# A STUDY OF THE CHLORINATION OF MILK

by

G. Albin Matson

B.A. University of Utah, 1927

Submitted to the Department of Bacteriology and the Faculty of the Graduate School of the University of Kansas in partial fulfillment of the requirements for the degree of Master of Arts.

Approved:

Noble P. Sherwood

Chr. Dept Bact.

June 3/29
date

Acknowledgement

I wish here to acknowledge my gratitude to Dr. Noble P. Sherwood, chairman of the Bacteriology Department, University of Kansas, for his kind and helpful suggestions, and to the other members of his staff for their encouragement to me in this work.

## Table of Contents

# A STUDY OF THE CHLORINATION OF MILK

## Historical

From time immemorial milk and other dairy products have made up an important part of the diet of man. Of the limited knowledge that we have of prehistoric man, acquired, as it is, largely by means of the implements and tools that he has left us, nothing is more sure than that dairy products constituted a considerable portion of his food. Pre-eminent among the implements that he used is the churn. This useful tool was used by early tribes in Africa, America, Australia, the South Sea Islands, Asia and Europe.

Heinemann (1), who draws freely from the book of Benno Martiny, "Kirne and Girbe", gives a brief yet comprehensive summary of the history and development of the dairy industry. He points out that it has been a rather slow development and that until a relatively recent date, no sanitary precautions in the production of milk have been very seriously attempted if we overlook a few sporadic cases in earlier times.

It has been supposed by earlier bacteriologists that milk, freshly drawn from the udder, is sterile; and would keep indefinitely were it not polluted subsequent to withdrawal. Lister (2), who in 1878 attempted to obtain milk without contamination, is perhaps largely responsible for this supposition. He succeeded in obtaining two sterile tubes out of twelve which contained carefully drawn milk. The organisms in the ten tubes that were not sterile, he concluded to be air contaminants, which gained entrance for want of sufficient care on his part and that the milk in the udder is sterile.

Prior to this, in 1874, Roberts (3) also succeeded in obtaining three sterile tubes out of ten.

In 1891, Schulz (4) reported that the first drawn milk contained numerous bacteria and that as the milking proceeded the numbers decreased. But it remained an open question until Moore (5) and later Moore and Ward (6) published their work which established the fact that normal udders and lactiferous ducts harbour bacteria which contaminates the milk as soon as it is secreted.

The types of bacteria which can grow within the udder are, however, limited. Rogers (7) states: "There are only three general groups of organisms that commonly grow in healthy udders--staphylococci, streptococci, and a group of minute rod forms of which the organism of contagious abortion is the type species."

After the milk is drawn there is further chance of contamination. Pathogenic bacteria gain access to milk in a variety of ways. The milker or handler of milk may be suffering from a mild infection, or may himself be well, but a carrier. Particularly may this be true in the case of Typhoid fever or Diphtheria. Utensils may infect milk if they are washed or rinsed in polluted water, or handled by an infected person or a carrier. Flies and rodents may be agents in carrying infectious material into milk, if they gain access to the milk or the utensils which handle it. Once in this medium par excellence they may reproduce and multiply, or, if kept at low temperatures, will remain viable for a long time. Thus many milk borne epidemics have been caused.

From 1881 to 1907, according to Trask (8), the following number of milk borne epidemics occurred:

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 179 | epidemics | of | Typhoid, 107 of which | were | in | the | U.S. |
| 51 | " | " | Scarlet Fever, 25 | " | " | " | " |
| 23 | " | " | Diphtheria, 15 of which | " | " | " | " |
| 7 | " | " | Sore Throat. | | | | |

Since 1907 other epidemics of sore throat have been reported by Bray (9), Capps and Miller (10), Hamburger (11), and others (11a). Only recently a large milk borne epidemic of Typhoid fever has occurred in Montreal.

It seems axiomatic to say that if sufficient precautions were taken to make sure that milk comes from healthy cows, and is handled in a sanitary manner by persons free from infectious disease, that no such epidemics would occur. Rogers (7) states that only two epidemics spread by certified milk have been reported in this country since 1892. Most of the milk consumed however, is not certified and much of that which is consumed in large cities does not reach the consumer until it is nearly, if not quite, a day old. For this reason milk is treated in various ways to insure longer keeping qualities and to render it more free from dangerous bacterial pollution.

Preliminary to a consideration of the chlorination of milk, it seems desirable to state briefly some of the present methods of treating milk.

Boiling:

Boiling, according to Minett (12), Mansell (13), and others, is the usual method of rendering milk safe for domestic consumption in the tropics. Boiling milk for infant feeding has its advocates both in this country and abroad and it is claimed that it forms a softer, more easily digested curd in the stomach than does the raw milk.

Pasteurization:

The word "pasteurization" originated from the classical experiments of Louis Pasteur, 1860-1864, on the so-called "diseases" of wine. He found that by heating to temperatures from 122° to 140° F. he was able to prevent abnormal fermentation of wine. Later, by the same process, beer was preserved from souring. The application of the process has given rise to the term "pasteurization."

As applied to milk there are three methods of Pasteurization. The first, in which milk is heated to a temperature of $165^{\circ}$ F. for 1/2 to 1 minute, is the flash or continuous process. The second, in which milk is maintained at a temperature of $145^{\circ}$ F. for 30 minutes, is known as the holding process. The third is a method in which milk is pasteurized in the final container. As a complete pasteurizing process, the flash method is obsolete. It is used frequently to pre-heat milk for the holding process. The tendency at present seems to be toward a wider use of the holding process, but, from a bacteriological standpoint, pasteurization in the final container seems to be the most desirable; since, by this method, the possibility of contamination of milk, after treatment is reduced to a minimum.

Treatment by Electricity:

Zeit (14), in 1901, demonstrated the fact that continuous electric currents produced a bactericidal effect partly by heat, but principally by the chemical action of the products of electrolysis. Alternating currents of low frequency favored growth and pigment production, but high frequency, high potential currents sterilized the medium though with an odor of ozone.

Anderson and Finkelstein (15), Chilson (16), and others have come to the conclusion that bacteria are really killed by the heat and that the apparatus merely furnished a means of creating a sudden high temperature for a brief period.

Beattie and Lewis (17), working in England under the direction of the Medical Research Council and with the collaboration of the eminent Sir Oliver Lodge, concluded that both the current and heat had to do with the sterilizing effect.

Prescott and Holmes (18) inspected and gave favorable reports of a plant in Boston that handles 3000 quarts of milk daily from November 1925 to January 1926.

Theoretically, the treatment of milk by electricity seems promising. It is yet in the experimental stage, however, and it has comparatively few advocates in this country.

Treatment by Ultraviolet Light:

The destructive action of light on micro-organisms was first described by Downes and Blunt (19) in 1877. These writers believed that oxygen was essential for the germicidal action of light. Arloing (20), Duclaux (21), and Roux (22) all had the same idea. Ward (23), however, writing in 1892 and 1893, presented the view that is pretty much general today, namely, that the bactericidal action of ultra-violet light is direct and not due to a secondary action involving oxygen. Norton (24), in a recent writing, comments as follows: "The evidence that bacterial protoplasm and many proteins will absorb ultra-violet rays is indisdisputable, and the facts concerning lipoids are well known. I think we can safely assume a direct photochemical action on the bacterial cell."

Ultra-violet light finds practical application in treatment of water in a number of municipal supplies in Europe but so far it has found little favor in this country. To be effective, the water must be clear. The treatment obviously would lend itself more efficiently to clear water than to milk, in which the proteins seem to shield the bacteria. Ayers and Johnson (25) have shown that not all the bacteria were killed when milk was treated in very thin layers for several minutes, and what was more serious, it imparted an unpleasant taste to the milk.

Treatment by Carbon Dioxide:

Carbon dioxide treatment has been advocated from time to time, but according to Rogers (7), this seems to be unsuccessful theoretically because of the buffers contained in the milk.

Freezing:

Milk has been frozen for exportation, as a low temperature prevents bacterial multiplication. The milk when frozen does not decompose to any appreciable extent, but undergoes serious physical changes. The thawed product is never the same as the original milk. This process of preservation has been extensively used in Denmark.

## Difficulties in Pasteurization

Of the above methods of treatment, that of pasteurization is by all odds the most widely employed in this country. It is not, however, without its drawbacks. The desirability on the part of dairymen to maintain the cream line sometimes causes them to employ too low temperatures and to pasteurize for too short a time. Ayers and Johnson (26) by means of a circular letter sent to pasteurizing plants, in nearly all cities over 25,000 population in this country in 1923, learned that the average temperaturé used was 62.8° C (145° F.) with the "holder" process and 71.1° C. (160° F.) with the "flash" process. They report their results as follows: "The reports from 219 milk plants which pasteurized showed that 75 used the "holder" process and 144 the "flash" process." They concluded that "as pasteurization is practiced the milk might have been heated from 1 minute at 60° C. (140° F.) to 30 minutes at 76.7° C. (170 F.) and it would all be known as pasteurized."

Leslie C. Frank (27), in a paper read before the Sanitary Engineering section of the American Public Health Association, St. Louis, October 1925, said, "Milk control in the United States is now in a chaotic non-uniform state." Referring to pasteurization, he continued, "Some ordinances require a pasteurization temperature of 140, some 142, some 143, and some 145$^{\circ}$ F. Some ordinances require a holding time of 20 minutes and some of 30 minutes." It seems obvious, then, that there is no uniform method of pasteurization.

