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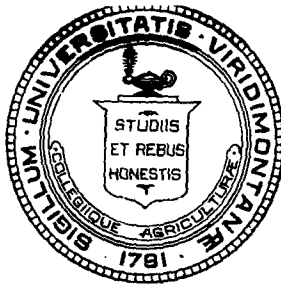
THE USE OF CHLORIN PRODUCTS AS
GERMICIDES ON DAIRY FARMS

By W. G. LOVELESS

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Concentrated compounds containing "available chlorin" deteriorate in storage. If held in the dark the rate of deterioration is lessened.

When foreign organic matter contacts a germicidal chlorin solution the concentration of its available chlorin is lowered. The rate of available chlorin loss increases as the temperature of the solution rises from 70° F. to 212° F.

Laboratory tests show chloramine-T solutions to be slower in action than hypochlorites. Acidification and raising the temperature of chloramine-T solutions increases germicidal activity but not to the degree shown by hypochlorites.

Corrosion of metal is a factor which deserves consideration in selecting the type of compound to use and in setting up practical procedure.

The danger of off-flavors caused by traces of chlorin adhering to utensils after treatment appears negligible providing the solution is carefully used and the utensils thoroughly drained.

Laboratory tests and practical experiments indicate definite improvement of the bacteriological quality of milk produced in farm utensils treated with chlorin solutions as compared to milk produced in utensils not so treated.

The convenience and low cost of hypochlorite treatment of farm utensils as compared with steam are practical advantages in its favor.

BULLETIN 369: THE USE OF CHLORIN PRODUCTS AS GERMICIDES ON DAIRY FARMS

W. G. LOVELESS

INTRODUCTION

Steam and hot water, the accepted standard agents of bacterial control in the dairy industry, have limitations. Their production in adequate quantities is often costly and frequently impracticable, especially on dairy farms. Furthermore, the source of supply can not always be located near the place where milk utensils are washed. Chlorin germicides, on the other hand, possess certain properties which seem to make them logical supplements to steam and hot water; hence a study has been conducted to determine how efficiently they fulfill the purpose for which they may be employed on the dairy farm. The limitations which restrict their usefulness have been examined and an attempt made to formulate a method of so applying them that a maximum of efficiency may be obtained. Their general properties are discussed and a theoretical study of the subject is also included. The practical applications deal more with the production than with the processing of milk.

REVIEW OF LITERATURE

Several studies dealing with the use of chlorin germicides in the dairy industry have been made during the past decade. Their introduction was at first opposed on the ground that chlorin might be intentionally placed in the milk as a preservative or gain access by accident, thus impairing its quality. Hale and Blecher (6) state that active chlorin—in hypochlorites—acts as a germicide when put directly into milk and that bacteria counts are reduced in proportion to its concentration in the milk. Zoller (32) studied the rate of decomposition of hypochlorite and showed that sterile milk reduced the active chlorin content as rapidly as did milk containing large numbers of bacteria, from which fact he concluded that chlorin in the form of hypochlorite when placed in milk exerted no selective action upon the chemical complexes of the bacterial cell. His further researches (33) tended to refute the findings of Hale and Blecher (6), indicating that the germicidal action of chlorin in milk was negligible in concentrations which did not affect its flavor and odor. The studies of Lochhead and Johns (13d) and of Prucha (26) tend to support the conclusion that the germicidal action of chlorin is relatively low in the presence of milk. The former subjected it to treatment with various amounts of

hypochlorite for 18 hours without materially reducing the bacteria count or changing the curdling time of treated and untreated milks. The latter states that five percent of milk in a chlorin rinse solution practically destroys its germicidal action.

Baker (2), studying sewage disposal, states that active chlorin showed greater germicidal powers than did other oxidizing agents such, for example, as permanganate which possesses a higher oxidation potential. He concluded that its ability to kill bacteria was probably the result of reactions other than oxidation. Hetler (7) analyzed bacterial cell structures and cites typical chemical reactions by the component compounds. Wright (31) studied the action of proteins and amino acids on hypochlorites and observed that alkalinity increased oxidation while acidity increased chlorination. Ayyar (1), Lochhead and Johns (13 a, b), and Myers and Johnson (17) found that chlorin compounds decrease in germicidal effectiveness with increased alkalinity.

Mudge and Lawler (15) found that the extent of the germicidal action of alkaline solutions was determined in large measure by time, temperature and the pH values. Myers (16) found that increasing the pH of alkali washing compound solutions augmented whereas a similar increment in chlorin solutions reduced their germicidal effectiveness. He also compared the effectiveness of neutral sodium hypochlorite solutions containing 100 parts per million of active chlorin in water with a solution of 0.5 normal sodium hydroxid and found chlorin to be considerably more effective than the alkaline solution. A neutral sodium hypochlorite proved to be distinctly more active than an alkaline solution, destroying 99 percent of suspended spores in less than a minute, whereas it took 22 minutes to accomplish the same reduction when alkaline hypochlorite was used.

Chloramine products have always proved less efficient than hypochlorites. Prucha (26) reports that 170 parts per million of chlorin from chloramine-T compounds gave good results in practical tests but that equally good results were secured when only 50 parts per million of hypochlorite chlorin were used. Lochhead and Johns (13 a, b) tested representative chlorin compounds by observing their action in reducing the numbers of viable bacteria derived from pure cultures of the types commonly present in milk and found chloramine products much slower in action than hypochlorites. Myers and Johnson (17), and Myers (18) secured similar results and advise greater concentration and longer contact periods where chloramine products replace hypochlorites.

Corrosion of metals by chlorin in solution has been studied by Hunziker, Cordes and Nissen (9), Prucha (27), Powers (21) and Johns (10). Hunziker, Prucha and Powers were concerned chiefly with the actual corrosive effect of the various types of chlorin compounds on metals commonly used in the fabrication of dairy equipment. Corrosion, as measured by loss in weight, was usually greatest when solutions of low alkalinity were employed. Strongly alkaline hypochlorite solutions and chlorin in the form of chloramine corroded most metals but slightly. Certain metals, particularly of the chromium-steel alloy type, were highly resistant to corrosion, whereas aluminum and galvanized iron were considerably affected even by alkaline hypochlorites. Johns, on the other hand, studied the effect of the addition of alkali to hypochlorite solutions with the view of determining what degree of alkalinity may be expected to lessen corrosion without seriously lowering germicidal effectiveness. He compared the corrosive action of solutions of varying pH values with their germicidal efficiencies and concluded that factors other than pH were to be considered, since various solutions of equal pH values to which sundry compounds had been added in order to increase alkalinity did not show parallel results, either as to their corrosive effects or the speed of their germicidal action.

Apparently the available chlorin content of a solution as determined by chemical test does not afford a true picture of its potency as a germicide. Myers and Johnson (17) recently studied the germicidal effectiveness of 12 commercial chlorin compounds relative to their chlorin concentrations. Their observations substantiate those of Lochhead and Johns (13 a, b) as well as the earlier studies made by Myers (16), indicating that those hypochlorites which approach neutrality are as effective as are the more alkaline types although their chlorin concentration may be less than half as great. The organic compound studied, although acid in reaction, required double the strength of chlorin to give results as effective as those afforded by hypochlorites of low alkalinity.

Tonney, Greer and Leibig (29) found that extremely low concentrations of chlorin may destroy bacteria when foreign organic matter is absent, *B. coli*, the most resistant of 503 strains of 48 species studied, succumbing in from 15 to 30 seconds when 0.25 parts per 1,000 were used.

Posson and Hotis (20) recommend 200 parts chlorin per million of water for treating farm milk utensils. They use the term "treat

to kill bacteria" rather than "sterilize," since the latter implies total destruction, which they feel can only be accomplished successfully by the use of steam under pressure or of hot air for prolonged periods.

Several extensive studies of the use of chlorin compounds in connection with milking machine sanitation have been made as, for example, by Parfitt (19), Lochhead and Johns (12) and Macy (14) while Black (3), Myers (18), Hunziker (8), Johnson (11), Powers (21), Scales (28), Prucha (25) and Fay (4) have made many helpful suggestions touching their use.

Fouts (5), comparing the relative values of hypochlorite and steam and hot water in treating milk plant equipment, states that rinsing with scalding water does not "sterilize" creamery equipment whereas sodium hypochlorite solutions proved very satisfactory when used as a rinse in concentrations of 45 parts per million.

Attention may be called to the work done by Prucha and his associates (22, 23, 24)—which had no relation to the use of chlorin since it anteceded its use in dairy technic—because of the stress they laid on the rôle of dairy utensils as factors in influencing the bacterial content of milk. They very properly point out the limitations of steam and hot water as germicidal agents.

Apparently little work has been done on the use of chlorin on the dairy farm. Lochhead and Johns (13 c) report that the quality of milk from 39 farms as delivered to city plants was much improved after the use of a chlorin rinse in addition to the procedure ordinarily followed. No other conclusive studies of the value of chlorin on the dairy farm have come to the writer's attention.

Theophilus and Atkeson (30) have published estimates of the cost of producing a supply of hot water and steam on the dairy farm and of operating such units. Their statements are referred to in the comparison of the cost of steam and hot water with chlorin compounds in succeeding pages.

THE CHEMISTRY OF CHLORIN GERMICIDES

The utility of chlorin compounds commonly used in dairy sanitation depends upon what is said to be their "active" or "available" chlorin content. All such compounds contain more or less chlorin in a form which, in solution, is free to act as a germicidal agent. In general there are six types which possess an "available chlorin content" as a common and distinctive property. All types may be used as germicides and certain of them represent those in general use. Some variations

exist within the type-classes, but for the purpose of this study the following empirical classification seems sufficiently comprehensive: 1. Chlorin gas. 2. Liquid sodium hypochlorite. 3. Crystalline sodium hypochlorite. 4. Calcium hypochlorite. 5. Chloramine-T. 6. Dichloramine-T. The latter agent does not dissolve readily and its germicidal action is slow. It is seldom used in the dairy industry and is not included in this study.

Chemicals employed in food processing must, of course, be non-toxic. No foreign substance which may impart an objectionable odor or flavor to milk or its products can be used. The products of decomposition induced by a chemical reaction must not affect the edibility or healthfulness of the product. Chlorin meets these three requirements, whereas certain other chemical germicides, such as formaldehyde, phenol or corrosive sublimate, do not and obviously can not be used.

INDIVIDUAL TYPES OF CHLORIN COMPOUNDS

1. *Chlorin Gas*.—Chlorin gas, compressed in steel cylinders, passed into and dissolved in water by means of special measuring apparatus, works well as a rinse and spray solution, particularly in mechanical bottle washing machines. Where large quantities of liquid sodium hypochlorite are required the concentrated stock solution may be prepared by passing chlorin gas into a soda ash solution. Considerable equipment is needed with which to measure the proportionate amount of gas into the soda ash solution but in large milk plants sodium hypochlorite may be thus inexpensively prepared. Where smaller volumes are required, as on producing farms, the home-made solutions from chlorid of lime or proprietary compounds are more convenient. For this reason chlorin gas was not used in the practical phases of this study.

2. *Liquid Sodium Hypochlorites*.—Some of the better known and most popular chlorin germicides belong to this class. A concentrated solution may be obtained in either of two ways; chlorin gas may be passed into a solution of soda ash, as has just been mentioned, or it may be made directly from sodium hypochlorite, which is a by-product of electrolysis of brine. Indeed, bleach liquor and germicides are thus prepared in great quantities. The percentage of available chlorin contained in liquid sodium hypochlorite may range from one to 12 percent. Liquid sodium hypochlorites are sold under many trade-names.

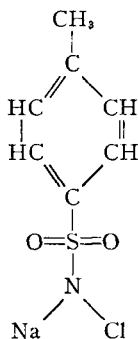
3. *Crystalline Sodium Hypochlorite*.—In this patented product chlorin is combined with the water of crystallization in tri-sodium

phosphate, resulting in a solid product which, dissolved in water, gives a sodium hypochlorite solution. About 2.5 to 3 percent of active or available chlorin is contained in this compound. The relative stability of the available chlorin in this product tends to ensure dependable strength of solutions.

4. *Calcium Hypochlorite*.—Calcium chlorid, from which hypochlorite solutions may be prepared, is one of the oldest sources of chlorin for germicidal purposes. Commercial “bleaching powder” or chlorid of lime, when fresh, contains up to 24 percent of available chlorin. However, it rapidly deteriorates and solutions may fail to work well because of deficiency in available chlorin.

A new manufacturing process now makes a product containing about 65 percent of available chlorin. Combined with a solution of soda ash this gives a highly concentrated sodium hypochlorite solution. Some manufacturers have combined this concentrated calcium chlorid with a suitable amount of soda ash to form a powder from which sodium hypochlorite stock solutions may be made. The 65 percent calcium hypochlorite is sold under the trade-name H T H (High Test Hypochlorite). The products representative of soda ash plus calcium hypochlorite are H T H—15, a powder containing about 15 percent chlorin and B K, a powder containing approximately 50 percent of available chlorin.

5. *Chloramine-T*.—Sodium paratoluenesulphonchloramid, having the structural formula



contains chlorin in an available form for germicidal purposes. In water solution the chlorin is liberated to form hypochlorous acid, the germicidal agent which destroys bacterial life. The chloramine-T compounds are very stable and relatively non-corrosive, but they are slower in action than the inorganic hypochlorites. The available chlorin

content of chloramine compounds ranges from 4 to 15 percent. They are sold under such trade-names as Sterilac, Santamine, Sterichlor.

CHEMICAL PROPERTIES

Hypochlorites are comparatively simple compounds, the structure of sodium hypochlorite, for example, consisting of an atom of sodium linked by an atom of oxygen to one of chlorin (Na-O-Cl). This compound is somewhat unstable and chemically reactive, in which connection note statements touching the stability of hypochlorites and their efficiency relative to chloramines as germicides on pages 12-28.

When hypochlorites react with organic matter—as, for example, with milk protein—one end product of the reaction is sodium chlorid and, undoubtedly, small quantities of other products such, for example, as chlorinated amino acids are also formed.

Calcium hypochlorite, in the form of chlorid of lime, is a highly complex and unstable compound. The structure is believed to be $\text{CaO-CaCl (OCl)-H}_2\text{O}$. Only the chlorin located in the (OCl) form is “available.” The other components form an insoluble sludge. The percentage of available chlorin present in the form of chlorid of lime averages about 24 percent in freshly prepared stock. Lochhead and Johns (13) found great variation in the available chlorin content of lots purchased at local supply houses.

The form of calcium hypochlorite containing up to 65 percent available chlorin is believed to be a true dehydrated calcium hypochlorite, its formula probably being Ca(OCl)_2 . Like bleaching powder it contains much insoluble calcium. When used to prepare germicidal solutions this insoluble material is generally allowed to settle and the clear liquid siphoned off. When combined with soda ash the sodium hypochlorite so formed is similar in reaction to those compounds already mentioned in the sodium hypochlorite class.

The structural formulæ of the chloramine-T compounds indicate that the chlorin is present in a side-chain on the benzene nucleus (p. 8). When in solution the chloramines release hypochlorous acid. However, the chlorin in these forms is somewhat more stable than that in hypochlorites, apparently because of the slow rate of liberation from the molecular structure. The truth of this assertion seems borne out by the fact that acidification of chloramine solutions gives results comparable to hypochlorites in so far as speed of action is concerned, in which connection see pages 21-28.

PROBABLE ACTION OF CHLORIN ON BACTERIA

The essential property possessed by hypochlorites and chloramines important in connection with dairy sanitation is their ability to destroy bacterial life. Biological chemists and bacteriologists are not fully agreed as to the manner in which this destruction takes place. The protoplasmic proteins of the bacterial cell may be expected to react chemically in a manner similar to that of protein in general [Hetler (7)]. Proteins are oxidized in the presence of hypochlorites; hence it is logical to expect the chemical composition of the bacterial cell to change and the result of such a change may be the destruction of the living organism. If this be so, one might conclude that oxidation of the protein in the living cell was the direct cause of the germicidal action of hypochlorites. On the other hand, again referring to the general reactions between proteins and hypochlorites, it seems that not only are the former oxidized but the amino acids which constitute protein structure may become chlorinated.

Wright (31), in a study of the reactions of hypochlorites with various proteins, found that as a general rule alkalinity increased oxidation while acidity increased chlorination. Johns (10) and Myers (16) have observed that an increase in the alkalinity of hypochlorite solutions tended to retard their germicidal action. Baker (2) states that such substances as, for example, permanganate, having a greater oxidizing potential than chlorin, are less effective than chlorin as germicides.

The conclusion which may be drawn from such evidence indicates that the germicidal action of hypochlorites is probably not directly the result of oxidation but, rather, that bacterial destruction may be due to a change in the protein structure of the bacterial cell brought about by chlorination.

CHEMICAL STABILITY

The degree of availability of the "active" or "available" chlorin is not uniform for all types of chlorin preparations. As has been stated, chloramines release available chlorin slowly as compared with liquid sodium hypochlorites. Trials have further demonstrated that some liquid hypochlorites are not as stable as are others. Thus crystalline hypochlorites are much more stable than are the liquid preparations. Now if stock preparations are to be stored the practical importance of stability is evident. On the other hand, the compounds which remain stable in their concentrated form require greater contact periods and stronger solutions to produce equivalent results,

The effect of storing a number of representative samples of compounds over a period of 12 months was determined. The original tests were made on samples taken from the containers in which the products were bought directly from their makers. Samples from each original supply were placed in clear bottles with ground-glass stoppers and stored in total darkness at room temperature. At the same time the residues in their original containers were placed on the laboratory reagent cupboard shelf where they were subjected to diffused light and to changing room temperatures. The use of autoclaves, etc., in the laboratory caused considerable variations in humidity and temperature, a condition somewhat similar to that obtaining in the average creamery. The strengths of both lots were checked from time to time and their available chlorin contents after four, six and 12 months' storage were determined (tables 1 and 2). The several compounds were trade prod-

TABLE 1.—STABILITY OF CHLORIN IN CONCENTRATED COMPOUNDS STORED IN TOTAL DARKNESS AT ROOM TEMPERATURE
(Sodium thiosulphate titration method)

Storage period in months	Compound							
	A		B		C		D	
	% Cl	% loss	% Cl	% loss	% Cl	% loss	% Cl	% loss
0	3.65	0	3.12	0	65.97	0	4.22	0
4	3.50	4.10	3.12	0	63.00	4.53	4.20	.47
6	3.00	17.80	2.95	5.44	4.00	5.18
12	2.87	20.13	2.90	7.05	58.12	7.05	4.00	5.18

TABLE 2.—STABILITY OF CHLORIN IN CONCENTRATED PRODUCTS STORED IN THE DIFFUSED LIGHT OF THE LABORATORY REAGENT CUPBOARD AT ROOM TEMPERATURE
(Sodium thiosulphate titration method)

Storage period in months	Compound									
	A		B		C		E		D	
	% Cl	% loss	% Cl	% loss	% Cl	% loss	% Cl	% loss	% Cl	% loss
0	3.65	0	3.12	0	65.97	0	15.8	0	4.22	0
4	2.95	19.18	3.00	3.84	58.00	12.05	4.00	5.18
6	2.30	37.31	2.95	5.44	55.65	15.64	15.7	.66	4.00	5.18
12	1.40	60.16	2.87	8.01	41.50	37.10	3.95	6.39

ucts which adequately represented the several classes and are hereinafter designated by letter as follows: A, sodium hypochlorite; B, crystalline sodium hypochlorite; C, calcium hypochlorite; D, chloramin-T; E, calcium hypochlorite with soda ash. Those held in darkness showed but slight reduction in their available chlorin contents. Hypochlorites are known to decompose when subjected to sunlight and heat and even when

stored in the dark some decomposition takes place. Myers and Johnson (17) observe that solutions having relatively high percentages of available chlorin decompose more readily than those of lower initial concentration. The results in table 1, while not comparing different concentrations of the same product, tend to bear out Myers and Johnson's statement. Evidence was observed in connection with solutions of a calcium hypochlorite and of soda ash. Two solutions, one of one and another of six percent chlorin content, were exposed to the laboratory light on an open shelf. The latter lost 20 percent of its chlorin content in four weeks, the former only four percent. A quart bottle of a liquid sodium hypochlorite remained unopened in the laboratory cupboard for 10 months, when it contained 93 percent of its original content. The stock solution in another bottle, from which supplies were withdrawn from time to time during the same 10 months, contained but little more than two percent of available chlorin and had lost more than 40 percent of its strength.

