. By examination of the way in which milk or water sprayed into a glass cylinder substituted for the regular pail, it was discovered that this resulted in securing a sample fairly representative of the whole milking. Samples from the pail itself gave results which indicated the total amount of contamination.

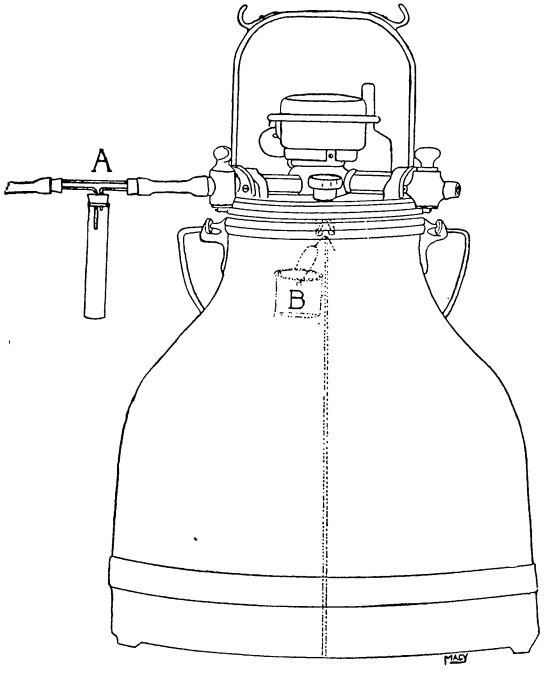

FIG. 6.—B-L-K MILKER SHOWING SAMPLING DEVICES.

Collection of samples, and methods of analysis used.—The samples were collected in sterile test tubes by means of sterile pipettes which were first used for agitating the water to be sampled. The plating of the samples was usually completed within an hour of the time of collection, or within three hours when collected at a distance from the Station. In the latter cases the samples were kept cold, when taken in summer, by immersion in water containing floating ice.

The medium used in plating had the following composition:

Agar, in shreds	15 grams.
Witte's peptone	10 grams.
Liebig's beef extract	5 grams.
Lactose, c. p	10 grams.
Distilled water	1,000 c.c.

The reaction of the medium was not adjusted, as the acidity varied between 0.9 and 1.2 per ct. normal acid to phenolphthalein. Experience at this laboratory has shown that a medium made of the above materials and of the above reaction to phenolphthalein has a hydrogen ion concentration between $P_H = 6.8$ and $P_H = 7.4$.

The colonies on the petri plates were counted under a hand lens (Engraver's lens no. 146) [43] after they were incubated for five days at 21° C and reincubated for two days more at 37° C. Whenever dilutions were made, the material was plated in triplicate in each dilution. Only such plates as showed more than 30 and less than 300 colonies per plate were used in computing the count per c.c. In a considerable portion of the work no dilutions were necessary. In these cases five plates were poured and all satisfactory plates counted.

It should be recognized (as is discussed in Bulletin No. 439) that all of the agar plate counts given in this bulletin, are counts of colonies which develop on petri plates. They are not counts of individual bacteria, but are rather counts of such single bacteria or groups of bacteria as grow into visible colonies under the conditions specified. While these counts may be compared with one another thus giving what is believed to be a fairly correct idea of the relative amount of contamination from each source, it should not be forgotten that the actual number of bacteria present was probably several times the figures given in each case.

PRELIMINARY SURVEY OF CONDITIONS AT THE STATION.

When this study of the amount and source of the bacterial contamination of milking machines was started (March, 1916), milking machines had been in use at the Station for ten years, and B-L-K machines for nine years. Soon after the original installation, the operation of the machines was placed in the hands of the regular dairy attendants under the supervision of one of us (S); but the attendants did all of the work of operating and caring for the machines.[44] The men chiefly involved in this work have not changed

[43] Sold by Bausch and Lomb Opt. Co.

[44] The two men, Mr. William Casey who has had charge of the cleaning and operation of the machines at the stable and Mr. William Lydon, who has had charge of the cleaning of the machines at the dairy, have done their work so efficiently that no small part of the credit for the successful operation of the machines during all of these years really belongs to them.

during the ten years and the work has been done as a regular routine. No regular bacteriological tests of the Station milk have been made during the time, but numerous analyses made at irregular intervals have invariably shown that the milk had a low germ content, that is, usually under 10,000 per c.c.

During this time the routine method of cleaning the machines has been as follows: Immediately after each milking, a large pailful of clean cold water was drawn into each of the machines thru the teat-cups. This was followed by a pailful of hot soda water, and finally by a pailful of clean hot water. After the evening milking, the pails of the machines were emptied, rinsed with cold water, and left until the following morning; but after each morning milking the pails and heads of the machines were sent to the dairy where they were more thoroly washed and steamed for a few minutes in a steam chest (with the exception of the pulsator piston). The pails then stood in a warm room with the heads on them until the evening milking, a procedure which was later found to be unsatisfactory because of the frequent presence of a small amount of moisture in the pail.

In all of the earlier work as discussed on pages 119–120, the teat-cups and rubber tubes were kept in a brine solution between milkings; but just before the more detailed experimental work reported in this bulletin was started the solution of brine was discarded in order to test the efficiency of chloride of lime. The strength of the solution used was carefully maintained and tested for available chlorine. This was maintained between 35 and 266 parts per million.[45]

On Tuesday of each week these tubes were sent to the dairy and scrubbed with spiral brushes and a warm soda solution, after which they were again placed in the chloride of lime solution.

In making tests to determine the amount of contamination of the milk which was derived from the machines themselves, five liters of sterile water were " milked " from the artificial udder as

[45] This solution was prepared by adding one quart of a strong stock solution to twenty-five gallons of water in a large earthenware jar. The stock solution was prepared by adding a 12 ounce can of dry bleaching powder to one gallon of water in a tall glass jar. This was allowed to stand for twenty-four hours before use, when the clear, greenish-colored solution could be decanted as needed. The solution in the large crock in which the tubes were kept was reinforced with one pint of fresh stock solution once per week, thus maintaining the amount of available chlorine in the solution. The tubes were suspended in the solution from hooks in the cover of the crock.

described (page 129). The results are given in Table I. The first two of the twenty-two tests were made on separate units, but in the later tests, two successive milkings, or later still four successive milkings were carried out with each unit. Test No. 5 was omitted in computing the averages as the difference between the two milkings was so much greater than that noted in the other cases that its inclusion in the averages would lead to erroneous conclusions.

TABLE I.— AMOUNT OF BACTERIAL CONTAMINATION DERIVED FROM STATION MACHINES.

Agar plate counts per c.c. of sterilized water, "milked" thru machines cared for in ordinary way.

TEST NUMBER.	Date.	First milking.	Second milking.	Third milking.	Fourth milking.
	1916.				
1	Mar. 1	*6 700	.....	.....	.....
2	Mar. 1	*3 100	.....	.....	.....
3	Mar. 2	1 320	3 890	.....	.....
4	Mar. 3	1 230	1 430	.....	.....
5	Mar. 7	*35 800	*22 200	.....	.....
6	Mar. 8	2 500	2 010	.....	.....
7	Mar. 9	10 800	10 720	.....	.....
8	Mar. 10	4 800	1 900	.....	.....
9	Mar. 11	680	380	.....	.....
10	May 11	3 200	2 400	.....	.....
11	May 12	113	171	.....	.....
12	May 13	460	320	.....	.....
13	May 20	710	870	970	335
14	May 23	2 500	4 200	2 720	2 250
15	May 24	1 110	950	1 860	2 260
16	May 25	2 420	520	1 190	880
17	May 26	337	676	438	505
18	May 27	582	1 800	928	747
19	June 6	3 300	3 000	2 100	2 300
20	June 7	2 000	800	1 100	3 000
21	June 8	800	585	600	500
22	June 9	1 390	4 900	3 400	1 240
Average of tests number 13–22		1 515	1 830	1 330	1 400
Average of tests number 3–22		2 118	2 183	.....	.....

* Omitted from averages.

In the first place it will be noted that the machines were not badly contaminated since but four tests showed counts in excess of 10,000 per c.c. If Test No. 5 is excluded, the average counts of the four milkings are all less than 2200 per c.c. Contaminations

of the amount noted would not be troublesome in the production of Grade A milk.

In these tests there was no progressive decrease in the germ content of the water in successive milkings even where four successive milkings were made on the same unit. Thus in the 19 tests where two successive milkings were made, the average counts were 2118 and 2183 respectively. In 10 of the 19 tests where four successive milkings were made, the averages were 1515, 1830, 1330 and 1400, respectively.

Source of the bacteria in the Station machine.— Before attempting any measures for improving the bacterial condition of the machines, or allowing the attendants to know of the purpose of the work, an attempt was made to discover the exact source or sources of the bacteria. As explained on page 129, thirteen tests of four milkings each were made, and samples taken of the rinse water (1) after passing thru the teat-cups and rubber tubes, (2) after it had passed thru the head of the machine, and (3) after it had collected in the pail. The results are given in Table II.

The results secured from the analyses were somewhat irregular and the counts from the water drawn into the pail of the machines are larger than those given in Table I, the averages from the four successive milkings in this case being 8473, 3038, 3606 and 2831, respectively.

There is a very noticeable tendency for the counts from the teat-cups and rubber tubes to be less than those taken later in the passage of the water into the machine and they remained at all times less than 5000 per c.c. At first these relatively low counts were explained as indicating that many fewer bacteria came from the teat-cups and rubber tubing than from the head and pail; but later it was realized that this result may have been caused by unrepresentative sampling (see page 129). It was surprising to secure occasionally almost sterile plates from the samples taken from the teat-cups and rubber tubes.

At times there is indication of a significant contamination from both the head of the machine and the pail. The latter may be explained by the fact that they were insufficiently steamed, and were allowed to stand in a moist condition for some hours before use at a temperature which favored bacterial growth.

TABLE II.— SOURCES OF THE BACTERIA DERIVED FROM STATION MACHINE.

Agar plate counts per c.c. of sterilized water "milked" thru machines cared for in ordinary way.

TEST No.	Date.	FIRST MILKING.			SECOND MILKING.			THIRD MILKING.			FOURTH MILKING.		
		Teat-cups only.	Teat-cups and head.	Entire machine.	Teat-cups only.	Teat-cups and head.	Entire machine.	Teat-cups only.	Teat-cups and head.	Entire machine.	Teat-cups only.	Teat-cups and head.	Entire machine.
	1916												
1...	June 22	2	38	2 530	20	15	2 230	5	5	730	13	5	710
2...	June 24	2	230	10 367	16	1 140	787	6	710	1 570	14	.....	1 187
3...	July 5	86	1 270	2 910	191	37	1 670	14	19	37	154	44	30
4*..	July 6	14	370	8 600	80	18	9 300	.....	.....	.....	.....	.....	.....
5...	July 7	3	43	184	57	3 855	4 900	2	3 080	10 300	28	407	10 900
6...	July 10	6	400	8 700	28	1 260	5 100	2	1 640	13 000	7	.....	1 350
7...	July 11	740	8 400	23 600	500	1 020	2 275	146	450	4 900	1 710	600	430
8...	July 19	95	940	840	2 340	11 200	1 260	58	44 400	4 070	129	340	4 100
9...	July 20	1 680	11 400	8 000	1 510	2 140	3 700	1 730	4 100	5 200	4 370	1 220	1 930
10...	July 24	549	7 200	6 000	346	1 570	1 820	105	540	1 010	265	247	11 300
11...	July 25	2 080	18 400	25 000	1 810	1 750	1 710	680	1 130	760	750	550	610
12...	July 26	12	125	141	185	253	65	20	24	36	67	.....	49
13...	Aug. 4	1 500	1 110	4 800	1 150	520	1 640	203	16 000	1 660	1 520	380	1 380
	Ave.....	564	4 144	8 473	686	2 065	3 038	248	6 008	3 606	752	†421	2 831

* Test No. 4 omitted from averages as it was incomplete.

† Only nine tests in average.

Sterilization of the machines.— At this time, the cleaning and handling of the machines was taken over by one of us (R) and an attempt made to completely sterilize one of the units. The results showed that by taking extreme precautions and by separating the rubber parts completely and placing them in relatively strong chloride of lime solution or in a 2 per ct. montanin solution, sterile water could in most cases be drawn into the machines and still remain practically sterile. A few tests however showed counts between 350 and 500 per c.c. (Tables XVII and XVIII) when there was no apparent reason for the increase in count. A suggestion made at the time that these increases might be due to the occasional failure of the trap (Fig. 8) to entirely prevent the return of condensation water from the vacuum piping into the pail of the machine, led to further tests.

Entrance of condensation water from the vacuum pipes into the pail as a source of bacteria.—As has been stated (pages 123–4), the investigations made by Stocking and Mason on B-L-K machines resulted in the insertion of a cotton filter at the junction between the connecting hose and the vacuum pipes. These investigators report that the use of the filters led to a lessening of the germ content of the milk drawn, which they attribute in their papers to the filtering effect of the cotton on the air which enters when the vacuum is broken, altho from private correspondence it is clear that they also had in mind the fact that the presence of cotton in the filter prevented the return of iron rust from the piping system.

In the earlier work at this Station, machines were used which had cotton filters of the type used by Stocking and Mason; and as a result of new analyses made at that time these filters were enlarged.

After this work and before the present studies were undertaken, Moak had found that badly contaminated condensation water from the vacuum piping was still a real cause of trouble. As a result of experimental work by the manufacturers of the machines, the cotton filter at this point (Fig. 7) was entirely discarded and a suction trap (Figs. 8 and 9) inserted. The condensation water which returns from the pipes collects in the bottom of this trap and no air is allowed to pass back into the pail thru this opening. A valve (Fig. 9, vacuum relief valve) placed on the head of the machine at another place is used in relieving the vacuum in the pail when milking is finished.