Engineering difficulties of some pasteurizing plants make for improper pasteurizing temperatures. Defects found in commercial equipment which will prevent proper pasteurization of all the milk have been grouped by Putman (28) as follows: (1) dead ends, (2) leakage through valves, (3) foam and splash, (4) defective continuous-flow units, and (5) unsatisfactory thermometers.

In dead ends, i.e., sections of pipes or other pockets in which the milk is not agitated and heated with the bulk of the milk, pasteurizing temperatures are not reached.

Leaky inlet valves permit the passage of raw milk into the holder and leaky outlet valves allow the milk to escape before it is completely pasteurized. Practically all milk valves, according to Putman (28), leak to some degree.

In 1899 Theobald Smith (29) working on the thermal death point of the tubercle bacillus indicated that the foam above the milk in the test tubes contain floating bacilli that are not subjected to the same condition as the milk beneath unless completely submerged. That the air and foam above the milk does not reach pasteurizing temperatures has been pointed out by Schorer and Rosenau (30) in 1912 and by Whittaker, Archibald, Leete and Miller (31) in 1927. The last named authors point out from their studies that pasteurization is not effective in raising the foam to a satisfactory pasteurizing temperature or in always accomplishing as great a destruction of bacteria in foam as in milk. Obviously, then, this non-pasteurized foam may furnish an inoculum for the milk.

Continuous flow holders sometimes fail to hold all of the milk at the pasteurizing temperature for thirty minutes. Instances where part of the milk in such a process was held for only three, ten and twelve minutes have been reported by Huelings, Grim, and Horn (32) and by Chilson and Wisler (33).

Putman (28) points out that the recording thermometer in general use in pasteurizing plants is not to be relied upon because, he asserts, they are not accurate. He states that, "it is not uncommon to find one registering from one degree to four degrees off." He suggests an accurate mercury-indicating thermometer as a remedy.

Certain milk borne epidemics have been traced to improper pasteurization. Such epidemics have been reported by Winslow (34), Capps and Miller (10), and others.

Again, it has been pointed out by Eddy (35), Hess and Fish (36), and others, that pasteurization, while removing dangerous germs, eliminates the anti-scorbutic vitamin C. Such anti-scorbutics as orange juice, tomato juice, etc., are prescribed for infants when feeding them pasteurized milk; so this objection to pasteurized milk is not considered of so much importance. The other drawbacks to pasteurization enumerated above, I think are not without foundation and from a public health standpoint are without doubt objectionable features.

Grant, however, that pasteurization is properly done; even then there is danger of contamination by subsequent handling. It is suggested by Ayers (37) that to prevent this "bacteriologically clean coolers, bottle fillers, bottles, and sterilized caps are necessary; and what is of greatest importance is to see that the pasteurized milk does not come in contact with human hands, or with apparatus including bottles and caps, touched by the hands after being sterilized." Pasteurization in the final container would, of course, eliminate much of the contamination incident to handling.

## Chlorination. Is it Possible?

In view of the objectionable features of pasteurization and other methods of treating milk enumerated above, it was thought that many if not all of these undesirable features might be overcome if a harmless chemical means of sterilizing milk could be evolved. Chlorination, which process is now so well established with respect to the treatment of water, suggests itself as a possible means of sterilizing milk.

History of Chlorination of Water

Race (38), from whose book "The Chlorination of Water" I am drawing freely, has summed up the history of chlorination with respect to water. Though chlorine was discovered by the Swedish chemist Scheele in 1774, it was not until 1810 that Sir Humphrey Davy established in the minds of scientists the elemental character of this substance. Its first use seems to have been that of a bleeching powder.

Chlorine was first used as a disinfectant in 1800 by de Morveau in France, and in England, by Gruikshank. It was prepared by heating a mixture of hydrochloric acid and potassium bichromate. In 1854, chlorine lime was used to deodorize the sewage of London. The American Public Health Association reported in 1885 that lime chloride was the best disinfectant available when cost and efficiency were considered. In Vienna chlorine was employed in 1895 in stamping out puerperal fever. Electrolysed sea water and salt solutions known as "Hermite Fluid","Electrozone", etc., have been tried for sewage treatment both in this country and in Europe. Phelps (39) in 1906-07, called attention to the unnecessary stringent standards of European practice and indicated the dosages necessary for crude sewage and filter effluents. Race (38) states that this "marks the commencement of a new era in sanitary science."

Credit for the first systematic use of chlorine in water as a disinfectant, according to Race (38), is due to Houston and McGowan (40), who carried out some rather extensive work at Lincoln in 1904 and 1905. The dosage they used was 1 part per million of available chlorine in the form of sodium hypochlorite.

The first commercial successful attempt at chlorination was made by G. A. Johnson (41) who, in 1908, eliminated the copper sulphate treatment of the effluent of the Union Stock Yards of Chicago at the Bubbly Creek filter plant, and substituted in its stead chlorine treatment in the form of bleaching powder with a dosage of 1.5 p.p.m. About the same time Johnson and Leal (41) began treatment of a Jersey City, N.J. water supply, using .2 p.p.m. of available chlorine. This became the cause of a law-suit in which the Court decided in favor of Chlorination.

Since 1911, municipal water chlorination has become almost universal in this country. Water is being treated at present both alone and in conjunction with filter plants.

## History of Milk Chlorination

The history of milk chlorination is short and very limited. No commercial attempts in this direction have been reported, and laboratory experiments date from 1922 when Mansell (13) undertook to chlorinate milk for the purpose of discovering whether or not milk could be kept from souring in the tropics by some easier method and more reliable than boiling. Bleaching powder solution having a titer of 1.87% available chlorine was used.

Amounts ranging from .5 cc to 5.5 cc of this solution were added to 100 cc amounts of milk. He detected the presence or absence of chlorine by means of a color reaction using starch solution and zinc iodide as indicator. The chlorine was allowed to act one half hour before plating on agar plates for colony count. The effect of varying amounts of bleaching powder solution was tried on B. typhosus, B Flexner, B. Gartner, and B cholerae in milk.

Mansel's results are interesting. With regard to smell and taste, when 2.5 cc of bleaching powder solution was introduced in 100 cc of milk, chlorine could be detected at the end of two hours but not at the end of three. With the addition of 3 cc of solution, chlorine was noticeable at the end of three hours, but not long after that, though available chlorine could be demonstrated chemically to be present. When 100 cc amounts of milk were treated with 2 cc of the bleaching powder solution, it kept sweet for from thirty to thirty-six hours at 85° F. His conclusions were as follows:

1. Milk may be kept fresh by simple and inexpensive process without any special precautions, the only precaution necessary is that of thoroughly stirring the milk when the bleaching powder solution is added.

2. Milk treated by this method is not appreciably altered as to taste or fat content. After allowing time for the

action of the chlorine.

3. Infected milk may be rendered safe for consumption. The most commonly surviving organisms are B. acidi lactici and B. lactis aerogenes.

In 1923, Hale and Bleecker (42) did some work on the chlorination of milk using various chlorinating agents. They tried out sodium hypochlorite in both neutral and alkaline solutions, calcium hypochlorite, and chlorine water. Their results seem to be in good agreement with the results of Mansell. They sum up their experiments as follows:

1. "Active chlorine does act as a germicide in milk and in ice cream with a reduction in the number of bacteria in general proportional to the amount of active chlorine present.

2. "Chlorine water gives as satisfactory results in 45 minutes as sodium hypochlorite does in 90 minutes, or as calcium hypochlorite does in 19 hours. The chlorine water could be used in higher concentration than the other two without an effect upon the flavor." The authors express themselves that it is not their wish for their paper to be considered as a recommendation for the chlorine treatment of market milk.

The same year (1923) Zoller and Eaton (43), and Zoller (44) published a series of articles on the action of sodium hypochlorite in cow's milk. They are quite definite in stating that "there are many natural reasons outside of the Food and Drugs Acts which preclude the possibility of its use to sterilize milk."

One study seemed to prove that sodium hypochlorite loses chlorine to some of the constituents of the milk, and that there is a limit to the quantity of the substance which can disappear in the milk in a given time. To test the bactericidal action of sodium hypochlorite in milk, varying concentrations, ranging from .1% to .0001% available chlorine were added to 100 cc quantities of milk at 40° C. and allowed to act from 5 to 15 minutes.

Their conclusions on one part of this work is stated thus: "In regard to cow's milk, we can say that sodium hypochlorite has a relatively low bactericidal effect, in view of its vigorous bactericidal action on the bacterial cells themselves. Only when an excess of sodium hypochlorite was present (as indicated by starch sodium iodide test) did we consistently obtain more than 50% reduction in the plate count; and then the highest obtained was 80.8%. The milk is always a pinkish-yellow color in the presence of 0.1% of added available chlorine".

These authors further conclude that the presence of the organic matter in the milk seems to protect the vitality of the organisms, as regards their power to reproduce on a milk agar plate, even after exposure to an excess of sodium hypochlorite in the milk.

In full agreement with this are the conclusions of Minett (12) who, in 1925, conducted some experiments in Hong Kong. He used chlorine in the form of 4% solution of chlorinated lime.