Table 2 shows the losses from preparations stored in diffused light in the laboratory cupboard. The liquid sodium hypochlorite lost the most chlorin. The solid form of sodium hypochlorite and the chloramine compound parted with relatively small percentages. Calcium hypochlorite lost the most heavily of the dry compounds, for it is packed in hermetically sealed metal cans and the seal had to be broken when the package was opened. The can was a poor storage container, having an ill-fitting cover, hence its contents were placed in a brown glass bottle with a cork stopper. It is meant for use in preparing stock solutions and its manufacturers recommend the preparation of a liquid stock hypochlorite as soon as the package is opened. It was bought at a later date than the other compounds and was observed under fall and winter storage conditions, whereas the compounds stored for 12 months were subjected to the higher summer temperatures.

THE STABILITY OF DILUTE SOLUTIONS WHEN HEATED

Chlorin preparations are used in dilute solution in dairy sanitation and conditions which may affect their stability and effectiveness when diluted are important considerations. Recommendations for using hypochlorites generally advise cold or lukewarm solutions, whereas it is suggested that chloramines be used at temperatures above 140° F. In order to determine the effect of heating upon freshly prepared chlorin

solutions several series of trials were conducted. The results of a series of typical trials are presented in table 3.

TABLE 3.—EFFECT OF BOILING ON THE AVAILABLE CHLORIN CONTENT OF SOLUTIONS
Concentration of chlorin given in parts per million of water.
Solutions A—boiled in open flasks with the loss from evaporation made up with distilled water before making the tests.
Solutions B—boiled under a water condenser to prevent evaporation.

Time in minutes	Compounds							
	A		B		C		D	
	A	B	A	B	A	B	A	B
0	150	177	149	149	291	291	241	241
30	142	163	149	142	241	256	200	198
60	140	163	144	134	240	240	198	170

Four chlorin compounds were dissolved in distilled water at room temperature. One 500 c.c. portion of each solution was boiled in an open flask, the loss by evaporation being restored with distilled water before making tests after 30 and 60 minutes of boiling. Another 500 c.c. of each solution was boiled under a condenser. The resulting reduction in available chlorin in this second series of trials seems to point to a change in the form of rather than a loss of chlorin. Hypochlorites decompose to form chlorides and chlorates, in which forms they manifest no germicidal properties. Temperature increments are paralleled by augmented decomposition, which fact may account for the larger losses when boiling took place under the condenser. Since there is reason to believe that chloramines produce sodium hypochlorite when dissolved, a similar explanation may hold with chloramine solutions when boiled.

The chlorin losses in solutions subjected to boiling are apparently small when there is no foreign organic matter present. Solutions were held at 80°, 120°, 140° and 160° F. for time periods up to two and one-half hours and in practically every case, provided no foreign organic matter was present, their initial chlorin contents were maintained.

Another series of similar tests were made during the summer of 1933. Fresh supplies of four commercial products were obtained from their manufacturers. Distilled water at desired temperatures, indicated in tables 4 to 6, ranging from 120° to 212° F. was used to make up solutions from each compound, calculated to contain approximately 200 parts of available chlorin per million of water. The actual content of available chlorin varied slightly from 200 p.p.m. when determined by the sodium thiosulphate test.

Ten 250 c.c. portions of solutions of each product were placed in a steam oven and kept at a temperature of 212° F. for an hour. After 10 minutes and 30 minutes samples were taken from each flask and tested for available chlorin and again at the end of an hour all samples were tested. The average results are shown in table 4. Since the

TABLE 4.—EFFECT OF BOILING TEMPERATURES ON THE AVAILABLE CHLORIN CONTENT OF FOUR COMMERCIAL COMPOUNDS MADE UP IN DILUTE SOLUTION WITH DISTILLED WATER
Average of 10 portions

Time in minutes	Parts per million of available chlorin			
	B	E	A	D
0	213	213	205.7	234.3
10	213	213	197.	234.3
30	213	213	169.7	234.3
60	213	213	159.7	234.3

liquid sodium hypochlorite solution was the only one showing appreciable chlorin loss, table 5 depicts the data from 10 solutions of this product subjected to this treatment.

TABLE 5.—DATA FROM 10 PORTIONS SHOWING THE EFFECT OF BOILING TEMPERATURES ON AVAILABLE CHLORIN CONTENT OF LIQUID SODIUM HYPOCHLORITE MADE UP IN DILUTE SOLUTIONS WITH DISTILLED WATER, ORIGINAL TEST AVERAGING 205.7 PARTS PER MILLION

Time in minutes	Parts per million of available chlorin										
	1	2	3	4	5	6	7	8	9	10	Ave.
10	198.8	191.7	177.5	205.7	198.8	191.7	198.8	198.8	213.	213.	198.8
30	170.4	177.5	142.	191.7	177.5	191.7	163.3	170.4	156.2	156.2	169.7
60	177.5	177.5	142.	177.5	142.6	177.5	142.	177.5	142.	142.	159.8

Table 6 sets forth the results obtained when similar solutions were prepared and held at temperatures of 180° F., 160° F. and 120° F. The data in this table were obtained on duplicate solutions, a larger number being deemed unnecessary as no loss in chlorin was evident from any solution.

Solutions at boiling temperature are never recommended for use in actual practice and temperatures over 120° F. are not suggested save in the case of the chloramines. Since little or no loss occurred from solutions held at from 80° to 160° F., it seems safe to say that the effect of temperature alone on the stability of available chlorin in dilute solutions is practically nil. Hence recommendations that hypochlorites be used at temperatures below 120° F., if justifiable, must be based on other premises.

TABLE 6.—EFFECT OF TEMPERATURES LOWER THAN BOILING ON THE AVAILABLE CHLORIN OF SOLUTIONS MADE UP IN DISTILLED WATER

A—Temperature 180° F.

Time in minutes	Parts per million of available chlorin			
	B	E	A	D
0	213	213	213	205.7
10	213	213	213	205.7
30	213	213	213	205.7
60	213	213	213	205.7

B—Temperature 160° F.

0	213	213	198.8	205.7
10	213	213	198.8	205.7
30	213	213	198.8	205.7
60	213	213	198.8	205.7

C—Temperature 120° F.

0	220.1	213	198.8	213
10	220.1	213	198.8	213
30	220.1	213	198.8	213
60	220.1	213	198.8	213

THE EFFECT OF ORGANIC MATTER ON CHLORIN STABILITY

The results of a series of trials to determine the effect of traces of milk which may get into chlorin solutions when used in dairy work are set forth in tables 7 to 10. Prucha (26) reports that large losses

TABLE 7.—CHLORIN SOLUTIONS HELD AT 160° F. FOR ONE HOUR

A—1,000 c.c. solutions made up in distilled water.

B—1,000 c.c. solutions made up in distilled water to which 1 c.c. of whole milk was added.

Minutes	Hypochlorites						Chloramine	
	B		A		E		D	
	A	B	A	B	A	B	A	B
0	134.9	110.05	99.4	71.	237.85	230.75	104.72	104.72
10	134.9	60.35	99.4	63.3	237.85	152.65	104.72	103.
20	134.9	35.50	78.1	42.4	237.85	124.25	104.72	103.
30	134.9	28.40	78.1	21.3	237.85	113.60	104.72	103.
60	134.9	10.65	78.1	7.1	237.85	102.90	104.72	101.17

ensue when from one to five percent of milk is added to chlorin solutions. One c.c. of milk was added to each 1,000 c.c. of solution, making the percentage of milk to solution 0.1 percent in all cases. The results when this modicum was added were so conclusive that trials with larger quantities seemed quite unnecessary.

As set forth in tables 7 and 8 two parallel series of solutions were used, A being milk-free and B being contaminated with 0.1 percent of milk. The chlorin contents of each lot were determined at ten-minute intervals, the initial test on Series B being made the moment the milk was added. Although it took less than two minutes to make a test, this

TABLE 8.—CHLORIN SOLUTIONS HELD AT 70° F. FOR ONE HOUR

A—1,000 c.c. solutions made up in distilled water.

B—1,000 c.c. solutions made up in distilled water to which 1 c.c. of whole milk was added.

Minutes	Hypochlorites						Chloramine	
	B		A		E		D	
	A	B	A	B	A	B	A	B
0	149.10	145.55	85.20	81.65	212.40	208.85	113.60	113.60
10	149.10	138.45	85.20	71.	212.40	208.85	113.60	113.60
20	149.10	134.90	85.20	67.45	212.40	208.85	113.60	113.60
30	149.10	134.90	85.20	63.90	212.40	208.85	113.60	113.60
60	149.10	134.90	85.20	56.80	212.40	208.85	113.60	110.05

elapsed time accounts for the drop in strength recorded as the initial test of series B. The extreme rapidity with which the chlorin disappears from hypochlorite types is readily apparent. Charts 1 to 4 show graphically the losses of available chlorin when organic matter is present and compare the fall in parts per million of chlorin at 70° F. with that at 160° F.

The actual reduction of available chlorin in solutions to which organic matter, in this case 0.1 percent of whole milk, is added is further illustrated by results presented in tables 9 and 10.

Solutions of four commercial compounds were made up in distilled water and, divided into 500 c.c. quantities, placed in Erlenmeyer flasks and heated to the required temperature. The computed amount of concentrated compound to produce 200 p.p.m. was used but some variation from this standard was evident when the sodium thiosulphate test was applied. One-tenth of one percent of whole milk was added to each 500 c.c. portion and tests made at the intervals set forth in tables 9 and 10.

The actual tests in all trials are recorded in table 9, whereas table 10 sets forth the averages of these triplicate trials at temperatures of 212° F., 160° F. and 120° F. The considerable reduction in the available chlorin content of solutions adulterated by organic matter is evident. Raising the temperature of the solution in all cases hastens the rate of chlorin loss. One flask of each solution was included in each trial to which no milk was added. These samples served as a

check on the possible loss from heat alone and in all cases, except for a small loss in liquid sodium hypochlorite at 212° F., no losses of chlorin occurred.

TABLE 9.—THREE TRIALS AT VARIOUS TEMPERATURES TO DETERMINE THE EFFECT OF THE ADDITION OF 0.1 PERCENT OF WHOLE MILK ON THE AVAILABLE CHLORIN CONTENT OF DILUTE SOLUTIONS OF FOUR COMMERCIAL CHLORIN GERMICIDES

Exposure time	Parts per million of available chlorin								
	212° F.			160° F.			120° F.		
Minutes	1	2	3	1	2	3	1	2	3
B Compound									
0	213.	213.	220.1	213.	205.9	213.	213.	234.4	213.
1	191.7	170.4	184.6	170.4	184.6	198.8	205.1	227.2	213.
2	170.4	170.4	170.4	149.1	117.8	184.6	198.8	191.7	205.7
5	163.3	149.1	134.9	142.	106.5	134.9	188.2	191.7	191.7
10	149.1	106.5	85.2	113.6	49.7	85.2	188.2	170.4	149.1
20	85.2	71.	28.4	85.2	35.5	28.4	184.6	142.	134.9
30	42.6	71.	213.	42.6	35.5	7.1	198.8	138.4	120.7
40	28.4	63.9	7.1	35.5	28.4	trace	177.5	113.6	92.3
60	trace	42.6	trace	trace	21.3	0	170.4	85.2	63.9
E Compound									
0	198.8	198.8	198.8	205.9	205.9	213.	248.4	198.8	220.1
1	198.8	191.7	177.5	198.8	205.9	213.	248.4	198.8	220.1
2	177.5	170.4	163.5	198.8	191.7	205.9	241.4	198.8	220.1
5	142.	149.1	156.2	184.6	177.5	198.8	241.4	191.7	213.
10	99.4	113.6	120.7	163.3	163.3	184.4	220.1	191.7	213.
20	63.9	92.3	106.5	142.	134.9	163.3	220.1	191.7	205.9
30	49.7	71.	78.1	127.8	127.8	142.	220.1	184.6	205.9
40	14.2	42.6	49.7	106.5	99.4	113.6	220.1	184.6	198.8
60	trace	21.3	35.5	85.2	63.9	99.4	220.1	163.3	191.7
D Compound									
0	198.8	198.8	205.9	191.7	205.9	205.9	213.	191.7	213.
1	177.5	170.4	205.9	191.7	205.9	198.8	205.9	191.7	213.
2	177.5	170.4	205.9	184.6	205.9	198.8	205.9	191.7	213.
5	170.4	170.4	198.8	177.5	198.8	191.7	205.9	184.6	213.
10	170.4	163.3	184.6	177.5	184.6	184.6	205.9	184.6	205.9
20	170.4	142.	163.3	163.3	184.6	177.5	205.9	177.5	205.9
30	163.3	142.	163.3	163.3	177.5	163.3	205.9	177.5	191.7
40	142.	127.8	142.	163.3	177.5	156.2	205.9	177.5	191.7
50	127.8	120.7	134.4	163.3	170.4	156.2	205.9	170.4	191.7
A Compound									
0	184.6	205.9	168.4	191.7	220.1	213.	243.1	213.	198.8
1	168.4	184.6	168.4	184.6	205.9	205.9	243.1	205.9	198.8
2	149.1	184.6	134.9	184.6	198.8	198.8	227.2	205.9	191.7
5	113.6	127.8	106.5	168.4	184.6	198.8	213.	205.9	177.5
10	71.	92.3	92.3	177.4	99.4	168.4	205.9	198.8	177.5
20	35.5	49.7	49.7	134.9	71.	134.9	205.9	184.6	163.3
30	7.1	trace	trace	113.6	49.7	106.5	198.8	170.4	156.2
40	trace	0	0	113.6	35.5	99.4	198.8	163.3	134.9
60	0	0	0	106.5	21.3	85.2	184.6	142.	120.7

TABLE 10.—SUMMARY OF DATA IN TABLE 9

Time in minutes	Average parts per million available chlorin			Average parts per million available chlorin		
	212° F.	160° F.	120° F.	212° F.	160° F.	120° F.
	B			E		
0	215	211	220	199	208	222
1	182	185	215	189	206	222
2	170	150	199	170	199	220
5	149	128	190	149	187	215
10	114	83	169	111	170	208
20	62	50	154	88	147	206
30	109	28	153	66	132	204
40	33	21	128	36	106	201
60	14	7	106	19	83	192
	A			D		
0	186	208	218	201	201	206
1	174	199	216	185	199	204
2	156	194	208	185	196	204
5	116	184	199	180	189	201
10	85	148	194	173	182	199
20	45	114	185	158	175	196
30	2	90	175	156	168	192
40	0	83	166	137	166	192
60	0	71	149	128	163	189

The importance of recognizing the effect of organic matter on the efficacy of hypochlorite solutions as germicides is essential to the establishment of practical principles. All the preparations studied, save the chloramine products, showed some loss of chlorin at 70° F. and much greater losses at higher temperatures. Prucha (26) states that even the stable chloramines lost all of their available chlorin in 30 minutes at 160° F. when five percent of milk was present. Two of the hypochlorites used showed greater stability at 70° F. than did another, the results being quite in line with those secured by Prucha (26), Myers and Johnson (17) and Lochhead and Johns (13 a), indicating that increased alkalinity by buffering solutions with alkaline salts yields a product of greater stability.

The results in tables 3 to 10 have a direct practical bearing. It was pointed out in tables 3 to 6 and in the discussion following that high temperatures alone do not materially lower the available chlorin content of a solution but that in the presence of organic matter the losses at the higher temperatures are very great and are noticeable at room temperature. Obviously all traces of grease or milk film should be thoroughly removed from utensils which are to be treated with chlorin of whatever origin. Of course, the prime object in view in using

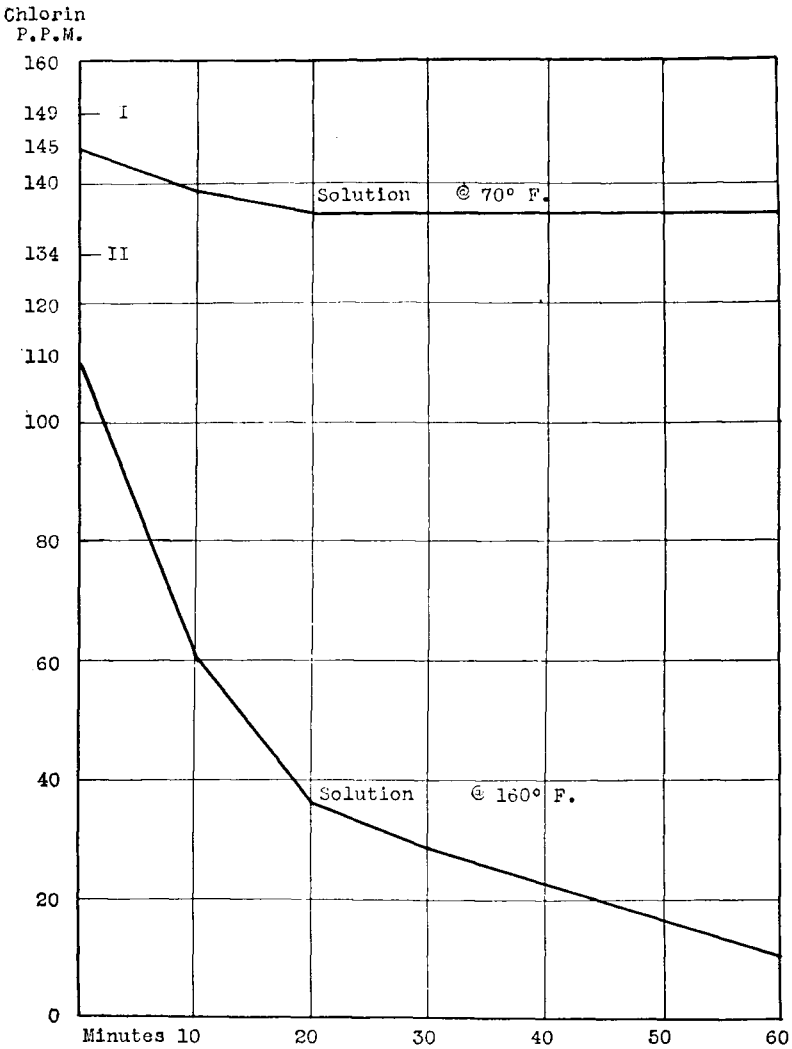


CHART 1.—Effect of an addition of 0.1 percent of milk on the available chlorin of a solution of compound B. Points I and II indicate the strength of solutions before the addition of milk.

chlorin is to destroy bacterial life. When it alone is present very dilute concentrations will serve the purpose, as is shown by Tonney, Greer and Leibig (29). The solution concentrations usually recommended should be strong enough to ensure efficient results, if no more organic matter is left on the utensils than should be left after thorough cleansing. If, however, organic detritus of any kind is present, be it milk residue, grease, or worse, this may use up all the available chlorin, in which case the bacteria life is not affected.