In order to satisfy ourselves that this trap is necessary, heads of the older type were used on our machines where the pail of the machine was replaced by a glass jar. The glass jar and all other parts of the machines were sterilized as perfectly as possible, and the success of the sterilization was tested by analysing samples taken from five liters of sterile water drawn into the machine from the artificial udder. On the first trial, the germ count was 15 per c.c., and on the second trial 9 per c.c. showing that the sterilization was practically complete.

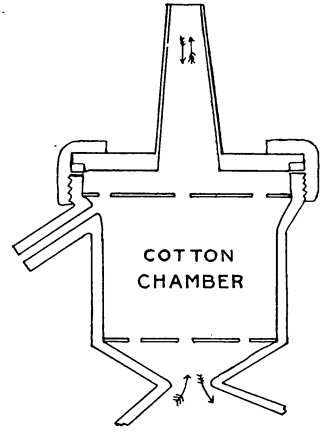

FIG. 7.—SUCTION TRAP—OLDER TYPE.

The procedure followed in carrying out the two tests was to break the vacuum thru the teat-cups after the five liters of sterile water was drawn into the glass jar and take a sample from the jar; then the milking was started again and stopped by breaking the vacuum thru the stanchion hose in the ordinary way after which a second sample was taken.

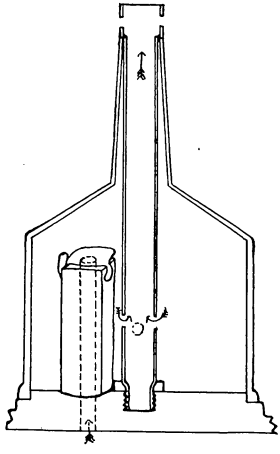

FIG. 8.—SUCTION TRAP—NEW TYPE.

Observations made thru the glass jar when the vacuum was broken thru the stanchion hose at the end of the first test showed that between 3 and 5 c.c. of condensation water was forced back thru the cotton filter by the inrush of air and the cotton was left nearly dry. The germ content of the water in the jar was raised by this contamination from 15 per c.c. to 39,000 per c.c. showing that this contamination was a serious one.

On the second trial the amount of water forced back thru the cotton filter was much less than in the first trial and the germ content was only raised from 9 per c.c. to 320 per c.c.

These variable conditions agree with other observations made in the Station stable where it has been found that the traps collect

very variable quantities of water during milking, the amounts varying from a few drops to as much as 10 c.c. The drain pipes on the vacuum lines discharge as much as 50 to 100 c.c. of water each day after milking is completed.

Numerous analyses were made of this drip water and of the condensation water in the trap. In almost all cases the germ content of the drip water and of the condensation water exceeded 1,000,000 per c.c. and frequently exceeded 10,000,000 per c.c. (maximum, 95,000,000). Because of these findings, an attempt was made to sterilize the piping by drawing a solution of chloride of lime thru the pipes. After repeated tests extending over several weeks, it was found that a certain improvement in conditions could be secured in this way; but that the bacteria very quickly reestablished themselves in the piping.

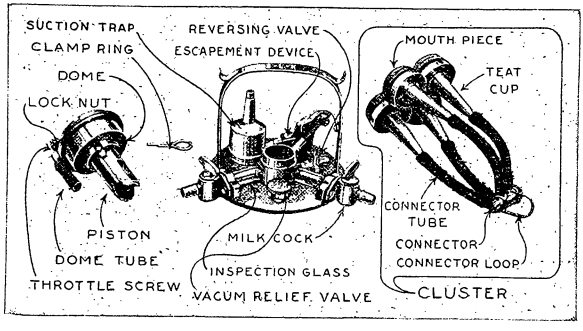

FIG. 9.—DETAILS OF THE BURRELL-LAWRENCE-KENNEDY MILKER.
Pulsator, Head, Teat Cups and Connector.

Meanwhile, tests were made to determine whether the trap (Fig. 8) absolutely prevented the return of this condensation water. In these tests, water was milked thru the machine from the artificial udder, while at the same time varying amounts of a methylene blue solution were placed in the trap. A piece of clean cotton was placed below the trap in the pail of the machine in order to reveal the presence of any methylene blue which might pass thru into the pail.

In no one of forty tests was there the slightests evidence of leakage. On the contrary the methylene blue solution largely passed up thru the connecting hose into the iron piping. While this makes it therefore very probable that this trap is efficient under all conditions, yet there remains a slight possibility that the unexplained increases in count of a few hundred per c.c., which were noted when all sources of contamination were supposed to be eliminated, came

from such leakage. The increases came at very irregular intervals and were only noted four times in making 144 tests (see Tables X, XI, XVII and XVIII).

Every effort should be made in milking machine construction to protect this opening into the pail, not only in machines of the B-L-K type but in all suction type machines. Undoubtedly the improvements in germ content after the introduction of a cotton filter at this point noted by Stocking and Mason and in the earlier work done at this Station were due to the partial protection from the condensation water offered by the cotton.

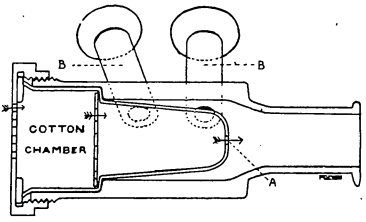

FIG. 10.— B-L-K TEAT-CUP CONNECTOR IN SECTIONAL VIEW

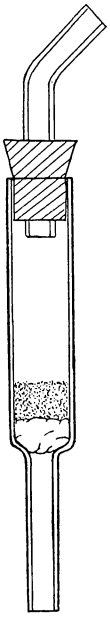

FIG. 11.— STANDARD AEROSCOPE.

Stable air as a source of bacteria.— Air has frequently been regarded as a source of a significant contamination of machine-drawn milk. In the present type of B-L-K machine, air comes in contact with the milk in two places. One at the relief valve placed on the "connector" at the base of the teat-cups (Fig. 9 and Fig. 10). Here a pin hole opening (Fig. 10, A) allows air to enter continuously during milking. When the pulsator on the head of the machine shuts off the vacuum in the connecting tube, the vacuum in the teat-cups is entirely relieved thru the air entering at this point. The second point of entrance for air is the "vacuum relief valve" (Fig. 9) placed on the head of the machine. This is opened at the completion of the milking process in order to relieve the vacuum in the pail and allow the cover to be raised.

Four series of tests were conducted to determine the amount of the contamination of the milk from these two sources. Aeroscopes developed in earlier investigations at this Station [46] (Fig. 11) were used in the tests as filters for removing all of the bacteria from the air as it entered the machine at the two release valves mentioned

[46] See footnote 1, fourth reference.

above. Two to three times the standard amount of fine sand was used as a filtering layer as it was feared that the passage of the rapid air currents thru the sand would spoil the efficiency of the filters; and in the first ten tests the aeroscopes were used in tandem in order to make sure that the first sand layer was an efficient filter.

The first series of tests were made in the stable with the cows in their stalls; but not while actual milking was in progress. The results given in Table III were secured during artificial milking.

TABLE III.—AMOUNT OF CONTAMINATION DERIVED FROM THE AIR DURING ARTIFICIAL MILKINGS.

Test No.	Air Sampled at Vacuum Relief Valve in Head of Machine.				Air Sampled at Relief Valve on Teat-cup Connector.			
	Plate Count		Total count per machine	Count per c.c. milk.†	Plate Count		Total count per machine	Count per c.c milk.†
	First aero-scope.	Second aero-scope.*			First aero-scope.	Second aero-scope.*		
1.....	29	0.7	290	.06	231	0.3	2310	.46
2.....	51	0.3	510	.10	129	2.0	1290	.26
3.....	99	2.0	990	.20	85	1.0	850	.17
4.....	99	0.7	990	.20	47	0.7	470	.09
5.....	107	0.7	1070	.21	100	0.3	1000	.20
6.....	85	0.7	850	.17	53	0.7	530	.11
7.....	38	1.0	380	.08	62	0.0	620	.12
8.....	22	0.0	220	.04	54	0.7	540	.11
9.....	30	0.3	300	.06	28	0.0	280	.06
10.....	72	0.0	720	.14	45	0.0	450	.09
11.....	100	.....	1000	.20	120	.....	1200	.24
12.....	89	.....	890	.18	730	.....	7300	1.46
13.....	137	.....	1370	.27	700	.....	7000	1.40
14.....	49	.....	490	.10	550	.....	5500	1.10
15.....	46	.....	460	.09	63	.....	630	.12
16.....	31	.....	310	.06	230	.....	2300	.46
17.....	54	.....	540	.11	250	.....	2500	.50
18.....	38	.....	380	.08	260	.....	2600	.52
19.....	175	.....	1750	.35	980	.....	9800	1.96
20.....	88	.....	880	.18	470	.....	4700	.94
21.....	2	.....	20	.004	1	.....	10	0.02
22.....	11	.....	110	.02	15	.....	150	.03
23.....	126	.....	1260	.25	139	.....	1390	.28
24.....	55	.....	550	.11	233	.....	2330	.46
Ave..	68	0.6	680	.14	233	0.6	2330	.46

* Figures given in this column were obtained from an aeroscope attached to first aeroscope in tandem.

† Computed for five liters of milk.

As shown in the third and fifth columns the counts obtained from the second aeroscope prove the first aeroscope to have been an efficient filter, and after ten trials the use of the second aeroscope was discontinued. The total germ content of the air which entered the machine thru the " vacuum relief valve " on the head of the machine was found to vary from 20 (Test No. 21) to 1750 (Test No. 19), with an average germ content of 680. The total germ content of the air entering a single machine during milking thru the vacuum relief valve on the teat-cup connector (column 4) was found to vary from 10 (Test No. 21) to 9800 (Test No. 19), with an average germ content from both sources of 2330. If all of the bacteria from both sources were added to the five liters of water drawn into the machine the average increase would be less than one per c.c. (0.6 per c.c.).

Since this series of analyses was not carried out under actual milking conditions, another series was made during milking, the results of which are given in Table IV. Certain difficulties in securing tests under these conditions compelled the making of separate series of analyses for each of the two valves thru which the air entered. Four to five tests were made each day, and analyses of the stable air were made at the same time.

In 33 tests of the air entering thru the relief valve on the head of the machine, the lowest count obtained was 40 (Test No. 27) while the highest was 11,400 (Test No. 9), the average number being 1590. The stable air at the same time showed a germ content which varied between 49 and 670 per liter, figures which are similar to those obtained in previous work.

In thirty tests taken at the relief valve on the " connector," the germ count per machine was found to vary from 6100 (Test No. 30) to 157,000 (Test No. 19), the average count being 37,300. The stable air showed a germ content varying between 93 and 890 per liter. When the maximum and minimum figures from both valves are added the maximum figures become 168,400, and the minimum, 6140. Under average conditions a count of 38,890 would be secured per machine. These numbers when taken by themselves seem to show that the air is a significant source of contamination; but when the figures are transformed so that they may be compared with the standard unit of measurement — a cubic centimeter of milk — it is found that this impression is only an apparent one. If the number of germs given in the minimum figures are

added to five liters of milk computation shows that the germ content of the milk would be increased by 1.2 germs per c.c., while if the

TABLE IV.—AMOUNT OF CONTAMINATION DERIVED FROM THE AIR DURING ACTUAL MILKINGS.

Test No.	Air Sampled at Air Relief Valve in Head of Machine.				Test No.	Air Sampled at Relief Valve on Teat-cup Connector.			
	Aeroscope plate count.*	Total count per machine.	Count per c.c. milk.†	Count per liter air.‡		Aeroscope plate count.*	Total count per machine.	Count per c.c. milk.†	Count per liter air.‡
1...	5	50	.01	79	1..	323	32300	6.5	890
2...	25	250	.05	.....	2..	810	81000	16.2	.....
3...	87	870	.17	.....	3..	668	66800	13.4	.....
4...	79	790	.16	.....	4..	634	63400	12.7	458
5...	97	970	.19	49	5..	772	77200	15.4	.....
6...	86	860	.17	.....	6..	190	19000	3.8	.....
7...	8	80	.02	.....	7..	385	38500	7.7	250
8...	38	380	.08	.....	8..	439	43900	8.8	.....
9...	114	11400	2.28	.....	9..	226	22600	4.5	.....
10...	57	5700	1.14	290	10..	285	28500	5.7	.....
11...	41	4100	.82	.....	11..	839	83900	16.8	.....
12...	30	300	.06	.....	12..	36	36000	7.2	93
13...	58	5800	1.16	.....	13..	26	26000	5.2	.....
14...	29	290	.06	.....	14..	26	26000	5.2	.....
15...	139	1390	.28	213	15..	502	50200	10.0	.....
16...	31	3100	.62	.....	16..	42	42000	8.4	770
17...	25	250	.05	.....	17..	47	47000	9.4	.....
18...	157	1570	.31	.....	18..	44	44000	8.8	.....
19...	133	1330	.27	.....	19..	157	157000	31.4	.....
20...	32	3200	.64	670	20..	19	19000	3.8	.....
21...	100	1000	.20	.....	21..	105	10500	2.1	530
22...	13	130	.03	.....	22..	275	27500	5.5	.....
23...	23	2300	.46	.....	23..	179	17900	3.6	.....
24...	106	1060	.21	.....	24..	328	32800	6.6	.....
25...	145	1450	.29	53	25..	69	6900	1.4	.....
26...	77	770	.16	.....	26..	385	38500	7.7	100
27...	4	40	.008	.....	27..	44	44000	8.8	.....
28...	22	220	.04	.....	28..	210	21000	4.2	.....
29...	49	490	.10	.....	29..	202	20200	4.0	.....
30...	79	790	.16	.....	30..	61	6100	1.2	.....
31...	129	1290	.26	.....	....	.....	.....	.....	.....
32...	24	240	.05	.....	....	.....	.....	.....	.....
33...	5	50	.01	.....	....	.....	.....	.....	.....
Ave.	.....	1590	.32	225	Ave.	.....	37300	7.4	442

* Sand suspension prepared in 10 c.c. sterile water. Some plates were prepared directly for this, others were made from dilutions of 1:10 and 1:100.