The chlorinated lime gave an analysis of 29.9% available chlorine. Of this he used from .5 to 2.5 cc per 100 cc of milk. From his experiments he came to the conclusion that chlorine as a milk purifier was of no value, even if allowed under the Food and Drugs Act. He grants that it keeps milk from going sour and from clotting for a considerable time, but that it does not kill off the organisms as it does in water. He attributes this to the inability of the chlorine to penetrate the fat globules, and possibly other constituents of the milk. He assumes that chlorine, to be efficient, must get at the organisms quickly and in a free state. "Any fatty or albuminous substance present," he asserts, " seems to eat up the free chlorine and so prevent its action on the organisms themselves."

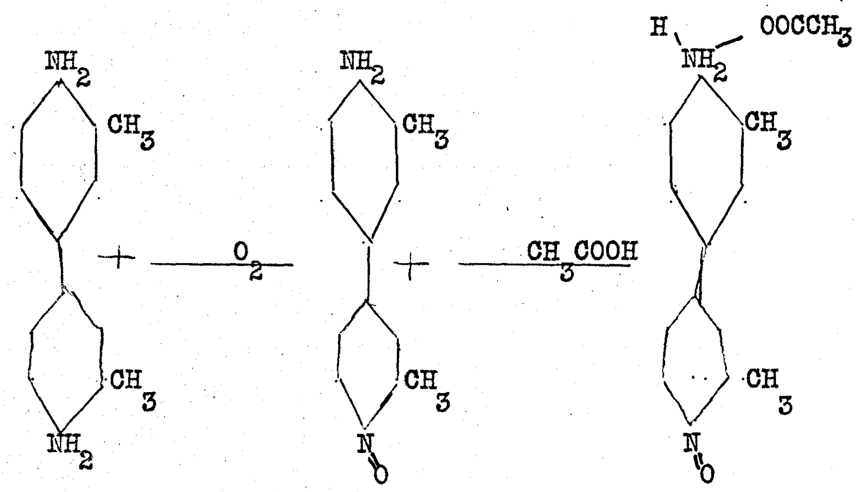
After a perusal of the literature it seems evident that no one has yet devised a harmless chemical means of sterilizing market milk. Those who have worked on the chlorination of milk have come to various conclusions. Mansell (13), who seems to be the first to have recorded any attempts at milk chlorination reports rather favorably. Hale and Bleecker (42) gave a favorable report of its possibility, but they did not wish to be understood as recommending chlorine treatment of market milk. Zoller and Eaton (43) do not consider it practical, and Minette (12) is of the same opinion.

## The Ortho-Tolodine Test

Before attempting the following experiments on the chlorination of milk, means for determining the amount of chlorine introduced into the milk were considered. The ortho-tolodine test, devised by Ellms and Hauser (46) was decided upon. The authors of this test claim that it is specific for chlorine in the absence of other oxidizing agents and that it has a sensitivity of 0.005 parts per million of free chlorine. The reagent as perfected by Ellms and Hauser consisted of 0.1% o-tolodine in 10% solution of Hydrochloric acid. This reagent is stable and produces with small quantities of chlorine a yellow color, though its preparation is somewhat tedious.

Ortho-tolodine is a colorless reagent, which, in the presence of large amounts of chlorine turns red in color and precipitates. In the presence of small amounts of chlorine, a yellow color is produced, the intensity of which is directly proportional to the amount of chlorine present.

Suggestions for the causes of these color changes are made by Ellms and Hauser (46) in the following formulae:




O-Tolodine
p-2-diamide-m-2
dimethylphenyl

Nitroso compound
<u>blue</u>

Acid salt
<u>Yellow</u>

mixture of these two probably green

"The red color and red precipitate produced by large amounts of chlorine may be a substitution product of the nitroso compounds resulting from the complete oxidation of all the o-tolodine present."

Permanent color standards were made up according to the specifications of the originators of the test, using copper sulphate and potassium bichromate in sulphuric acid solution. These standards were made to cover a range from 0.01 p.p.m. to 10. p.p.m. of available chlorine as follows:

Potassium bichromate solution: 0.025 gr. plus 0.1 cc concentrated sulphuric acid, diluted to 100 cc with distilled water.

Copper Sulfate solution: 1.5 gr. $Cu{\cdot}SO_4{\cdot}5H_2O$ plus 1 cc concentrated sulphuric acid, diluted to 100 cc with distilled water.

| Parts per Million | Copper Sulphate cc | Potassium bichromate cc |
|---|---|---|
| 0.01 | -- | 0.8 |
| 0.02 | -- | 2.1 |
| 0.03 | -- | 3.2 |
| 0.04 | -- | 4.3 |
| 0.05 | 0.4 | 5.5 |
| 0.06 | 0.8 | 6.6 |
| 0.07 | 1.2 | 7.5 |
| 0.08 | 1.5 | 8.7 |
| 0.09 | 1.7 | 9.0 |
| 0.10 | 1.8 | 10.0 |

Standards higher than 0.1 parts per million require a stronger potassium bichromate solution; i.e. 0.25 gr. plus 1 cc concentrated sulphuric acid, diluted to 100 cc with distilled water.

| Parts per Million | $CuSO_4$ solution cc | Potassium bichromate solution cc |
|---|---|---|
| 0.10 | 1.8 | 1.0 |
| 0.20 | 1.9 | 2.0 |
| 0.30 | 1.9 | 3.0 |
| 0.40 | 2.0 | 3.8 |

continued---

| Parts per million | $CuSO_4$ solution cc | Potassium bichromate solution cc |
|---|---|---|
| 0.50 | 2.0 | 4.5 |
| 0.60 | 2.0 | 5.1 |
| 0.70 | 2.0 | 5.8 |
| 0.80 | 2.0 | 6.3 |
| 0.90 | 2.0 | 6.7 |
| 1.00 | 2.0 | 7.2 |
| 2.00 | 2.0 | 12.0 |
| 3.00 | 2.0 | 21.0 |
| 4.00 | 2.0 | 30.0 |
| 5.00 | 2.0 | 39.0 |
| 6.00 | 2.0 | 46.0 |
| 7.00 | 2.0 | 56.0 |
| 8.00 | 2.0 | 63.0 |
| 9.00 | 2.0 | 70.0 |
| 10.00 | 2.0 | 75.0 |

## Detection of Chlorine in Milk

There is no test yet reported for the detection of chlorine in milk that is sensitive enough for the purpose desired in this work. The test devised by Phillip Rupp (46) which seems to be the most sensitive colorimeteric test reported in the literature for the determination of chlorine in milk, is claimed to be sensitive to 1 part in 50,000 of milk. This, of course, is much out of the range for a satisfactory reagent. Keister (47) has pointed out that the Rupp test is eight times as sensitive to copper as it is to chlorine and that in running the test the presence of copper should be ruled out. Perhaps a more serious objection to the test for the purpose of these experiments lies in the fact that this test purports to test the chlorine both free and combined with the constituents of the milk, by first releasing the chlorine from combination with the milk proteins. What is wanted here is a test to detect tiny amounts of free chlorine and not so much that which is combined. Since in water such small amounts as 0.01 p.p.m. of available chlorine is injurious to bacterial growth, obviously, a test that detects chlorine no closer that 1 part in 50,000 would be of little value.

Unsuccessful attempts were made to detect minute quantities of available chlorine in chlorinated milk by separating the whey and solid portion and testing for chlorine in the whey by the ortho-tolodine test. A yellow-green water soluble pigment (lactochrome) interferes with the test. This pigment has the following properties according to Rogers (7). "It is composed of C,H,O and N. Its composition is still unknown. It contains free amino groups. It is not salted out of its aqueous solutions. It gives no color reactions except those of phenylalanine. It reduces ammonical silver solutions in the cold, It is precipitated by silver nitrate, mercuric acetate (both acid and alkaline solutions), mercuric nitrate, basic lead acetate, copper salts, and phosphotungstic and phosphomolybdic acids, the last two precipitates being soluble in dilute acids. On heating with acids, amorphous brown or black precipitates of melanin are formed. Lactochrome is soluble only in water, dilute ethyl alcohol, methyl alcohol, and in mixtures of chloroform and ethyl alcohol. Its solutions readily bleach in the light."

In view of the fact that this pigment, because of its color, masks the o-tolodine test in the whey, attempts were made to remove it by precipitation and still leave the chlorine in solution. Phosphotungstic and phosphomolybdic acids both looked promising for this purpose because each precipitated the lactochrome and no precipitate was formed when dropping chlorine water into either of these acids. After removing the precipitate by filtration, however, and the o-tolodine reagent was added, another precipitate formed, which masked the color. This is by no means conclusive and further work may reveal that the o-tolodine test for available chlorine in milk can be run on the whey; although Hale and Bleecker think that the o-tolodine test is valueless when applied to milk.

## OBJECTS OF THIS STUDY

The study herein reported was undertaken with the idea of repeating some of the work that has been done, and extending the study wherever it seemed desirable to do so.

First of all, it was decided to study the effect of varying concentrations of sodium hypochlorite, calcium hypochlorite, chlorine water, "Diversol", and free chlorine on the number of bacteria in milk as determined by the colony count, and further to study the effect of varying concentrations of chlorine on appearance, smell, taste, and reaction of milk.

The second object of this work was to study the effect of chlorination on the per cent of butter fat.

A third object was to study the effect of chlorination on various pathogens, and upon Streptococcus lactis.