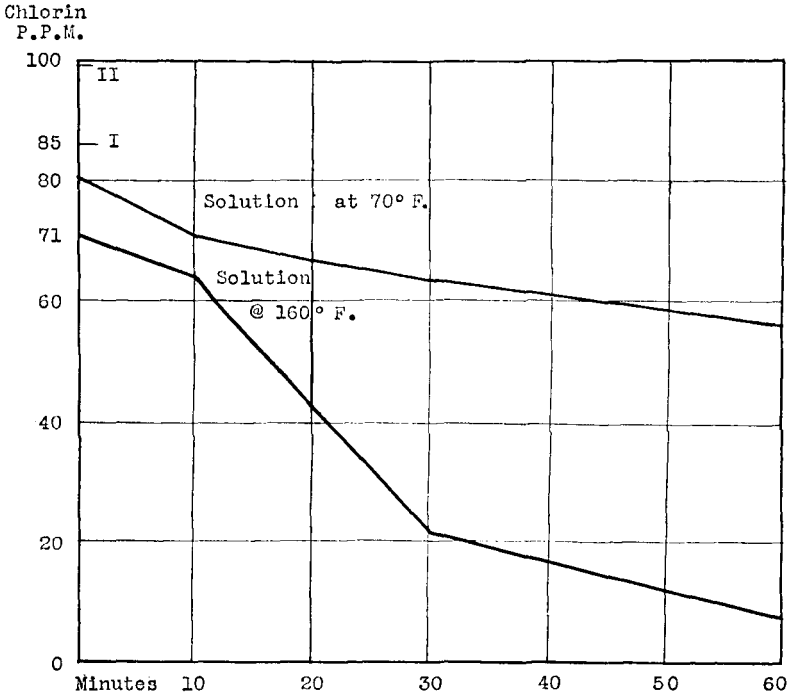


CHART 2.—Effect of an addition of 0.1 percent of milk on the available chlorin of a solution of sodium hypochlorite, compound A. Points I and II indicate the strength of solutions before the addition of milk.

STABILITY IN RELATION TO GERMICIDAL EFFICIENCY

There appears to be a definite relationship between the stability of hypochlorite chlorin and its effectiveness in destroying bacterial life. High alkalinity increases the stability of this class of compounds. Myers and Johnson (17) recently measured the germicidal potency of 12 commercial preparations and report a negative correlation between the alkalinity of chlorin compounds and the effectiveness with which they destroy bacteria. Eleven hypochlorites were used. About 100 parts per million of chlorin were needed to destroy the test organism when six compounds of low alkalinity were tried, whereas in the case of five compounds of high alkalinity it took from 275 to 300 parts to accomplish the same end. Two hundred forty-seven parts were needed when an organic chlorin compound, acid in reaction, was used.

The writer has not made an exhaustive study of laboratory methods of determining germicidal effectiveness. However, no evidence was found in literature touching certain aspects of the problem; hence trials

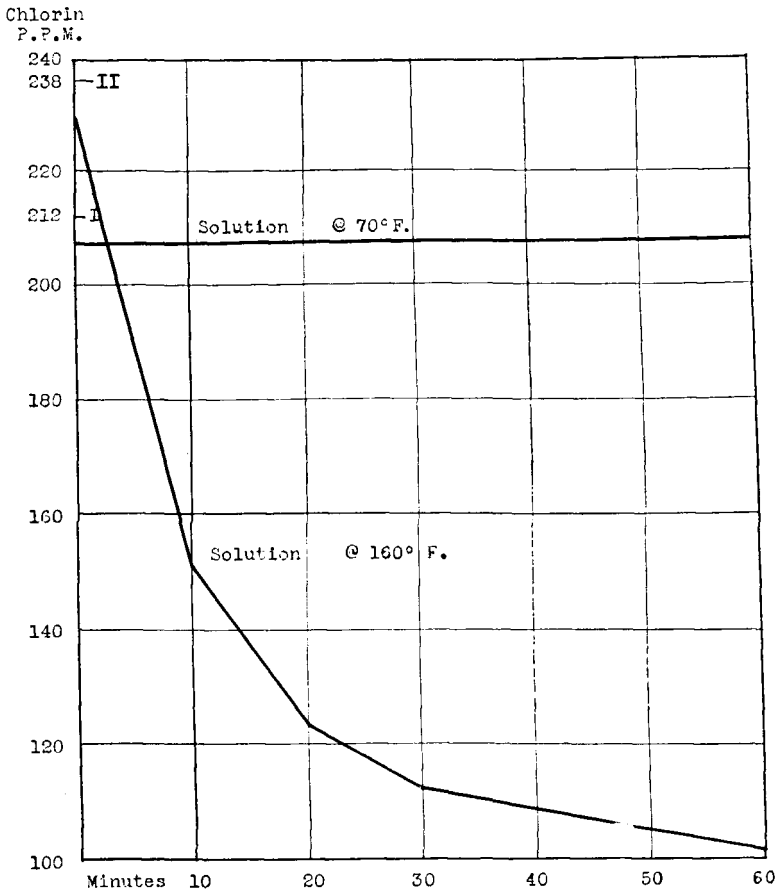


CHART 3.—Effect of addition of 0.1 percent of milk on the available chlorin of a solution of sodium hypochlorite, compound E. Points I and II indicate the strength of solutions before the addition of milk.

were instituted with a view of checking the accuracy of the conclusions of other workers as well as to clear up certain moot points.

SPEED OF GERMICIDAL ACTION

Some manufacturers of chloramine products have held that the slow action of organic chlorin compounds as compared with hypochlorites of low alkalinity may be partially overcome by acidification of the solution. As is set forth in table 11 and chart 5 there seems to be some justification for such claims.

A commercial organic chlorin compound, D, was dissolved in sterile distilled water at 70° F., the solution containing 106 parts per million of available chlorin. Two 200 c.c. portions were placed in sterile

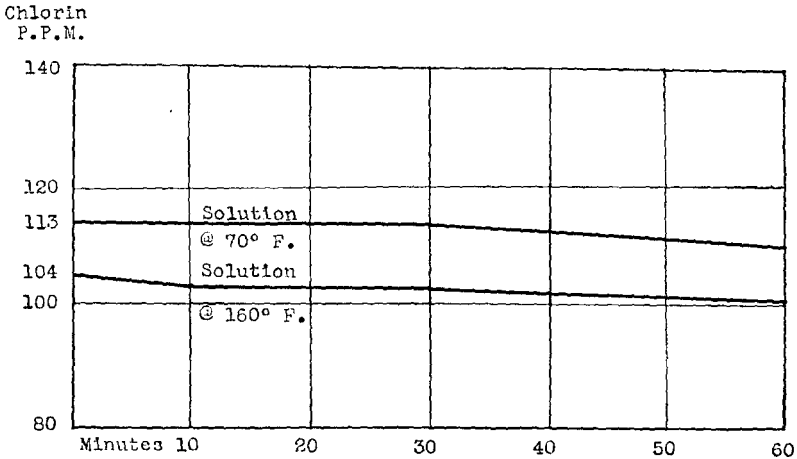


CHART 4.—Effect of the addition of 0.1 percent of milk on the available chlorin of a solution prepared from an organic chloramine compound D.

Erlenmeyer flasks, 0.1 percent of commercial sulphuric acid was added to one flask and a suspension of a known bacterial organism (a strain of *B. coli* obtained from the University of Vermont College of Medicine) placed therein after the manner described by Lockhead and Johns (13 a). Broth cultures were incubated for 18 hours at 37° C. prior to use. The culture was diluted 1:50 in sterile water and 1 c.c. of this dilution used.

Following Lochhead and Johns' (13 a) plating method, the acidified compound and the flask containing the unacidified solution were plated for comparison. Triplicate tests were made and the results averaged. At the same time a similar solution at 160° F. without acidification was plated as well as a water blank at 160° F. which was also contaminated and plated at 15-second intervals. The results secured with the hot solution appear in curve B on chart 5. The plates poured from the water blank showed practically no decrease in count after 60 seconds contact but were so crowded that accurate counts could not be made.

Table 11 shows the percentages of survival at the end of each exposure period. Either acidification of chloramine solutions or increased

TABLE 11.—GERMICIDAL POTENCY OF CHLORIN AGAINST *B. COLI*
Initial count 578,000

Germicidal solution 106 parts per million	Percentages surviving			
	15 seconds	30 seconds	45 seconds	60 seconds
D 70° F.	85.46	42.73	30.20	29.23
D 70° F. plus 0.1 percent H ₂ SO ₄	17.57	3.63	0.3114	0.0345
D 160° F.	50.51	18.20	13.34	4.01

temperature appear materially to have increased the speed at which bacteria are destroyed. Aside from the increased germicidal potency resulting from acidification, certain other changes were noted in the properties of the acidified compound. When subjected to a temperature of 160° F. in the presence of 0.1 percent of whole milk the

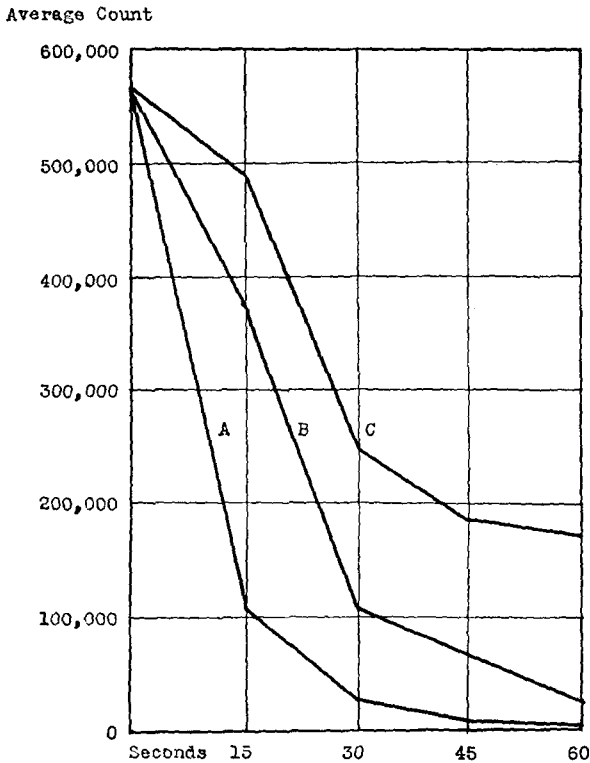


CHART 5.—The germicidal potency of an organic chlorin, compound D. Solution A—106 parts per million chlorin at 70° F. plus 0.1 percent H_2SO_4 . Solution B—106 parts per million chlorin at 160° F. Solution C—106 parts per million at 70° F.

chlorin disappeared rapidly, whereas the loss of chlorin from an unacidified solution was extremely small (table 7). However, the acidification of a solution treated in the manner set forth in table 4 resulted in a drop from 220 to 98 parts per million of chlorin after 30 minutes exposure.

Table 12 records the results of tests made of three types of hypochlorite compounds to determine their comparative effectiveness in killing the same test organism. For purposes of comparison the results

TABLE 12.—GERMICIDAL POTENCY OF VARIOUS TYPES OF CHLORIN PREPARATIONS AGAINST *B. COLI**

Seconds exposed	Percentage of bacteria surviving			
	Germicidal solutions 100 parts per million			106 parts per million
	A	B	E	D†
15	0	2.0454	3.1761	17.57
30	0	0.8181	1.0122	3.63
45	0	0.0000	0.0000	0.3114
60	0	0.0000	0.0000	0.0346

* Initial counts of the diluted broth culture used: A, 165,000; B, 440,000; E, 573,000.

† D, 578,000; solutions at 70° F.; results from table 11.

secured with acidified chloramine are included. The solutions contained approximately 100 parts per million of active chlorin. Following the technic employed in testing the effectiveness of chloramine on *B. coli*, triplicate tests were made and the average percentages of survival determined. The results seem to indicate that the hypochlorites act very rapidly and that in so far as this organism is concerned, they are quite effective. The efficiency of a highly alkaline commercial compound, B, appears to better advantage in this test than was reported in somewhat similar tests made by Lochhead and Johns (13 a). On the other hand, E, calcium hypochlorite with soda ash, gave results more in accord with the results obtained by their test of solutions prepared from soda ash and a calcium hypochlorite in proportions similar to E, than with the findings of Myers and Johnson (17), who, however, employed a very different technic.

During the course of practical farm experiments conducted in the summer months of 1933, the strength of solutions used for germicidal purposes was increased to approximately 200 parts of available chlorin per million parts of water. Tests were made in the laboratory to determine the relative efficiency of different compounds at such strength. Tests were also conducted to determine the efficiency of organic chloramine compounds under various conditions.

Johns' (13 a) plating technic was followed in making all tests of germicidal speed. A culture of *B. coli* was obtained from the University College of Medicine and 18-hour beef broth cultures were used as inocula. This broth suspension was further diluted 1:100 in sterile water to provide a count low enough to prevent overcrowding of plates. The testing technic employed was similar to that described above. Some plates, however, were much overcrowded and an estimated count was made. The comparative value of such counts was believed to be reasonably accurate since the same method of estimation applied to all tests.

TABLE 13.—PLATE COUNTS ON B. COLI INOCULUM AFTER TREATMENT WITH A CHLORAMINE-T SOLUTION CONTAINING 200 PARTS PER MILLION OF AVAILABLE CHLORIN. INITIAL COUNT 2,000,000

A. Room temperature (70° F.). B. Room temperature + 1 percent commercial H₂SO₄. C. At 180° F. D. Sterile water at 180° F.

Trial number	Colony plate counts after exposure to chlorin			
	15 sec.	30 sec.	45 sec.	60 sec.
1-A	338,000	175,000	25,200	5,800
2-A	325,000	143,000	34,600	14,800
3-A	403,000	223,000	70,800	42,800
4-A	260,000	163,000	52,800	14,800
5-A	76,000	6,400	800	0
6-A	291,000	11,000	1,400	0
7-A	19,000	400	400	0
8-A	154,800	20,800	3,600	0
9-A	143,000	23,800	2,400	0
Average count	223,311	85,156	21,333	8,689
1-B	0	0	200	0
2-B	0	0	0	0
3-B	200	200	0	0
4-B	0	0	0	0
5-B	0	0	0	0
6-B	0	0	200	0
7-B	0	0	0	0
8-B	0	0	0	0
9-B	200	0	0	0
Average count	44	22	44	0
1-C	0	0	0	0
2-C	0	200	0	0
3-C	0	0	0	0
4-C	0	0	0	0
5-C	0	0	0	0
6-C	200	0	0	0
7-C	0	0	0	0
8-C	0	0	0	0
9-C	0	0	0	0
Average count	22	22	0	0
1-D	0	0	200	0
2-D	0	0	0	0
3-D	200	0	0	0
4-D	0	0	0	0
5-D	0	0	600	0
6-D	0	200	0	200
7-D	0	0	0	0
8-D	0	0	0	0
9-D	6,200	200	0	0
Average count	711	44	89	22

Chloramine-T was again tested as previously described to determine the value of acidification of solutions or of heat on the efficiency and speed of action on the test organism. The actual and average counts obtained on plates poured in nine trials are given in table 13. The percentage of surviving bacteria after various contact periods appear in

table 14. These percentages were calculated from the average numbers surviving as set forth in table 13.

TABLE 14.—GERMICIDAL POTENCY OF A SOLUTION OF CHLORAMINE-T CONTAINING 200 PARTS PER MILLION OF AVAILABLE CHLORIN TESTED AGAINST B. COLI
Initial count of B. coli inoculum—2,000,000 per c.c.

Germicidal solution	Percentage of bacteria surviving			
	15 sec.	30 sec.	45 sec.	60 sec.
	%	%	%	%
D at 70° F.	11.1656	4.2578	1.0666	0.4344
D at 70° F. + 1% H ₂ SO ₄	0.0022	0.0011	0.0022	0.0000
D at 180° F.	0.0011	0.0011	0.0000	0.0000
Sterile water at 180° F....	0.0356	0.0022	0.0044	0.0011

TABLE 15.—PLATE COUNTS ON B. COLI INOCULUM AFTER TREATMENT WITH VARIOUS CHLORIN GERMICIDES CONTAINING APPROXIMATELY 200 PARTS PER MILLION OF AVAILABLE CHLORIN IN COLD SOLUTION
Initial count 15,400,000

Compound and trial number	Colony plate count after exposure to chlorin			
	15 sec.	30 sec.	45 sec.	60 sec.
E				
1	18,200	8,400	200	0
2	0	0	0	0
3	5,400	800	0	0
4	13,200	0	200	200
5	0	0	0	0
6	0	0	0	0
Average count	6,133	1,533	67	33
B				
1	0	200	0	0
2	0	200	0	400
3	0	0	0	200
4	0	0	0	0
5	0	0	200	200
6	0	0	0	0
Average count	0	67	33	133
A				
1	0	0	0	0
2	0	200	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
Average count	0	33	0	0
D				
1	1,352,000	247,000	81,600	1,200
2	689,000	97,600	15,800	800
3	1,040,000	57,600	10,000	800
4	585,000	77,200	0	200
5	247,000	12,800	0	0
6	1,721,000	41,800	2,000	200
Average count	939,000	89,000	18,233	533

Comparison of the results obtained in these later trials with those in which a solution of approximately 100 parts per million was used shows a similar trend but not to the same degree. The increased concentration of available chlorin gave increased germicidal efficiency, even in cold solutions. Acidified and hot solutions were very efficient in the destruction of *B. coli*. It would seem from the results obtained with sterile water at 180° F., that some of the increased efficiency in the hot solution is probably due to its temperature as well as to its active chlorin content.

Tests were also made on the four germicidal products used in practical farm experiments. Following the technic outlined by Johns (13 a), cold water solutions were made up in 200 c.c. sterile water blanks to

TABLE 16.—PLATE COUNTS ON MILK CAN RINSE INOCULUM AFTER EXPOSURE TO GERMICIDAL SOLUTIONS CONTAINING 200 PARTS PER MILLION OF AVAILABLE CHLORIN IN COLD SOLUTION
Initial count of inoculum—3,110,000 per c.c.

Compound and trial number	Colony plate count after exposure to chlorin			
	15 sec.	30 sec.	45 sec.	60 sec.
E				
1	5,200	400	0	0
2	0	0	0	0
3	3,800	2,600	600	0
4	6,400	2,800	0	0
5	46,000	22,200	12,200	2,600
6	1,800	0	0	0
Average count	10,533	4,667	2,133	433
B				
1	0	0	0	0
2	0	400	0	0
3	800	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
Average count	133	67	0	0
A				
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
D				
1	22,400	8,400	5,600	1,400
2	84,800	56,200	39,200	18,600
3	16,800	4,400	0	0
4	82,000	75,200	22,000	15,200
5	42,400	56,200	18,800	6,400
6	22,800	12,600	4,400	0
Average count	45,200	35,500	15,000	6,933

contain approximately 200 parts per million of available chlorin. The same inoculum was used and was grown in an 18-hour broth suspension in one series of trials and in a later series rinsings from unwashed milk cans were used. Tables 15 and 16 set forth the actual counts obtained from six trials from which the percentage survivals were calculated and set forth in tables 17 and 18.

