A STUDY OF DETERGENTS AND DETERGENT-SANITIZERS USED IN IN-PLACE CLEANING AND SANITIZING OF MILK PIPE LINES ON DAIRY FARMS

by

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I. INTRODUCTION

The use of cleaned-in-place milk pipe lines has been increasing for the past several years, particularly in conjunction with the farm bulk tank. It offers an opportunity for increasing operating efficiency by reducing the amount of labor needed for the milking operations. For many dairymen who have either outgrown or worn out existing facilities it is the least expensive and most efficient way of improving their milk handling facilities.

The use of pressure in circulation cleaning of permanent milk pipe lines has been developed to give satisfactory results in the dairy plant. This method involves the use of considerable extra equipment which may be used for other purposes. Pipe line milker installations on most dairy farms are cleaned by the use of vacuum circulation rather than by pressure circulation cleaning. This enables the dairy farmer to utilize the same vacuum pump for circulation cleaning of the pipe line that is used for the milking operation. This eliminates the purchase of extra equipment that would be of no value except in the cleaning operation.

Our present knowledge of the factors involved in the installation, the maintenance, and the care of cleaned-in-place milk pipe lines on the farm is limited. Dairymen who are now using such a system, and those considering its use, need more scientific information on the cleaning and sanitizing aspects of the farm milk pipe line installations. Cleaning compounds which have been designed for hand cleaning operations are commonly used for pipe line cleaning. It is impossible to obtain maximum cleaning efficiency of pipe lines with these compounds because they are milder in their alkaline action than compounds designed for in-place cleaning. The chemical activity of compounds designed for in-place cleaning is increased by the maintenance of a temperature of 140 °F. or higher. The circulation temperature range of 140 to 160 °F. as recommended by the several detergent manufacturers is difficult to maintain in actual practice. Many detergents recommended for circulation cleaning are not suitable for use in all types of farm in-place installations due to the excessive foaming of the detergents in solution when placed under vacuum. This excessive foaming causes considerable difficulty in circulation operations and prevents proper solution contact with the pipe line surface.

The problem of cleaning and sanitizing cleaned-in-place pipe lines on dairy farms is confounded by the absence of standardization in the engineering aspects of cleaned-in-place systems. The introduction of short-cuts or laxity in following the proper cleaning and sanitizing procedures may result in the lowering of milk quality.

The primary objective of this investigation was to evaluate the cleaning and sanitizing ability of a number of cleaning compounds used in the vacuum circulation cleaning of a pipe line milker in a stanchion type dairy barn. A second objective was to evaluate the effect of circulation temperature ranges on the cleaning efficiency of the detergents.

II. REVIEW OF LITERATURE

Permanent milk pipe lines were introduced into the dairy industry as a means of increasing operating efficiency and reducing costs without impairing milk quality according to Nelson, Ormiston, and Alexander[22]. They further state that it was demonstrated that milk lines in manufacturing plants and on farms can be cleaned effectively in-place.

Fleischman and Holland[10] reported that an approximate 50 per cent saving could be made in labor for cleaning and sanitizing pipe lines in a dairy plant utilizing at least 100 feet of cleaned-in-place lines. Their data indicated that the break-even point of take-down versus permanent pipe lines, as far as cleaning was concerned, was about 45 feet. According to Parker et al.[24], cleaning pipe lines in-place in one Oregon plant resulted in a savings of 70 per cent in cleaning and sanitizing time. Members of the cleaning crew became less fatigued in operating the in-place cleaning system than when they hand cleaned the pipes and fittings. The cleaning crew maintained a higher degree of efficiency because the in-place cleaning system appealed to them and many undesirable working conditions were eliminated.

Information on the amount of labor that can be saved by the use of cleaned-in-place pipe lines on dairy farms is limited. Calbert[5] reported that the amount of labor saved by this method of cleaning pipe lines was quite variable and depended on many factors. Results of a study made at the University of Illinois by Nelson, Ormiston, and Alexander[22] showed that premilking, milking, and clean-up operations in pipe line systems required more clock hours than did conventional milking systems. The data showed that a more efficient utilization of labor (man-minutes) occurred during the milking operation when a pipe line system was used than when two- and four-unit conventional milking systems were used. A four-unit, two-man conventional milking system modified to the extent that the helper prepared the cows and used the strip cup during the time he was not engaged in carrying milk and a two-unit, one-man pipe line system had approximately equal labor requirements (man-minutes) for accomplishing the premilking, milking, and clean-up operations.