## THE EFFECT OF CHLORINATION ON THE BACTERIAL COUNT, APPEARANCE, SMELL, TASTE, AND REACTION OF MILK.

Methods:

The following methods were employed:

One hundred cc amounts of raw milk were measured and put into 110 cc oil bottles that had previously been sterilized in an autoclave at 15 pounds pressure for 20 minutes. The chlorine solution which had been previously titrated for available chlorine was then added to the milk in varying amounts. The milk was then agitated thoroughly and allowed to stand for 30 minutes at room temperature with frequent agitation before plating. One untreated bottle was used as a control in every case.

After thorough agitation the milk was then plated out as follows: 1 cc of the sample was removed with a sterile 1 cc pipette and put into a 99 cc water blank which had previously been sterilized in an autoclave at 15 pounds pressure for 15 minutes. This made a 1 to 100 dilution of the milk. This dilution was thoroughly shaken and then with another sterile 1cc pipette, 1 cc of this dilution was removed and put into another 99 cc sterile water blank. This made a 1 to 10,000 dilution which was also shaken. After thorough mixing 1 cc amounts were transferred with sterile pipettes from each of the dilutions to two sterile petri dishes for each dilution. Duplicate plates were always made. Plate and water controls were run with each sample. Melted nutrient agar was poured into the plates at 42° C. and the contents well mixed.

The plates were then incubated at 37.5° C. for 48 hours after which a plate colony count was made. The 1 to 100 and 1 to 10,000 dilutions proved to be sufficient so that the plate count could be conveniently made from one or the other of these dilutions. Only in few cases was it necessary to count part of the plate and multiply the average number by the area of the plate. Jeffer's plate counter was used in counting the plates as was also a hand lens of the type specified in "Standard Methods of Milk Analysis."

## Action of Calcium Hypochlorite

A solution of calcium hypochlorite (bleaching powder) was first used as a means of chlorination. The solution as used titrated 2.5% available chlorine by the o-tolodine method. Three different samples were chlorinated, the results of which are tabulated in Table I.

Table I. Action of Calcium Hypochlorite (Bleach)

| Amount of Bleach Sol. 2.5% Av. Chlorine added to 100 cc milk | Time of acting | Bacteria | Gas in Lactose Broth |
|---|---|---|---|
| Sample 1. Untreated | 30 minutes | 60,000 | None |
| 1 cc | " | 90,000 | " |
| 2 cc | " | 50,000 | " |
| 3 cc | " | 57,000 | " |
| 4 cc | " | 40,000 | " |
| 5 cc | " | 26,000 | " |
| 6 cc | " | 11,000 | " |
| 7 cc | " | 20,000 | " |
| 8 cc | " | 18,000 | " |
| 9 cc | " | 3,700 | " |
| 10 cc | " | 6,500 | " |
| 11 cc | " | 6,000 | " |
| Sample 2. Untreated | " | 110,000 | " |
| 1 cc | " | 100,000 | " |
| 2 cc | " | 90,000 | " |

Table I. continued--

| Amount of Bleach Sol. 2.5% av. Chlorine added to 100 cc Milk | Time of acting | Bacteria | Gas in Lactose Broth |
|---|---|---|---|
| Sample 2. 3 cc | 30 minutes | 25,000 | None |
| 4 cc | " | 37,000 | " |
| 5 cc | " | 40,000 | " |
| 6 cc | " | 20,000 | " |
| 7 cc | " | 41,000 | " |
| 8 cc | " | 7,000 | " |
| 9 cc | " | 11,000 | " |
| 10 cc | " | 10,000 | " |
| 11 cc | " | 6,800 | " |
| Sample 3. Untreated | " | 90,000 | " |
| 1 cc | " | 28,000 | " |
| 2 cc | " | 30,000 | " |
| 3 cc | " | 40,000 | " |
| 4 cc | " | 19,000 | " |
| 5 cc | " | 27,000 | " |
| 6 cc | " | 26,000 | " |
| 7 cc | " | 20,000 | " |
| 8 cc | " | 18,000 | " |
| 9 cc | " | 8,700 | " |
| 10 cc | " | 600 | " |
| 11 cc | " | 100 | " |

That the quality of the milk used in these tests so far as the bacteria are concerned, is fairly good may be assumed by the moderately low bacterial count and the absence of gas in lactose broth. It will be noticed too, that while there is in general a germicidal effect proportional to the amount of the chlorine added, yet there is considerable inconsistency. Hale and Bleecker (42) had somewhat similar results using sodium hypochlorite and they suggested that "this variation is probably due to differences in composition and in the quality in the milk used." This perhaps was true under the conditions of their experiment, but it is difficult, under the conditions of the work recorded above, to see how that a supposedly homogeneous solution such as milk would vary so greatly in composition within the same sample. I do not attempt to explain the variation but it may be within the range of technical error.

## Action of Bleaching Powder

Bleaching powder was next applied in the form of straight bleach which was well mixed when put into the milk. The following results, given in Table II were obtained.

Table II. Action of Bleaching Powder.

| Amount of Bleach added to 100 cc of milk | | Time for acting at 25° C. | Bacteria | Gas in Lactose Broth |
|---|---|---|---|---|
| Sample 1. | Untreated | 30 minutes | 1,540,000 | None |
| | 1 gram | " | 30,000 | " |

Table II continued--

| Amount of Bleach added to 100 cc Milk | Time for acting at 25° C. | Bacteria | Gas in Lactose Broth |
|---|---|---|---|
| 2 grams | 30 minutes | 2,800 | none |
| 3 " | " | 9,200 | " |
| 4 " | " | 1,400 | " |
| 5 " | " | 100 | " |
| 6 " | " | 400 | " |
| 7 " | " | sterile | " |
| 8 " | " | 400 | " |
| 9 " | " | 400 | " |
| 10 " | " | 300 | " |

The bleaching powder used in this case titrated 25% available chlorine, thus each gram made a dilution of 0.25% available chlorine when diluted in 100 cc volume of milk. This, of course, is an overwhelming amount of chlorine and one gram reduced the bacterial count about 98%. The bleaching powder was certainly bactericidal, but here again, as in Table I, there is some inconsistency. The appearance of the milk was also changed. One gram of the bleaching powder produced a slight cream color and it graded from this to light brown in the succeeding bottles, getting darker with each higher concentration. A peculiar smell and taste accompanied the color. Chlorine could not be tasted or smelled until the bottle to which three grams had been added was tasted.

## Action of Sodium Hypochlorite "B.K."

Sodium hypochlorite in the form of commercial "B.K." was tried, using the same methods indicated above. The product was titrated for available chlorine by the ortho-tolodine method and varying amounts of a solution containing 2.5% available chlorine was introduced into the milk which was allowed to stand 30 minutes at room temperature, plated out, and colony count made 48 hours later. The results are given in Table III.

Table III. Action of Sodium Hypochlorite (B.K.)

| Amount of B.K. 25% av. Chlorine added to 100 cc milk | Time acting at 25° C. | Bacteria | Gas in Lactose. |
|---|---|---|---|
| Untreated | 30 minutes | 600,000 | none |
| ½ cc | " | 105,000 | " |
| 1 cc | " | 120,000 | " |
| 2 cc | " | 82,000 | " |
| 3 cc | " | 136,000 | " |
| 4 cc | " | 110,000 | " |
| 5 cc | " | 70,000 | " |
| 6 cc | " | 6,800 | " |
| 7 cc | " | 500 | " |
| 8 cc | " | 300 | " |
| 9 cc | " | sterile | " |
| 10 cc | " | 800 | " |
| Plate control | -- | sterile | --- |
| Water | -- | sterile | --- |

Here again it will be noticed that there is in general a germicidal effect proportional to the amount of available chlorine used. The milk did, however, have a decidedly altered and unpleasant taste even where the smallest amount of "B.K." was used. From 2 cc up there was an apparent change in smell, where 3 cc or more had been used the color of the milk changed, (ranging from slight creamy color to pale pink), and where 5 cc or more had been used a precipitate was apparent. The reaction to litmus was neutral in all the milk after 72 hours in the refrigerator at $8^{\circ}$ C.

## Action of "Diversol"

The next substance tried was "Diversol" a product of the Diversey Manufacturing Company of 53 W. Jackson Boulevard, Chicago. Diversol, according to its manufacturers, is a combination of an alkaline sodium phosphate combined with sodium hypochlorite in the form of a solid solution, the hypochlorite being contained in the former's water of crystallization. It contains about 94% alkaline sodium phosphate, 3% sodium hypochlorite, and 3% sodium chloride. Titration showed the Diversol to contain 3% available chlorine.

Varying amounts of Diversol were added to the milk in the powder form and mixed well. The results are given in Table IV.

Table IV. Action of "Diversol"--3/4 to 3% available Chlorine

| Amount of Diversol 3% available chlorine added to 100 cc milk | Time acting at 25° C. | Bacteria | Gas in Lactose |
|---|---|---|---|
| Untreated | 30 minutes | 1,580,000 | None |
| 1 gram | " | 630,000 | " |
| 2 " | " | 350,000 | " |
| 3 " | " | 40,000 | " |
| 4 " | " | 830,000 | " |
| 5 " | " | 20,000 | " |
| 6 " | " | 110,000 | " |
| 7 " | " | 15,000 | " |
| 8 " | " | 90,000 | " |
| 9 " | " | 70,000 | " |
| 10 " | " | 2,000 | " |

Here again the results are much the same as in the previous tables. A germicidal effect will be noticed, in general, proportional to the concentration of the hypochlorite, but variations occur. The increase in concentration of "Diversol" was accompanied by a corresponding increase of yellow to brown coloration, granular appearance, peculiar odor, bad taste, and separation of whey which was colored from golden to brown.