† Computed for five liters of milk.

‡ Counts obtained by using standard method of air analysis.

maximum number were added the increase would be less than 34 per c.c. These figures are insignificant when compared with the much larger number of bacteria invariably present from other sources and show that machine-drawn milk is no more abundantly contaminated with bacteria from the air than is hand-drawn milk (Compare with results obtained on hand-drawn milk as given in Bulletin No. 409).

In the Empire milker because of the use of double-lined teat-cups, no air enters the machine except as the vacuum is relieved in the pail at the end of milking. As the pails are roughly comparable in size to those used in the B-L-K machines, it is presumable that the air contamination in this case compares with that found here as entering thru the relief valve on the head of the machine. The Hinman machine is so constructed that there is little entrance of air into the interior of the vacuum chamber. It is therefore apparent that the total contamination from the air in these machines is even less significant, if such a thing is possible, than in the case of the B-L-K machine. All of the air contaminations are so small in amount that they would be quite undetectable in milk.

Efficiency of cotton as an air filter.— Two series of analyses have been made to test the efficiency of the cotton filters for which provision is made in the B-L-K machines and which were designed to remove the germs from the air, entering the machine during milking. In the first series of fourteen tests, two aeroscopes attached in tandem were used; and these were connected with the vacuum relief valves in the head of the machine. A mass of cotton was placed in the first aeroscope which was similar in amount to that used in the vacuum relief valve at this point. The second aeroscope contained sterile sand as usual. After milking five liters of water thru the machine, for each of four successive milkings, the aeroscopes were brought to the laboratory and counts made in the usual way. The results given in the second column of Table V show at once that the cotton is not a perfect filter. The counts obtained, however, are not as high as those secured under similar conditions where no cotton was used (see Table III), the average germ count per machine being 680 where no filters were used as contrasted with an average of 190 per machine where filters were used. These figures indicate that the cotton removed more than two-thirds of the bacteria. An examination of the cotton itself showed that coarse particles of dust and dirt were stopped by it.

TABLE V.— EFFICIENCY OF COTTON FILTERS IN PREVENTING THE ENTRANCE OF BACTERIA DURING ARTIFICIAL MILKING.

Cotton in first aeroscope of tandem pair.

TEST NO.	AIR SAMPLED AT AIR RELIEF VALVE IN HEAD OF MACHINE.		AIR SAMPLED AT RELIEF VALVE IN TEAT-CUP CONNECTOR.	
	Plate count second aeroscope.	Total count per machine.	Plate count second aeroscope.	Total count per machine.
1	7	70	17	170
2	1	10	121	1210
3	36	360	123	1230
4	33	330	244	2440
5	0.7	7	10	100
6	1	10	8	80
7	3	30	157	1570
8	42	420	200	2000
9	4	40	10	100
10	41	410	5	50
11	50	500	88	880
12	43	430	131	1310
13	1	10	1	10
14	6	60	1	10
Averages	19	190	80	800

Fourteen similar tests were made at the same time with aeroscopes attached at the relief valve on the teat-cup connector. The results are given in column four of Table V. In general they show conditions similar to those just discussed, the average germ count per machine where no cotton was used (Table III) being 2330 which was reduced to 800 per machine (Table V) where the cotton was used.

A second series of nine analyses was made in which the aeroscopes were attached to the teat-cup connectors of the machines during actual milking. In order to make conditions more nearly comparable to those which actually occur in milking, the first aeroscope in the tandem series was replaced by a teat-cup connector. The four small openings on the side of this were plugged and cotton placed in the cotton chamber (see Fig. 10). The extra connector and the attached aeroscope were then joined with the air relief valve on the teat-cup connector in actual use.

Five tests were made on the first day, the same cotton being retained in the cotton chamber during each of the five milkings; but a new aeroscope with sterile sand was inserted for each milking. Four similar tests were carried out the second day. The results of analysing the sand in the aeroscopes are given in the right and left halves of Table VI.

TABLE VI.— EFFICIENCY OF COTTON FILTERS IN PREVENTING THE ENTRANCE OF BACTERIA DURING ACTUAL MILKING.

Cotton placed in teat-cup connector attached in tandem to aeroscope.

TEST No.	Plate count from aeroscope.	Total count per machine.	TEST No.	Plate count from aeroscope.	Total count per machine.
1*	120	1200	6†	350	3500
2	186	1860	7	260	2600
3	123	1230	8	140	1400
4	92	920	9	51	510
5	780	7800	....	....	....
			Average		2340

* Cotton in connector unchanged during tests 1–5.
† Cotton in connector unchanged during tests 6–9.

The germ counts obtained, while higher than those obtained during artificial milking (compare the average of 2340 per machine, Table VI with the average of 800 per machine in Table V), are nevertheless lower than those obtained under similar circumstances where no cotton was used (compare the average of 2340 per machine in Table VI with the average of 37,300 per machine in Table IV).

The results again indicate that more than two-thirds of the bacteria were stopped by the cotton filter. Thus it is evident that the cotton filters serve a useful purpose in protecting the milk from contamination with dust and bacteria; but their importance is usually greatly overestimated. It should be remembered that the total contamination from the air is so slight when compared with the excessive contamination possible from other sources, that even the removal of all of the bacteria from the air produces an effect quite undetectable when measured by the germ content of the milk itself.

Dropping teat-cups as a source of bacteria.— In using milking machines, it inevitably happens that the teat-cups occasionally fall to the floor. Because of the suction, dirt is drawn into them at once. Those investigators who have noted this fact have agreed that accidents of this sort are a prolific source of bacteria; but apparently no direct measurements have been made to determine the numbers of bacteria thus introduced into the pails of the machines.

Experiments were therefore made in the Station stable in which five liters of sterile water was drawn from the artificial udder into carefully sterilized machines. Sterilization of the machines was accomplished by steaming the metal parts and by disinfecting the rubber parts in a strong chloride of lime solution (containing approximately 20,000 parts per million of available chlorine). The teat-cups were assembled by an operator who handled them with sterile gloves and who rinsed the assembled parts in practically sterile hot water to remove the disinfectant.

The results secured in the first milking were used as a check to determine the perfection of the sterilization of the machines. During the second milking on each day, the teat-cups were held for 30 seconds over and lightly touching the stable floor. This was previously prepared with stable materials so that it represented conditions comparable to those found in the less sanitary commercial dairies. Detailed descriptions of the materials used are given in Table VII together with the results of the analyses made. In some cases it was necessary to clean out the teat cups by rinsing them in water before the third milking on account of stoppage of the tubes with dirt. During the fourth milking the teat cups were dropped on the floor and allowed to lie on their sides in the bedding for 15 seconds.

It is believed that the tests were so arranged that they give a rough indication of the upper and lower limits of contamination to be expected when the cups are accidentally dropped on the floor. In these tests it was found that the amount of foreign matter which could be introduced into the machine, in the case of the B-L-K tubes was limited by the small bore of the 4 individual metal tubes which join the rubber tubes to the teat-cup connector (Fig. 10 B). Any appreciable amount of foreign matter in these tubes greatly retards or entirely stops the action of the machine, and they must be cleaned before milking can be resumed.

The results obtained from the analyses of the water taken from the pail after the first milking (Column 4 of Table VII) show that the machines were practically sterile since only 5 to 12 colonies developed on the agar plates per c.c. of water.

TABLE VII.—AMOUNT OF BACTERIAL CONTAMINATION DERIVED FROM DROPPING THE TEAT CUPS ON THE STABLE FLOOR.

Agar plate counts per c.c. of sterile water, "milked" thru carefully sterilized machines.

TEST No.	Date.	Water before milking.	First milking.*	Second milking.†	Third milking.‡	Fourth milking.§	Notes.
	1918.						
1....	Mar. 20	2	8	15 900	310	250	Teat-cups cleaned after second milking. Bedding a mixture of dried cow manure, dry feed, haydust, straw, fine sawdust and fine dry soil.
2....	April 2	4	12	24 400	2 835	2 415	Teat-cups not cleaned after second milking. Bedding a mixture of sawdust and dirt.
3....	April 3	2	8	12 800	550	830	Teat-cups cleaned before second milking could be completed. Bedding of hay dust and dirt from floor in loft of barn.
4....	April 9	1	12	18 500	2 530	610	Machine not clogged and not washed out. Dirty bedding with dry manure.
5....	April 11	2	5	4 600	490	60	Teat-cups cleaned after second milking. Bedding, very dusty chopped hay.

* Sterile water "milked" thru sterile machines.

† Teat-cups held vertically over and lightly touching the bedding for 30 seconds.

‡ Teat-cups not dropped to the floor during this milking. Cleaned out where it was made necessary by the dirt clogging the tubes.

§ Teat-cups again dropped to the floor and allowed to lie on their sides in the bedding for 15 seconds.

On the other hand the number of colonies developing after the teat-cups had been dropped to the floor (Column 5) was between 4600 and 24,400 per c.c. in the five tests which were made. In the

case of the milkings made on April 9 and 11, the amount of dry matter per liter of water in the pail was determined by filtering the water thru a Gooch filter and drying to constant weight. On April 9, the amount of sediment was .014 grams per liter, while on April 11 it was .026 grams per liter. Thus it will be seen that considerable quantities of dirt were drawn into the machines. If such accidents had happened during actual milking, the milk would have been classed as very dirty. Yet from the standpoint of germ counts, the counts obtained, while large, are not excessive and do not support the idea that excessive numbers of bacteria are introduced into the machines when the teat-cups are dropped on the floor.

The conclusion that dropping the teat-cups does not produce excessively high counts is further strengthened by the results secured from the third milking (Column 6). All of these counts were much lower than those obtained during the milking in which the teat-cups were dropped on the floor (counts vary from 310 to 2835 per c.c.) showing that the numbers of bacteria were much reduced either by the rinsing of the teat-cups in water or merely by the passage of the water thru them. The highest counts were obtained during the two milkings in which the teat-cups were not rinsed out with water.

During the fourth milking (Column 7) in which the teat-cups were allowed to lie on their sides in the bedding less dirt entered the machines and the germ counts are correspondingly low, varying from 60 to 2415 per c.c.

These results appear to justify the conclusion that dropping the teat-cups on the floor of the stable produces a measurable but variable increase in the number of germs in the milk, depending upon the length of time the teat-cups lie on the floor, and upon the kind of material on the floor.

Yet after all, the number of bacteria in the water in the pails gives a very inadequate idea of the dirty conditions present. The dropping of the teat-cups produces results which are objectionable more because of the dirt and filth thus introduced into the milk than because of the contamination of the milk with bacteria.

Discussion of results.— The foregoing results indicate that, at the Station, no one part of the milking machine was constantly the most important source of bacteria, the total amount of bacterial contamination never becoming very bad at any time. Frequently

the rubber tubing and teat-cups contributed a large part of the total contamination, but the pails and heads of the machines were usually fully as important as sources of bacteria.

There is no good reason why the metal parts of the machines should contribute large numbers of bacteria under any condition as they may be kept practically free from bacteria either by scalding and thoro drying, or by steaming. The sterilization of the rubber tubing, on the other hand, is only possible by means of special methods, since scalding and steaming are not applicable to ordinary rubber.

The stable air which enters the B-L-K machines during the milking process was found to contribute relatively insignificant numbers of bacteria to the milk, the increase in count never exceeding 34 per c.c. under the conditions tested. It is probable that even this small number would be still less in some other types of machines where smaller amounts of air come in contact with the milk. The cotton filters used on the B-L-K machines as a means of removing bacteria from the air were found to be partially effective as filters, removing the coarse dust particles and more than two-thirds of the bacteria.

On the other hand it was found that the very noticeable effect of the large cotton filters used on the head of the older types of machines should be attributed to their action in partially preventing the return of the highly contaminated condensation water from the vacuum piping. The entrance of small quantities of this condensation water into the pail was found to cause very noticeable increases in count. The suction trap which has been substituted for the cotton filters on the latest types of B-L-K machines appears to be entirely efficient in preventing the return of the condensation water, severe tests having failed to reveal any positive proof of leakage.

Dropping the teat-cups on the floor, with the resultant introduction of dirt into the pails of the machines, was found to be objectionable both because of the amount and character of the dirt and filth introduced in this way as well as because of the increase in the germ content thus produced. The germ counts did not increase as much as might have been expected as they did not exceed 25,000 per c.c. under very bad conditions.

EFFECT OF MILKING MACHINES IN USE ON ORDINARY DAIRY FARMS UPON THE GERM CONTENT OF THE MILK DRAWN.

In the course of this work opportunity was presented to study the germ content of milk drawn by machine under practical dairy conditions. Thru cooperative agreement with two local milk companies, the Geneva City Board of Health, and the dairy farmers of the vicinity, the Station undertook an experiment in the control of city milk supplies which began Feb. 1, 1915. A test has been made of the microscopic method of analysing milk samples in the preliminary portion of this work, and the results secured have already been discussed in Bulletin No. 443 of the Station. During the period previous to April 1, 1917, milking machines were in operation for part or all of the time on six of the 44 dairy farms which sent milk to the City of Geneva. Between April 1, 1917, and Dec. 1, 1917, four additional farmers installed milking machines, making a total of ten farmers using mechanical milkers who have come under the direct observation of the Station.

Detailed accounts of the conditions noted on the original six farms are given in Bulletin No. 443 and will not be repeated. A comparatively small percentage (14.22 per ct.) of the 11,851 cans of milk examined [47] were found to contain bacteria in excess of 1,000,000 individual bacteria per c.c. (approximately the same as an agar plate count of 200,000 per c.c.). About one-fifth (345 cans) of the 1682 cans containing this relatively large number of bacteria were found to contain nearly pure cultures of bacteria of the type associated with infected udders (garget). The excessive numbers of bacteria in the remaining 1337 cans appeared to be largely derived from the direct contaminating effect of utensils, tho the growth of bacteria in the milk played a noticeable role in increasing the numbers of bacteria under favorable conditions.