TABLE 17.—PERCENTAGE AVERAGE SURVIVAL OF BACTERIA IN SIX TRIALS TO DETERMINE THE GERMICIDAL SPEED AND EFFECTIVENESS OF SOLUTIONS OF FOUR COMMERCIAL CHLORIN COMPOUNDS IN COLD SOLUTION AGAINST THE TEST ORGANISM B. COLI

Time in seconds	Percent of bacteria surviving exposure to chlorin			
	E	B	A	D
15	0.0398	0.0000	0.0000	6.0974
30	0.0100	0.0004	0.0002	0.5779
45	0.0004	0.0002	0.0000	0.1184
60	0.0002	0.0009	0.0000	0.0034

TABLE 18.—PERCENTAGE AVERAGE SURVIVAL OF BACTERIA IN SIX TRIALS TO DETERMINE THE GERMICIDAL SPEED AND EFFECTIVENESS OF SOLUTIONS OF FOUR COMMERCIAL CHLORIN COMPOUNDS IN COLD SOLUTIONS AGAINST A MIXED CULTURE FROM CAN RINSINGS

Time in seconds	Percent of bacteria surviving exposure to chlorin			
	E	B	A	D
15	0.3387	0.0042	0.0000	1.4533
30	0.1500	0.0021	0.0000	1.1414
45	0.0685	0.0000	0.0000	0.4823
60	0.0139	0.0000	0.0000	0.2229

The results obtained in these trials further illustrate the more rapid action of inorganic chlorin compounds as compared with the organic forms. Liquid sodium hypochlorite shows the greatest speed and efficiency but the small survival in the case of any hypochlorite tested appears to make this type of germicide preferable for rapid rinsing with cold solutions, in so far as speed of action and efficiency is concerned.

THE CORROSIVE ACTION OF CHLORIN COMPOUNDS

Active chlorin, such as is found in germicidal preparations, is corrosive in varying degrees to most metals. The degree of corrosion is minimized by buffering solutions, but, as has been pointed out, increased alkalinity is detrimental to germicidal potency and non-corrosive properties are gained at the expense of germ-killing efficiency. Further-

more, all metals are not equally protected by buffer salts, as has been shown by Hunziker, Cordes and Nissen (9).

The corrosive action of the several types was also studied in connection with the trials made to determine the germicidal potency of the various sorts of chlorin compounds. Samples of six metals commonly used in the construction of dairy equipment, namely, galvanized iron, copper, aluminum, tinned copper, Monel metal (nickel-copper alloy) and Allegheny metal (chromium-steel alloy) were subjected to test. Solutions of the four chlorin preparations, namely, liquid hypochlorite of low alkalinity (A), sodium hypochlorite buffered with tri-sodium phosphate (B), calcium hypochlorite buffered with soda ash (E), and an organic chlorin preparation (D) were made, each containing 100 parts per million of available chlorin. Metal strips, four inches by one inch, polished to a clean brightness were half immersed in these four solutions in covered mason jars, which stood in the ordinary diffused light of the laboratory at room temperature for five days. They were then removed, washed and examined for visible corrosion (table 19).

TABLE 19.—EFFECT OF VARIOUS TYPES OF CHLORIN COMPOUNDS IN SOLUTION ON METALS USED IN THE CONSTRUCTION OF DAIRY EQUIPMENT

Solutions containing 100 parts per million of chlorin
Exposure by immersion at room temperature for five days

Amount of visible corrosion	Chlorin compound			
	A	B	E	D
None	Chromium-steel alloy	Chromium-steel alloy Nickel-copper alloy	Chromium-steel alloy	Chromium-steel alloy Nickel-copper alloy
Slight		Tinned copper Copper	Nickel-copper alloy Copper Aluminum	Copper Aluminum
Heavy	Nickel-copper alloy Tinned copper Aluminum Copper Galvanized iron	Aluminum Galvanized iron	Tinned copper Galvanized iron	Tinned copper Galvanized iron

The outstanding metal from the standpoint of resistance to corrosion was the chromium-steel alloy. No visible corrosion was apparent on any strip of this metal immersed in any solution. Alloys of this type have received little attention from the manufacturers of dairy equipment until recent years, during which considerable advance has

been made in fabricating processes and the future may find much of the corrosion problem solved by the use of non-corroding metal. However, most of the equipment now in use in the dairy industry is made either of tinned copper or tinned steel. Aluminum is becoming more popular and nickel-copper, copper and galvanized iron are found in many milk plants, notwithstanding the fact that off-flavors in milk attributed to contact with these metals has resulted in their elimination whenever possible in the manufacture of modern dairy equipment.

The alkalinity of the liquid sodium hypochlorite, A, is relatively low, and every metal save the chromium steel was heavily corroded thereby.

The sodium hypochlorite combined with tri-sodium phosphate did not visibly corrode either alloy and it affected tinned copper and copper but slightly. Aluminum and galvanized iron were heavily corroded. The corrosive effect of alkaline hypochlorites on aluminum is believed to be caused by the alkali and the particular type present in this compound, B, appears profoundly to affect aluminum.

The calcium hypochlorite buffered with soda ash, E, showed less corrosive effects than did hypochlorites of low alkalinity. The presence of soda ash in this concentrated compound increases the alkalinity of solutions prepared therefrom which probably accounts for its reduced corrosive effect.

The chloramine solution, D, caused little corrosion of four of the metals, the exceptions being galvanized iron and tinned copper. Galvanized iron was corroded by every solution tested.

Hunziker, Cordes and Nissen (9), Johns (10), Powers (21) and Prucha (27) made extensive trials on strips of metal immersed in chlorin solutions and accurately weighed before and after immersion. Large losses in weight and visible corrosion resulted from immersion of some common metals in solutions of low alkalinity. As alkalinity increased the amount of corrosion from hypochlorites was lowered. Chloramine solutions showed little corrosive action. They indicate in general that corrosion is reduced as alkalinity increased and that all metals are not equally protected by buffering solutions. Some metals of the stainless steel alloy type showed relatively great resistance to corrosion by all types of chlorin solutions.

SUMMARY DISCUSSION OF THEORETICAL PRINCIPLES

1. *Storage.* Concentrated chlorin preparations deteriorate on prolonged storage. Stability appears to be improved by buffering with alkaline salts. Compounds of low alkalinity are least stable. Chlor-

mine-T compounds deteriorate but little in storage. Compounds to be used on the farm should be stored in a cool, dark place. If this is done no serious losses of available chlorin are likely to occur during six months; however, supplies need not be secured in quantities necessitating prolonged storage.

2. *Temperature of solutions.* High temperatures alone do not greatly reduce the content of available chlorin but tend to augment losses when organic matter is present.

3. *Speed of germicidal action.* Chloramines do not appear to give as efficient results as hypochlorites of equal chlorin concentration even when acidified or used in solutions at a temperature of 160° F. Hypochlorite solutions of low alkalinity killed *B. coli* with somewhat greater rapidity than did hypochlorite solutions of higher alkalinity.

4. *Corrosiveness.* Rapid acting, slightly alkaline solutions are more corrosive to various metals than are highly alkaline, slow-acting hypochlorites. Chloramine-T compounds caused very slight corrosion. A chromium-steel alloy resisted the corrosive action of all chlorin compounds.

PRACTICAL APPLICATIONS

The primary object of this study was to determine the possibilities and limitations of chlorin germicides when used to improve the quality of milk on producing farms. With this objective in mind, the results of the tests reported upon in the preceding pages may be considered in their relation to farm practice.

As has been pointed out, concentrated preparations may be expected to give satisfaction if bought in quantities which can be consumed within a few months. However, care should be exercised in purchasing the supply to secure a fresh product. Certain manufacturers of chlorin germicides date the label at the time of packaging, which practice affords an added safeguard to the buyer.

The aphorism "time is money" holds upon the farm as it does elsewhere; hence a quick-acting hypochlorite is indicated. However, its corrosive action should be taken into account. If used, the method of application should be so adjusted as to lessen or eliminate damage to utensils by corrosion. One way of achieving this end is to rinse off the utensils with the solution just before they are to be used. As has been pointed out, the milk or other organic film uses up the chlorin, destroys its activity as it were, and hence the danger from corrosion is reduced.

The destructive effect exerted by organic matter, such as milk film, etc., on chlorin solutions has an important bearing in relation to use on the dairy farm. Unfortunately, some manufacturers of commercial chlorin germicides have insisted in the past that chlorin was a cure-all for unsanitary conditions. For example, they have said that chlorin "cleans while it sterilizes," "cuts milk film," etc. As a matter of fact, satisfactory results are not often secured in this manner since chlorin in available germicidal form rapidly disappears as such, even when as little as 0.1 percent of milk is present. Modern authorities agree that if satisfactory results are to be attained by the use of chlorin germicides, thorough mechanical cleansing must precede their use since otherwise the lethal effect may not be obtained.

THE EFFECT OF A CHLORIN RINSE ON THE FLAVOR AND ODOR OF MILK

Objection has been raised to the use of chlorin on the ground that traces may get into and cause undesirable flavors in milk, which statement obviously is important if true.

Six pint milk bottles were thoroughly washed and allowed to dry, and five of them were then rinsed with chlorin solutions with concentrations of 100, 200, 300, 400 and 500 parts of chlorin per million, the sixth being an untreated check. The bottles were inverted and allowed to drain for two minutes after rinsing and then filled with fresh, whole milk, which was placed in and held for 18 hours in the refrigerator at below 50° F. They were then removed and allowed to reach room temperature, between 70° and 80° F. Several people tasted the milks. No distinction whatever could be detected in the initial trial between the flavors of the five chlorin-rinsed bottles, but an inexplicable off-flavor was observed in the contents of the check bottle. Two similar series were run, the bottles being sterilized in the hot air sterilizer after washing and before receiving the chlorin rinse. No differences whatever in flavor were detected in the contents of the two sets of six bottles. It seems fair to conclude that the chlorin odor was volatile since a distinct odor could be detected in the milks immediately after the bottles in which the stronger rinses were used were filled, whereas no odor whatever was perceptible 18 hours afterward, a period which may be held to represent the lapse of time ordinarily occurring between bottling and delivery to the consumer. All the concentrations used in rinsing these bottles were in excess of the recommendations generally made for bottle rinsing.

Now while farm utensils are less readily treated than are milk bottles, and while the volume of rinse used in such utensils as cans or pails is much larger than in a pint milk bottle, the proportion of rinse to milk would not likely be greater, nor does it seem that the danger of imparting off flavors or odors would be increased. Bearing out this contention are the observations made on the odor of milk deliveries from 20 farms where chlorin was used as a rinse in practical trials. Every can of milk which was delivered by the farmers using chlorin was carefully examined at the weigh tank of the creamery and special attention was paid to any odors which might be present. Throughout the period during which all utensils used in the production of milk received chlorin treatment each morning and evening, no odor whatever could be detected which seemed associated with chlorin. Similar attention was given for a long time to all milk which came in contact with utensils thus treated at the University farm, with similar results.

THE PRACTICAL APPLICATION OF CHLORIN IN TREATING UTENSILS TO KILL BACTERIA

Prucha, Weeter and Chambers (24) concluded, after an extensive study of the influence of the utensil upon the bacteria content of milk, that "in an attempt to produce milk with low germ content too much stress has been laid on practices of minor importance and the influence of utensils poorly steamed and not dried has been commonly neglected." They state that as compared with the extent of contamination caused by barn conditions, milk pails exerted approximately 11 times as great an effect, the strainer a one and one-half times greater effect and shipping cans an enormously greater effect.

Shipping cans are usually washed at the receiving plant and then are generally steamed in order to kill such bacteria as survived the washing process. Now the cans when returned to the farm are often kept covered until they are again to be used. The dairyman himself does nothing to them, assuming that the plant treatment leaves them in satisfactory sanitary condition. The advisability of this *laissez-faire* practice is debatable. If cans are thoroughly dried after they are steamed, no material bacterial growth is likely to occur before they are again put to use. However, many mechanical can-washing machines do not leave the can in a perfectly dry condition. Enormous bacterial growth may develop in a closed, moist can in the hot summer months, as is shown by Prucha and his associates (24). Thus Prucha

and Harding (23) examined 52 milk pails and 206 milk cans after they had undergone the usual hot water washing and rinsing procedure and had stood for some time in the sun and air. The average pail contained 10,000,000 and the average can 24,000,000 bacteria.

Three methods of eliminating germs from utensils after they had been thoroughly washed were studied by Prucha and his associates (22, 23).

1. Cans were rinsed with varying amounts of water ranging in temperature from 70° F. to 210° F. Cans rinsed with four quarts of water at 208° F. were found to contain an average bacteria content of 339,524. When smaller quantities of water were used at lower temperatures, the figures were in the millions.

2. Cans placed in the sun and air after washing for from 8 to 24 hours were found to contain from ten thousand up to hundreds of millions of bacteria.

3. Steaming cans over a live steam jet after washing reduced their germ content more than any other method. A minimum of 12 cubic feet of steam, the jet playing into an eight-gallon can from 15 to 30 seconds, proved effective. The writers remark that "the cans in commercial plants are not as a rule steamed uniformly nor are they steamed efficiently." Such evidence of extreme contamination suggests the use of chlorin as an alternative to other methods of bacterial destruction.

Chlorin has now become very popular in the dairy industry. Research workers, health officers and plant operators have given much thought and study to its use in milk and dairy products plants, but one finds little evidence of its use upon the producing farm except in connection with milking machines. These mechanisms, if carelessly handled, may so readily cause great bacterial contamination that much study has been given to the best methods of combating the difficulty. Lochhead and Johns (12) and Parfitt (19) in particular have sought to determine the place of chlorin in the technic of machine milking.

Steam and hot water are rarely available on the farm in quantities large enough to assure thorough treatment of utensils. Although municipal milk ordinances and standards have brought about much improvement in the methods used on dairy farms in treating utensils to kill bacteria, they are not always complied with and there is still much to be accomplished.

The age-old method of heating water on the kitchen stove and scalding all utensils after thorough washing should be effective if conscientiously carried out. However, in a survey of farms made in

connection with the work now being discussed and as a result of the writer's observations on farms visited in Vermont and elsewhere, it may safely be claimed that thorough germicidal treatment of utensils following washing is none too common and that the washing process itself is often done in a perfunctory manner. Clearly efficient supplementary practices are necessary. The problem in the writer's mind shapes itself as follows:

1. Can chlorin, which is an effective bactericide, be used on the dairy farm in a simple and practical way?
2. Can farmers use chlorin preparations effectively without instruction, other than that afforded by the directions printed on the package label, and will they profit by such usage?

METHOD OF STUDY

Utensils.—The University farm dairy plant and utensils were used to determine the efficiency of chlorin solutions in reducing the bacterial content of both utensils and the milk. The milk pails, cans and strainer in daily use were well constructed and in good condition. New pails were used in certain laboratory trials.

Washing.—The milk pails and strainer were rinsed with cold water immediately after milking and then in warm water containing sodium carbonate washing powder, a stiff brush being used to scrub them. The cans were similarly treated later in the day. Each utensil was then rinsed with clear water at about 130° F. and variously handled as indicated in connection with the several trials.

Analyses.—One liter of sterile tap water was used as a rinse with the cans and 200 c.c. with the pails.¹ The water was poured into the utensil, thoroughly shaken about for a minute, collected and one c.c. portions—or dilutions thereof—promptly plated in duplicate on standard beef extract agar. In most cases the sample itself was used but occasionally, if high counts were to be expected, suitable dilutions were made. The colony numbers, calculated to the volume of the rinse water, gave the germ content of the utensil. The technic employed was that indicated in "Standard Methods of Milk Analysis."

Whenever possible, the strength of the chlorin solutions used was determined by sodium thiosulphate titration. Occasionally, when this was not feasible, and in order to check their strength by a test which may be used outside of the laboratory, recourse was had to the Orthotolidine test. This chemical, when placed in chlorin solutions, produces

¹ In one instance sterile skim milk replaced water.

a yellow color of varying intensities depending upon the concentration of the chlorin. The hues are compared with color standards which correspond with the colors produced in solutions of known strength. Myers and Johnson (17) found that this test gave accurate results when carefully performed with hypochlorite solutions but that with chloramines the results were lower than those afforded by the titration method.

BACTERIOLOGICAL EXAMINATION OF UTENSILS RINSED WITH CHLORIN SOLUTIONS

A preliminary examination of the milk utensils in use at the University farm showed that as a result of the regular washing and steaming procedure, their bacterial content was relatively low as compared with that found in utensils studied by Prucha and associates (24). Ten cans selected at random on different days in July showed an average bacterial content of 198,000 per can when each was rinsed with a liter of sterile water. The average number of bacteria present in ten pails was 78,000 per pail. Theoretically, the average can would add five and the average pail eight bacteria per c.c. to milk placed in them. Preliminary can and pail rinsing experiments, in which the milk handled in utensils rinsed with chlorin was compared by plate counts and methylene blue reduction tests with milk handled in steamed pails, failed to show any noticeable variation in quality. Artificial contamination was therefore used to determine to what extent a chlorin rinse would destroy bacteria in milk pails.

Pails.—Two new pails were thoroughly washed and rinsed with hot water, placed over a steam jet for 10 minutes, immediately covered with heavy wrapping paper and allowed to cool. Ten c.c. of contaminated rinse water (obtained by rinsing dirty milk bottles) was swished around the inner surface of each pail with a view of getting as much as possible of the rinse water to adhere. The pail was then allowed to stand for 10 minutes, a quart of a quick-acting hypochlorite solution containing approximately 200 parts per million of available chlorin was poured in and rinsed around the pail for 30 seconds, poured out, the pail was inverted and allowed to drain for 30 seconds. Two hundred c.c. of sterile skimmilk were poured into the pail and shaken around its interior for one minute, skimmilk replacing water in order to limit the length of time in which the chlorin solution was allowed to carry on a germicidal action. It was felt that the milk would immediately use up the active chlorin for the reason that the small amount

of chlorin solution left adhering to the sides of the pail would lose its germicidal potency in the presence of organic matter. The milk was promptly plated on standard agar and the plates incubated for 48 hours at 37° C.

The number of bacteria taken from each pail by the sterile milk rinse is shown in table 20. No attempt is made to average the counts

TABLE 20.—NUMBER OF BACTERIA REMOVED FROM PAILS BY 200 C.C. OF STERILE SKIMMILK FOLLOWING TREATMENT WITH CHLORIN RINSE SOLUTIONS

Average contamination—3,502,800

Number of pail	Bacterial content	Number of pail	Bacterial content
1	0	15	300
2	0	16	0
3	200	17	0
4	4,200	18	0
5	6,500	19	200
6	0	20	1,400
7	450	21	26,600
8	0	22	450
9	0	23	0
10	0	24	0
11	0	25	200
12	0	26	450
13	0	27	0
14	5,400	28	0

since no contamination was evident in more than half of the cases and hence are reported as zero. Samples of the rinse water used to contaminate the pails were plated concurrently with each trial and the average number of bacteria present in 10 c.c. was 3,502,800. The sterility of the pails before contamination was checked by rinsing eight pails following the 10-minute steaming, but when cool. Control samples of the sterile milk were plated concurrently with each trial.

No explanation is vouchsafed as to the irregularity of the counts obtained on the milk rinses. Perhaps the rinse method of determining the germ content of utensils may be more conclusive where natural contamination is studied but marked variations and irregularities were apparent in all utensil rinsing experiments in this study.

Cans.—The cans used at the University farm are washed daily late in the afternoon, steamed for from 30 to 60 seconds over a jet following washing and then inverted on a draining rack until used (p. 35). Cans thus treated were selected at random from time to time during January and February. After rinsing with clear water at about 130° F., one gallon of chlorin solution containing approximately 100 parts per million of available chlorin was thoroughly shaken in each can for one minute and the can was then inverted on the draining rack. The

next morning all chlorin-rinsed cans and several cans picked from those which had received the regular steam treatment were rinsed each with one liter of sterile water, a sample of which rinse was later plated on standard agar and incubated for 48 hours at 37° C.