Hale and Bleecker (42) had better success with chlorine water than they had with either sodium or calcium hypochlorite. By using starch iodide as an indicator they showed that active chlorine disappeared much more readily when introduced as chlorine water than when they used sodium or calcium hypochlorite. The probable reason for this, as they suggest, is that the chlorine is more readily available in the form of chlorine water and hence acts more rapidly.

## Action of Chlorine Water

The chlorine water used in this work was prepared by allowing liquid chlorine from a drum to bubble through a bottle of distilled water at 28° C. The first chlorine water so prepared titrated 0.6% available chlorine by the o-tolodine method. Varying amounts of this was put into the milk and allowed to stand 30 minutes, as in the tests above, after which it was plated out and incubated at 37.5° C. for 48 hours. The results so obtained are given in Table V.

Table V. Action of Chlorine Water --0.6% Av. Chlorine

| Amount of Chlorine water-.6% av. Cl. added to 100 cc milk | | Time acting 26° C. | Bacteria | Gas in Lactose- |
|---|---|---|---|---|
| Series A. | Untreated | 30 minutes | 300,000 | none |
| | 0.5 cc | " | 110,000 | " |
| | 1.0 cc | " | 180,000 | " |
| | 1.5 cc | " | 120,000 | " |
| | 2.0 cc | " | 150,000 | " |
| | 2.5 cc | " | 70,000 | " |

Table V--continued--

| Amount od Chlorine water .6% av. Cl. added to 100 cc milk | | Time acting $26^{0}$ C. | Bacteria | Gas in Lactose |
|---|---|---|---|---|
| Series A. | 3 cc | 30 minutes | 110,000 | none |
| | 3.5 cc | " | 200,000 | " |
| | 4.0 cc | " | 68,000 | " |
| | 4.5 cc | " | 30,000 | " |
| | 5.0 cc | " | 15,000 | " |

In the next series, the chlorine water used only titrated 0.5% available chlorine. The results of this series is given in Table VI.

Table VI. Action of Chlorine Water--0.5% available Chlorine

| Amount of Chlorine water .5% av. Cl. added to 100 cc milk | | Time acting $27^{0}$ C. | Bacteria | Gas in Lactose |
|---|---|---|---|---|
| Series B. | Untreated | 30 minutes | 60,000 | none |
| | 0.5 cc | " | 9,700 | " |
| | 1.0 cc | " | 50,000 | " |
| | 2.0 cc | " | 21,000 | " |
| | 3.0 cc | " | 14,000 | " |
| | 4.0 cc | " | 10,000 | " |
| | 5.0 cc | " | 6,500 | " |
| | 6.0 cc | " | 20,000 | " |
| | 7.0 cc | " | 20,000 | " |

Table VI--continued

| Amount of Chlorine Water 0.5% av. Cl added to 100 cc milk | Time acting 27° C. | Bacteria | Gas in Lactose |
|---|---|---|---|
| Series B. 8.0 cc | 30 minutes | 40,000 | none |
| 9.0 cc | " | 30,000 | " |
| 10.0 cc | " | 25,000 | " |

Another series of tests were made in which chlorine water titrating 0.3% available chlorine was used. The results of these tests are given in Table VII.

Table VII. Action of Chlorine Water 0.3% available chlorine

| Amount of Chlorine water 0.3% av. Cl. added to 100 cc milk. | | Time acting 25° C. | Bacteria |
|---|---|---|---|
| Series C | | | |
| Sample 1 | Untreated | 30 minutes | 430,000 |
| | 1 cc | " | 380,000 |
| | 2 cc | " | 380,000 |
| | 3 cc | " | 290,000 |
| | 4 cc | " | 280,000 |
| | 5 cc | " | 330,000 |
| | 6 cc | " | 290,000 |
| | 7 cc | " | 270,000 |
| | 8 cc | " | 250,000 |
| | 9 cc | " | 150,000 |

Table VII --continued---

| Amount of Chlorine Water 0.3% av. Cl. added to 100 cc milk | Time acting 25° C. | Bacteria |
|---|---|---|
| Sample 1 | | |
| 10 cc | 30 minutes | 350,000 |
| 20 cc | " | 121,000 |
| Sample 2 | | |
| Untreated | " | 1,590,000 |
| 1 cc | " | 1,050,000 |
| 2 cc | " | 1,160,000 |
| 3 cc | " | 650,000 |
| 4 cc | " | 1,500,000 |
| 5 cc | " | 880,000 |
| 6 cc | " | 930,000 |
| 7 cc | " | 1,500,000 |
| 8 cc | " | 1,430,000 |
| 9 cc | " | 1,170,000 |
| 10 cc | " | 870,000 |
| 20 cc | " | 820,000 |
| Sample 3 | | |
| Untreated | " | 3,900,000 |
| 1 cc | " | 3,400,000 |
| 2 cc | " | 2,140,000 |
| 3 cc | " | 230,000 |
| 4 cc | " | 3,300,000 |
| 5 cc | " | 2,700,000 |

Table VII continued---

| Amount of Chlorine Water 0.3% Av. Cl. added to 100 cc milk. | Time acting 25° C. | Bacteria |
|---|---|---|
| Sample 3 | | |
| 6 cc | 30 minutes | 1,200,000 |
| 7 cc | " | 3,700,000 |
| 8 cc | " | 1,940,000 |
| 9 cc | " | 3,700,000 |
| 10 cc | " | 2,900,000 |
| 20 cc | " | 3,400,000 |

A discussion of the results recorded in Tables V and VI would be much the same as that for the results obtained by using the various hypochlorites. There is in general a germicidal effect but some of the results are inconsistent with the amount of chlorine used. An interesting observation may be made however, by comparing Tables I and VI. It will be noticed that about the same quality of milk was used in each case, the untreated milk having a colony count of 60,000. In general the reduction in the count compared to the amount of solution used is somewhat similar. It will be observed, however, that the calcium hypochlorite solution used titrated 2.5% available chlorine, whereas the chlorine water titrated only 0.5% available chlorine by the o-tolodine method. It would seem that the chlorine in the chlorine water gets at the bacteria more quickly and perhaps more efficiently than it does in calcium hypochlorites.

In Table VII, where chlorine water titrating 0.3% available chlorine was used, and where the milk had a higher bacterial count, the germicidal effect is not so pronounded and is not definitely proportional to the amount of available chlorine used.

Another feature that was noticed with respect to chlorine water treatment is that in no instance was there any noticeable change in the normal appearance of the milk, whereas, with the hypochlorites there was marked change both in color and consistency.

The smell of chlorine was not apparent after standing two hours, except where more than 8 cc of 0.6% and 9 cc of 0.5% chlorine water had been added to 100 cc of milk. It was not detected after 24 hours in any of the bottles; neither was it detected at all in any bottles to which 0.3% chlorine water had been added. Although the smell of chlorine was not apparent there was an odor, different from untreated sweet milk, and strangely peculiar to all the milk that had been chlorinated.

The taste of the milk to which chlorine water had been added was peculiar and, in higher concentrations, unpleasant. Above 6 cc of 0.6% chlorine water and 8 cc of 0.5% chlorine water added to 100 cc of milk, chlorine could be detected by taste.

Milk to which 7 cc or more of chlorine water had been added did not turn sour in 24 hours at $25^{\circ}$ C., but there was none that did not turn sour in 48 hours at this temperature.

## The Effect of Free Gaseous Chlorine

The next attempt was to chlorinate milk by simply bubbling through it chlorine gas released from a drum containing liquid chlorine. The chlorine was obtained from the Lawrence City Water Plant and is a product of the Wallace and Tiernan Company.

Method:

To the valve that was attached on the drum was connected a small flexible copper pipe. To this was fastened a piece of rubber tubing on the end of which was affixed a 1 cc pipette. The valves were turned and so adjusted that only a small volume of gas was allowed to escape. This was bubbled into a bottle of water and the number of bubbles per minute were counted. The gas was then bubbled into a 100 cc of chlorine-free distilled water for 60 seconds and the solution then titrated by the o-tolodine method for the amount of available chlorine that it contained. The gas was then bubbled into 100 cc amounts of milk in oil bottles for varying lengths of time. The milk was allowed to stand for 30 minutes after chlorination, then plated out and incubated for 48 hours. An untreated control was run with each bottle and a plate and water control was run for each set. Duplicate plates were made throughout and the same dilutions used here as in the earlier tests.

The amount first tried was 118 bubbles per minute. This amount of chlorine when bubbled through chlorine-free distilled water at 25° C. consistently gave an o-tolodine test of 70 parts per million of available chlorine. One hundred cc amounts of milk were thus chlorinated in oil bottles for 1 minute, 2 minutes, 3 minutes, 4 minutes, 5 minutes, 10 minutes, 15 minutes, 20 minutes, 30 minutes, and 1 hour respectively. The effect of this on the colony count is recorded in Table VIII.