The milk cans appeared to be the utensils which ordinarily caused the trouble; but there was reason for thinking that the milking machines frequently caused very large bacterial contamination of the milk.

In three instances, records were secured by weekly inspection of each can of milk delivered which indicated the bacterial quality of both hand-drawn and machine-drawn milk from the same farm.

[47] Analysis principally made by J. D. Brew. A few were made by W. D. Dotterrer.

In the case of Dairyman A, the installation of a milking machine caused no marked change in the quality of the milk produced. There was on the other hand a very noticeable improvement in the quality of the milk sent by Dairyman B, after the machines were discarded; but since this was associated with improved cooling of the milk and other changes, it is probable that the improvement was in part due to other things than discarding the machines. Dairyman D sent milk of much improved quality after machine milking was discontinued and the surrounding circumstances indicated that this improvement was connected with the change in the method of milking.

In a list of 44 men, the highest rank secured by any of those who used machines was 17th place. The others ranked 21, 33, 40, 41, and 42, respectively. A comparison between the bacterial quality of 3013 cans of milk from the *nine farms sending the poorest quality of hand-drawn milk*, and that of the 3051 cans of milk examined from the *six farms sending machine-drawn milk* showed that the two groups of farmers sent milk containing approximately equal numbers of bacteria. As a class, those men who had milking machines sent milk containing more bacteria than the milk sent from the farms where hand milking was practised.

Another fact worth noting in this connection is that in the case of all but one of these farms (and that the one that stood the highest in rank) the milking was all done by hired labor. In general the machines were installed on the largest and best equipped farms where there were herds of more than 15 and less than 50 cows.

" Two of the four herds from which milk containing streptococci (bacteria associated with garget) were milked by hand and two were milked by machine. The machine milker was discarded at one of the two latter farms partly because of a belief that it caused garget. Nevertheless, so far as the records of the milk examinations show, the garget cleared up at the farm where the machine milking was continued just as quickly as at the farm where it was discarded. Both farms occasionally brought milk containing many streptococci, during the year that followed."

During a supplementary period from April 1, 1917, to Dec. 1, 1917, not discussed in Bulletin No 443, these farms and four additional farms where milking machines have been used have con-

tinued under observation.[48] Dairyman A, who stood in 17th place in the first list of 44 dairymen, dropped to 28th place in a new list of 36 men, having delivered milk which graded both absolutely and relatively poorer in bacterial quality. On the other hand, Dairyman E who stood in 41st place in the old list, secured 29th place in the new list, having improved the quality of his milk both absolutely and relatively. Dairyman C who had 33d place in the old list dropped to the very bottom of the new list of 36 men, having delivered milk of much poorer quality than during the previous period. Dairymen B, D and F either discontinued the sale of milk or the use of the machine during this period.

The six men who have used machines during part or all of the 8 months ranked 6, 28, 29, 34, 35 and 36 in the list of 36 men, thus showing a very decided tendency to deliver milk containing more numerous bacteria than did their neighbors who did their milking by hand. A seventh dairyman (J) using a milking machine, who delivered milk for only five months of the period, produced the poorest quality of milk that has been received at any time during 34 months.

In addition to the information gathered in the course of the experimental milk control work done in the City of Geneva, one or more visits have been made to eight of these farms on which machines were in use, for the purpose of testing the antiseptic solutions in use and to determine the amount of bacterial contamination of the machines. Process samples of the milk itself were taken at some of these places, while at all of them from four to eight milkings of sterile water from the artificial udder were made.

Dairyman A was visited Aug. 22, 1916, and was found to be using a Hinman milking machine and keeping the teat-cups and tubes in a solution of brine and chloride of lime. Tests showed only a trace of available chlorine in the solution. At this time, two milkings of sterile water were made successively thru a single unit after it was prepared as usual for milking. This unit was in good condition as the water from the pail showed a plate count on agar of only 1050 and 1190 per c.c. (see Table VIII). Samples taken as this water entered the pail (Fig. 3) showed that the greater part of these bacteria were already in the water before it entered the pail (see Table VIII).

Process samples taken at this time gave a count of 1770 per c.c. for the milk as drawn from the udder of a cow (see Table IX) while milk from the same cow gave a count of 5800 per c.c. as taken from the pail of the machine. The mixed milk of several cows gave a count of only 6100 per c.c. as taken from the final container, a 40-quart can.

Samples of the antiseptic solution taken on Oct. 13, 1917, showed only a trace of available chlorine in a solution of about 14 per ct. salt.

[48] Analyses of milk during the period were made by J. D. Brew and H. Macy.

On a third visit made, Oct. 31,1917, two successive milkings of sterile water thru two different units showed agar plate counts varying from 45,600 to 124,600 per c.c. (Table X). Again the larger part of the bacteria were found to be in the teat-cups and tubes. These were not in as satisfactory a condition as at the time of the first visit. This finding corresponds likewise with the fact that this dairyman made a poorer showing in the quality of his milk as delivered in the City in 1917 than in 1916. Tests of the antiseptic solution showed about 12 parts per million of available chlorine in a 14 per ct. brine.

Dairyman C was visited August 29, 1916, and found to be using a Hinman milker and keeping the teat-cups and tubes in a solution prepared from a proprietary germicide (Bacilli-Kil). Two milkings of sterile water thru the same unit gave counts of 170,000 and 36,000 per c.c. for the water in the pail, while samples taken as the water entered the pail showed counts even higher than these (Table VIII) which indicated that the bacteria were from the teat-cups and head of the unit.

Process samples of milk showed an agar plate count of 90 per c.c. for the milk as drawn from the cow into a second milking machine unit while the milk in the pail had a count of 37,000 per c.c. (Table IX). The mixed milk after reaching the 40-quart can gave a count of 51,000 per c.c. indicating that the teat-cups and tubes of the machines were responsible for the largest part of the germ content of this milk.

A sample of the antiseptic solution taken on Oct. 13, 1917, showed about 14 per ct. of salt with a very strong chloride of lime solution which tested about 1800 parts per million of available chlorine. Acting on the theory that if a little was good, more was better, this dairyman had added the entire contents of a 12 oz. can of bleaching powder to the jar in which the tubes were kept.

Two days later, two successive milkings of sterile water thru two different units showed agar plate counts varying between 66,300 and 96,800 per c.c. for the water after it reached the pail (Table X). In this case samples from the teat-cups and head of the machine indicated that only part of the bacteria came from this source and that the pail itself added bacteria in measurable numbers. The antiseptic solution tested as before except that the available chlorine had become reduced to 1700 parts per million. These results are interesting as they show that even very strong solutions of chloride of lime and brine are not in themselves sufficient to keep the tubes free from bacteria.

Dairyman E.—A visit was made August 18, 1916, and tests made of two successive milkings of a Hinman machine in use by this dairyman. The sterile water, after passing into the pails of the unit tested, gave agar plate counts of 3,174,000 and 2,516,000 per c.c., while practically identical counts were obtained from the water before it reached the pail (Table VIII), showing that these very large numbers of bacteria were practically all derived from the teat-cups and head of the machine. A chloride of lime solution containing no salt was in use. A sample tested 18 parts per million of available chlorine.

Process samples were taken from two other units at the same time. The first cow milked with one unit showed a germ content of 1130 per c.c. for her milk as drawn into a sterile test tube and 1,003,000 per c.c. (Table IX) after reaching the pail of the machine. The second cow gave milk with a germ content of 780 per c.c. which was increased to 900,000 per c.c. by the time it reached the pail of the machine. A sample taken from the 40-quart can used as the final container gave a count of 1,350,000 per c.c.

On Oct. 26, 1917, a sample of the antiseptic solution was found to contain about 14 per ct. of salt and enough chloride of lime to give a solution with 106 parts per million of available chlorine.

The following day two successive milkings of sterile water were made with each of two units and gave very irregular results (Table X) which are difficult to interpret. These varied between 1500 and 600,000 per c.c. for the samples taken from the pails of the machines while those taken as the water entered the pail varied from 6,400 to 19,400 per c.c. It is not certain, however, that these lower counts indicate that the principal contamination came from the pails themselves because of the fact that the dairyman had kept his teat-cups and tubes in a much stronger chloride of lime solution than that ordinarily used. Since it was not the practice on this farm to rinse the

teat-cups and tubes after being taken from the solution and before use, some of this strong chloride solution was present in the tubes when milking was started and must have effectively reduced the bacterial count in the first milking of water thru each unit, at least. However, only a trace of chloride could have been present, as otherwise the water would have been much more completely sterilized.

TABLE VIII.—AMOUNT OF BACTERIAL CONTAMINATION DERIVED FROM COMMERCIAL DAIRY FARM MACHINES.

Agar plate counts per c.c. sterilized water milked thru machines.

DAIRYMAN.	Farm No.*	Date.	FIRST MILKING.		SECOND MILKING.		Type of machine.
			Teat-cups, tubes and head.	Entire machine.	Teat-cups, tubes and head.	Entire machine.	
		1916					
A.....	17	Aug. 22	2 170	1 050	400	1 190	Hinman
C.....	33	Aug. 22	195 000	170 000	40 000	36 000	Hinman
E.....	41	Aug. 18	3 500 000	3 174 000	2 310 000	2 516 000	Hinman
F.....	42	Aug. 25	1 200 000	1 188 000	743 000	629 000	B-L-K
J.....	....	Aug. 24	.....	1 462 000	.....	621 000	Empire

* As given in Bulletin No. 443.

While it is probable that these counts are somewhat too low for the reasons just mentioned, it should be noted that the lower counts found on this visit as compared with those found the year previous correspond also with the fact that the milk sent from this farm was of better quality during 1917 than in 1916.

TABLE IX.— BACTERIAL CONTAMINATION OF MILK DERIVED FROM MACHINES AND OTHER FARM UTENSILS.

Agar plate counts per c.c. of milk. Not from the units used for results given in Table VIII.

DAIRYMAN.	Date.	Strippings of cow.	From pail of machine.	From carrying pail.	After passing thru strainer and over aerator.	From 40-quart can.
	1916					
A.....	Aug. 22	1 770	5 800	4 800	.....	6 100
C.....	Aug. 22	90	37 000	34 800	59 000	51 000
E.....	Aug. 18	*955	†952 000	1 016 000	1 180 000	1 350 000
F.....	Aug. 25	2 170	239 000	243 000	291 000	273 000
J.....	Aug. 24	4 090	1 095 000	1 267 000	1 046 000	1 012 000

* Average from two cows; 1130 and 780 per c.c.

† Average from two machines; 1,003,000 and 900,000 per c.c.

Dairyman F.— Only one visit was made to this farm for the purpose of testing the machines. This was made on August 25, 1916, and two tests were made on a single unit of the B-L-K machine. The water was caught and sampled as it entered the pail by the use of the device shown in Fig. 8, B. The water after passing the teat-cups, tubes and head of the machine gave counts of 1,200,000 and 743,000 per c.c., respectively (Table VIII), while counts from the pail were of about the same size. The antiseptic solution was stated to contain about 12 per ct. of salt and chloride of lime. Tests, however, showed only a trace of available chlorine. This herd was sold soon afterward and no further tests were made.

Dairyman G.— An Empire milking machine was in use at this place. A sample of the antiseptic solution in use was collected June 11, 1917. This contained no salt but did contain available chlorine from the chloride of lime added to the extent of 888 parts per million.

A visit was also made on November 3, 1917, and two successive milkings of sterile water were drawn into each of two units. The count obtained varied from 200 to 5500 per c.c. (Table X) showing the machines to be in excellent condition. This

TABLE X.—AMOUNT OF BACTERIAL CONTAMINATION DERIVED FROM COMMERCIAL DAIRY FARM MACHINES.

Agar plate counts per c.c. sterilized water milked thru machines. Two units tested in each case.

DAIRYMAN.	Date.	FIRST MILKING.		SECOND MILKING.		Type of machine.
		Teat-cups, tubes and head.	Entire machine.	Teat-cups, tubes and head.	Entire machine.	
	1917					
A.....	Oct. 31	33 800	45 600	24 100	95 300	Hinman
		139 600	124 600	27 500	57 700	
C.....	Oct. 15	16 400	90 000	66 300	83 700	Hinman
		41 300	96 800	6 300	66 200	
E.....	Oct. 27	6 600	34 500	6 400	600 000	Hinman
		19 400	1 500	9 000	27 900	Hinman
G.....	Nov. 3	.....	5 500	.....	1 090	Empire
		.....	480	.....	200	
H.....	Nov. 8	.....	16 600	.....	.....	Empire
		.....	6 000	.....	6 300	
I.....	Nov. 14	.....	Less than 600	.....	Less than 400	Empire
		.....	Less than 450	.....	Less than 150	
J.....	Nov. 2	.....	59 300 000	.....	16 000 000	Empire
		.....	4 200 000	.....	8 300 000	

corresponds with the excellent record of this farm which was the best of any of those that came under observation.

Dairyman H.— A visit was made to this farm on November 8, 1918, and tests made on two units of the Empire milker as before. The water after reaching the pail

of the machine gave counts which varied in three milkings from 6000 to 16,600 per c.c. (Table X). The antiseptic solution consisted of brine to which chloride of lime was added. A sample showed the available chlorine present to be 35 parts per million.

Dairyman I.— One visit was made to this farm on November 14, 1917, and two units of the Empire milker, tested. Two successive milkings of sterile water thru each gave the lowest counts secured on any farm, all of them being less than 600 per c.c. (Table X). The brine and chloride of lime solution showed about 16 per ct. of salt and about 35 parts per million of available chlorine.

Dairyman J.— A visit was made on August 24, 1916, and two successive milkings of sterile water thru a single unit of the Empire machine were made. The water in the pail was found to give counts of 1,462,000 and 621,000 per c.c. (Table VIII). The tubes were said to have been boiled once daily and kept in an antiseptic solution at night.