Moore, Tracy, and Ordal[21] reported no measurable contamination of milk as a result of milk transfer through permanent pipe lines installed in a dairy plant. Although sterility was not attained, swab counts on the pipe line were low and the results would be judged excellent by the standards recommended for restaurant utensils. Under the conditions of this study, stainless steel and glass pipe lines were equally satisfactory from a sanitation aspect. Bacteriological results of a study by Alexander, Ormiston, and Nelson[1] on several different milking systems, including pipe line milking, showed that milk of acceptable quality could be produced by all of the systems studied. Alexander, Nelson, and Ormiston[2] reported no significant difference in the bacteriological quality of milk handled by either conventional methods or permanently installed pipe lines constructed of stainless steel or pyrex glass. Hunter, Marth, and Frazier[13] compared the dismantled cleaning of stainless steel pipe lines in a milking parlor with the cleaning of stainless steel pipe lines by vacuum recirculation procedures in a stanchion dairy barn. They found that the milk from both pipe lines was of excellent quality. Swab tests indicated that the pipe lines were maintained in a satisfactory sanitary condition. The bacterial population on the inside surface of the dismantled pipe line increased as the frequency of brushing decreased, but the milk passing through the pipe line did not increase in bacterial count.

Calbert[6] asserted that pipe line milkers presented a problem whenever cleaning and sanitizing of the pipe line were involved. As yet, no method of cleaning and sanitizing milk lines in-place has been devised that will work satisfactorily in all installations. The engineering and installing of farm milk pipe lines should be standardized to simplify the cleaning procedures. According to Haskell[12], the proper engineering of cleaned in-place pipe line milker systems is a factor bearing heavily on the operation of the equipment in a sanitary manner. Early attempts to clean by recirculation procedures did not yield consistently satisfactory results. It was found that many cleaning failures were not due to chemical weaknesses, but rather to engineering faults of the installations. In referring to results obtained by cleaning pipe lines in-place, Weber[30] stated that without proper design and installation of the piping and with inadequate application of poor solutions, the results tended to be unacceptable. The results were excellent when a careful study had been made of the problem and sound principles applied.

Nelson, Ormiston, and Alexander[22] reporting work conducted at the University of Illinois, stated that the operator was a key factor in the production of high quality milk with the use of pipe lines. The bacteria counts of milk varied often with the different operators involved in the milking operations. Milk produced under the supervision of one herdsman employed in this study contained one-half the number of bacteria of milk produced when another herdsman was in charge, even though the same equipment and procedures were used by both men. The first operator was apparently more careful and followed instructions more closely.

The cleaning and sanitizing agents are a major consideration in the cleaning and sanitizing of cleaned in-place milk pipe lines. Mallmann(18) asserted that in order to kill microorganisms, the killing agent must either enter the organism or it must come into intimate contact with the cell wall of the organism. If the organism is completely covered with organic or inorganic soil, such as milk proteins, milk stone or water stone, it is practically impossible to reach the cell with any acceptable chemical or physical sanitizer. The cleaning of dairy equipment and the type of cleaner used are two important factors in the production of quality milk.

According to Dunn(8), the over-all purpose of a detergent is to facilitate the removal of soil by means of water, with or without the use of heat, and with or without the application of scrubbing, brushing, or other physical procedure. It may accomplish this purpose by a combination of several or all of the following actions: dissolving or increasing the solubility of the soil in the water; wetting the surface, as a result of decreasing the surface tension between the soil and the equipment surface, and thus aiding in the penetration of the soil; conditioning the water to increase its effectiveness;

deflocculating or breaking up the soil particles; emulsifying the lipids; and preventing redeposition of the soil and aiding in its removal by rinsing. Minkin[20] reported that a demonstration of detergent properties, using the basic chemicals found in a balanced detergent, illustrated dramatically that no single chemical has a high degree of all the desired properties. Lehn[16] stated that in the formulation of a scientifically balanced cleaner a definite function must be assigned to each compound. The elements in the formula must not only fill their own functions but must also support and reinforce each other, so that the cleaner will be more active and more stable in cleaning activity than any of the component parts used separately.