The individual bacterial contents of 25 chlorin rinsed and 25 steamed cans are shown in table 21. Three commercial compounds were used as chlorin rinses. They are of the minimum corrosive type

TABLE 21.—COMPARISON OF THE EFFECT OF TREATMENT WITH STEAM AND CHLORIN SOLUTIONS ON THE BACTERIAL CONTENT OF MILK CANS

Chlorin solution—100 parts per million of available chlorin, 1 gallon per can

Compound used	Cans treated with chlorin rinse		Cans treated with steam	
	Can number	Bacteria in sterile rinse water	Can number	Bacteria in sterile rinse water
B	1	2,000	26	91,000
B	2	3,000	27	15,500
B	3	6,500	28	24,000
B	4	500	29	12,500
B	5	29,000	30	23,000
B	6	97,500	31	142,500
B	7	175,000	32	38,000
B	8	17,000	33	233,000
B	9	0	34	6,000
B	10	17,000	35	11,500
D	11	1,500	36	99,000
D	12	25,000	37	121,000
D	13	6,500	38	88,000
D	14	33,000	39	85,000
D	15	15,000	40	125,000
D	16	22,500	41	106,000
D	17	6,500	42	81,500
D	18	12,000	43	199,000
D	19	107,500	44	61,000
D	20	39,000	45	35,000
E	21	17,500	46	48,000
E	22	17,000	47	20,000
E	23	8,000	48	37,500
E	24	4,500	49	12,000
E	25	29,000	50	31,500

so the cans were left with some chlorin solution adhering to their inside surfaces. This could not have been done had corrosive types been used since the tinned surfaces would have been injured. The slower action of the compounds used has little significance since the adhering film presumably remained in contact with any bacteria present long enough to permit germicidal action.

Ten cans were rinsed with one liter of sterile water immediately after washing as a control estimate of the probable normal germ content of cans. The average number was 2,636,000.

The averages (geometric mean) of the groups of cans treated with different chlorin compounds and the averages of all counts are

shown in table 22. The average bacterial content of three series of steamed cans in the three groups which were run as checks are also included for comparison. No comparison of the effectiveness of the three compounds is made. The results seem to indicate that all are effective as bactericides as is steam when applied to physically clean cans.

TABLE 22.—AVERAGE (GEOMETRIC MEAN) BACTERIAL CONTENT OF CANS EXAMINED AFTER TREATMENT WITH CHLORIN SOLUTIONS OR STEAM
(From individual counts as shown in table 10)

Number of cans examined	Chlorin solutions 100 parts per million from compound	Average number of bacteria removed from can by one liter of sterile rinse water after treatment with:	
		Chlorin rinse	Steam
10	B	4,400	31,100
10	D	15,600	91,600
5	E	12,500	27,700
Average of all cans (25).....		9,000	46,400

Conclusions based on the study of counts obtained by the can rinsing method must take into account wide variations in individual counts. The initial contamination was comparative, if one may judge by the average contamination of eight check cans. The University farm method of handling milk does not simulate creamery practice, since it is subjected to efficient cooling methods from the time it enters the cans until it reaches the bottle filler. The time during which the milk is subject to other than optimum cooling conditions is only about 15 minutes, or the period during which it passes from the barn to the creamery. Practically all milk receiving stations are located at some distance from the sources of supply and the difference obtaining between the milk handling methods at the University farm and those applied by the average dairy farmer must be taken into account. However, 23 of the 25 cans after rinsing with chlorin solution contained less than the maximum 100,000 per can content set up as a provisional standard in the laboratory manual of the International Association of Milk Dealers. On the other hand, 19 of the 25 steamed cans failed to meet this standard. The averages for both chlorin rinsed and steamed cans is well below the 100,000 mark, the lower average pertaining to the chlorin rinse.

The results secured in these pail rinsing and can rinsing trials indicate that the application of solutions carrying from 100 to 200 parts per million of chlorin to previously washed utensil surfaces reduced their bacteria contents to a satisfactory extent.

QUALITY OF MILK PRODUCED UNDER CONTROLLED CONDITIONS

Attempts were made to show how efficacious an application of chlorin solution to utensils immediately before their use would be in reducing bacterial contamination of milk handled therein as compared with milk handled in utensils which had been either steamed or rinsed with water at 150° F. The conditions under which milk was produced in these trials were such that the bacteria count rarely exceeded 10,000 per c.c. The cows were hand milked by clean and careful milkers and the milk promptly cooled to below 50° F. The regular routine was as follows: Immediately after morning milking the pails and strainer were rinsed with cold water and allowed to stand until after breakfast, when they were thoroughly washed, rinsed, steamed over a jet, and set out in the sun and air, inverted on a rack until the evening milking. After the evening milking they were rinsed with cold water and inverted on the outdoor rack, receiving no washing until the next morning. Cans were washed and steamed in the afternoon and were inverted on the draining rack in the laboratory until the following morning when they were taken to the barn.

Beginning in April samples of morning and evening milk—the former cooled to 50° F. or less—were subjected to bacteriological analysis by the plate count and methylene blue reduction tests. Samples were obtained for one week under the usual routine and then a chlorin rinse was substituted for the steam treatment, no change, however, being made in the method of washing. The solution used was made up from a concentrated compound of the quick acting type and contained about 150 parts per million of available chlorin. Immediately before milking approximately one gallon of the solution was swished about in the pails, poured through the strainer and shaken thoroughly in the can, care being taken to have all surfaces on the inside of the can and lid exposed to the bactericide. About one minute was taken to rinse each utensil, the total time required to treat three pails and one can, including the time taken to prepare the solution, approximating 10 minutes. The milk produced during alternate weeks of steam and chlorin treatment was tested for four weeks. Following these trials the utensils were rinsed, after the regular washing, with water at 150° F. and were then inverted on the draining rack in the open air until milking time. Between the evening milking and morning milking they received no treatment other than a rinse with cold water. Samples of the milk produced under these conditions were taken as before. These five weeks brought the work into the month of May but there had

been no really hot weather. In July, during a very warm period, two weeks of trials were run using a hot water rinse during the first and a chlorin treatment during the second week. Table 23 sets

TABLE 23.—AVERAGE, MAXIMUM AND MINIMUM STANDARD PLATE COUNTS AND THE AVERAGE MINIMUM AND MAXIMUM REDUCTION TIMES OF MILK PRODUCED IN UTENSILS WHICH RECEIVED DIFFERENT TREATMENTS TO KILL BACTERIA FOLLOWING WASHING

Treatment of utensils following washing	No. of samples	Plate counts			Reduction time		
		Average (geometric mean)	Maximum	Minimum	Average hrs. min.	Maximum hrs. min.	Minimum hrs. min.
Rinsed with hot water 150° F.	13	13,800	38,000	5,000	7 59	10 15	6 15
Steamed over a jet	17	4,900	8,900	1,450	9 15	11 30	6 30
Rinsed with chlorin solution immediately before milking...	40	4,500	21,500	1,200	9 31	14 30	6 15

forth the average plate counts and reduction time of samples of milk thus obtained. The maximum and minimum counts obtained and hours necessary to reduce methylene blue show that the milk was of excellent bacteriological quality under all conditions and that the range of variation was at all times narrow. Incidentally, the milk produced during the periods in which the chlorin rinse was used exhibited slightly lower average counts and slightly longer reduction times than when the steam treatment was in use. The weeks during which the utensils were rinsed with hot water were intended as checks on the possible contamination of the milk from the treated utensils. The procedure on many dairy farms is to pour water, which originally was hot but ultimately becomes barely lukewarm, from one utensil to another after washing, in the belief that such cursory attention will put utensils into satisfactory sanitary condition. The milk obtained under these conditions, while not showing a tremendous increase in bacterial content, was consistently of inferior bacteriological quality to that produced in utensils which were either steamed or rinsed with chlorin solution. The results secured in these tests are less conclusive than those which presumably would obtain in cases where the milk produced is normally of poorer quality than that made in these trials where utensil washing was thorough and the milk was almost always of low bacterial content. Efficient cooling and cleanly surroundings minimized the possibility of great increases in bacterial count from sources other than the utensils; hence the increase which repeatedly occurred during periods

of rinsing with hot water seemed justly attributable to inadequate germicidal treatment. On the other hand, utensils which received no such treatment following washing but which were chlorin rinsed just before usage were responsible for no more contamination than were utensils which were thoroughly steamed.

Now since steam is not readily available on most farms, it is obvious that much is to be gained if a chlorin rinse can replace it without impairment of the quality of the product. The comparison of the average counts and reduction times in table 23 indicate that chlorin is dependable, when carefully used, in the production of low count milk.

PRACTICAL APPLICATION OF CHLORIN GERMICIDES ON DAIRY FARMS

Having obtained satisfactory results from the use of a chlorin rinse solution under experimental conditions, trials were conducted to determine the value of chlorin germicides when used under practical conditions on Vermont milk producing farms. Thanks to the courtesy by the managers of a local milk producers' organization and of a Burlington retail milk company, access was secured to 20 farms from which they bought milk for city distribution and the cooperation of the 20 dairymen secured. It was the writer's desire to include 40 farms in this trial, but half of the number of farmers approached would not agree to the use of the hypochlorite rinse.

The 20 farms were divided into three lots. Ten farms producing milk which ranged in quality from good to very poor were placed in group 1. Groups 2 and 3 each consisted of five farms and included producers who delivered milk which varied widely in quality during the control periods, as judged by plate counts and reduction times.

The routine methods of handling milk on these farms were not uniform. Electric cooling tanks were used to cool the night's milk in some cases, ice in others and flowing spring water in others. The morning's milk was often not adequately cooled, but it was commonly delivered within two or three hours after milking. Milking machines were used on some of the farms, but hand milking generally prevailed. The average daily production per farm ranged from 80 to 500 pounds. Pail and strainer washing on 19 farms was done by the women in their kitchens. There was a wash room at the barn in one farm, hot water being supplied by an exhaust heater on a gasoline engine used to operate the milking machine. The pails were usually but not always scalded after washing. The milk cans were cleansed at the city creamery in a rotary can washer. During the period of the trials now under discus-

sion this mechanism needed repair and the cans were sometimes returned to the farms in very unsanitary condition. Some of the farmers washed the cans at the farm and others were in the habit of rinsing them with hot water before using them as milk containers. In conducting these trials, no attempt was made to alter any particular practice and the usual method of treatment at the farm obtained throughout the control period prior to the installation of the hypochlorite rinse.

Beginning on July 12, samples were drawn at the milk plant from the supplies brought in from the 10 farms in group 1, held in the plant refrigerator at 40° F. until all were collected, iced and taken to the laboratory where they were promptly plated and tubed, about 30 minutes elapsing between the time when the last sample was taken and the plating and tubing was completed. Samples were thus taken for six days during this control test period. Then each of the 10 dairymen was supplied with concentrated hypochlorite powder in package form, together with directions for making up a gallon of rinse solution containing between 150 and 160 parts per million active chlorin. Each man was asked to see to it that the utensils were washed in the usual way but that they were not scalded; that a gallon of rinse solution was prepared and that pails, cans and strainer were rinsed with the freshly prepared hypochlorite just before milking. The importance of thoroughly shaking the solution within each utensil in order that every part of the interior be reached was stressed. The rinsing of the pails first and then of the strainer and cans was advised in order that the chlorin rinse film adhering to the metal surfaces might have time to act on the bacteria before milk was put into them. The rinsing solution would thus have time in which to act on the bacteria in the pails while the cans and strainer were being handled and its action in the cans would continue until the milk began to be strained into them. Where several milkers were employed the farmer was advised to commence the rinsing process five minutes before milking time. On all farms where milking machines were installed some system of chlorin soaking of the tubes and cups was already in use, but suggestions looking toward improvement of technic were made and rinsing of machine heads and pails was proposed along with the rinsing of other utensils.

Samples were taken in the same manner as in the control week but their numbers were increased. The test period lasted for 15 days in order to determine whether the initial enthusiasm of the farmer in carrying out the rinsing practice fell off to such an extent as might be detected by increment in the bacterial contents of the milks. In

some cases such lapses did occur, and inquiries resulted in such admissions as "I forgot to rinse my pails and cans yesterday." Control samples were taken on the five farms in group 2 during the latter portion of the test period with group 1 and their owners then supplied with the hypochlorite and directions and sampling continued during the next 15 days. The four-day control period in group 3 was concurrent with the first four days of the hypochlorite period on group 2 and then the rinse was put into operation and samples collected for 15 days.

The writer was in daily contact with all producers throughout these trials and from time to time made inquiries to determine their attitude towards the use of the rinse. The methylene blue test was in daily use at the milk plant and when several of the cooperating farmers found that their milks were "standing up" better in the plant tests after they commenced to rinse with hypochlorite, they became enthusiastic and discussed its use with their neighbors. Two cooperating farmers were known to have "forgotten" to use the rinse several times during the test period. However, in the analysis of results all counts were included, some of which were exceedingly high.

ANALYSIS OF RESULTS

Comparison of the average plate counts and average reduction times of milk sampled from each of the 20 farms under control and test conditions as presented in table 24 indicate that improvement in quality after the adoption of hypochlorite rinsing is most evident in cases where the average plate count originally lay between 200,000 and 1,000,000 bacteria. The average count in three cases was lower during the control week than after the hypochlorite was used. No definite explanation can be advanced, yet it should be noted these producers made a comparatively low count milk to begin with and that there was very little change in reduction times. Certain individual cases, as for example No. 3, showed an outstanding improvement in quality as determined by the plate count after the hypochlorite rinse was used. Conditions on this particular farm and No. 20 were investigated to find out if any change in procedure had occurred, other than the method of treating utensils, which would account for such marked reductions in bacteria counts. So far as could be learned by questioning, no other change had been made in the usual routine.

TABLE 24.—QUALITY OF MILK PRODUCED ON 20 VERMONT FARMS UNDER THE USUAL METHOD OF UTENSIL SANITATION AS COMPARED WITH THAT PRODUCED ON THE SAME FARMS AFTER A SYSTEM OF RINSING UTENSILS WITH CHLORIN HAD BEEN ADOPTED. CHLORIN RINSE—ONE GALLON APPROXIMATELY 150 PARTS PER MILLION CHLORIN

Group I

Control				Chlorin rinse			
Farm No. 1	Plate count	Logarithms	Reduction time	Farm No. 1	Plate count	Logarithms	Reduction time
Days			Minutes	Days			Minutes
1	745,000	5.87	120	1	40,000	4.60	435
2	190,000	5.28	150	2	100,000	5.	405
3	70,000	4.85	285	3	35,000	4.54	390
4	210,000	5.32	360	4	17,500	4.24	330
5	400,000	5.60	285	5	103,500	5.01	270
6	645,000	5.81	405	6	112,000	5.05	345
				7	191,000	5.28	270
				8	230,000	5.36	315
				9	614,000	5.79	285
				10	710,000	5.85	210
				11	216,500	5.34	180
				12	272,000	5.43	332
				13	366,500	5.56	235
				14	630,000	5.80	195
				15	316,000	5.50	270
Average	284,000*	5.453	4 h., 27 m.	Average	167,000*	5.223	4 h., 58 m.
Farm No. 2				Farm No. 2			
1	81,500	4.91	495	1	3,410,000	6.53	330
2	91,700	4.96	390	2	1,045,000	6.02	502
3	96,000	4.98	390	3	50,000	4.70	375
4	176,000	5.25	300	4	20,000	4.30	570
5	3,145,000	6.50	270	5	60,000	4.78	435
6	1,655,000	6.22	360	6	56,500	4.75	375
				7	77,000	4.89	360
				8	37,000	4.57	315
				9	75,000	4.88	315
				10	160,000	5.20	300
				11	203,000	5.31	285
				12	447,000	5.65	255
				13	5,750,000	6.76	285
				14	378,000	5.58	360
				15	981,500	5.99	360
Average	295,000*	5.47	6 h., 7 m.	Average	212,000*	5.327	6 h., 1 m.
Farm No. 3				Farm No. 3			
1	715,000	5.85	435	1	545,000	5.74	450
2	2,275,000	6.36	420	2	10,000	4.	435
3	2,090,000	6.32	502	3	10,000	4.	495
4	247,000	5.39	540	4	6,000	3.78	555
5	3,835,000	6.58	142	5	15,500	4.19	495
6	2,180,000	6.34	225	6	14,800	4.17	450
				7	17,700	4.25	435
				8	21,250	4.33	495
				9	20,000	4.30	495
				10	196,000	5.29	420
				11	3,055,000	6.49	120
				12	74,000	4.87	495
				13	58,500	4.77	480
				14	645,000	5.81	367
				15	966,000	5.98	270
Average	1,380,000*	6.14	6 h., 17 m.	Average	63,000*	4.798	7 h., 10 m.

* Geometric mean.

TABLE 24.—QUALITY OF MILK PRODUCED ON 20 VERMONT FARMS UNDER THE USUAL METHOD OF UTENSIL SANITATION AS COMPARED WITH THAT PRODUCED ON THE SAME FARMS AFTER A SYSTEM OF RINSING UTENSILS WITH CHLORIN HAD BEEN ADOPTED. CHLORIN RINSE—ONE GALLON APPROXIMATELY 150 PARTS PER MILLION CHLORIN—*Continued*

Control				Chlorin rinse			
Farm No. 4	Plate count	Logarithms	Reduction time	Farm No. 4	Plate count	Logarithms	Reduction time
Days			Minutes	Days			Minutes
1	6,600,000	6.82	90	1	3,450,000	6.54	165
2	6,170,000	6.79	150	2	3,895,000	6.59	180
3	2,030,000	6.31	150	3	600,000	5.78	157
4	1,862,000	6.27	150	4	2,665,000	6.43	285
5	1,595,000	6.20	225	5	60,000	4.78	450
6	1,895,000	6.28	195	6	110,000	5.04	450
				7	100,750	5.	405
				8	300,000	5.48	405
				9	425,000	5.63	330
				10	370,000	5.57	315
				11	812,500	5.91	390
				12	41,000	4.61	270
				13	51,000	4.71	420
				14	57,000	4.76	420
				15	217,000	5.34	300
Average	2,790,000*	6.445	2 h., 40 m.	Average	301,000*	5.478	5 h., 29 m.
Farm No. 5				Farm No. 5			
1	1,620,000	6.21	90	1	450,000	5.65	510
2	5,940,000	6.77	75	2	850,000	5.93	300
3	1,795,000	6.25	105	3	4,550,000	6.66	135
4	3,700,000	6.57	150	4	10,320,000	7.01	105
5	15,675,000	7.19	60	5	1,365,000	6.13	240
6	1,350,000	6.13	270	6	11,000,000	7.04	135
				7	11,050,000	7.04	105
				8	11,800,000	7.07	75
				9	1,560,000	6.19	210
				10	1,375,000	6.14	255
				11	4,390,000	6.64	270
				12	1,100,000	6.04	375
				13	990,000	6.	375
				14	2,755,000	6.44	225
				15	2,350,000	6.37	180
Average	3,310,000*	6.52	1 h., 48 m.	Average	2,650,000*	6.42	3 h., 53 m.
Farm No. 6				Farm No. 6			
1	1	10,000	4.	525
2	56,850	4.75	345	2	10,000	4.	495
3	11,300	4.05	480	3	8,700	3.94	570
4	11,850	4.07	540	4	2,750	3.44	570
5	40,000	4.60	480	5	7,800	3.89	525
6	10,000	4.	510	6	11,700	4.07	375
				7	17,200	4.24	360
				8	8,350	3.92	540
				9	9,800	3.99	465
				10	18,750	4.27	465
				11	19,750	4.30	450
				12	17,750	4.25	510
				13	11,700	4.07	360
				14	49,900	4.70	465
				15	29,000	4.46	420
Average	19,700*	4.294	5 h., 25 m.	Average	12,300*	4.09	7 h., 53 m.

* Geometric mean.