Process samples taken on the same day showed a germ content of the milk from a single cow to be 4090 per c.c. as drawn into a sterile test tube and 1,095,000 per c. c. after reaching the pail of the milking machine unit (Table IX). The mixed milk in the final container gave a count of 1,012,000 per c.c.

On November 1, 1917, a sample of the antiseptic solution then in use was secured and tests showed it to contain about 14 per ct. of salt with 106 parts per million of available chlorine. On the next day two successive milkings of sterile water were made with each of two units which gave counts varying between 4,200,000 per c.c. and 59,300,000 per c.c. The reason for these very high counts was not evident but they correspond exactly with the condition noted in the milk itself, as it was delivered in the city. At this time nearly every can of milk from this farm showed millions of bacteria in every c.c. It is worth noting that these counts, which were the highest obtained under any conditions, were obtained from the same type of machine with which the best results were obtained.

From the tests of commercial dairies it appears that dairies using milking machines tended to produce milk containing more bacteria than dairies where the milking was done by hand. On six farms where direct comparisons between hand-drawn and machine-drawn milk were possible, only two of them (Dairymen A and G) produced milk by machine with as few germs as were contained in the milk produced by them by hand. The milk from these farms would have satisfied the bacterial standards ordinarily set for Grade A pasteurized market milk. The remaining four men (Dairymen B, D, H, and I) delivered milk of poorer bacterial quality during the period when they practiced machine milking than when they practiced hand milking. In one of these cases, however, the deterioration in quality was not great (B) and the improvement noticed when hand milking was reintroduced was associated with other changes which may have been in large part responsible for the lessened number of bacteria in the milk. In the remaining three cases the deterioration in quality, associated with the period of machine milking, was marked. It is significant that not one of the dairymen produced milk of markedly better quality when machine milking was practised.

Looked at superficially, these results indicate that machines are even yet scarcely successful from the standpoint of a bacteriologist since these farmers, to a certain extent, had the benefit of personal advice from members of the Station staff. Observation has shown, however, that because of lack of convenient facilities, cleaning the machines with hot water, or even with cold water, has been inadequately done, and that the teat-cups and rubber hose have not been taken apart and given a thoro cleansing as frequently as once a week, the period used at the Station and which is known to give satisfactory results (see pages 177–9). The machines have been found in very uncleanly condition on all of the farms where poor results were obtained.

Likewise, directions in regard to using antiseptics have not been followed, the dairyman's unfamiliarity with the action and purpose of these solutions frequently leading him to neglect essential directions. This was particularly true in regard to chloride of lime solutions where failure to keep the solutions up to the proper strength was frequently observed. The attempt of one dairyman (C) to meet this situation by using a very strong solution of chloride of lime resulted in showing that strong germicides alone are not sufficient, a result which is entirely satisfactory as all will agree that physical cleanliness as well as freedom from bacteria is highly desirable.

It is significant that this failure to keep the machines clean is associated with another condition, namely that on the farms of Dairymen B, D, H, and I, as well as on all of the other farms where relatively poor results were secured, the milking was done by hired labor in almost all cases. On some of the larger farms this was distinctly high grade labor but on others it consisted of very ordinary and indifferent persons who had no direct interest in maintaining the machines in good condition. The best results were secured on the farms where both the dairyman and his wife were thoroly interested in securing good results, and did the work themselves.

IV. MILKING MACHINES: METHODS OF MAINTAINING IN A BACTERIA-FREE CONDITION.

INTRODUCTION.

As shown in the previous pages, milking machines as used by dairymen under practical conditions are sometimes maintained in excellent bacteriological condition; but frequently are badly contaminated so that milk drawn thru them contains very large numbers of bacteria. Methods for overcoming this difficulty by means of antiseptics have been previously studied and are in practical use on the majority of the farms where machines are in operation. In spite of this, however, not all practical dairymen have succeeded in getting as good results with the antiseptic solutions as were indicated under experimental conditions; and there has also been some confusion brought about by the fact that different investigators have not secured entirely harmonious results.

Because of these things comparative tests have been made of a number of the suggested antiseptics or antiseptic measures that have been recommended. References to the previous work have already been made (pages 116–125). It will be sufficient for our purpose here to mention the various substances which have been tried and found sufficiently valuable to have been used under practical conditions.

As was natural, when suction machines were first introduced attempts were made to keep the rubber tubes and teat-cups in satisfactory condition by using hot water or steam, or by boiling them. There is almost universal agreement that these methods were either ineffective or were destructive of the rubber parts. This caused a search for more useful methods, and led to immersing the tubes in water, and to the use of harmless antiseptics.

Both early workers (Erf,[49] and Stocking and Mason [50]), as well as later ones (Wing,[51] and Larsen, White and Fuller [52]), have found that formaldehyde will keep the tubes in a satisfactory condition; but agree in declaring it to be unsuited for use because of its poisonous nature. Erf [53] and others have found lime water a satisfactory

[49] See footnote 7.
[50] See footnote 9.
[51] See footnote 20.
[52] See footnote 21.
[53] See footnote 7.

antiseptic and this has been extensively used under practical conditions. Hastings and Hoffman [54] have made the most extensive tests of this substance tho Hooper and Nutter [55] have also reported favorably upon it in a more recent work.

Brine was apparently first brought into use in connection with milking machines by the manufacturers of the B-L-K machine and was reported upon favorably by Stocking and Mason,[56] and later in more extended tests made at this Station.[57] This solution has also been used extensively under practical conditions. Hoffman-Bang [58] has used hot brines.

The work of Miss Wing [59] called attention to the usefulness of bleaching powder (chloride of lime or calcium hypochlorite) and, since her report, this substance, because of its known effectiveness as a germicide and known harmlessness has been generally used, sometimes alone and sometimes in brines. Moak,[60] in particular, has tried brine and chloride of lime in connection with the production of certified milk. He has likewise obtained good results from the use of a proprietary product "montanin," but has not recommended its general use.

In this connection it should be noted that two recent European reports (Williams, Golding and McIntosh,[61] and Burri and Hohl [62]) upon tests of milk drawn by a suction type of milking machine show good results even where no antiseptics are used. In the machine tested (the Omega) the amount of rubber tubing and of parts not sterilizable by steam is reduced to a minimum.

In recent years, without recommendation from any of the investigators mentioned, there have come into quite extensive use certain proprietary antiseptics or germicides which have been so used because of liberal advertising. The majority of these are in reality solutions of calcium or sodium hypochlorites similar in their action to the much cheaper chloride of lime or bleaching powder (calcium hypochlorite).

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[54] See footnote 10.
[55] See footnote 23.
[56] See footnote 9.
[57] See footnote 16.
[58] See footnote 18.
[59] See footnote 20.
[60] See footnote 26.
[61] See footnote 19.
[62] See footnote 27.

PRESENT STUDIES.

The usefulness of brine as a solution in which to keep the teat-cups and rubber tubing.— Since 1909, when a report on the action of 10 per ct. brine was published, brine solutions were in use at this Station until 1916. In fact the original solution was kept in use all of these years, being maintained in volume and strength by adding more salt or water as needed. The cleanliness of the solution was maintained by drawing off the precipitate or sludge from the bottom of the tank. The original solution contained 10 per ct. of salt but the amount was gradually increased until the solution contained between 12 and 13 per ct. of salt, at which strength it was maintained until discarded.

Between 1907 and 1916 nearly five hundred analyses were made of the germ content of this brine, about one-half of which were made by J. K. Wilson, formerly assistant bacteriologist at the Station, and about one-half by one of us (R). The results of these analyses will not be given in detail since no good purpose would be served by so doing, but several conclusions can be drawn as a result of the work which are of general interest.

The germ counts were found to vary greatly from day to day, showing less than one thousand colonies developed on agar per c.c. of the brine on one day, while on the succeeding day, the analyses might show more than 10,000 or even 100,000 colonies per c.c. The number of colonies as found by the agar plate method varied between none and 11,000,000 per c.c. of brine, usually, however, being more than 10,000 and less than 200,000 per c.c.

The numbers of colonies appearing on plates made from the same dilution were usually approximately the same; but counts made from plates prepared from successive dilutions frequently did not agree. Thus on Feb. 5, 1913, the brine was plated on lactose agar in dilutions of 1:10, 1:100, and 1:1000. The resulting count from the first dilution was 108,550, that from the second dilution was 58,400, while that from the third dilution 3000 per c.c. On Nov. 13, 1913, the brine was plated on gelatin and many very fine colonies developed on the plates made from the undiluted material, the number as estimated by means of a microscope being 134,000 per c.c. However, the count from the plates made from a dilution of 1:10 was only 560 per c.c., while no colonies developed on the plates

made from the 1:100 dilution and only an occasional colony on the plates made from the 1:1000 dilution.

Numerous results similar to these were obtained from lactose agar, 10 per ct. salt agar, and 10 per ct. salt gelatin. These results indicate the presence of large numbers of organisms which do not grow. No plating technique was developed which proved satisfactory, nor could satisfactory microscopic preparations be made because of the presence of the salt. The real number of bacteria in the solution was therefore undetermined, but the results showed that large numbers of living organisms were present.

A true brine flora was present as was shown by fishing colonies from the plates. Three types of bacteria seemed to predominate which were found to be capable of growth on agar slants containing from 10 to 25 per ct. of salt. The less numerous flora did not grow on agars containing more than 5 per ct. of salt. The true brine organisms isolated were (1) a short thick rod (2), a long slender rod, and (3) a large long rod. The first two were the predominant organisms on the plates from which they were isolated, while the third was abundant, but not the predominant organism on the plates from which it was isolated. Another predominant type was a large coccus which usually occurred in pairs, or tetrads with the adjacent sides of the individuals somewhat flattened. Microscopic examination showed it to be abundant in the brine from which it was obtained.

Organisms similar to these were also isolated directly from a lump of salt from a barrel of salt in use at the time. The organisms were secured by putting a small piece of salt, taken from the interior of a lump with aseptic precautions, into a tube of sterile broth. When the latter became turbid, it was plated on salt-agar and the colonies fished and examined. This suggests that the source of the brine flora was the salt itself. (Compare also Wolff [63].)

The organisms isolated as described above when inoculated into sterile milk and sterile litmus milk and incubated for three weeks at ordinary temperatures, did not produce any noticeable changes and there was but little evidence of growth. Presumably, then, even if these organisms are present on the teat-cups and tubes they are of no significance in the milk, and may not even grow on the agar plates from which milk counts are ordinarily made.

[63] Wolff, A. Prüfung des Molkerei-Salzes. *Milchw. Zentralbl.*, 43:545-551. 1914.

During all of the period in which this brine was in use at the barn, milk of high keeping quality was continuously produced at the Station, and such analyses as were made of the general supply rarely gave agar plate counts in excess of 10,000 per c.c. Owing to this fact, and to the extensive studies which have been made upon brine, no further studies have been made since 1916. In passing, it should be noted that our observations, while agreeing with the two tests given by Hooper and Nutter [64] in showing the brine solution to contain many bacteria, do not give support to their contention that brine is therefore unsuited for use in connection with milking machines.

Methods used in study of antiseptics other than brine.— In comparing the efficiency of various antiseptics other than brine, one or more pairs of teat-cups have been kept in the solutions under test, for periods varying in time from a few weeks to several months. The teat-cups were kept in daily use for milking while the tests were in progress, were rinsed daily as described (page 132) and were thoroly scrubbed once per week.

In determining whether any particular solution was satisfactory, tests were made by " milking " five liters of sterile water thru the machines, the metal parts of which had been steamed. Frequent testing showed this steaming to be sufficient to practically sterilize the metal parts. The count obtained by making agar plates from the water drawn into the pail of the machine was therefore assumed to indicate the bacterial condition of the tubes. No attempt was made to protect the sterile water from air contamination other than to do the work in a moist milk room where the air was known to be nearly sterile. Under these conditions, the number of bacteria derived from this source must have been negligible (see pages 139–143).

Complete sterilization of machines by use of steam and chloride of lime.— For the sake of comparison and in order to satisfy our own curiosity, an attempt was made in 1916 to draw sterile water into a sterile machine. In order to secure a sterile machine a combination of steam and special cleanliness was used on the metal parts and all of the rubber parts were separated and placed in antiseptic solutions.

The machines, which had been in use for months previously, were first taken apart by one of us (R) and the separate metal and rubber

[64] See footnote 23.

parts given a thoro cleansing. The metal parts, except the pail, were then sterilized under 15 pounds pressure in an autoclave, while the rubber tubing was placed in a very strong chloride of lime solution (the solution in this case probably contained about 20,000 parts of available chlorine per million).

Just before the regular afternoon milking, the rubber parts were rinsed thoroly in boiling water and then the various parts of the teat-cups were assembled, sterile rubber gloves being used to protect these parts from contamination from the operator's hands. At the two places where air entered the machines (relief valve on teat-cup connector and vacuum relief valve on the head, Figure 9), aeroscopes were attached so that the air was filtered thru a layer of sterile fine sand which had been tested and found to be an effective bacterial filter.

After five liters of sterile water had been drawn into the machine, samples were taken and analysed, the results being given in Table XI.

TABLE XI.— AMOUNT OF BACTERIAL CONTAMINATION DERIVED FROM STATION MACHINES WHEN THESE WERE CAREFULLY STERILIZED. CHLORIDE OF LIME USED AS GERMICIDE ON RUBBER TUBES.

Agar plate counts per c.c. sterilized water milked thru machines.

TEST NUMBER.	Water before milking.	First milking.	Second milking.	Water before milking.	Third milking.	Fourth milking.
1.....	1	5	2	1	4	5
2.....	5	63	13	10	13	9
3.....	1	7	6	.....	5	4
4.....	½	4	8	5	6	16
5.....	4	13	16	4	43	14
6.....	2	1	2	15	3	6
7.....	3	7	.....	1	5	16
8.....	3	25	15	3	15	8
9.....	0	2	1	1	10	6
Averages....	2	14	8	5	12	9

In the third and sixth columns of this Table, the counts obtained from samples of the sterile water are given. These show that a few colonies developed on the agar plates. It should be remembered, however, that these plates were held in the incubator first for five days at 21° C. and later for two additional days at 37° C.