Table VIII. The Action of Free Chlorine Gas-118 bubbles per Minute

| Time acting 118 bubbles per min. in 100 cc milk. | Bacteria in treated | -untreated | Color | Consistency | Smell | Taste | Reaction litmus |
|---|---|---|---|---|---|---|---|
| | | | After four hours | | | | |
| 1 minute | 39,000 | 40,000 | normal | norm. | norm. | norm. | neut. |
| 2 minutes | 32,000 | 330,000 | " | " | " | peculiar | " |
| 3 " | 90,000 | 200,000 | sl. creamy | ppt | " | " | " |
| 4 " | 55,100 | 265,000 | " | " | " | grainy | " |
| 5 " | 35,600 | 152,000 | " | " | " | " | flat acid |
| 10 " | 95,000 | 735,000 | " | " | cooked | " | " |
| 15 " | 60,000 | 105,000 | " | " | " | " | " |
| 20 " | 196,000 | 700,000 | " | " (heavy) | " | " | " |
| 30 " | 260,000 | 730,000 | " | " | cl." | " | " |
| 60 " | 120,000 | 1190,000 | " | " | " | " | CL. " |

After the milk was thus chlorinated it was kept at about 27° C. and observed at 24 hour and 48 hour intervals. In 24 hours, it was observed that the milk that had been five minutes or less had coagulated whereas none was coagulated above that.

In discussing the results expressed in Table VIII, it should be pointed out that there is no definite information given as to the amount of available chlorine that was introduced into the milk per minute. Although the O-tolodine test showed that water had taken up 70 parts per million in one minute, it does not necessarily follow that the milk would absorb a like amount. There is, in fact, good reason for believing that much more chlorine would be taken up by the milk. Zoller (44) has shown that the absorption of chlorine by milk and sodium caseinate is very rapid. He suggests that the possible influence of the SH groups in the casein molecule may be partially responsible for the rapid absorption of chlorine. He infers, probably correctly, that much of the chlorine absorbing power of milk is due to the proteins and nitrogenous bodies contained therein.

Although the amount of chlorine that the milk takes up in one minute is not known, still the means employed does give, I think, a basis of comparison suitable for the purpose of this work, since the point of primary interest is not to determine the amount of chlorine absorbed by the milk, but whether or not milk can be successfully chlorinated.

There are two things that may be worthy of note in the results recorded in Table VIII. First, that there is in each instance from 2 minutes upward a considerable (in some cases as high as 90%) decrease in the colony count; and second, that concurrent with this there is a peculiar taste, grainy feel to the tongue and pallate, a cooked smell, a precipitate that ranges from a slight one with small amounts of chlorine to a heavy one in the bottles to which large amounts of chlorine were added, and that there is a change in color that ranges from a slight creamy to a rich creamy color depending upon the amount of chlorine added to the milk.

It was learned, as a point of technique, that by agitating the milk vigorously during the chlorinating process that the amount of precipitate formed can be considerably reduced.

Another sample of milk was chlorinated by the free chlorine gas method. This time 150 bubbles per minute or about 90 parts per million per minute (in water) were added. The milk was chlorinated at 25° C. and the chlorine allowed to act for 30 minutes before plating. The results after 48 hours are recorded in Table IX.

Table IX. Action of Free Chlorine Gas--150 Bubbles per minute.

| Amount 150 Bubbles per min. in 100 cc milk. | Colony Count | Color | Consistency | Smell | Taste | Reaction to litmus |
|---|---|---|---|---|---|---|
| Untreated | 243,000 | normal | normal | normal | normal | neutral |
| ½ minute | 202,000 | " | " | " | " | " |
| 1 minute | 140,000 | " | " | " | " | " |

Table IX--continued--

| Amount 150 bubbles per minute in 100 cc of milk. | Colony Count | Color | Consistancy | Smell | Taste | Reaction to Litmus |
|---|---|---|---|---|---|---|
| $1\frac{1}{4}$ minutes | 79,000 | normal | sl.ppt | normal | peculiar | neut. |
| $1\frac{1}{2}$ " | 70,000 | " | " | " | " | v.sl. acid |
| $1\frac{3}{4}$ " | 50,000 | " | " | " | " | " |
| 2 " | 17,000 | " | " | " | " | " |
| $2\frac{1}{2}$ " | 55,000 | " | " | " | " | " |
| 3 " | 54,000 | sl.creamy | " | " | " | " |
| 4 " | 70,000 | " | " | sl. Cl | acid | " |

The milk was allowed to stand at 26° C. for 24 hours when it was observed that all the milk was sour up to the one that had been chlorinated $1\frac{3}{4}$ minutes which was sweet to the smell but had a peculiar taste due to the action of the chlorine on the milk.

The same general discussion that applied to table VIII may apply to Table IX. It will be observed, however, that in the range where the milk appears to be normal there is not so great a decrease in the bacterial count. The question arose as to whether or not there is an inhibition of growth in these dilutions even though the germicidal effect is not so pronounced. Another sample was therefore chlorinated and plated out. The chlorinated milk was held a week and plated out again. The results of this experiment are recorded in Tables X and XA.

Table X--The Action of Free Chlorine Gas--128 bubbles

| Amount 128 bubbles per min. into 100 cc milk. | Colony count | Color | Consistency | Taste | Smell | Reaction to Litmus |
|---|---|---|---|---|---|---|
| Untreated | 2,400,000 | normal | normal | normal | normal | neutral |
| ½ minute | 800,000 | " | " | " | " | " |
| 1 " | 1,000,000 | " | " | " | " | " |
| 1¼ " | 1,850,000 | " | " | sl. pec. | sl.pec. | " |
| 1½ " | 1,700,000 | " | " | " | " | v.sl. acid |
| 1¾ " | 470,000 | " | " | " | " | " |
| 2 " | 330,000 | " | sl. ppt. | " | " | " |
| 2½ " | 660,000 | " | " | grainy | " | " |
| 3 " | 530,000 | " | " | " | " | " |
| 4 " | 132,000 | " | " | Cl. Acid | " | " |

The milk was placed in the refrigerator and kept at about $10^{\circ}$ C. for 7 days and then plated out again. The results obtained after 7 days are recorded in Table X a.

Table X a. Milk recorded in Table X plated 7 days later.

| Amount 128 Bubbles per min. in 100 cc milk. | Colony Count | Color | Consistency | Smell | Taste | Reaction to Litmus. |
|---|---|---|---|---|---|---|
| Untreated | 12,000,000 | normal | normal | sour | sour | acid |
| ½ Minute | 2,500,000 | " | " | normal | normal | neutral |
| 1 " | no growth | " | " | " | " | " |
| 1¼ " | 200,000 | " | " | sl.pec | sl.pec | " |

Table X a--Continued--

| Amount 128 bubbles per min. in 100 cc milk. | Colony Count | Color | Consistency | Smell | Taste | Reaction to Litmus |
|---|---|---|---|---|---|---|
| 1½ minutes | 380,000 | normal | normal | sl.pec | sl.pec | sl.acid |
| 1¾ " | 400,000 | " | " | " | " | " |
| 2 " | 350,000 | " | sl.ppt. | " | " | " |
| 2½ " | 70,000 | " | " | " | " | " |
| 3 " | 80,000 | " | " | " | " | " |
| 4 " | 130,000 | " | " | " | " | " |

It will be observed by comparing the two Tables X and Xa that even the greatest dilution of chlorine did markedly inhibit the growth of bacteria. Whereas the untreated milk was sour and had a bacterial count of 12,000,000 per cc, the milk that had been chlorinated for 1/2 minute only was still sweet and neutral to litmus. The milk was replaced in the refrigerator and observed when 10 days old, at which time 1/2 minute milk was sour, but the 1 minute and 1 ¼ minute milk was still neutral to litmus. Above this there was acid due, no doubt, to the excessive amount of chlorine which probably produced HCl and hypochlorous acid in the milk.

After the milk had been chlorinated, and, having been in the refrigerator for 24 hours it was tasted. A peculiar, though not unpleasant taste was discernable in 1 minute milk. No difference could be detected in the 1/2 minute milk however, and when samples of this milk were given together with samples of the untreated milk to different persons (six): three guessed wrong and three guessed right as to the one that had been treated. Neither of them felt sure about their decision. Much more work will need to be done before any conclusion can be made on this point.

## The Effect of Chlorination on the Amount of Butter Fat

The second object of this study was to determine the effect of chlorination on the per cent of butter fat. Does chlorination effect the per cent of butter fat? In determining the per cent of butter fat in the milk the Babcock test was used.

### Methods:

The technique observed was as follows:

1. The sample which was between 15° and 20° C. was thoroughly mixed.

2. A Babcock pipette of a 17.6 cc capacity was filled with milk to the mark and the milk emptied into the test bottle.

3. The acid measure which is calibrated for 17.5 cc was filled to the mark with commercial sulphuric acid sp. gr. 1.82 to 1.83.

4. The acid and milk was thoroughly mixed by a rotary motion with the hand and let stand three or four minutes, when

it was mixed again.

5. The contents of the bottle were centrifugalized for 4 minutes.

6. The test bottles were filled to the base of the neck with hot water, and again centrifugalized at full speed for 2 minutes.

7. Hot water was again added to bring the fat column up into the neck of the bottle and again centrifugalized for 1 minute.

8. The bottles were put in a water bath at a temperature of about 120 to 130° F. for a few minutes and the butter fat read from the top of the upper meniscus to the bottom of the lower meniscus.

The results obtained are recorded in Table XI.