According to Jacobsen[14], the higher phosphates contribute considerable more water conditioning power to a cleaner than do the basic alkalis. This water conditioning is known as sequesting since the water hardness is not precipitated but only held in solution and in such a form that it does not interfere with detergent action. Metasilicate and trisodium phosphate are more effective detergents than sodium carbonate. With the addition of wetting agents, also called synthetic organic detergents, to these detergents a greater penetration of soil films and an improved rinsing of the equipment is obtained. The real problem is to keep these three components in the proper balance so that equipment can be cleaned quickly and completely with reasonable economy. If an unnecessarily high proportion of the higher phosphates or wetting agents are used, the cost of the cleaner will be increased without a commensurate increase in cleaning value. They will also crowd out the more alkaline products which contribute the alkalinity for cleaning. The higher phosphates have relatively low alkalinity and thus larger quantities are required to develop the required active alkalinity for washing.

Mallmann[(19)] reported that strong alkaline cleaners, at pH 10.5 to 11, generally have good saponifying action. The alkaline cleaners, for example trisodium phosphate and sodium metasilicate, at pH 10.5 are good deflocculating and protein dissolving agents. At pH values above or below this point, they lose their value to a large extent.

Smith[(27)] stated that although many products are effective to some extent with regard to a number of cleansing properties, they are often classified according to the property for which they exhibit the greatest degree of activity. Thus, one product may be spoken of as a detergent, another as wetting agent, and so on. Frequently, a product will succeed in lowering surface and interfacial tensions but will fail to emulsify or hold the dirt in suspension. Such compounds are better classed as wetting agents or penetrants than detergents.

Jacobsen(14) further stated that wetting agents, in general, are the most expensive components of dairy cleaners. It is possible to clean with a wetting agent alone, but the cost would be excessive if compared with the cost of blends of wetting agents with alkalis and higher phosphates. The greatest efficiency in cleaning equipment is obtained by using the blended products which contain the best features of the basic products available.

Scales(25) placed foaming second in importance in a list of desirable detergent qualities because it is just a physical variation of wetting quality. Foams have good wetting properties because the outside surfaces of the bubbles have higher surface tension than the inside and so flatten out against the surface of contact. Foam in a detergent solution is a valuable quality since particles of soil or dirt that are detached by the wetting may be carried away by the foam. White(31) stated that it is important for the cleaners used in permanent pipe line installations to contain a minimum of those wetting agents which leave films on contact surfaces. An excess of a wetting agent produces copious foam, making cleaning solutions difficult to pump through the system and prevents proper solution contact. The addition of chlorine to dairy cleaners aids in the removal of films left by wetting agents. The added chlorine is used to function as a cleaner rather than a sanitizer. The admixture of chlorine and detergent in no way substitutes for a separate sterilizing procedure.

Data obtained by MacGregor, Elliker, and Richardson(17) indicated that varying concentrations of up to 100 parts per million of sodium hypochlorite significantly increased the cleaning efficiency of three cleaners when added to them. The increased cleaning efficiency was possibly due to the solubilizing action of the hypochlorite on the protein fraction of the soil.

Chaplin and Johns(7) reported that survival curves showed, under conditions likely to be found in practice, hypochlorite solutions of low alkalinity disinfected milk films on surfaces more rapidly than those of greater alkalinity. Dehydration of the milk film resulted in slower disinfection rates by both neutral and alkaline solutions. Increasing the milk content in the film reduced the rate of disinfection and reduced the effect of the neutral solution to a greater extent than an alkaline solution. A 99.9 per cent kill of organisms was obtained in four seconds with a neutral solution of hypochlorite while at pH 10.5, eight seconds were required for a similar destruction of organisms. In dried milk films ten and twenty seconds were required for destruction of the organisms by neutral and alkaline solutions, respectively.

Results of a study by Scales and Kemp[26] of the sterilizing quality of chlorine solutions under different conditions indicated a greater germicidal efficiency of chlorine solutions when the pH was adjusted to around six. Laboratory and plant tests showed that a solution containing 50 parts per million of available chlorine at a pH around six would produce just as satisfactory germicidal results as a solution which contained 255 parts per million of chlorine at a pH around ten.