Under these conditions some contaminations introduced from the air during plating are sure to develop no matter how perfectly the plates are protected. The averages of 2 and 5 colonies per c.c. on these plates represent the total contaminations from these sources.

In the columns given under the headings, "First," "Second," "Third" and "Fourth" Milkings, the figures represent the number of colonies developed per c.c. of the sterile water after it was drawn into the pail of the machine. It will be seen at once that there was a slight increase in number of colonies above the 2 and 5 per c.c. developed from the contaminations just mentioned. The largest number of colonies found was 63 per c.c., this number being developed from a sample of the first milking made on Oct. 4. In some cases, however, no colonies developed from the water in the pail, showing that our object had been accomplished and that the water had been drawn into the pail without the slightest contamination. The averages secured for the four successive milkings of 14, 8, 12, and 9 per c.c., moreover, show that contamination in all cases was practically absent. The averages from the six most successful tests of 4, 4, $5\frac{1}{2}$, and 9 per c.c. were scarcely higher than the average counts from the plates of sterile water.

Not being satisfied even with these nearly perfect results and still wishing to know how great precautions were required to secure absolutely perfect results, a second series of milkings of the same sort was made in which even more extreme precautions were taken. These precautions consisted (1) in doing the work in the milk room where a moist atmosphere and freedom from dust would lessen chances of contamination from dust, (2) in making the plates, greater care was used to exclude all chances of dust contamination, (3) a stronger solution of chloride of lime was used which was tested and found to contain between 20,000 and 30,000 parts of available chlorine per million.

The results of this series of 10 milkings are given in Table XII. It will be seen at once that these extreme measures cut the amount of bacterial contamination even lower than in the former series of tests. The average count of colonies from the sterile water was only one per c.c. The highest count obtained from the water after being drawn thru the machine was obtained on the fourth milking on November 14, when 11 colonies developed per c.c. In 12 instances out of the 40 milkings no more colonies developed

from the water drawn thru the machine than from the water taken before milking, and in all of the other cases the increase was insignificant, showing that our purpose had really been accomplished and that it was possible by the use of extreme precautions repeatedly to draw sterile water into the machines and have it remain sterile.

TABLE XII.— AMOUNT OF BACTERIAL CONTAMINATION DERIVED FROM STATION MACHINES WHEN THESE WERE STERILIZED WITH EXTREME PRECAUTIONS. CHLORIDE OF LIME USED AS GERMICIDE ON RUBBER TUBES.

Agar plate counts per c.c. of sterilized water milked thru machines.

TEST No.	Date.	Water before milking.	First milking.	Second milking	Water before milking.	Third milking.	Fourth milking.
	1916.						
1.....	Sat., Nov. 11	2	2	2	0.8	2	3
2.....	Mon., Nov. 13	0.2	2	2	1.0	2	2
3.....	Tue., Nov. 14	2	5	5	2.0	3	11
4.....	Fri., Nov. 17	0.6	1	1	0.4	1	0.6
5.....	Wed., Nov. 22	0.8	0.4	0.2	0.6	0.4	0.6
6.....	Thur., Nov. 23	0.0	0.4	0.4	0.2	0.4	0.4
7.....	Sat., Nov. 25	2	0	1	0.6	0.2	1
8.....	Tue., Nov. 28	2	5	4	2	3	3
9.....	Wed., Nov. 29	0.4	0.4	1	0.2	1	0
10.....	Thur., Dec. 7	0.4	4	3	2	2	3
Averages.....		1	2	2	1	1.5	2.5

However, because the extreme precautions used are impracticable even for use on farms producing certified milk, tests were then made to find how great the increase in contamination would be under practical conditions.

Sterilization of the machines by steam and chloride of lime under practical conditions.— In the first series of tests in which chloride of lime was used as the disinfecting agent, the same machines were used; but the procedure was modified as follows: (1) the metal parts were steamed for 15 minutes daily in a steam chest and care was taken to see that they were thoroly dry as soon as removed from the steam chest, (2) the regular attendants, who cared for the daily washing of the pails and heads, and the weekly washing of the teat-cups, were asked to exercise care in cleaning these parts, (3) between milkings, the teat-cups rinsed by the usual methods (page 132) were kept at the barn in a crock containing a solution of chloride

of lime which held from 497 to 745 parts per million of available chlorine as shown by frequent tests.

In each series of tests of the bacteriological condition of the tubes, they were first rinsed thoroly with practically sterile hot water to remove all traces of the disinfectant. Eight series of milkings were made between December 19, 1916, and January 12, 1917, the first of the tests being taken after the tubes had been in use for 12 days since the preceding tests, given in Table XII. The results of the new series of tests are given as the first series in Table XIII.

TABLE XIII.— AMOUNT OF BACTERIAL CONTAMINATION DERIVED FROM STATION MACHINES, CAREFULLY CLEANED AND STERILIZED BY PRACTICAL METHODS. CHLORIDE OF LIME USED AS GERMICIDE.

Agar plate counts per c.c. of sterilized water milked thru machines.

TEST No.	Date.	Water before milking.	First milking.	Second milking.	Water before milking.	Third milking.	Fourth milking.
	1916.		First series.				
1.....	Dec. 19	0.2	2	7	2.0	5	6
	1917.						
2.....	Jan. 4	0.8	16	6	0.2	2	3
3.....	Jan. 6	0.8	4	3	1.0	6	5
4.....	Jan. 8	0.2	12	3	0.2	2	157
5.....	Jan. 9	4.0	12	10	2.0	8	11
6.....	Jan. 10	0.6	10	19	1.0	27	31
7.....	Jan. 11	0.4	5	2	0.6	3	4
8.....	Jan. 12	2.0	3	8	3.0	30	8
			Second series.				
1.....	Mon., Mar. 26	0.0	36	7	1.0	7	42
2.....	Tue., Mar. 27	1.0	46	22	0.2	18	21
3.....	Mon., April 2	0.4	13	4	0.2	35	53
4.....	Mon., April 16	0.4	15	10	0.8	21	33
5.....	Tue., April 17	1.6	15	3	0.6	7	24
6.....	Mon., May 14	5.0	79	25	6.0	32	54
7.....	Tue., May 15	3.0	71	49	5.0	40	58
8.....	Mon., May 28	0.2	14	12	1.6	7	53
9.....	Tue., May 29	0.6	11	7	.....	.....	.....
10.....	Mon., June 11	1.2	629	345	1.2	193	224
11.....	Tue., June 12	1.0	23	4	0.0	3	2
12.....	Mon., June 25	0.4	3 008	1 637	0.2	1 082	1 139
13.....	Tue., June 26	0.6	1 526	12 306	1.2	11 620	9 632
14.....	Mon., July 9	0.8	25	680	1.4	1 020	920
15.....	Tue., July 10	3.0	11 470	4 890	1.6	3 950	2 960
16.....	Mon., July 23	3.4	49 700	25 800	1.6	28 230	24 230
17.....	Tue., July 24	0.4	179 430	92 600	1.4	53 200	55 300
18.....	Mon., Aug. 6	0.7	25 000	16 100	0.7	15 100	10 600
19.....	Tue., Aug. 7	0.3	16 900	5 100	0.3	11 800	8 600

The results of these milkings carried out under conditions regarded as practicable on dairy farms gave results which to our surprise were nearly as perfect as those obtained by the use of extreme measures. Exclusive of one count of 157 colonies per c.c. (fourth milking, January 8), the highest count obtained in any of the 32 milkings was only 31 per c.c. (fourth milking, January 10). Contamination of this amount would be insignificant even in the highest grade milk.

Feeling, however, that this short-time test was not sufficiently severe to show that equally good results could be obtained under practical conditions, the same methods of handling the machines were continued from this time until the middle of August, 1917. The steaming of the pails and heads was reduced to five minutes. Milkings of sterile water were made on Monday and Tuesday afternoons of each week. These days were chosen as the day before and the day on which the thoro weekly cleaning was given the tubes. The whole of the routine was in the hands of the regular dairy attendants except that one of us (R) cared for the strength of the chloride of lime solution. Frequent tests showed that this was maintained between 177 and 710 parts per million of available chlorine.

The results of the analyses are given as the second series in Table XIII. Inspection of this table shows that the results secured were nearly as good as those given in Table XI and in the First Series of Table XIII *until the warm summer weather began.* Late in June counts of increased size began to appear, at first somewhat irregularly, and later, as the hot summer weather came, the counts became large even reaching 180,000 per c.c. in the first milking made on July 24, 1917. This unexpected result was not correlated in any evident way with any change in conditions except the warmer temperature, the strength of the chloride solution having been constantly maintained as shown by tests of the available chlorine present.

Just as these results were secured, in a personal conference, Dr. M. J. Prucha, of the University of Illinois, suggested that in similar tests he had found that the strength of the chloride of lime solution was much weaker in the interior of the tubes than in the surrounding solution, and that this was due to the chemical action of the chloride of lime on the rubber, the reaction taking place more rapidly at warmer temperature. A preliminary account of this work has since

been published,[65] and a more complete account will be published later.

Accordingly, on August 9, a set of teat-cups was removed from the crock just before the evening milking in such a way as to retain the liquid in the tubes. By disconnecting the long tube from the teat-cups, the liquid in each of the two parts was collected separately. The solution in the crock showed 213 parts per million, the solution in the interior of the teat-cups, 168 parts per million, and the solution in the interior of the long tubes only 35 parts per million of available chlorine. Samples of these solutions plated on agar were sterile.

A second test made on the following day gave similar results showing that the solution in the long tubes was materially weaker than that outside of the tubes.

On August 1, 2, 3, and 10, tests were made to determine whether this solution was so weak that it failed to sterilize the rubber. Two sets of tubes where lifted out of the crock each day just before use in milking and brought to the laboratory, where the outer sides of the tubes were carefully rinsed with hot water, the operator using sterile rubber gloves in handling the tubes. The rubber tubing and metal parts were then disconnected and tests made as follows:

a. A liter of sterile water was poured thru each of the long rubber tubes. On August 1, the counts from this water were 229,000 and 97,000 per c.c., respectively. On the following day, the counts were 689,000 and 61,000 per c.c. while on August 3 they were 579,000 and 208,000 per c.c. In contrast to these, the August 10 tests showed less than 300 colonies per c.c. for each tube.

b. The eight short rubber tubes were placed in a flask with a liter of sterile water on each day. By pouring from one sterile flask to another these tubes were rinsed as thoroly as possible before agar plates were made. This rinse water on August 1 proved to be sterile, while the count on the following day was only 5 per c.c. On August 3, however, a count of 2570 per c.c. was obtained from this rinse water. On August 10, the count was 2 per c.c.

c. The two metal teat-cup connectors and the eight metal teat-cups were washed as thoroly as possible on each day in a liter of sterile water in a sterile pail. The counts from this rinse water were 1 per c.c. on August 1, 0 per c.c. on August 2, 2.5 per c.c. on August 3, and 4.5 per c.c. on August 10.

d. The small metal rings and rubber disks used in the mouth pieces of the B-L-K teat-cups and the rubber mouth pieces were all separated and washed in a liter of sterile water. The counts from these were 2 per c.c. on August 1, 3 per c.c. on August 2, 37 per c.c. on August 3, and 4 per c.c. on August 10.

e. The heads of the machines were tested at the same time in order to determine whether any bacteria came from these. The piston was rinsed in a liter of sterile water and one liter of sterile water was poured thru each of the two milk cocks on the head of the machine. The results in all but one case were practically sterile, the count never exceeding 7.5 per c.c. In the single exception referred to the rinse water gave a count of 148 per c.c.

[65] Prucha, M. J., Weeter, H. M., and Chambers, W. H. Hypochlorites as a disinfectant for rubber. *Abs. of Bact.*, **2**:19. 1918.

From these tests it is evident that the principal source of the bacteria was the interior of the rubber tubes, the long rubber tubes being especially contaminated with them. It is noteworthy, however, that even these were not in very bad condition on August 10. Apparently on this date the chloride of lime retained its strength in the tubes sufficiently to largely prevent growth.

The above results clearly indicate that chloride of lime solutions when used alone become weakened too quickly to make them suitable for use under practical farm conditions. This weakness is especially troublesome in hot summer weather.

Sterilization by steam and a mixture of saturated brine and chloride of lime.— In an effort to secure the advantages of both brine and chloride of lime, Miss Wing has suggested the use of a combined solution which has met with much favor. It has since been discussed by Moak and has been used extensively by practical dairymen in the State and elsewhere. In this combined solution all brine organisms are killed by the chloride of lime making a sterile solution, while the stability of the solution is secured by the fact that the strength of brines does not alter during use.

Tests were therefore made between August 20, 1917, and January 14, 1918, of solutions containing both salt and chloride of lime. A saturated brine solution was used to which one quart of a stock solution of chloride of lime was added twice per week (available chlorine in stock solution about 20,000 parts per million). In order to make the test a severe one, warm weather conditions were maintained until November 21, by keeping the crock containing the tubes in a warm place.

The temperature of the solution in the crock was usually between 20° C. and 28° C. during this period, and the chloride of lime solution lost strength rapidly, testing between 18 and 400 parts per million of available chlorine. After November 21, the crock was put in a cold place so that the temperature of the solution was reduced to a few degrees above freezing. The available chlorine increased rapidly under these conditions so that it never fell below 400 parts and even reached 1200 parts per million.

Four successive milkings of five liters each of sterile water were made on each Monday and Tuesday as in the previous work, the results of the analyses being given in Table XIV. Inspection of these results show that even under the severe test of warm conditions

the counts obtained from these tubes exceeded 100 per c.c. in only 11 instances out of a possible 64. The highest count obtained was 1920 per c.c. on the fourth milking of August 21, the second day after starting the experiment. On August 31 the water was sterile as received into the pail of the machine, the plates showing an average of less than one colony per plate.