Table XI. The Effect of Chlorine on the Butter Fat.

| Minutes exposure to Chlorine Gas. 118 Bubbles per minute in 100 cc milk. | Original percentage of fats. | Percentage of Fat six hours after chlorination |
|---|---|---|
| Untreated | 3.2 | 3.2 |
| 1 minute | 3.2 | 3.2 |
| 2 " | 3.2 | 2.8 |
| 3 " | 3.2 | 2.8 |
| 4 " | 3.2 | 2.9 |
| 5 " | 3.2 | 2.8 |

Table XI continued---

| Minutes exposure to Chlorine Gas. 118 Bubbles per minute in 100 cc milk | Original percentage of Fats | Percentage of Fat six hours after chlorination. |
|---|---|---|
| 10 minutes | 3.2 | 2.8 |
| 15 " | 3.2 | 2.8 |
| 20 " | 3.2 | 2.7 |
| 30 " | 3.2 | 3.2 |
| 60 " | 3.2 | 3.1 |
| Untreated | 4.2 | 4.2 |
| 1 minute | 4.2 | 4.0 |
| 2 " | 4.2 | 4.1 |
| 3 " | 4.2 | 3.9 |
| 4 " | 4.2 | 4.2 |
| 5 " | 4.2 | 3.9 |
| 10 " | 4.2 | 4.0 |
| 15 " | 4.2 | 3.9 |
| 20 " | 4.2 | 4.2 |
| 30 " | 4.2 | 4.1 |
| 60 " | 4.2 | 4.1 |

From the results of this experiment it would seem that chlorination by this method does not alter the percentage of butter fat to an appreciable extent. Mansell (13) got somewhat similar results using calcium hypochlorite.

## The Effect of Chlorination on Pathogens in Milk

A third purpose of this study was to determine the effect that chlorination has on pathogens in milk.

The organisms used were B. para typhosus , B., Brucella abortus, and B. tuberculosis. It was also thought advisable in this connection to study the effect of chlorination on Streptococcus lactis in milk.

### Methods:

The methods outlined in the earlier part of this work were again followed here. Milk was sterilized in 100 cc amounts in oil bottles, in an autoclave at 10 pounds pressure for 13 minutes. A suspension of a 24 hour culture of the organism used was made in a sterile 100 cc water blank. From this suspension each bottle of milk was inoculated with 1cc by means of a sterile pipette. The milk containing the organisms was well shaken and chlorinated. The chlorine was allowed to act 30 minutes after chlorination and then plated out in the usual manner using a 1 to 100 and a 1 to 10,000 dilution. The plates were all run in duplicate. Controls were run on milk, water, and plates. The organisms used were bona fide stock cultures of B. Paratyphosus B., Brucella abortus, and B. tuberculosis. The B. tuberculosis culture is an old stock culture that has been growing in this laboratory on plain agar for about 13 years. Grain stains were made of the colonies that developed an the plates and when compared with the stock culture, were seen to be identical in morphology and in the appearance of the colonies.

Chlorine gas was bubbled through each 100 cc amount of milk at the rate of 148 bubbles per minute, at 26$^{o}$ C.

Table XII. The Effect of Chlorine on Pathogens in Milk

| Mins. of Cl. gas, 148 bubbles per min in 100 cc milk | B. paratyphosus 30 min. | B. 3 hours | acting 24 hours | B. abortus 30 min. | B. tuberculosis 2 hours |
|---|---|---|---|---|---|
| Untreated | 1,800,000 | 5,000,000 | | 5,000,000 | 22,000 |
| $\frac{1}{4}$ minute | 1,340,000 | 1,420,000 | 110,000 | 4,640,000 | 13,900 |
| $\frac{1}{2}$ " | 1,400,000 | 1,020,000 | 520,000 | 3,600,000 | 16,500 |
| $\frac{3}{4}$ " | 940,000 | 250,000 | 10,000 | 1,980,000 | 11,700 |
| 1 " | 1,080,000 | 47,000 | 12,400 | 1,330,000 | 14,000 |
| $1\frac{1}{4}$ " | 1,000,000 | 680 | 400 | 2,120,000 | |
| $1\frac{1}{2}$ " | 530,000 | 800 | 300 | 1,080,000 | 2,400 |
| $1\frac{3}{4}$ " | 160,000 | 2,300 | sterile | 810,000 | |
| 2 " | 2,300 | 500 | " | 630,000 | 1,800 |
| $2\frac{1}{2}$ " | 11,700 | sterile | " | 643,000 | |
| 3 " | sterile | " | " | 500,000 | |

Milk controls, OK, Water controls, OK, Plate controls OK.

A bactericidal and inhibiting effect is seen to be exhibited on all three organism tested. There did not seem to be a great decrease in the colony count of B para typhosus B. when milk treated for 1/4 minute was allowed to stand for 30 minutes. After standing three hours, however, the untreated milk had increased from 1,800,000 to 5,000,000, whereas that treated for 1/4 minute remained about the same. That treated for $1\frac{3}{4}$ minutes was made sterile after being allowed to stand at 26° C. for 24 hours but it took 3 minutes treatment to make the same amount of milk sterile when allowed to act 30 minutes.

It will be seen that under the conditions of the experiment, sterility was not attained until enough chlorine had been added to produce a bad taste in the milk. Of course, it must be considered that this was an overwhelming dose of pathogens to begin with.

Streptococcus lactis was put into milk and subjected to the same treatment as that given the pathogens. The results are given in Table XIII.

Table XIII. Action of Chlorine Gas on Streptococcus Lactis.

| Mins. of $Cl_2$ gas. 148 bubbles per min. in 100 cc milk. | 30 minute colony count | 7 days colony count |
|---|---|---|
| | Time of acting | |
| Untreated | 18,000 | 370,000 |
| $\frac{1}{4}$ minute | 55,000 | 1, 600,000 |
| $\frac{1}{2}$ " | 55,000 | 670,000 |
| $\frac{3}{4}$ " | 91,000 | 48,000 |
| 1 " | 64,000 | 41,000 |
| $1\frac{1}{2}$ " | 66,000 | 52,000 |
| 2 " | 64,000 | 47,000 |

Table XIII--continued--

| Minutes of treatment | Count--acting 30 minutes | 7 days |
|---|---|---|
| $2\frac{1}{2}$ minutes | 51,000 | 24,000 |
| 3 " | 46,000 | 22,000 |
| 4 " | 18,000 | 6,000 |

From the results recorded in Table XIII, it will be noticed that there was no marked inhibition until 148 bubbles per minute had been bubbled through the milk for $\frac{3}{4}$ minute. That this amount of chlorine produced a decided inhibitory effect in the milk will be observed at once since the bacterial count after the chlorine had acted 30 minutes was 91,000 and the same milk, after standing 7 days in the refrigerator, gave a count of only 48,000. Another interesting observation is that sterility was not produced in any of the bottles to which chlorine had been added, where as, the milk containing B. paratyphosus B. was made sterile when treated with 148 bubbles of chlorine gas for 3 minutes and allowed to act for 30 minutes before plating. (Table XII). I think we can believe that according to these experiments, S. lactis is not inhibited in the milk to the same extent as are pathogens that have been here considered.

It is generally agreed that cocci are more resistant to disinfectants than are the bacilli. This may be due to the fact that cocci, being spheres, offer a minimum surface exposure for the amount of protoplasm that they contain. The difference in permiability of cocci and bacilli may be a factor.

It was thought that perhaps the acid produced by Streptococcus lactis had an effect upon the action of chlorine. Accordingly

100 cc of sterile milk was inoculated with Streptococcus lactis and the milk allowed to sour. Another 100 cc amount of sterile milk was then inoculated with Streptococcus lactis. Both bottles were treated with chlorine gas for 2 minutes at the rate of 148 bubbles per minute. The results were as follows:

Table XIV Effect of Lactic Acid on Action of Chlorine

| 2 minutes of $Cl_2$ gas 148 bubbles per min. in 100 cc milk. | Time acting | Colony count |
|---|---|---|
| Untreated sweet | 1 hour | 860,000 |
| Treated " | " | 120,000 |
| Untreated sour | " | 9,820,000 |
| Treated " | " | 28,000 |

The results do not indicate that the acid produced by Streptococcus lactis is an acid which makes for the resistance of Streptococcus lactis to the action of chlorine, since the greatest percentage reduction was in the milk that contained the largest amount of acid. The series is much too small however, and further work needs to be done before a conclusion can be made on this point.

## SUMMARY

1. A study of some features of the chlorination of milk has been presented.

2. Unsuccessful attempts were made to devise a means

whereby the ortho-tolodine test could be used to detect small amounts of chlorine in milk. The water-soluble pigment (lactochrome) interfered with the test by masking the color given in the whey. Attempts were made to remove the lactochrome, but without success.

3. The effect of calcium hypochlorite, sodium hypochlorite, "diversol", chlorine water, and free chlorine gas on the bacterial count of milk was studied. In general there was a bactericidal effect but which was in no way comparable to the action of chlorine in water, in that exceeding large amounts had to be used in milk to produce the same effect. This, in all probability, is due to the rapidity of absorption of chlorine by the proteins and other nitrogenous substances in the milk.