The suitability of swab tests for determining the bacterial content of dairy equipment was demonstrated by Thomas et al.[29]. They found that only one-third of the bacteria were removed from washed dairy equipment by a single swabbing. By carrying out two separate swabbings with a ribbon gauze swab 60 per cent of the bacteria were recovered, even in the case of neglected equipment covered with milk stone. Fellers, Levine, and Harvey[9] reported that the swab test recovered from 40 to 80 per cent of the organisms present on an artificially inoculated surface. Speck and Black[28] found that 21 per cent of the organisms recovered by a moistened swab were retained in the cotton. Barnes[4] reported that the addition of a wetting agent to the swabbing solution increased the estimated percentage of recovery of organisms.

In a study of in-place cleaning in a dairy plant, Kaufmann, Andrews, and Tracy[15] found that when the temperature of the alkaline cleaning solution was reduced from 160 to 120 °F. the joints and pipes remained in an excellent state of sanitation bacteriologically. Excellent bacteriological results were indicated by the fact that no contamination was observed in 35 per cent of the joints and in 60 per cent of the pipes. Although the bacteriological findings after a two-weeks test period on the permanent line indicated a satisfactory sanitary condition, swab counts which were made after operating for one year showed that some bacterial build-up occurred.

III. THE INVESTIGATION

A. Description of Facilities

The data were obtained by utilizing the facilities available in the college Holstein Dairy Barn. The barn is of cinder block construction 137 feet long and 40 feet wide. The ceiling is six-inch concrete slab. On one side and midway of the barn is a small feed storage. On the other side and midway of the barn is the milk house.

The interior of the barn is divided into four sections of equal size with a total capacity of 56 cows. Two sections on one side contain 15 conventional stanchions and 15 tie stalls. The two on the other side of the barn contain 14 tie stalls and 12 "comfort" stalls.

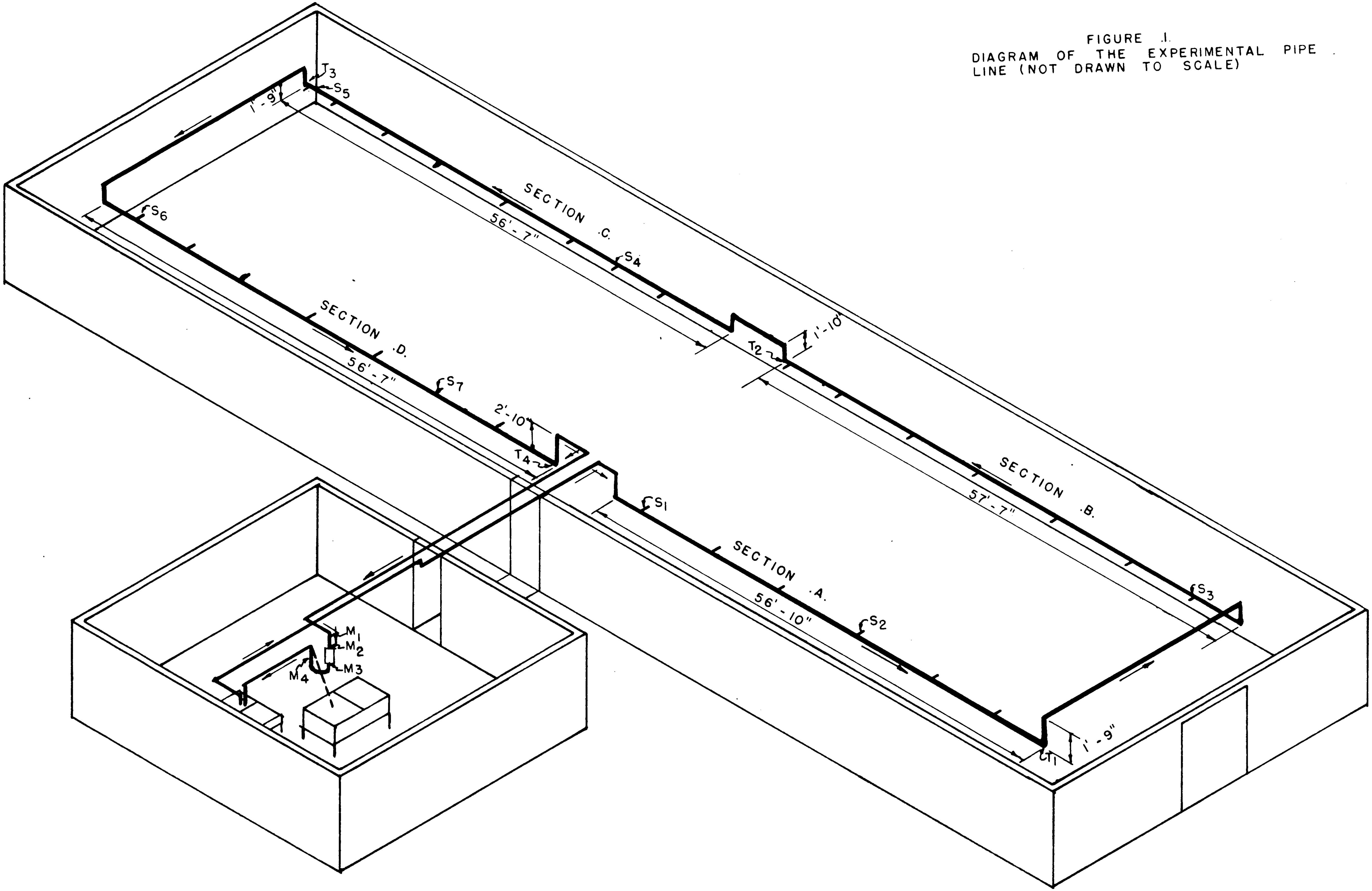
The milk house is of cinder block construction 34 feet long and 32 feet wide. A hallway runs the entire length of the milk house. The milk room contains a 400 gallon bulk tank and a two-compartment wash tank insulated with two-inch rock wool insulation. A 5000 watt electric water heater was installed in the wash tank to aid in the maintenance of high circulation temperatures of cleaning solutions. Attached to the wash tank is a washing manifold which holds the teat-cup assembly in position for cleaning. Above the wash tank is a recording thermometer for recording the circulation time and temperature of the cleaning and sanitizing solutions. The milk receiver, sanitary trap, and a diaphragm milk pump for pumping the milk from the receiver to the bulk tank and the cleaning and sanitizing solutions from the receiver to the wash tank during clean-up operations are located along one wall.