TABLE 24.—QUALITY OF MILK PRODUCED ON 20 VERMONT FARMS UNDER THE USUAL METHOD OF UTENSIL SANITATION AS COMPARED WITH THAT PRODUCED ON THE SAME FARMS AFTER A SYSTEM OF RINSING UTENSILS WITH CHLORIN HAD BEEN ADOPTED. CHLORIN RINSE—ONE GALLON APPROXIMATELY 150 PARTS PER MILLION CHLORIN—*Continued*

Control				Chlorin rinse			
Farm No. 7	Plate count	Logarithms	Reduction time	Farm No. 7	Plate count	Logarithms	Reduction time
Days			Minutes	Days			Minutes
1	272,500	5.44	300	1	30,000	4.48	360
2	250,000	5.40	265	2	10,000	4.	405
3	162,000	5.21	390	3	20,000	4.30	360
4	166,000	5.22	390	4	13,700	4.14	390
5	73,000	4.86	330	5	53,300	4.73	300
6	310,000	5.49	270	6	47,750	4.68	225
				7	165,750	5.22	285
				8	39,000	4.59	375
				9	80,000	4.90	435
				10	115,000	5.06	375
				11	378,000	5.58	225
				12	39,000	4.59	330
				13	56,500	4.75	420
				14	39,500	4.60	420
				15	113,000	5.05	390
Average	186,000*	5.27	5 h., 25 m.	Average	51,400*	4.711	5 h., 53 m.
Farm No. 8				Farm No. 8			
1	1,800,000	6.26	180	1	4,025,000	6.60	105
2	1,465,000	6.17	90	2	12,690,000	7.10	30
3	1,150,000	6.06	150	3	7,750,000	6.89	105
4	1,370,000	6.14	135	4	5,060,000	6.70	105
5	14,300,000	7.16	60	5	5,687,500	6.76	60
6	7,150,000	6.85	180	6	7,000,000	6.85	0
				7	7,850,000	6.89	15
				8	13,650,000	7.14	45
				9	6,500,000	6.81	90
				10	4,150,000	6.62	120
				11	1,255,000	6.10	210
				12	1,375,000	6.14	135
				13	460,000	5.66	315
				14	1,267,500	6.10	30
				15
Average	2,750,000*	6.44	2 h., 12 m.	Average	3,940,000*	6.596	1 h., 30 m.
Farm No. 9				Farm No. 9			
1	5,250,000	6.72	105	1	1,130,000	6.05	330
2	2,640,000	6.42	165	2	3,200,000	6.51	180
3	825,000	5.92	285	3	2,210,000	6.34	225
4	1,140,000	6.06	195	4	770,000	5.89	330
5	3,000,000	6.48	135	5	1,930,000	6.29	240
6	2,240,000	6.35	180	6	5,250,000	6.72	210
				7	365,000	5.56	375
				8	1,390,000	6.14	285
				9	2,690,000	6.43	180
				10	2,330,000	6.37	255
				11	410,000	5.61	255
				12	2,520,000	6.40	195
				13	4,240,000	6.63	180
				14
				15
Average	2,114,000*	6.325	2 h., 57 m.	Average	1,686,000*	6.227	4 h., 9 m.

* Geometric mean.

TABLE 24.—QUALITY OF MILK PRODUCED ON 20 VERMONT FARMS UNDER THE USUAL METHOD OF UTENSIL SANITATION AS COMPARED WITH THAT PRODUCED ON THE SAME FARMS AFTER A SYSTEM OF RINSING UTENSILS WITH CHLORIN HAD BEEN ADOPTED. CHLORIN RINSE—ONE GALLON APPROXIMATELY 150 PARTS PER MILLION CHLORIN—*Continued*

Farm No. 10	Control			Farm No. 10	Chlorin rinse		
	Plate count	Logarithms	Reduction time		Plate count	Logarithms	Reduction time
Days			Minutes	Days			Minutes
1	58,500	4.77	345	1	75,000	4.88	427
2	156,000	5.19	240	2	30,000	4.48	420
3	54,000	4.73	195	3	8,450	3.93	375
4	83,000	4.92	315	4	24,000	4.38	457
5	15,000	4.18	345	5	25,500	4.41	390
6	25,000	4.40	360	6	44,500	4.65	270
				7	95,500	4.98	405
				8	113,750	5.06	360
				9	50,000	4.70	450
				10	135,000	5.13	300
				11	282,500	5.45	360
				12	28,000	4.45	375
				13	188,000	5.27	285
				14	64,000	4.81	420
				15	27,500	4.44	360
Average	49,700*	4.696	5 h., 0 m.	Average	54,200*	4.734	6 h., 17 m.

Group II

Farm No. 11				Farm No. 11			
1	515,000	5.71	210	1	121,000	5.08	390
2	190,000	5.28	270	2	57,000	4.76	420
3	240,000	5.38	300	3	44,500	4.65	330
4	467,000	5.67	180	4	1,137,000	6.06	300
5	250,000	5.40	390	5	203,000	5.31	375
6	315,000	5.50	345	6	316,000	5.50	315
7	730,000	5.86	360	7	51,500	4.71	375
8	680,000	5.83	210	8	185,000	5.27	390
9	534,000	5.73	300	9	32,500	4.51	465
				10	61,000	4.79	405
				11	191,500	5.28	360
				12	68,500	4.84	360
				13	491,000	5.69	225
				14	480,000	5.68	450
				15	200,000	5.30	420
Average	394,000*	5.595	4 h., 45 m.	Average	145,000*	5.161	6 h., 16 m.

Farm No. 12				Farm No. 12			
1	9,330,000	6.97	120	1	2,950,000	6.47	420
2	8,675,000	6.94	105	2	11,970,000	7.08	225
3	8,287,000	6.92	60	3	1,207,000	6.08	90
4	8,450,000	6.93	105	4	6,460,000	6.81	285
5	6,500,000	6.81	120	5	4,990,000	6.70	315
6	11,375,000	7.06	120	6	8,450,000	6.93	30
7	4,900,000	6.69	120	7	3,640,000	6.56	120
8	22,400,000	7.35	15	8	5,460,000	6.74	165
9	8,190,000	6.91	150	9	1,852,000	6.27	60
				10	7,150,000	6.85	60
				11	40,000,000	7.60	45
				12	1,200,000	6.08	285
				13	16,850,000	7.23	90
				14	25,500,000	7.41	15
				15	4,500,000	6.65	90
Average	8,974,000*	6.953	1 h., 42 m.	Average	5,810,000*	6.764	2 h., 31 m.

* Geometric mean.

TABLE 24.—QUALITY OF MILK PRODUCED ON 20 VERMONT FARMS UNDER THE USUAL METHOD OF UTENSIL SANITATION AS COMPARED WITH THAT PRODUCED ON THE SAME FARMS AFTER A SYSTEM OF RINSING UTENSILS WITH CHLORIN HAD BEEN ADOPTED. CHLORIN RINSE—ONE GALLON APPROXIMATELY 150 PARTS PER MILLION CHLORIN—*Continued*

Farm No. 13	Control			Farm No. 13	Chlorin rinse		
	Plate count	Logarithms	Reduction time Minutes		Plate count	Logarithms	Reduction time Minutes
Days				Days			
1	306,000	5.49	345	1	710,000	5.85	255
2	130,000	5.11	330	2	467,000	5.67	405
3	227,500	5.36	300	3	57,000	4.76	360
4	322,500	5.51	315	4	495,000	5.69	360
5	1,500,000	6.18	330	5	900,000	5.95	360
6	820,000	5.91	375	6	747,000	5.87	330
7	4,225,000	6.63	240	7	70,000	4.85	330
8	1,275,000	6.11	240	8	192,000	5.28	405
9	493,000	5.69	285	9	176,000	5.25	390
				10	311,000	5.49	345
				11	1,230,000	6.09	255
				12	390,000	5.59	285
				13	630,000	5.80	285
				14	480,000	5.68	345
				15	395,000	5.60	390
Average	597,000*	5.776	5 h., 7 m.	Average	364,000*	5.561	5 h., 40 m.
Farm No. 14				Farm No. 14			
1	23,500	4.37	525	1	46,000	4.66	450
2	18,000	4.26	525	2	35,000	4.54	450
3	51,500	4.71	465	3	10,000	4.	405
4	28,000	4.45	465	4	59,000	4.77	450
5	21,500	4.33	510	5	55,000	4.74	495
6	30,500	4.48	405	6	70,000	4.85	480
7	24,500	4.39	480	7	71,500	4.85	450
8	38,000	4.58	480	8	33,500	4.52	405
9	7,500	3.88	450	9	32,000	4.51	480
				10	36,000	4.56	465
				11	16,000	4.20	480
				12	26,500	4.42	405
				13	46,000	4.66	420
				14	41,500	4.62	495
				15	193,500	5.29	435
Average	24,200*	4.383	7 h., 58 m.	Average	40,800*	4.612	7 h., 31 m.
Farm No. 15				Farm No. 15			
1	1,270,000	6.10	202	1	131,000	5.12	345
2	2,330,000	6.37	135	2	387,000	5.59	315
3	2,820,000	6.45	150	3	200,000	5.30	330
4	2,535,000	6.40	165	4	137,500	5.14	300
5	100,000	5.	270	5	220,000	5.34	315
6	330,000	5.52	240	6	70,000	4.85	240
7	605,000	5.78	180	7	40,000	4.60	345
8	342,000	5.53	225	8	7,000	3.85	405
9	326,000	5.51	270	9	138,500	5.14	495
				10	30,000	4.48	420
				11	73,000	4.86	435
				12	60,500	4.78	420
				13	190,000	5.28	405
				14	43,500	4.64	285
				15	50,000	4.70	420
Average	709,600*	5.851	4 h., 35 m.	Average	81,500*	4.911	5 h., 36 m.

* Geometric mean.

TABLE 24.—QUALITY OF MILK PRODUCED ON 20 VERMONT FARMS UNDER THE USUAL METHOD OF UTENSIL SANITATION AS COMPARED WITH THAT PRODUCED ON THE SAME FARMS AFTER A SYSTEM OF RINSING UTENSILS WITH CHLORIN HAD BEEN ADOPTED. CHLORIN RINSE—ONE GALLON APPROXIMATELY 150 PARTS PER MILLION CHLORIN—Continued

Group III

Control				Chlorin rinse			
Farm No. 16	Plate count	Logarithms	Reduction time	Farm No. 16	Plate count	Logarithms	Reduction time
Days			Minutes	Days			Minutes
1	85,000	4.93	375	1	67,500	4.83	420
2	128,000	5.11	420	2	23,000	4.36	405
3	192,000	5.28	330	3	31,500	4.50	435
4	171,000	5.23	360	4	36,000	4.56	375
				5	11,000	4.04	465
				6	7,000	3.85	390
				7	30,500	4.48	360
				8	660,000	5.82	300
				9	2,000	3.30	435
				10	8,000	3.90	255
				11	7,500	3.88	525
				12	4,500	3.65	465
				13	33,000	4.52	345
				14	9,000	3.95	435
				15	4,500	3.65	420
Average	137,100*	5.137	6 h., 11 m.	Average	16,600*	4.219	6 h., 42 m.
Farm No. 17				Farm No. 17			
1	110,000	5.04	435	1	53,000	4.72	480
2	25,000	4.40	480	2	38,000	4.58	540
3	200,500	5.30	345	3	110,000	5.04	540
4	14,500	4.16	450	4	845,000	5.93	315
				5	147,500	5.17	420
				6	417,000	5.62	510
				7	3,055,000	6.49	90
				8	113,500	5.05	405
				9	60,000	4.78	405
				10	180,000	5.26	495
				11	36,000	4.56	540
				12	153,000	5.18	375
				13	51,500	4.71	435
				14	58,000	4.76	510
				15	630,000	5.80	395
Average	53,100*	4.725	7 h., 8 m.	Average	150,000*	5.176	7 h., 10 m.
Farm No. 18				Farm No. 18			
1	3,153,000	6.50	75	1	35,000	4.54	405
2	1,380,000	6.14	210	2	19,000	4.28	390
3	52,000	4.72	120	3	114,000	5.06	300
4	2,570,000	6.41	135	4	780,000	5.89	135
				5	26,500	4.42	405
				6	30,500	4.48	345
				7	205,000	5.31	225
				8	1,480,000	6.17	60
				9	672,000	5.83	75
				10	380,000	5.58	315
				11	23,500	4.37	360
				12	295,000	5.47	255
				13	710,000	5.85	105
				14	100,000	5.	255
				15	150,000	5.18	210
Average	876,000*	5.942	2 h., 15 m.	Average	145,200*	5.162	4 h., 16 m.

* Geometric mean.

TABLE 24.—QUALITY OF MILK PRODUCED ON 20 VERMONT FARMS UNDER THE USUAL METHOD OF UTENSIL SANITATION AS COMPARED WITH THAT PRODUCED ON THE SAME FARMS AFTER A SYSTEM OF RINSING UTENSILS WITH CHLORIN HAD BEEN ADOPTED. CHLORIN RINSE—ONE GALLON APPROXIMATELY 150 PARTS PER MILLION CHLORIN—*Continued*

Control				Chlorin rinse			
Farm No. 19	Plate count	Logarithms	Reduction time	Farm No. 19	Plate count	Logarithms	Reduction time
Days			Minutes	Days			Minutes
1	745,000	5.87	420	1	120,000	5.08	480
2	262,000	5.42	435	2	116,000	5.06	480
3	150,000	5.18	375	3	100,000	5.	420
4	590,000	5.77	435	4	94,500	4.98	405
				5	258,000	5.41	465
				6	142,000	5.15	510
				7	45,000	4.65	480
				8	339,000	5.53	285
				9	86,000	4.93	465
				10	39,000	4.59	210
				11	108,000	5.03	480
				12	370,000	5.57	465
				13	450,000	5.65	375
				14	260,000	5.42	360
				15	108,000	5.03	322
Average	363,000*	5.60	6 h., 54 m.	Average	137,400*	5.138	6 h., 53 m.

Farm No. 20				Farm No. 20			
1	630,000	5.80	255	1	20,000	4.30	420
2	326,000	5.51	195	2	17,000	4.23	465
3	1,745,000	6.24	135	3	23,500	4.37	420
4	5,310,000	6.73	90	4	85,000	4.93	255
				5	26,000	4.42	435
				6	37,500	4.57	405
				7	47,500	4.68	360
				8	87,000	4.94	390
				9	600,000	5.78	180
				10	89,500	4.95	450
				11	94,500	4.98	240
				12	48,500	4.69	375
				13	110,500	5.04	225
				14	97,500	4.99	225
				15	12,500	4.10	270
Average	1,175,000*	6.07	2 h., 49 m.	Average	53,800*	4.731	5 h., 41 m.

* Geometric mean.

Consideration of the results obtained has thus far been confined to a comparison of quality in individual cases. Further comparisons may also be made between the averages of groups of counts and reduction times, or the counts may be grouped and the percentage distribution of low, medium and high counts used as a basis for comparison. Similarly, the reduction times may be considered according to group distribution. Tables 25 and 26 contain data presented in groups and

TABLE 24.—QUALITY OF MILK PRODUCED ON 20 VERMONT FARMS UNDER THE USUAL METHOD OF UTENSIL SANITATION AS COMPARED WITH THAT PRODUCED ON THE SAME FARMS AFTER A SYSTEM OF RINSING UTENSILS WITH CHLORIN HAD BEEN ADOPTED. CHLORIN RINSE—ONE GALLON APPROXIMATELY 150 PARTS PER MILLION CHLORIN—*Concluded*

Summary

Number of farm	Average (geometric mean) standard plate count		Reduction time averages			
	Control	Chlorin rinse	Control		Chlorin rinse	
			Hrs.	Min.	Hrs.	Min.
1	284,000	167,000	4	27	4	58
2	295,000	212,000	6	7	6	1
3	1,380,000	63,000	6	17	7	10
4	2,790,000	301,000	2	40	5	29
5	3,310,000	2,650,000	1	48	3	53
6	19,700	12,300	5	25	7	53
7	186,000	51,400	5	25	5	53
8	2,750,000	3,940,000	2	12	1	30
9	2,114,000	1,686,000	2	57	4	9
10	49,700	54,200	5	..	6	17
11	394,000	145,000	4	45	6	16
12	8,974,000	5,810,000	1	42	2	31
13	597,000	364,000	5	7	5	40
14	24,200	40,800	7	58	7	31
15	709,600	81,500	4	35	5	36
16	137,100	16,600	6	11	6	42
17	53,100	150,000	7	8	7	10
18	876,000	145,200	2	15	4	16
19	363,000	137,400	6	54	6	53
20	1,175,000	53,800	2	49	5	41

group averages. Since the surrounding air temperature has a direct influence on the growth of bacteria, the mean air temperature for the period in which each group was studied is included in table 25. The weather conditions during the trials were favorable for the collection of data, four degrees of difference in the mean daily air temperature during the testing of milk from farms in group 3 being the maximum. The maximum and minimum counts obtained in each group under control and test conditions and the maximum and minimum reduction times, as set forth in tables 25 and 26, respectively, indicate the wide range of bacterial content in every group. In grouping the data obtained during hypochlorite rinse periods, only the same number of tests as in the control period were used. For example, six samples in group 1 were tested from each farm during the control period and 15 samples were taken during the hypochlorite rinse period. When grouping the resulting data, only the plate counts and reduction times from the first six days of hypochlorite rinsing were included. The total average values, however, include all samples in each group.

TABLE 25.—DISTRIBUTION OF THE STANDARD PLATE COUNTS OF MILK PRODUCED IN UTENSILS RECEIVING THE ORDINARY FARM TREATMENT AS COMPARED WITH MILK PRODUCED ON THE SAME FARMS AFTER USING CHLORIN RINSE ON UTENSILS

Farms were divided into three groups, 10 in group 1, and 5 in each of groups 2 and 3

Plate counts	Percentage of distribution of standard plate counts of bacteria					
	Group 1		Group 2		Group 3	
	Control	Hypochlorite rinse	Control	Hypochlorite rinse	Control	Hypochlorite rinse
Under 30,000	8	31	13	4	10	25
30,000 to 200,000	25	27	13	51	35	65
200,000 to 1,000,000	17	8	38	22	30	10
Over 1,000,000	49	34	36	22	25	0
Maximum count	15,675,000	12,690,000	22,400,000	11,970,000	5,310,000	780,000
Minimum count	10,000	2,750	7,500	10,000	14,500	17,000
Average count (geometric mean)	535,700	187,300	514,700	227,100	312,400	57,800
Mean air tem- perature	66° F.	68° F.	70° F.	69° F.	72° F.	76° F.