TABLE XIV.— AMOUNT OF BACTERIAL CONTAMINATION DERIVED FROM STATION MACHINES, CAREFULLY CLEANED AND STERILIZED BY PRACTICAL METHODS. BRINE AND CHLORIDE OF LIME USED AS GERMICIDE.

Agar plate counts per c.c. of sterilized water milked thru machines.

TEST No.	Date.	Temperature of brine and chloride solution.	Water before milking.	First milking.	Second milking.	Water before milking.	Third milking.	Fourth milking.
	1917.							
1....	Mon., Aug. 20	24° C.	7	204	53	13	86	86
2....	Tue., Aug. 21	23° C.	0.8	70	235	0.6	870	1920
3....	Wed., Aug. 29	21° C.	0.3	16	4	0.7	1	1
4....	Fri., Aug. 31	20° C.	0.0	0.3	0.3	0.3	0.3	0.7
5....	Mon., Sept. 24	25° C.	0.7	12	14	1.3	20	73
6....	Tue., Sept. 25	22° C.	1.7	3	9	0.7	41	35
7....	Wed., Sept. 26	26° C.	1.7	7	4	65*	37	31
8....	Thur., Sept. 27	27° C.	0.7	77	53	0.7	12	9
9....	Fri., Sept. 28	27° C.	2.0	15	18	1.7	5	9
10....	Mon., Oct. 8	25° C.	1.0	5	5	1.3	5	5
11....	Tue., Oct. 9	26° C.	0.7	260	11	4.0	7	6
12....	Mon., Oct. 22	28° C.	0.0	27	621	0.3	359	65
13....	Tue., Oct. 23	27° C.	4.3	52	53	7.0	46	38
14....	Mon., Nov. 5	26° C.	1.7	162	108	1.0	167	380
15....	Mon., Nov. 19	25° C.	1.0	28	33	2.0	4	5
16....	Tue., Nov. 20	22° C.	0.3	9	35	0.7	2	3
17....	Mon., Dec. 10	2° C.	0.3	64	11	2.0	108	101
18....	Tue., Dec. 11	2° C.	0.3	81	76	2.0	58	20
	1918.							
19....	Mon., Jan. 14	2° C.	1.3	211	53	2.3	51	16

* Plates apparently contaminated.

The results obtained between November 21 and January 14 while the crock was kept in a cold place were similar to those obtained while it was in a warm place. Two out of twelve counts exceeded 100 per c.c., the highest count of 211 per c.c. being obtained from the first milking made on January 14.

In considering the significance of these figures, it should be kept in mind that while these milkings were all made in a milkroom where the air was free from dust, slight contaminations from this source are undoubtedly present in each case. The sterilization of the pails and head of the machine was carried out by the dairy attendants and while tests showed the steaming given to be sufficient to sterilize, there is always present the possibility that some of the bacteria were derived from these sources rather than from the teat-cups and tubes. In any case the total contaminations from the machines due to all sources was not great enough to be significant in practical dairying. It should be remembered that the entire care of the machines was in the hands of the attendants except for the maintenance of the strength of the antiseptic solution, and that nearly a year had elapsed in the course of the work since they had been requested to use special care in cleaning the machines.

Sterilization by steam and lime water.— As stated previously, several workers and many practical dairymen recommend the use of lime water as a solution in which to keep the teat-cups and tubes. Accordingly tests similar to those just described for the combination of brine and chloride of lime were carried out between August 24 and November 13, 1917.

The lime water solution was prepared by placing a relatively large amount (10 pounds) of quick lime in the bottom of a 25-gallon crock. This was slaked carefully and then the crock was filled with water. Because of this large excess of lime it was expected that a saturated solution of lime water would be maintained for a long period of time. Tests showed that when the solution was frequently stirred this purpose was accomplished.

Analyses to show the bacteriological condition of the teat-cups and tubes were usually made as before on Mondays and Tuesdays, five liters of sterile water being drawn thru the tubes into the machines. Summer conditions were maintained thruout the test by keeping the crock in a warm room where the temperature of the solution varied between 20° C. and 30° C. It was felt that this would make the test a severe one as lime is less soluble in warm water than in cold water. The results of the analyses are given in Table XV.

TABLE XV.— AMOUNT OF BACTERIAL CONTAMINATION DERIVED FROM STATION MACHINES, CAREFULLY CLEANED AND STERILIZED BY PRACTICAL METHODS. LIME WATER USED AS GERMICIDE.

Agar plate counts per c.c. of sterilized water milked thru machines.

TEST No.	Date.	Temperature lime water solution.	Water before milking.	First milking.	Second milking.	Water before milking.	Third milking.	Fourth milking.
	1917.							
1....	Fri., Aug. 24	24° C.	0.8	410	20	1.0	26	9
2....	Mon., Aug. 27	21° C.	1.0	244	30	0.8	41	28
3....	Tues., Aug. 28	22° C.	0.3	91	7	0.0	9	2
4....	Thur., Aug. 30	20° C.	0.3	30	4	0.7	2	2
5....	Sat., Sept. 1	22° C.	0.0	229	11	0.0	4	56
6....	Mon., Oct. 1	23° C.	1.0	2 270	970	2.0	1 010	1 240
7....	Tues., Oct. 2	22° C.	1.3	1 530	193	1.3	56	38
8....	Wed., Oct. 3	26° C.	0.7	910	57	3.0	24	104
9....	Thur., Oct. 4	27° C.	1.3	2 050	940	0.7	189	57
10....	Fri., Oct. 5	26° C.	3.0	580	53	.....	.....	12
11....	Sat., Oct. 6	24° C.	5.0	620	47	4.0	25	15
12....	Tues., Oct. 16	31° C.	1.3	124	25	5.0	69	110
13....	Mon., Oct. 29	26° C.	1.3	500	52	1.7	23	75
14....	Tues., Oct. 30	30° C.	0.7	73	32	0.7	28	23
15....	Mon., Nov. 12	28° C.	1.7	1 910	1 390	0.7	710	610
16....	Tues., Nov. 13	26° C.	0.7	41	35	4.0	14	13

Inspection of this table shows that practically sterile water was drawn thru the tubes in some instances, e. g. second, third and fourth milking on September 1. However, in the majority of cases, the bacteriological results, while excellent, were not quite as good as those secured when the brine and chloride of lime were used in combination under equally severe conditions. The highest counts were obtained on October 1 and 4 when two of the counts were slightly in excess of 2000 per c. c. Even counts of this size would not be significant in practical dairying so that the analyses uphold the idea that lime water is a satisfactory solution for use. No objectionable action on the metal or rubber was observed and the tubes appeared sweeter and cleaner than with the brine or with the chloride of lime solutions.

Cold running water as a means of keeping the tubes free from bacteria.— Because of the fact that it had been reported that certain practical dairymen were getting excellent results by keeping their

teat-cups and tubes in a jar which received the overflow from a cold spring, it was decided to test this system of caring for the tubes. Accordingly between April 14, 1917, and August 13, 1917, two pairs of teat-cups and tubes were kept in a crock which received a constant flow of water from a city water tap. The rate of flow was approximately 12 gallons per hour. In all other respects the tubes were handled as before. At the beginning, the temperature of the water in the jar was 6° C., but this gradually increased as the weather become warmer until in August the temperature rose to 19½° C.

After a preliminary series of analyses made daily, Monday and Tuesday analyses were made as before. The results are recorded in Table XVI. An inspection of these results shows that as long as

TABLE XVI.— AMOUNT OF BACTERIAL CONTAMINATION DERIVED FROM STATION MACHINES CAREFULLY CLEANED AND STERILIZED BY PRACTICAL METHODS. TUBES PLACED IN COLD RUNNING WATER.

Agar plate counts per c.c. of sterilized water milked thru machine.

TEST No.	Date.	Temperature. of water.	Water before milking.	First milking.	Second milking.	Water before milking	Third milking.	Fourth milking.
	1917.							
1....	Sat., Apr. 14	6° C.	1.8	132	97	1.6	113	49
2....	Wed., Apr. 18	.....	1.0	72	9	0.2	9	18
3....	Thur. Apr. 19	.....	3.0	263	10	0.6	7	4
4....	Fri., Apr. 20	.....	0.2	.....	28	.....	245	23
5....	Sat., Apr. 21	.....	0.6	51	12	0.6	77	378
6....	Mon., May 7	9° C.	7.0	385	60	1.0	98	53
7....	Tues., May 8	9° C.	0.8	231	21	0.4	18	75
8....	Mon., May 21	9½° C.	1.2	56	57	0.6	117	207
9....	Tues., May 22	.....	0.2	251	37	0.2	181	348
10†...	Mon., June 4	11° C.	0.4	39	18	0.6	16	36
11†...	Tues., June 5	11° C.	0.6	238	108	0.8	50	34
12†...	Mon., June 18	13° C.	1.0	48	135	1.0	13	6
13†...	Tues., June 19	13° C.	1.2	22	3	1.0	4	1
14....	Mon., July 2	15° C.	1.4	389	17	3.0	216	60
15....	Tues., July 3	15° C.	1.2	207	29	2.0	13	9
16....	Mon., July 16	17° C.	0.6	350	255	1.8	173	108
17‡...	Tues., July 17	16° C.	1.0	340	27	0.6	14	10
18‡...	Mon., July 30	18° C.	2.0	46,850	28,500	2.0	21,870	8,280
19‡...	Tues., July 31	.....	1.3	53,000	20,000	0.7	14,750	11,770
20‡...	Mon., Aug. 13	19½° C.	0.8	32,000	8,400	*19.0	38,200	10,200

* Plates apparently contaminated.

† Samples of water taken from crock gave plate counts of 9700, 3800, 4200 and 320 per c.c. respectively on these dates.

‡ Samples of water from the crock gave counts of 2585, 8500, 352,000 and 11,300 per c.c. on these dates. Samples of tap water taken on Jul. 30, Jul. 31 and Aug. 13 gave counts of 54, 61, and 136 per c.c. respectively.

the temperature of the water remained below 18° C. (62° F.), the cold water acted as an effective means of repressing bacterial growth in the tubes. The water in the pails after milking while never sterile, was in some cases almost so; e.g., the second, third and fourth milkings on June 19 where the counts were 3, 4, and 1 per c.c., respectively. The highest count obtained while the water was still colder than 18° C. was only 389 per c.c. These surprisingly low counts are as good as any obtained where chemical disinfectants were used under practical conditions.

However, as soon as the water reached 18° C., a complete change took place in the results; and beginning with July 30, much larger counts appeared. In the twelve milkings carried out under these conditions the lowest count obtained was 8280 per c. c. while the highest was 53,000 per c.c.

The plain indication from this series of tests is that this method of keeping the tubes is satisfactory *provided the water is cold enough to prevent the development of bacteria.* Where cold running water is available, this method of handling the tubes deserves further trial as it has the marked advantage of avoiding the use of antiseptics entirely. It should be noted that several previous investigators (e. g., Harrison, Hooper and Nutter, Ruediger) have experimented with cold water, but apparently not *running* water. They have in each instance reported unfavorably upon the use of water.

Sterilization of the teat-cups and tubes with "montanin."— Because of results reported by Moak with this proprietary germicide, and its entirely different chemical nature from any of the other germicides it was decided to include this in the series of tests. "Montanin" is in reality a by-product of the ceramic industries and was previously supplied from Germany, it is stated to be a mixture of hydro-fluor-silicic acid and fluor-silicates of zinc and aluminum[66]; and is used in the brewing industry for the disinfection of rubber hose, washing walls and woodwork and the like. The strength recommended for use is a 2 per ct. solution. This makes a clear, colorless, nearly odorless, slightly acid solution. No good data was secured regarding its toxicity to man, but it is probably not highly poisonous, if poisonous at all in small quantities.

[66] Wehmer, C. Versuche über die hemmende Wirkung von Giften auf Mikroorganismen. IV. Wirkung von Fluorverbindungen auf Hausschwamm, Schimmelbildung, Fäulniss und Gärung. *Chemiker-Zeitung*, 38:114–115, 122–123. 1914.

In the first series of analyses made with montanin, an attempt was made to completely sterilize the machines as in the series of analyses made where chloride of lime was used (see pages 162–5). The teat-cups and tubes were completely taken apart each day and each tube and metal piece thoroly scrubbed. The metal parts of the machine other than the large pail and piston plunger were sterilized in the autoclave under 15 lbs. pressure for fifteen minutes each day. The rubber tubing was placed in a 2 per ct. montanin solution, while the piston plunger was immersed in a 4 per ct. solution. Before testing, the operator (R) assembled the parts, using sterile rubber gloves, and rinsing each part separately in boiling hot water.

The tests were made as before, five liters of sterile water being drawn into the machines in each case. The results are given in Table XVII. On Feb. 23, the water after the first milking gave a count of 374 colonies per c.c., the reason for which is not evident;

TABLE XVII.—AMOUNT OF BACTERIAL CONTAMINATION DERIVED FROM STATION MACHINES WHEN THESE WERE STERILIZED WITH EXTREME PRECAUTION. MONTANIN USED AS GERMICIDE ON RUBBER TUBES.

Agar plate counts per c.c. of sterilized water milked thru machines.

TEST NO.	Date.	Water before milking.	First milking.	Second milking.	Water before milking.	Third milking.	Fourth milking.
	1917						
1.....	Feb. 2	0.8	0.6	0.6	0.0	1.0	0.4
2.....	Feb. 10	1.0	0.2	0.8	0.4	8.0	3.0
3.....	Feb. 14	0.4	0.6	0.0	1.0	2.0	1.0
4.....	Feb. 15	0.0	0.8	0.4	0.0	0.2	0.4
5.....	Feb. 16	1.0	0.2	0.4	0.2	0.6	0.2
6.....	Feb. 21	0.2	0.6	0.0	0.0	0.2	2 0
7.....	Feb. 23	0.2	374.0	0.2	0.2	1.0	2.0

but with this exception the results from the 28 milkings indicated that the machines were even more perfectly sterilized in this case than where the chloride of lime was used. (Compare with Tables XI and XII).