A sanitary glass pipe line is attached to a line manifold on the wall over the wash tank. The line leads from the wall manifold through the hallway of the milk house and into the barn. The pipe line makes a complete circuit around the barn in front of the stall sections, back through the hallway of the milk house and finally terminates at the milk receiver. A stainless steel line connects the milk receiver to the diaphragm pump and the pump to the bulk tank. An in-line filter is located in the line between the pump and bulk tank. An accessory stainless steel pipe line is connected to the stainless steel line leading from the pump to return the cleaning and sanitizing solutions to the solution tank during the cleaning and sanitizing operations. The total length of pipe line cleaned-in-place is approximately 400 feet of pyrex glass and 15 feet of stainless steel line. Twenty feet of the glass line is outside of the barn and is insulated with felt and wrapped with roofing material.

There are 20 glass 90° ells, five stainless steel 90° ells, and four drain tees in the glass and stainless steel lines. There are four risers in the glass line, one at the end of each of the four sections in the barn. The total height of risers in the line is eight feet two inches. A drain tee is located in the glass line at each one of these risers. The glass line located in front of each stall section slopes to a drain tee to facilitate removal of residual solutions from the line. Each drain tee is closed with a sanitary rubber bushing which is removed to drain the line.

The glass line in front of the tie stalls in section A, as shown in Figure 1, is approximately 56 feet ten inches long with a slope of approximately 1/10 of an inch per foot to a drain tee and a riser one foot nine inches high. Section B has approximately 57 feet seven inches of glass pipe with a slope of approximately 1/10 of an inch per foot to a drain tee and a one foot ten inch riser. The glass line in section C is approximately 56 feet seven inches long with a slope of approximately 1/12 of an inch per foot to a drain tee and a riser one foot nine inches high. In section D the line is approximately 56 feet seven inches long with a slope of approximately 1/14 of an inch per foot to a drain tee and a two feet ten inch riser.