When sufficient amounts of chlorine were introduced to produce a marked bactericidal effect, an unpleasant taste was produced in the milk, a peculiar odor resulted, a precipitate formed, and the milk became creamy to brown in color. Less of this undesirable effect was produced by chlorine gas than with any of the other means of chlorination. Chlorine water was better than the hypochlorites.

4. Chlorination of milk by the free chlorine gas method did not alter the per cent. of butter fat to an appreciable extent.

5. Free chlorine gas did produce a bactericidal and inhibiting effect on the pathogens inoculated into the milk.

Streptococcus lactis was inhibited but not to the extent as if they were pathogens.

## CONCLUSIONS

1. The ortho-tolodine test in its present form is valueless as a test for chlorine in milk.

2. Calcium hypochlorite, sodium hypochlorite, "diversol", chlorine water, and free chlorine gas do act as germicidal agents in milk, their action being in general, proportional to the amount of available chlorine added.

3. Free chlorine gas acts more rapidly than any of the other agents tried. Chlorine water is more efficient than are the hypochlorites.

4. Excessive amounts of available chlorine produces a creamy color, granular precipitate, peculiar odor, and unpleasant taste in milk. Agitation during the chlorinating process tends to lessen these effects.

5. Chlorination of milk by the free chlorine gas method does not alter the per cent of butter fat to an appreciable extent.

6. Free chlorine gas does produce an inhibiting and bactericidal effect upon B. para typhosus B., Brucella abortus, and B. tuberculosis. It inhibits Streptococcus lactis but not to the same extent that it does the pathogens.

Bibliography

1. Heinemann, P.G., "Milk," W.B. Saunders Co., 1921, p.17.

2. Lister, J., Quart. J. Microsc. Sci., 18:189, (1878): cited by Rogers, "Fund. of Dairy Sci." Monograph 41, Chem. Catalogue Co., p. 249, (1928).

3. Roberts, W., Phil. Tr. Roy. Soc., 164:457, (1874); cited by Breed, "Newer Knowledge of Bacteriology and Immunology", U. of Chicago Press, p. 378, (1928).

4. Schulz, L., Archl Hyg., 14:260, (1892): cited by Breed, ibid.

5. Moore, V.A., U.S. Dept. Agri. Ann. Rep. Bur. An. Ind., 12 and 13:261, (1899).

6. Moore, V.A., and Ward, A.R., Cornell Agric. Exp. Sta. Bull. 178, (1900). Breed, loc. cit.

7. Rogers, L.A., "Fundamentals of Dairy Science." Monograph #41, Chemical Catalogue Co., p.252, (1928).

8. Trask, J.W., "Milk as a Cause of Epidemics of Typhoid Fever, Scarlet Fever, and Diphtheria." U.S. Hyg. Lab. Bul. 56:25-149 (1909).

9. Bray, H.A., "A milk Borne Epidemic of Tonsilitis in Tuberculosis patients." Jour. Amer. Med. Assoc., 64:1127-1130 (1915).

10. Capps, J.A., and Miller, J.L., "The Chicago Epidemic of Streptococcus Sore Throat and its Relation to the Milk Supply." Jour. Amer. Med. Assoc., 58:1848-1852, (1912).

11. Hamburger, L. P., "An Epidemic of Septic Sore Throat in Baltimore and its Relation to a Milk-supply, A Preliminary

Report", Jour. Amer. Med. Assoc., 58:1109-1111 (1912)

11 a Am. Jour. of Pub. Health. 17:783 (1927).

12. Minett, E.P., "Notes on the Chlorination of Milk," Jour. of the Roy. Army.Med. Corps, 44:116-118, (Feb, 1925).

13. Mansell, R.A., "The Chlorination of Milk", Jour. of the Roy. Army Med. Corps, 39:372-377, (Nov. 1922).

14. Zeit, F.R., "Effect of Direct, Alternating, Tesla Currents, and X-Rays on Bacteria." Jour. Amer. Med. Assoc., 37: 1432, (1901).

15. Anderson, A.K., and Finkelstein, R. "A Study of the Electro-Pure Process of Treating Milk," Jour. Dairy Sci, 2:374-406, (1919).

16. Chilson, C.H., 10th Ann. Rept. Internat. Assoc Dairy and Milk Inspectors, p.308, (1921). Cited by Rogers, loc. cit.

17. Beattie, J.M., and Lewis, F.C., "Electric Current (Apart from Heat Generated) a Bacteriological Agent in the Sterilization of Milk andother Fluids", Jour. of Hyg. 24:123 (Oct. 1925).

18. Prescott, S.C., "The Treatment of Milk by an Electrical Method," Amer. Jour. of Public Health, 17:221 (1927).

19. Downes, A., and Blunt, T. P., Proc. Roy. Soc., 26:488, (1877), cited by Norton "New Knowledge of Bacteriology and Immunology," U. of Chicago Press., 371 (1928).

20. Arloing, M.S., "Influence de la lumiere sur la vegetation et les proprietes pathogenes du B. anthracis," Compt.

Rend. Acad. de Sci., 100:378, (1885): also "Influence du Soleil sur la vegetabilite des spores du B. anthracis," Compt. rend. Acad. de Sci., 101:511, (1885)

21. Duclaux, E., "Influence de la lumière du soleil sur la vitalité des gemmes de microbes," Compt. rend. Acad. de Sci., 100:119, (1885).

22. Roux, E., Ann. de l'Inst. Pasteur., 1:445, (1887), cited by Norton, loc. cit.

23. Ward, M.H., Proc. Roy. Soc., 52:393, (1892-93), cited by Norton, ibid.

24. Norton, J.F., "The Action of Ultra-Violet Light on Bacteria and their Products," ibid. p.377.

25. Ayers, S.H., and Hohnson, W.T. Jr., Centr. Bakt. Parasitenk., II Abt., 40:109, (1914), cited by Rogers, loc. cit., p.376.

26. Ayers, S.H., and Johnson, W.T. Jr., "A Study of the Bacteria which survive Pasteurization", U.S. Dept. Agri. Bur. An. Ind. Bull. 161, (1913).

27. Frank, L. C., "The Unification of Milk Control in the United States," Jour. Am. Public Health, 15:977-80,(Oct.22, 1925)

28. Putman, G.W., "Mechanical and Engineering Aspects of Pasteurization," Newer Knowledge, 419-436, (1928)

29. Smith, T., "The Thermal Death-point of Tubercle Bacilli in Milk and Some other Fluids," Jour. Exp. Med. 4:217, (1899).

30. Schorer, E.H., and Rosenau, M. J., "Tests of the Efficiency

of Pasteurization of Milk under Practical Conditions",
Jour. Med. Research, 26:127-158, (1912).

31. Whittaker, H.A., Archibald, R.W.; Leete, C.S. and Miller, L.F., "A Comparison of the Temperature and Bacterial Count of Milk and Foam During Certain Stages of the Pasteurization Process," U.S. Dept. Agr. Tech. Bul. 18, (1927).

32. Huelings, S. M., Grim, G.W., and Horn, D.W., 13th Ann. Rept., Internat. Assoc. Dairy and Milk Inspectors, p.197 (1924) cited by Putman, loc. cit.

33. Chilson, C.H., and Wisler, C.O., ibid. p.243.

34. Winslow, C.E.A., "An Outbreak of Tonsilitis or Septic Sore Throat in Eastern Massachusetts and its Relation to an Infected Milk Supply," Jour. Infect. Dis., 10:104-5, (May,'11)

35. Eddy, W.H., "The Vitamin Manual," Williams and Wilkins Co, (1921)

36. Hess, A.F., and Fish, M., "Infantile Scurvy: The Blood, The Blood Vessels, and the Diet," Amer. Jour. Diseases Children, 8:385-405, (1914).

37. Ayers, S.H., "The Present Status of the Pasteurization of Milk," U.S. Dept. Agri. Bul. 342, (Sept. 1926).

38. Race, Joseph, "The Chlorination of Water," Wiley and Sons, (1918)

39. Phelps, "Water Supply Paper #229," Dept. of Int. U.S. Geo. Survey, (cited by Race, ibid).

40. Houston and McGowan, 5th Rpt. Roy. Commission on Sewage Disposal. (cited by Race, ibid).

41. Johnson, G.A., "Hypochlorite Treatment of Public Water Supplies:

Its Adaptability and Limitations" Amer. Jour. Public Health, 1:562-574, (1911).

42. Hale, H., and Bleecker, W.L., "Active Chlorin as a Germicide for Milk and Milk Products," Jour. of Agri. Research, 26:375-382, (Nov.1923).

43. Zoller, H. F., and Eaton, S.M., "Some Studies in the Bactericidal Action of Sodium Hypochlorite in Cow's Milk," Jour. Dairy Sci., 6:131-134 (Mar. 1923)

44. Zoller, H.F., " The Rate of Decomposition of Sodium Hypochlorite in Cow's Milk," Jour. Dairy Sci., 6:310-19,(July '23)

45. Ellms, J.W., and Hauser, S.J.,"Ortho-tolodine as a Reagent for the Colorimetric Estimation of Small Quantities of Free Chlorine," Jour. of Indus. and Eng. Chem. 5:915-917 (1913).

46. Rupp, P., " The Detection of Hypochlorites and Chloranimes in Milk andCream," Bull. #1114, U.S. Dept. of Agri. (1922)

47. Keister, J.T., "The Rupp Method for the Detection of Chlorine in Milk," Amer. Jour. Public Health, 15:781-784 (Sept,'25).