Examination of the percentage distributions as shown in tables 25 and 26 indicate the quality of milk on an empirical grade basis. For example, there were 10 producers in group 1. Half of 59 plate counts made during the control period exceed 1,000,000, but during the rinse period only one-third of 60 samples exceed 1,000,000 in plate count. On the other hand, during the control period only one-twelfth of the counts were under 30,000, whereas after hypochlorite was used nearly three-eighths of the counts fell below 30,000. In group 2, the number of samples below 30,000 during the control period exceeds those in the test period. However, only one-sixth of the total number in the control column fall between 30,000 and 200,000, whereas the

TABLE 26.—DISTRIBUTION OF REDUCTION TIMES OF MILK PRODUCED IN UTENSILS RECEIVING THE ORDINARY FARM TREATMENT AS COMPARED WITH MILK PRODUCED ON THE SAME FARMS AFTER USING A CHLORIN RINSE ON UTENSILS

Farms were divided into three groups, 10 in group 1, and 5 in each of groups 2 and 3

Reduction times	Percentage distribution of reduction times					
	Group 1		Group 2		Group 3	
	Control	Hypochlorite rinse	Control	Hypochlorite rinse	Control	Hypochlorite rinse
Over 5½ hours	34	54	31	58	55	80
5½ to 2 hours	53	34	60	36	35	20
Under 2 hours	14	12	9	7	10	0
Maximum	9 hrs.	9 hrs. 30 m.	8 hrs. 45 m.	8 hrs. 15 m.	8 hrs.	9 hrs.
Minimum	1 hr.	0 m.	15 m.	30 m.	1 hr. 15 m.	2 hrs. 15 m.
Average	4 hrs. 25 m.	6 hrs. 30 m.	4 hrs. 37 m.	5 hrs. 43 m.	5 hrs. 4 m.	6 hrs. 44 m.

figure in the hypochlorite period is three-sevenths. Similarly, the grouping of reduction times in table 26 makes quality comparisons convenient. The grading system used is a modification of the classification recommended in "Standard Methods of Milk Analysis," the grade under 20 minutes being omitted. The significant reduction in average bacteria count during the hypochlorite test period from that of the control week is shown at the bottom of table 25. Expressed as percentage reduction, in group 1, the hypochlorite rinse method shows a decrease in average bacteria count of 44 percent, in group 2 of 51 percent and in group 3 of 78 percent. The average time required to reduce methylene blue was lengthened approximately an hour in each group after the adoption of the hypochlorite rinse method.

Table 27 presents plate count and reduction data from all participating farms. They show the same general increases as were apparent in assembling data from each group. The average plate count of the

TABLE 27.—PERCENTAGE DISTRIBUTION OF THE STANDARD PLATE COUNTS AND METHYLENE BLUE REDUCTION TIMES OF MILK PRODUCED ON 20 VERMONT FARMS UNDER THE CUSTOMARY METHODS OF UTENSIL SANITATION AS COMPARED WITH MILK PRODUCED ON THE SAME FARMS AFTER THE ADOPTION OF A SYSTEM OF HYPOCHLORITE RINSING

Number of samples: 124 control, 124 hypochlorite rinse

Plate counts	Control	Hypochlorite	Reduction times	Control	Hypochlorite
	%	%		%	%
Under 30,000	10	20	Over 5½ hrs.	36	60
30,000 to 200,000	23	42	5½ to 2 hrs.	52	32
200,000 to 1,000,000	27	14	Under 2 hrs.	11	8
Over 1,000,000	40	24			
Average plate count (geometric mean) all samples	580,500	175,600	Average reduction time all samples	4 hrs. 36 m.	5 hrs. 48 m.

hypochlorite samples showed a reduction of 71 percent from the average count of control samples, while the average methylene blue reduction time was lengthened an hour and a quarter when the hypochlorite rinsing system was in use. While other factors, such as a change to better cooling methods, may be responsible for the improved quality in individual cases, it seems clear that to the use of hypochlorite is due the better quality of these milks. The fact that the milk cans were poorly washed by the milk plant washing machine doubtless accounts, to some extent at least, for the excessive bacterial contamination during the trial period.

TRIALS CONDUCTED DURING THE SUMMER OF 1933

Trials of chlorin germicides on the farms as previously discussed in this study were subject to variable factors not controlled by the technic employed. Beginning in June and continuing until the end of August, 1933, additional trials were conducted in which an effort was made to eliminate some of the possible sources of error. Two distinct methods of study were adopted which, for purposes of identification, will be referred to as trial number one and trial number two.

TRIAL NO. 1.—A STUDY OF THE EFFECT OF CHLORIN GERMICIDES AS A RINSE FOR MILK CANS ON THE QUALITY OF MILK HANDLED

During the course of the previous farm work (p. 42-54) the milk shipping cans were found to be poorly washed when returned from the creamery to the farm. Milk residues and greasy material inside the cans made them unsightly and at times foul smelling. Such containers presented a problem to the farmer who wished to maintain a high quality product and in many cases the cans were rewashed at the farm. Great improvement in the condition of the milk cans resulted from the rebuilding of the washing machine at the creamery. However, the cans are generally considered a prime source of bacterial contamination to milk and the following trial was designed to ascertain to what extent a chlorin rinse at the farm might reduce can contamination.

Five farms were selected and the cooperation of their occupants secured. Included in this group was the University Farm, typifying modern efficient methods of milk production and handling and four average farms supplying milk to the local city market. The writer was allowed by a local creamery company to take samples from the patrons' cans on their arrival at its plant. Small cheese vats were taken to each farm to serve as reservoirs in which the milk produced at each milking could be thoroughly mixed.

The actual rinsing and labeling of cans was done by laboratory assistants who went out to each farm at each milking time. The number of cans used at each milking was divided, one-half of them being treated with chlorin, while the remainder were used without further treatment than that given at the creamery. At the University farm (E), the cans were washed and steamed at its creamery.

The utensils, other than the cans, received no special attention but were used as in the regular farm routine. The milk was strained into

the cheese vat reservoirs, thoroughly stirred, and a few quarts run through the outlet valve and returned to the vat in order to ensure uniform quality throughout.

Cans which were treated with chlorin were rinsed with a solution containing approximately 200 parts per million of available chlorin. Two gallons was used each time and at least one minute of rinsing was allowed for each can before transferring the rinse to the next can. The chlorin-rinsed cans were tagged with red tags, the untreated ones with white tags. At the end of each milking, half the contents of the reservoir was run into treated cans and half into untreated cans. Samples were drawn from each can on delivery at the creamery and standard plate counts and methylene blue reduction tests were made in the laboratory.

Table 28 sets forth the daily and average data from each farm and also the method by which averages were obtained, the geometric means being used for the reason that such figures give one a more accurate conception of the true quality of milk as received from a biological standpoint.

A summary of the average quality of the milk secured at each farm resulting from the five types of treatment, as determined by the plate count and reduction time indices, is given in table 29. Each milking period of each farm is averaged separately and the averages of evening and morning milkings of all five farms are also tabulated.

Tables 30 and 31 show the percentage of milk samples by treated or untreated groups which fall into empirical groupings as to quality. The limits of the groups selected are purely arbitrary but were suggested by public health classifications for market milk.

ANALYSIS OF RESULTS

The average difference in quality of milk handled in treated and untreated cans, as set forth in table 29, is noticeably greater in the case of evening's milk than in morning's milk. Since the milk handled in the morning remained in the cans only a brief time before it reached the creamery and was sampled, the incubation period was comparatively short, and there was less chance of increased counts due to can contamination. In the case of evening milk the quality of milk handled in treated cans is decidedly superior to that handled in cans receiving no special treatment. The average plate count for all five farms was reduced 42.9 percent in the case of treated cans. The average reduc-

TABLE 28.—DAILY AND AVERAGE (GEOMETRIC MEAN) OF STANDARD PLATE COUNTS AND THE AVERAGE METHYLENE BLUE REDUCTION TIMES OF MILK HANDLED IN CANS USED AS RETURNED FROM THE CREAMERY COMPARED WITH THOSE OF MILK HANDLED IN CANS RECEIVING A CHLORIN RINSE ON THE FARM IN ADDITION TO CREAMERY TREATMENT

Farm A—Evening milk						
Can number	Untreated cans			Chlorin treated cans		
	Plate count	Logarithms	Reduction time	Plate count	Logarithms	Reduction time
			Minutes			Minutes
1	143,000	5.16	345	78,000	4.89	345
2	101,000	5.	375	58,000	4.76	375
3	66,000	4.82	495	32,000	4.51	540
4	7,700	3.89	577	10,000	4.	570
5	28,500	4.45	435	18,500	4.27	405
6	195,000	5.29	210	74,000	4.87	360
7	325,000	5.51	135	300,000	5.48	180
8	51,000	4.71	345	45,000	4.65	352
9	450,000	5.65	135	260,000	5.42	180
10	380,000	5.58	457	167,000	5.22	300
Total		50.06	3,509		48.07	3,600
Average	101,400*	5.006	5 hrs., 51 min.	64,100*	4.807	6 hrs.
Morning milk						
1	71,500	4.85	570	33,000	4.52	577
2	7,600	3.88	667	7,800	3.89	570
3	4,500	3.65	750	2,700	3.43	615
4	3,600	3.56	562	3,700	3.57	615
5	5,800	3.76	615	4,650	3.67	607
6	14,000	4.15	495	11,500	4.06	495
7	18,000	4.26	345	15,000	4.18	360
8	262,000	5.42	360	203,000	5.31	405
9	37,000	4.57	390	31,000	4.49	405
Total		38.10	4,754		37.12	4,649
Average	17,100*	4.233	8 hrs., 48 min.	13,300*	4.124	8 hrs., 37 min.
Farm B—Evening milk						
1	200,000	5.30	390	105,000	5.02	405
2	40,000	4.60	330	35,000	4.54	330
3	1,500,000	6.18	30	1,350,000	6.13	30
4	1,070,000	6.03	45	675,000	5.83	45
5	70,000	4.85	405	20,000	4.30	495
6	45,000	4.65	375	25,000	4.40	360
7	800,000	5.90	180	90,000	4.95	240
8	450,000	5.65	150	90,000	4.95	210
9	160,000	5.20	330	20,000	4.30	390
10	45,000	4.65	435	20,000	4.30	495
11	55,000	4.74	435	76,000	4.88	435
12	52,500	4.72	495	60,500	4.78	450
13	58,000	4.76	375	55,000	4.74	405
14	99,000	5.	420	32,000	4.51	420
15	240,000	5.38	330	190,000	5.28	345
16	206,000	5.31	315	158,000	5.20	300
17	106,000	5.03	435	30,000	4.48	495
18	33,500	4.52	465	38,500	4.59	555
19	24,000	4.38	525	12,500	4.10	495
20	30,000	4.48	420	22,000	4.34	420
21	51,000	4.71	450	26,000	4.42	465
22	143,000	5.16	405	60,000	4.78	420
Total		111.20	7,740		104.82	8,205
Average	113,500*	5.055	5 hrs., 52 min.	57,600*	4.765	6 hrs., 13 min.

* Geometric mean.

TABLE 28.—DAILY AND AVERAGE (GEOMETRIC MEAN) OF STANDARD PLATE COUNTS AND THE AVERAGE METHYLENE BLUE REDUCTION TIMES OF MILK HANDLED IN CANS USED AS RETURNED FROM THE CREAMERY COMPARED WITH THOSE OF MILK HANDLED IN CANS RECEIVING A CHLORIN RINSE ON THE FARM IN ADDITION TO CREAMERY TREATMENT—*Continued*

Farm B—Morning milk

Can number	Untreated cans			Chlorin treated cans		
	Plate count	Logarithms	Reduction time	Plate count	Logarithms	Reduction time
			Minutes			Minutes
1	50,000	4.70	330	85,000	4.93	375
2	355,000	5.55	330	155,000	5.19	375
3	410,000	5.61	180	825,000	5.92	180
4	215,000	5.33	285	180,000	5.26	300
5	55,000	4.74	435	45,000	4.65	465
6	50,000	4.70	465	10,000	4.	487
	45,000	4.65	345	75,000	4.88	330
	20,000	4.30	360	90,000	4.95	367
	830,000	5.92	450	740,000	5.87	435
	40,000	4.60	555	5,000	3.70	645
	10,000	4.	525	5,000	3.70	435
	8,000	3.90	525	3,500	3.54	525
	6,000	3.78	577	3,500	3.54	472
	10,000	4.	607	15,500	4.19	495
15	15,500	4.19	587	20,000	4.30	585
16	9,000	3.95	525	6,500	3.81	540
17	4,500	3.65	570	8,000	3.90	592
18	7,500	3.88	600	3,500	3.54	630
19	13,500	4.13	577	11,000	4.04	592
20	5,000	3.70	465	6,000	3.78	540
21	16,000	4.20	585	7,500	3.88	600
22	8,000	3.90	637	9,000	3.95	585
Total		97.38	10,515		95.52	10,550
Average	26,700*	4.426	7 hrs., 58 min.	22,000*	4.342	8 hrs., 0 min.

Farm C—Evening milk

1	3,750	3.57	750	2,250	3.35	765
2	2,900	3.46	765	1,600	3.20	742
3	5,400	3.73	772	5,800	3.76	772
4	3,000	3.48	817	3,000	3.48	735
5	20,500	4.31	465	18,500	4.27	480
6	3,500	3.54	555	3,000	3.48	495
7	11,500	4.06	330	9,700	3.99	315
8	14,300	4.16	495	5,450	3.74	547
9	6,800	3.83	772	7,700	3.89	667
10	8,600	3.93	705	8,800	3.94	780
11	18,500	4.27	697	8,900	3.95	720
12	13,500	4.13	830	9,700	3.99	615
13	15,600	4.19	855	9,300	3.97	870
14	10,600	4.03	817	8,500	3.93	735
15	30,100	4.48	592	31,800	4.50	607
16	97,500	4.99	585	34,800	4.48	540
Total		64.16	10,802		61.98	10,385
Average	10,200*	4.01	11 hrs., 15 min.	7,500*	3.874	10 hrs., 49 min.

* Geometric mean.

TABLE 28.—DAILY AND AVERAGE (GEOMETRIC MEAN) OF STANDARD PLATE COUNTS AND THE AVERAGE METHYLENE BLUE REDUCTION TIMES OF MILK HANDLED IN CANS USED AS RETURNED FROM THE CREAMERY COMPARED WITH THOSE OF MILK HANDLED IN CANS RECEIVING A CHLORIN RINSE ON THE FARM IN ADDITION TO CREAMERY TREATMENT—*Continued*

Farm C—Morning milk

Can number	Untreated cans			Chlorin treated cans		
	Plate count	Logarithms	Reduction time	Plate count	Logarithms	Reduction time
			Minutes			Minutes
1	2,600	3.42	585	1,550	3.19	795
2	5,600	3.75	757	5,000	3.70	690
3	6,800	3.83	757	5,400	3.73	742
4	6,000	3.78	780	6,000	3.78	795
5	3,400	3.53	720	4,800	3.68	720
6	10,300	4.01	532	9,800	3.99	570
7	5,500	3.74	540	4,650	3.67	435
8	6,600	3.82	690	7,400	3.87	757
9	5,500	3.74	697	4,800	3.68	735
10	6,000	3.78	720	6,000	3.78	600
11	8,700	3.94	630	8,500	3.93	630
12	9,000	3.95	810	7,000	3.85	840
13	19,000	4.28	647	21,000	4.32	630
14	31,600	4.50	465	34,500	4.54	435
15	35,600	4.55	600	16,000	4.20	675
Total		58.62	9,930		57.91	10,049
Average	8,100*	3.908	11 hrs., 2 min.	7,200*	3.861	11 hrs., 10 min.

Farm D—Evening milk

1	1,090,000	6.04	525	1,300,000	6.11	525
2	5,200,000	6.72	202	3,700,000	6.57	240
3	30,000	4.48	510	15,000	4.18	510
4	2,800,000	6.45	412	397,000	5.60	337
5	835,000	5.92	97	585,000	5.77	135
6	1,200,000	6.08	345	500,000	5.70	390
7	98,500	4.99	637	142,000	5.15	675
8	487,000	5.69	540	213,000	5.33	600
Total		46.37	3,268		44.41	3,412
Average	625,100*	5.796	6 hrs., 48 min.	355,600*	5.551	7 hrs., 6 min.

Morning milk

1	290,000	5.46	435	195,000	5.29	465
2	480,000	5.68	345	255,000	5.41	420
3	290,000	5.46	540	52,000	4.72	450
4	545,000	5.74	570	40,000	4.60	645
5	80,000	4.90	607	81,000	4.91	577
6	600,000	5.78	510	450,000	5.65	517
7	106,000	5.03	570	107,500	5.03	517
8	124,000	5.09	510	149,000	5.17	480
Total		43.14	4,087		40.78	4,071
Average	246,500*	5.392	8 hrs., 31 min.	125,300*	5.098	8 hrs., 29 min.

* Geometric mean.

tion time showed a small increase. The difference between the average counts of morning's milk, though small, favored the rinsed cans.

The grouped counts as shown in table 30 show a larger percentage of high quality milk in chlorin-rinsed cans both morning and evening.

TABLE 28.—DAILY AND AVERAGE (GEOMETRIC MEAN) OF STANDARD PLATE COUNTS AND THE AVERAGE METHYLENE BLUE REDUCTION TIMES OF MILK HANDLED IN CANS USED AS RETURNED FROM THE CREAMERY COMPARED WITH THOSE OF MILK HANDLED IN CANS RECEIVING A CHLORIN RINSE ON THE FARM IN ADDITION TO CREAMERY TREATMENT—*Continued*

Farm E—Evening milk

Can number	Untreated cans			Chlorin treated cans		
	Plate count	Logarithms	Reduction time	Plate count	Logarithms	Reduction time
			Minutes			Minutes
1	18,500	4.27	420	14,500	4.16	450
2	2,700	3.43	660	1,850	3.27	615
3	3,200	3.51	570	6,100	3.79	517
4	4,000	3.60	600	6,500	3.81	765
5	6,500	3.81	660	1,950	3.29	697
6	8,900	3.95	510	2,250	3.35	502
7	9,300	3.97	495	3,300	3.52	630
8	1,100	3.04	585	1,600	3.20	585
9	1,200	3.08	757	1,050	3.02	810
10	11,300	4.05	555	9,100	3.96	585
11	2,600	3.42	720	1,500	3.18	720
Total		40.13	6,532		38.55	6,876
Average	4,450*	3.648	9 hrs., 54 min.	3,200*	3.505	10 hrs., 25 min.

Morning milk

1	1,200	3.08	615	800	2.90	860
2	8,500	3.93	682	9,000	3.95	615
3	6,200	3.79	540	3,750	3.57	480
4	1,250	3.10	660	900	2.95	682
5	4,500	3.65	450	3,650	3.56	510
6	2,200	3.34	532	2,500	3.40	495
7	10,000	4.	495	6,000	3.78	540
8	1,050	3.02	615	900	2.95	712
9	9,400	3.97	532	24,000	4.38	540
10	1,400	3.15	675	1,200	3.08	682
Total		35.03	5,796		34.52	6,116
Average	3,180*	3.503	9 hrs., 40 min.	2,830*	3.452	10 hrs., 12 min.

* Geometric mean.

Here, again, the variation is greater in the case of evening's milk. A slight increase in favor of chlorin rinsing is apparent in table 31 where the reduction times are classified in groups, but the difference appears only in the case of evening milk.

Table 28, containing the data from individual farms, indicates the variations in quality between farms and the day-to-day milk analysis.

TABLE 29.—AVERAGE (GEOMETRIC MEAN) OF STANDARD PLATE COUNTS AND THE AVERAGE METHYLENE BLUE REDUCTION TIMES OF MILK HANDLED IN CANS USED AS RETURNED FROM THE CREAMERY COMPARED WITH THOSE OF MILK HANDLED IN CANS RECEIVING A CHLORIN RINSE ON THE FARM IN ADDITION TO CREAMERY TREATMENT

Farm	Untreated cans				Chlorin treated cans			
	Average plate count	Average reduction time		Average plate count	Average reduction time			
		Hrs.	Min.		Hrs.	Min.		
A	101,400	5	51	64,100	6	1		
B	113,500	5	52	57,600	6	13		
C	10,200	11	15	7,500	10	49		
D	625,100	6	48	355,600	7	6		
E	4,450	9	54	3,200	10	25		
Average	170,930*	7	56	97,600*	8	7		

Morning milking						
Farm	Average plate count	Average reduction time		Average plate count	Average reduction time	
		Hrs.	Min.		Hrs.	Min.
A	17,100	8	48	13,300	8	37
B	26,700	7	58	22,000	8	0
C	8,100	11	2	7,200	11	10
D	246,500	8	31	125,300	8	29
E	3,180	9	40	2,830	10	12
Average	60,316*	9	12	34,126*	9	18

* Geometric mean.