Because of these excellent results it was decided to try out this germicide under practical conditions. Consequently this solution was put into use between Feb. 27, 1917, and March 13, 1917, at the barn, a 2 per ct. solution being used in the crock and the teat-cups and tubes being handled by the regular dairy attendants, as in the

other similar series. The tests were made before the important effect of temperature on the action of some of these germicides was fully appreciated and the solution was cold in all cases.

The results from a series of 38 milkings are given in Table XVIII. In none of these did the germ content of the water in the pail of

TABLE XVIII.—AMOUNT OF BACTERIAL CONTAMINATION DERIVED FROM STATION MACHINES CAREFULLY CLEANED AND STERILIZED BY PRACTICAL METHODS. MONTANIN USED AS GERMICIDE.

Agar plate counts per c.c. of sterilized water milked thru machines.

TEST No.	Date.	Water before milking.	First milking.	Second milking.	Water before milking.	Third milking.	Fourth milking.
	1917						
1.....	Tues., Feb. 27	0.8	5	1	0.2	3	2
2.....	Thur., Mar. 1	0.6	12	6	.....	.....	.....
3.....	Fri., Mar. 2	0.8	11	496	0.4	24	420
4.....	Tues., Mar. 6	0.8	19	8	0.0	4	7
5.....	Wed., Mar. 7	2.0	20	4	1.0	4	11
6.....	Thur., Mar. 8	4.0	93	16	1.0	9	63
7.....	Fri., Mar. 9	0.2	4	1	0.0	1	0.6
8.....	Sat., Mar. 10	1.0	4	2	1.0	2	1
9.....	Mon., Mar. 13	0.0	19	18	0.4	12	5
10.....	Tues., Mar. 14	2.0	2	490	0.2	2	0.6

the milker exceed 500 per c.c., and in only three instances did it exceed 100 per c.c. In many cases the count from the water after being drawn into the pail was so low as to indicate that the teat-cups and tubes were sterile. In general the bacteriological results were fully as good as any secured.

Certain other considerations, however, make the use of this otherwise excellent germicide of doubtful desirability. Tests showed that, if the tubes were not thoroly rinsed after being taken from the solution and before use in milking, the small quantity of solution left in the tubes produced a noticeable and undesirable " tinny " and somewhat " pungent " taste in the milk. If large quantities of the solution were left in the tubes there might even be enough of the acid solution to curdle the fresh milk in the interior of the tubes. In the face of the known carelessness of many practical dairymen in rinsing the tubes it was felt that these conditions make it undesirable to recommend this solution for general use. This is the more true because of the lack of satisfactory evidence to show the

harmlessness of montanin. Fortunately there appears to be little danger of the fraudulent use of this germicide in milk itself as very small amounts added to milk give unpleasant tastes which make the milk unsalable. Some metals are attacked by montanin tho the rubber remained unaffected even when used for a much longer period than that noted above.

Conclusions in regard to chemical antiseptics.— In the foregoing study it has been shown that by simple and inexpensive means, it is possible to keep the teat-cups and rubber tubes of milking machines in a germ-free or nearly germ-free condition. That is, by immersion in brine, in cold chloride of lime solutions, in brine and chloride of lime combined, in lime water, in cold running water, or in montanin, these tubes *may* be made as nearly sterile as an ordinary dairy utensil can be made by steaming or scalding accompanied by drying. It should be noted, however, that all of these tests were made on machines *thoroly and copiously rinsed after each milking by clean, cold water, hot soda water, and hot water and that regularly once per week the teat-cups and tubes were completely taken apart and thoroly washed.* Observations made under practical farm conditions give clear indications that the antiseptics described do not give equally good results on machines which are cared for in a less satisfactory manner than this.

Mechanical cleaning of the teat-cups and tubes.— Early in the experimental work on milking machines at this Station, after tests had been made upon the bacteriological condition of the machines, it became the custom to clean the teat-cups and tubes thoroly only once per week. Such apparent carelessness has been criticized by others who have based their judgment upon the fact that ordinary metal utensils must be thoroly cleansed after each use.

In view of this criticism, a large part of our tests with the various antiseptics have been made upon Mondays, the day before the thoro cleaning and Tuesdays, the day on which the thoro cleaning was given. These data have been retabulated in Table XIX. In all there are 31 comparisons given with eight milkings in each.

In 15 of these the counts obtained from the water in the pail were appreciably higher on Mondays than on Tuesdays. Ten pairs of counts show the reverse condition, while the remaining six gave practically identical counts on both days. This result might be held

TABLE XIX.— BACTERIAL CONTAMINATION OF STATION MACHINES ON THE DAY BEFORE CLEANING TUBES CONTRASTED WITH THAT ON THE DAY ON WHICH THE TUBES WERE CLEANED.

Agar plate counts per c.c. of sterile water milked thru machines.

TEST No.	Date.	Germicide in use.	MONDAY TESTS. DAY BEFORE CLEANING.				TUESDAY TESTS. DAY ON WHICH CLEANED.				AVERAGES.	
			MILKING NO.				MILKING NO.				Mon.	Tues.
			1	2	3	4	1	2	3	4		
	1916											
1.....	Jul. 10–11	Weak chloride of lime.....	8,700	5,100	13,000	1,350	26,000	2,275	4,900	430	7,038	8,401
2.....	Jul. 24–25	Ditto.....	6,000	1,820	1,010	11,300	25,000	1,710	760	610	5,033	7,200
3.....	Oct. 16–17	Strong chloride of lime.....	7	.....	5	16	25	15	15	8	9	16
	1917											
4.....	Jan. 8– 9	Ditto.....	12	3	2	157	12	10	8	11	44	10
5.....	Mar. 26–27	Weak chloride of lime.....	36	7	7	42	46	22	18	21	23	27
6.....	Apr. 16–17	Ditto.....	15	10	21	33	15	3	7	24	20	12
7.....	May 14–15	Ditto.....	79	25	32	54	71	49	40	58	48	54
8.....	May 28–29	Ditto.....	14	12	7	53	11	7	.....	.....	21	9
9.....	June 11–12	Ditto.....	629	345	193	224	23	4	3	2	348	8
10.....	Jnne 25–26	Ditto.....	3,008	1,637	1,082	1,139	1,526	12,306	11,620	9,632	1,716	8,771
11.....	July 9–10	Ditto.....	25	680	1,020	920	11,470	4,890	3,950	2,960	661	5,818
12.....	July 23–24	Ditto.....	49,700	25,800	28,230	24,230	179,430	92,600	53,200	55,300	31,990	95,132
13.....	Aug. 6– 7	Ditto.....	25,000	16,100	15,100	10,600	16,900	5,100	11,800	8,600	16,700	10,600
14.....	Aug. 20–21	Brine and chloride of lime..	204	53	86	86	70	235	870	1,920	107	774
15.....	Sep. 24–25	Ditto.....	12	14	20	73	3	9	41	35	30	22
16.....	Oct. 8– 9	Ditto.....	5	5	5	5	260	11	7	6	5	71
17.....	Oct. 22–23	Ditto.....	27	621	359	65	52	53	46	38	268	47
18.....	Nov. 19–20	Ditto.....	28	33	4	5	9	35	2	3	18	12
19.....	Dec. 10–11	Ditto.....	64	11	108	101	81	76	58	20	71	59
20.....	Aug. 27–28	Lime water.....	244	30	41	28	91	7	9	2	86	27
21.....	Oct. 1– 2	Ditto.....	2,270	970	1,010	1,240	1,530	193	56	38	1,372	454
22.....	Oct. 29–30	Ditto.....	500	52	23	75	73	32	28	23	162	39
23.....	Nov. 12–13	Ditto.....	1,910	1,390	710	610	41	35	14	13	1,155	26
24.....	May 7– 8	Cold running water.....	385	60	98	53	231	21	18	75	149	86
25.....	May 21–22	Ditto.....	56	57	117	207	251	37	181	348	109	204
26.....	June 4– 5	Ditto.....	39	18	16	36	238	108	50	34	27	108
27.....	June 18–19	Ditto.....	48	135	13	6	22	3	4	1	51	8
28.....	July 2– 3	Ditto.....	389	17	216	60	207	29	13	9	171	65
29.....	July 16–17	Ditto.....	350	255	173	108	340	27	14	10	222	98
30.....	July 30–31	Ditto.....	46,850	28,500	21,870	8,280	53,000	20,000	14,750	11,770	26,375	24,880
31.....	Mar. 12–13	Montanin.....	19	18	12	5	2	490	2	1	14	124
Ave. of 22 tests in which all counts were less than 1,000 per c.c.....											91	85
Ave. of remaining 9 tests.....											10,227	17,920
Grand ave. of 31 tests.....											3,033	5,263

to indicate that there was a slight advantage to be gained in washing the tubes more frequently.

On the other hand, the grand average of all the Monday tests was 3033 per c.c., while that of the Tuesday tests was 5263 per c.c. An examination of the table will show that the difference between the averages is very largely produced by the tests made on Monday, July 23, and Tuesday, July 24, 1917. The four Tuesday counts in this case averaged 95,132 per c.c. as contrasted with an average for the four Monday milkings of only 31,990 per c.c. However, even if this test is omitted in the average, the results were still slightly higher for Tuesday than for Monday.

In the face of these analyses, the only conclusion which can be drawn is that there was no measurable difference between the bacteriological condition of the tubes on the days when they were thoroly washed and the condition of the same tubes after six days of use. Observation showed, however, that the tubes were cleaner on Tuesdays than on Mondays, and that there was real reason for the thoro cleaning as frequently as once per week. More frequent cleaning requires extra labor and is at the same time destructive of the rubber tubing.

Germ content of water after the first, second, third and fourth milkings. — In our observations on the bacteriological condition of milk from farms where milking machines were in use, it has been frequently noted that the germ content of all the different cans of milk produced would be approximately equal. This was surprising as it might be expected that because of the rinsing of the machines by the milk, the cans last filled would have many fewer bacteria. In the course of our experimental work, four successive milkings have been made of sterile water thru the machines in almost all instances.

The results obtained from all of these successive milkings have been divided into two groups. One group in which none of the counts exceeded 1000 per c.c. and a second group in which were placed counts in excess of 1000 per c.c. The grand averages of the four successive milkings of the 81 series of counts in the first group were 114 per c.c. for the first milking, 49 for the second milking, 37 for the third milking and 50 for the fourth milking. This would indicate a slight reduction in the number between the first and second milkings, with but little further reduction in the case of the later milkings. In the case of the results from the more badly

contaminated machines it is seen that there was a regular reduction noted when the averages of the 16 tests are examined. The average count from the first milking was 26,671 per c.c., from the second milking, 13,734, from the third milking 12,728 and from the fourth 9218. This percentage reduction is not as great, however, as might have been expected and is less than that noted by Prucha, Weeter and Chambers,[67] who have made tests of successive rinsings of badly contaminated milk cans. These findings indicate that the bacteria are not as readily loosened from the surfaces of the teat-cups and tubing as from smooth metal surfaces.

DISCUSSION AND CONCLUSIONS.

In the foregoing studies it has been found that while the bacterial condition of the machines in use at the Station and at some of the commercial dairy farms in the vicinity of Geneva was always at least reasonably satisfactory and usually excellent, the bacterial condition of the machines at the remaining commercial farms was very unsatisfactory. In many of the latter cases the machines themselves were found to contribute millions of organisms per c.c. of sterile water drawn thru them as in ordinary milking.

The chief sources of this heavy seeding with bacteria are the teat-cups and rubber tubes. The difficulty in maintaining these in proper condition lies in the fact that, being partially made of rubber tubing, they cannot be satisfactorily cleaned by ordinary methods of washing and scalding.

During the course of the experimental work the usefulness of the commonly used and recommended antiseptic solutions have been tested upon the station machines. In this connection, it cannot be too strongly emphasized that our machines were constantly maintained in a cleanly condition. While satisfactory results were obtained with these clean machines it does not necessarily follow that equally good results would have been obtained with dirty machines. In fact all of the observations indicate that physical cleanliness of the machines cannot be neglected if good results are desired.

The antiseptic solutions tried have been (1) 10–13 per ct. brine, (2) chloride of lime solutions of varying strength, (3) chloride of

[67] See footnote 2.

lime in a saturated brine, (4) lime water, (5) cold running water, and (6) a proprietary germicide sold under the trade name of "montanin."

Satisfactory results have been secured with all of these. However, with both ordinary chloride of lime and with running water, it was found that they became ineffective during hot weather. Certain considerations, moreover, make some of the solutions less desirable than others.

In the case of brine it has been found to contain large numbers of bacteria. Since, however, these appear to be very largely salt organisms which do not have any significance in milk, very satisfactory results are secured from its use. The great advantages of brine are that it is easily and cheaply prepared and does not lose its strength during use. Sterile brine solutions can be obtained by adding chloride of lime to the brine, and in this way the advantages of both substances are secured without additional disadvantages. Constant attention is required, however, if the strength of the chloride of lime solution is to be maintained, and this is especially true in hot weather.

Excellent results were secured by the use of running water so long as its temperature remained lower than 18° C. (62° F.). Under certain circumstances where the overflow of a cold spring of pure water is available this method of keeping the tubes is worthy of further trial.

The montanin was only tested to a limited extent and not under the most severe conditions. It was found to be very effective so far as tried. Because of unpleasant tastes in the milk where a montanin solution was carelessly used, its action on many metals, and doubtful desirability, this solution is not recommended for general use.

The usefulness of lime water was found to be much as reported by others. The bacterial contamination in the tubes was low at all times, tho not quite as low as where the mixture of brine and chloride of lime was used. For this as well as for other reasons, the authors of this paper believe the mixture of chloride of lime and saturated brine to be the most satisfactory one to recommend for general use.