TABLE 30.—PERCENTAGE OF STANDARD PLATE COUNTS OF MILK PRODUCED IN CANS AS RETURNED FROM THE CREAMERY AND CANS RECEIVING AN ADDITIONAL CHLORIN RINSE AT THE FARM BY GROUPS

Number of comparisons—Morning 64—Evening 67

Bacteria counts	Cans used as returned from the creamery		Cans rinsed with chlorin germicidal solution	
	Morning milking	Evening milking	Morning milking	Evening milking
	%	%	%	%
Under 30,000	66	42	69	52
30,000 to 200,000...	19	34	23	33
200,000 to 1,000,000.	16	15	8	10
Over 1,000,000	9	..	4

TABLE 31.—PERCENTAGE OF METHYLENE BLUE REDUCTION TIMES OF MILK PRODUCED IN CANS AS RETURNED FROM THE CREAMERY AND CANS RECEIVING AN ADDITIONAL CHLORIN RINSE AT THE FARM BY GROUPS

Methylene blue reduction times	Cans used as returned from the creamery		Cans rinsed with a chlorin solution on the farm	
	Morning milking	Evening milking	Morning milking	Evening milking
	%	%	%	%
Over 5½ hours	94	79	95	82
5½ to 2 hours	6	16	5	15
Under 2 hours	4	..	3

The fact that a greater number of samples were obtained from some farms than from others was due to differences in the volume of daily production; for example, farm A supplied two cans per milking, farm B four.

Taking the summarized results as a whole, the rinsing of cans with a chlorin solution containing 200 parts per million of available chlorin immediately before using the cans as milk containers appears to aid materially in improving the bacteriological quality of the milk handled.

TRIAL NO. 2.—A STUDY OF THE EFFECT ON THE QUALITY OF MILK HANDLED IN UTENSILS RINSED WITH CHLORIN GERMICIDES AS COMPARED WITH MILK HANDLED IN UTENSILS RECEIVING ROUTINE FARM CARE

At the conclusion of trial number one, five farms in addition to those included in the can-rinsing trial were selected in order to test chlorin rinsing as a help in the sanitary care of all milk utensils. The plan followed in making this study was as follows: The complete set of utensils used on each farm of the 10 was rinsed on alternate days with a solution of chlorin germicide containing approximately 200 parts per million of available chlorin. The rinsing was done by laboratory assistants who went to five of the 10 farms at each milking time and thoroughly rinsed all utensils just before they were used in handling the milk. Thus, utensils on five farms received chlorination every other day. On the alternate days the farmers were requested to use their utensils in the ordinary farm routine manner. They were particularly requested to conform as closely as possible to the average practice which they ordinarily employed. Occasional unexpected visits by the writer convinced him that all the farmers cooperating endeavored to comply with this request. The assistants who did the rinsing observed conditions which might influence the quality of milk other than utensils and reported their observations to the writer. Temperatures of the evening milk were taken the following morning. Samples for bacteriological analysis were taken at the creamery, a composite sample being obtained daily from each of the 10 farms. As in previous studies, plate counts and methylene blue reduction tests were made.

ANALYSIS OF RESULTS

Table 32 contains the day-to-day data on each farm and illustrates the variation of quality produced on different farms and also the method of analysis of results.

TABLE 32.—DAILY AND AVERAGE (GEOMETRIC MEAN) OF STANDARD PLATE COUNTS AND THE AVERAGE METHYLENE BLUE REDUCTION TIMES OF MILK PRODUCED IN CHLORIN RINSED UTENSILS AS COMPARED WITH THOSE OF MILK PRODUCED IN UTENSILS RECEIVING ONLY THE USUAL FARM SANITATION TREATMENT WITHOUT A CHLORIN RINSE

Farm A

Number	Untreated utensils			Chlorin treated utensils		
	Plate count	Logarithms	Reduction time	Plate count	Logarithms	Reduction time
			Minutes			Minutes
1	605,000	5.78	165	300,000	5.48	210
2	81,000	4.91	345	61,500	4.79	345
3	158,000	5.20	345	32,000	4.51	457
4	21,000	4.32	465	33,000	4.52	450
5	42,000	4.62	450	15,000	4.18	442
6	260,000	5.42	285	45,500	4.66	255
7	370,000	5.57	315	190,000	5.28	330
8	881,000	5.94	255	67,000	4.83	405
9	89,000	4.95	345	268,000	5.43	285
10	43,000	4.63	315	150,000	5.18	240
Total		51.34	3,285		48.86	3,419
Average	136,200*	5.134	5 hrs., 28 min.	76,900*	4.886	5 hrs., 42 min.

Farm B

1	60,000	4.78	435	1,500	3.18	457
2	227,500	5.36	210	29,100	4.46	382
3	237,500	5.38	330	24,000	4.38	390
4	160,000	5.20	300	123,000	5.09	390
5	783,000	5.89	225	62,500	4.80	390
6	1,830,000	6.26	90	66,500	4.82	255
7	220,000	5.34	240	155,000	5.19	255
8	1,590,000	6.20	150	260,000	5.42	240
9	985,000	5.99	45	380,000	5.58	210
10	970,000	5.99	120	290,000	5.46	180
Total		56.39	2,145		48.38	3,149
Average	435,500*	5.639	3 hrs., 34 min.	68,900*	4.838	5 hrs., 15 min.

Farm C

1	29,200	4.47	360	94,200	4.97	457
2	157,000	5.20	360	71,500	4.85	457
3	436,000	5.64	390	17,200	4.24	457
4	64,500	4.81	585	152,000	5.18	555
5	27,300	4.44	510	23,000	4.36	660
6	41,500	4.62	405	12,000	4.08	555
7	405,000	5.61	465	58,500	4.77	375
8	227,000	5.36	420	91,000	4.96	525
9	353,000	5.55	300	78,000	4.89	465
10	123,000	5.09	495	26,000	4.42	450
Total		50.79	4,290		46.72	4,956
Average	119,900*	5.079	7 hrs., 9 min.	47,000*	4.672	8 hrs., 16 min.

* Geometric mean.

TABLE 32.—DAILY AND AVERAGE (GEOMETRIC MEAN) OF STANDARD PLATE COUNTS AND THE AVERAGE METHYLENE BLUE REDUCTION TIMES OF MILK PRODUCED IN CHLORIN RINSED UTENSILS AS COMPARED WITH THOSE OF MILK PRODUCED IN UTENSILS RECEIVING ONLY THE USUAL FARM SANITATION TREATMENT WITHOUT A CHLORIN RINSE—*Continued*

Farm D

Number	Untreated utensils			Chlorin treated utensils		
	Plate count	Logarithms	Reduction time	Plate count	Logarithms	Reduction time
			Minutes			Minutes
1	703,000	5.85	390	1,500	3.18	630
2	1,950,000	6.29	315	565,000	5.75	465
3	2,000,000	6.30	135	219,000	5.34	480
4	1,100,000	6.04	465	96,500	4.98	540
5	20,000	4.30	510	15,000	4.18	675
6	8,450,000	6.93	105	35,000	4.54	587
7	1,495,000	6.17	165	350,000	5.54	480
8	140,000	5.15	360	170,000	5.23	525
9	1,500,000	6.18	270	35,000	4.54	330
10	1,250,000	6.10	330	23,000	4.36	480
Total		59.31	3,045		47.64	5,192
Average	853,200*	5.931	5 hrs., 4 min.	58,100*	4.764	8 hrs., 39 min.

Farm E

1	29,200	4.47	750	1,300	3.11	645
2	4,000	3.60	787	800	2.90	660
3	2,300	3.36	562	1,400	3.15	892
4	15,600	4.19	405	5,400	3.73	600
5	18,600	4.27	360	4,350	3.64	705
6	8,350	3.92	465	3,400	3.53	720
7	12,500	4.10	375	5,200	3.72	660
8	4,700	3.67	450	6,200	3.79	652
9	28,250	4.45	390	2,350	3.37	765
10	10,800	4.03	595	7,900	3.90	450
Totals		40.06	5,139		34.84	6,749
Average	10,100*	4.006	8 hrs., 34 min.	3,050*	3.484	11 hrs., 15 min.

Farm F

1	3,410,000	6.53	167	460,000	5.66	345
2	504,000	5.70	255	2,320,000	6.37	165
3	830,000	5.92	390	40,000	4.60	420
4	650,000	5.81	315	180,000	5.26	300
5	135,000	5.13	420	320,000	5.51	285
6	690,000	5.84	270	845,000	5.93	225
7	2,270,000	6.36	262	910,000	5.96	285
8	590,000	5.77	487	550,000	5.74	375
9	405,000	5.61	315	295,000	5.47	285
10	110,000	5.04	405	50,000	4.70	450
Total		57.71	3,286		55.20	3,135
Average	590,100*	5.771	5 hrs., 29 min.	331,200*	5.52	5 hrs., 14 min.

* Geometric mean.

TABLE 32.—DAILY AND AVERAGE (GEOMETRIC MEAN) OF STANDARD PLATE COUNTS AND THE AVERAGE METHYLENE BLUE REDUCTION TIMES OF MILK PRODUCED IN CHLORIN RINSED UTENSILS AS COMPARED WITH THOSE OF MILK PRODUCED IN UTENSILS RECEIVING ONLY THE USUAL FARM SANITATION TREATMENT WITHOUT A CHLORIN RINSE—*Continued*

Farm G

Number	Untreated utensils			Chlorin treated utensils		
	Plate count	Logarithms	Reduction time Minutes	Plate count	Logarithms	Reduction time Minutes
1	11,300	4.05	585	8,500	3.93	757
3	18,300	4.26	630	17,700	4.25	667
3	11,100	4.05	630	16,800	4.23	742
4	5,150	3.71	570	18,250	4.26	495
5	8,000	3.90	600	18,500	4.27	480
6	200,000	4.30	390	40,000	4.60	420
7	22,500	4.35	495	38,000	4.58	555
8	42,000	4.62	562	21,000	4.32	390
9	40,000	4.60	405	29,500	4.47	495
10	65,000	4.81	420	43,500	4.64	495
Total		42.65	5,287		43.55	5,496
Average	18,200*	4.265	8 hrs., 49 min.	22,600*	4.355	9 hrs., 10 min.

Farm H

		Minutes		Minutes	
187,000	5.46	450	116,000	5.06	420
108,000	4.99	435	94,000	4.97	480
10,000	5.40	345	110,000	5.04	405
1,000	5.44	480	124,000	5.09	480
100	5.44	465	239,000	5.38	480
100	5.68	390	188,000	5.27	465
100	5.47	450	440,000	5.64	210
100	5.80	300	211,000	5.32	375
	5.56	362	59,000	4.77	330
	5.05	420	20,000	4.30	450
4.29		4,095		50.84	4,095
					084 6 hrs., 50 min.

TABLE 32.—DAILY AND AVERAGE (GEOMETRIC MEAN) OF STANDARD PLATE COUNTS AND THE AVERAGE METHYLENE BLUE REDUCTION TIMES OF MILK PRODUCED IN CHLORIN RINSED UTENSILS AS COMPARED WITH THOSE OF MILK PRODUCED IN UTENSILS RECEIVING ONLY THE USUAL FARM SANITATION TREATMENT WITHOUT A CHLORIN RINSE—*Concluded*

Farm K

Number	Untreated utensils			Chlorin treated utensils		
	Plate count	Logarithms	Reduction time	Plate count	Logarithms	Reduction time
1	325,000	5.51	285	57,500	4.76	322
2	111,000	5.05	390	90,000	4.95	375
3	70,000	4.85	285	40,000	4.60	435
4	167,000	5.22	300	20,000	4.30	450
5	32,000	4.51	405	233,000	5.37	270
6	780,000	5.89	135	565,000	5.75	255
7	1,385,000	6.14	105	435,000	5.64	300
8	560,000	5.75	172	880,000	5.94	180
9	250,000	5.40	210	645,000	5.81	180
10	620,000	5.79	270	155,000	5.19	255
Total		54.11	2,557		52.31	3,022
Average	257,700*	5.411	4 hrs., 16 min.	170,200*	5.231	5 hrs., 2 min.

* Geometric mean.

Table 33 presents a summary of the tests obtained from each under control and test conditions. The geometric mean, used in comparing the standard plate counts, shows a decided improvement in the quality of milk on most farms, although, in the case of farm K, the control shows a lower average count than the test period. Similar conditions have appeared throughout the practical work in this study. It is worthy of note that in the study conducted during the summer

TABLE 33.—AVERAGE (GEOMETRIC MEAN) OF STANDARD PLATE COUNTS AND AVERAGE METHYLENE BLUE REDUCTION TIMES OF MILK PRODUCED IN CHLORIN RINSED UTENSILS AS COMPARED WITH THOSE OF MILK PRODUCED IN UTENSILS RECEIVING ONLY THE USUAL FARM SANITATION TREATMENT WITHOUT A CHLORIN RINSE—*Continued*

(see page 44), the same irregular results were noted at this farm. The quality of milk produced there is comparatively good at all times and the writer believes that during the chlorin rinsing period the washing and care of utensils may have been somewhat neglected in the belief that the rinse would take care of all necessary sanitation. This supposition can not be substantiated but is offered as a possible explanation.

The total averages of all farms show a marked reduction of 63.9 per cent in the geometric mean of all plate counts, while the methylene blue reduction time was lengthened an average of one hour.

TABLE 34.—PERCENTAGE DISTRIBUTION OF THE STANDARD PLATE COUNTS AND METHYLENE BLUE REDUCTION TIMES OF MILK PRODUCED ON 10 VERMONT FARMS UNDER REGULAR METHODS OF UTENSIL SANITATION AS COMPARED WITH MILK PRODUCED ON THE SAME FARMS ON ALTERNATE DAYS WHEN THE SYSTEM OF CHLORIN RINSING OF UTENSILS WAS EMPLOYED
Number of samples—100 control, 100 chlorin rinsed

Bacteria counts	Chlorin treated		Methylene blue reduction times	Chlorin treated	
	Control	%		Control	%
Under 30,000	24	39	Over 5½ hours	61	73
30,000 to 200,000 . . .	28	38	5½ to 2 hours	35	27
200,000 to 1,000,000	36	22			
Over 1,000,000	12	1	Under 2 hours	4	..
Total	100	100		100	100

In table 34 the percentage of high quality milk under utensil rinsing conditions distinctly shows in the grouping of plate counts and reduction times. The percentage increase of counts under 30,000, from 24 percent under usual farm sanitation to 39 percent under rinsing conditions, is borne out further in the increased percentage of reduction times over five and one-half hours.

COST OF GERMICIDAL TREATMENT

The cost per day of the concentrated compound used to prepare the hypochlorite rinse in the farm experiment was approximately one-third of a cent. Considerable differences were noted in the actual cost of "available chlorin" present in each chemical in purchasing several different types of proprietary compounds from local stores. The costs per pound of the purchases did not differ to a noticeable extent but since the chlorin contents were only 3.5 percent in two cases and ranged as high as 15 percent in another, the latter supplied "available chlorin" at a lower cost.

Table 35 shows the actual initial cost per pound in quantities suitable for farm distribution, the calculated cost per pound of available chlorin and an estimate of the cost per day of practical rinse solutions

TABLE 35.—THE COST OF FOUR COMMERCIAL CHLORIN GERMICIDES BASED ON THE PRICE TO FARM TRADE

Compound number	Available chlorin	Price per pound	Calculated cost per pound of available chlorin	Cost of four gallons per day of 200 parts per million solution
	Percent	Cents	Dollars	Cents
1	15.	40.	2.67	1.77
2	3.5	13.4	3.83	2.54
3	3.5	25.	7.14	4.74
4	4.	30.	7.50	4.98

of four chlorin compounds obtainable at local stores. The cost per pound of all compounds is calculated from quotations obtained from the manufacturer as applicable to creameries which purchase wholesale quantities for distribution to patrons on a non-profit basis. Compound number two is in liquid form and the per pound price is calculated from the per gallon cost.

Certain materials appear to advantage over others when their cost is compared in this manner, but a true analysis of value must take into consideration the suitability of a compound according to the purpose for which it is to be used. Corrosive solutions may be low in price but costly in the long run if they ruin expensive equipment. Slow acting compounds may be cheap but if rapid germicidal action is required this type may not give satisfactory results. Because of the individual limitations of some compounds, a thorough knowledge of their behavior under specific conditions seems necessary in a comparison of values.

From a practical farm standpoint, treatment to kill bacteria with chlorin germicides appears to be less expensive than steam or hot water. The estimated cost of four gallons per day of 200 parts per million available chlorin derived from the most costly chemical is approximately five cents. This would amount to about \$18 per year and is, perhaps, considerably higher than necessary since the amount of solution and its strength are in excess of general recommendations.

Theophilus and Atkeson (30) state that hot water may be supplied on an Idaho farm for germicidal purposes in adequate quantities at a minimum of \$38 when an electric heater is used and of \$23 when an oil burner is used. Vermont farm rates for electricity and the local cost of kerosene during the past year are about the same as those obtain-

ing in Idaho. These minimum figures cover only the costs of actual operation and do not include the initial cost of installation or labor costs. Now, if chlorin compound No. 1 is taken as providing hypochlorite at minimum cost, the yearly expenditure for material would be less than \$6.50 if careful rather than wasteful use prevailed.

SUMMARY OF PRACTICAL EXPERIMENTS

Neither milk held in bottles for 18 hours nor that held in cans rinsed with chlorin solutions showed off-flavors.

Artificially contaminated milk pails when rinsed with a quick acting hypochlorite carried relatively few bacteria on their surfaces.

Chlorin-rinsed cans compared favorably with well steamed cans in respect to their bacterial content.

The quality of milk produced under experimental conditions where hypochlorite solutions were employed in treating utensils to kill bacteria compared favorably with that produced in steamed utensils.

The utensils on 20 Vermont farms were rinsed with hypochlorite solution twice daily immediately before milking. The average quality of the milk as measured by plate counts and methylene blue reduction tests was materially improved following this procedure.

Milk handled in cans which were treated at the farm with a chlorin rinse in addition to the treatment given at the creamery showed less contamination than did portions of the same lot of milk handled in unrinsed cans.

Milk produced on 10 Vermont farms averaged materially better in bacteriological quality when all utensils were chlorin rinsed by laboratory assistants than when utensils received only the usual farm treatment without a chlorin rinse.

The actual cost per pound of available chlorin as bought in different proprietary compounds varied widely.

CONCLUSIONS

1. Concentrated compounds containing "available chlorin" deteriorate in storage. If held in the dark the rate of deterioration is lessened.

2. When foreign organic matter contacts a germicidal chlorin solution the concentration of its available chlorin is lowered. The rate of available chlorin loss increases as the temperature of the solution rises from 70° F. to 212° F.

3. Laboratory tests show chloramine-T solutions to be slower in action than hypochlorites. Acidification and raising the temperature of chloramine-T solutions increases germicidal activity but not to the degree shown by hypochlorites.

4. Corrosion of metal is a factor which deserves consideration in selecting the type of compound to use and in setting up practical procedure.

5. The danger of off-flavors caused by traces of chlorin adhering to utensils after treatment appears negligible providing the solution is carefully used and the utensils thoroughly drained.

6. Laboratory tests and practical experiments indicate definite improvement of the bacteriological quality of milk produced in farm utensils treated with chlorin solutions as compared to milk produced in utensils not so treated.

7. The convenience and low cost of hypochlorite treatment of farm utensils as compared with steam are practical advantages in its favor.