FIGURE .1.
DIAGRAM OF THE EXPERIMENTAL PIPE LINE (NOT DRAWN TO SCALE)



B. Methods and Procedures

Three methods are generally used for the appraisal of cleaning compounds. They are: (a) field tests, (b) laboratory tests which simulate field tests, and (c) laboratory tests of certain properties which are generally assumed to contribute to good cleaning action[11]. In this study field tests, supplemented by laboratory tests, were used for evaluating the cleansing abilities of six compounds which were employed in the vacuum circulation cleaning of a pipe line milker installation in a stanchion dairy barn.

The residual bacterial contamination of the pipe line after circulation cleaning served as an index for evaluating the cleaning and sanitizing ability of the compounds studied. The residual bacterial contamination was determined by the swab contact method as outlined in "Standard Methods for the Examination of Dairy Products"[3].

The experiment was designed to study six cleaning compounds under dairy herd operating conditions for two 15-day periods each. Compounds A and B were studied for 15 days each with a circulation temperature range of 160 °F. to 140 °F. and 15 days each with a circulation temperature range of 140 °F. to 110 °F. Compounds C and D were studied for two 15-day periods each with a circulation temperature of 140 °F. to 110 °F. Compounds E and F were studied for five days each with a circulation temperature of 140 °F. to 110 °F. The use of compound E was discontinued after a trial period of five days due to excessive foaming of the solution under vacuum. The use of compound F was discontinued after a trial period of five days because of the difficulty encountered in getting the compound into solution.

The cleaning procedure closely paralleled the recommendations of the respective detergent manufacturers for their products. Some modifications were necessary to obtain uniformity in procedure. Since surface contact time is one of the many factors upon which the actual cleaning ability of a solution depends, a standard 15-minute circulation time for all compounds tested was chosen. This standard was adopted with the approval of the respective detergent manufacturers involved. Some manufacturers recommended that periodically, usually once a week, an acid cleaner be substituted for the alkaline cleaner in the cleaning procedure. In this study the acid cleaner was circulated for a 30-minute period once a week, in addition to the alkaline cleaning procedure.

The cleaning of the line was accomplished in the following manner: the line was rinsed with warm water immediately after milking until the rinse water was free of milk. The temperature of the water was 140 °F. as it left the wash tank but, by the time the first rinse water reached a section of pipe line which had been in contact with milk, the temperature had dropped to approximately 120 °F. The temperature of the first rinse water reaching the return outlet was approximately 90 °F. This higher-than-average rinse water temperature was used for warming the line, thereby preventing a large initial drop in temperature at the beginning of circulation of the cleaning solution. The rinse water was drained and a detergent solution circulated for 15 minutes. At the end of circulation the pipe line was drained, rinsed with warm water to remove the remaining cleaning solution, and again drained. During circulation of the cleaning solution the milk valves at the stanchions were cleaned by hand brushing. Just before milking, the line was sanitized by circulating a 200 parts per million chlorine solution for ten minutes. When the cleaner-sanitizer (compound A) was used, the line was drained after the solution was circulated for 15 minutes then used at the next milking without further treatment.

When swab samples were taken, the pipe line was not rinsed immediately after circulation of the cleaning solution. The cleaning solution was drained from the line, then approximately one hour before the next milking, swab samples were taken at different locations in the line and of different milk valves. After securing the samples the line was rinsed and sanitized.

Six times during the study, the four drain tees were swabbed after rinsing and again after circulation of the chlorine solution.

Swab samples were taken at 15 different locations three times during each 15-day period except in four instances. Samples were taken twice, rather than three times, during two periods. Samples were obtained one time during each of the two five-day periods when compounds E and F were being used.

It was believed that if any bacterial build-up were to occur it would be most evident at the four drain tees and in the milk valves. Figure 1, page 22, shows where the samples for bacteriological analyses were taken in four drain tees (T_1, T_2, T_3, and T_4), in seven milk valves (S_1 to S_7), two places in the glass line above the milk receiver (M_1 and M_2), in the stainless steel line under the milk receiver (M_3), and the stainless steel line connected to the diaphragm pump outlet (M_4). The equipment manufacturer recommended that the stainless steel line under the milk receiver (M_3) and the stainless steel line leading from the pump (M_4) be disassembled each day and hand cleaned with a brush. In this study location M_3 was cleaned in-place by the different cleaning solutions being pulled along by the suction action of the diaphragm pump. Location M_4 was cleaned in-place by the different cleaning solutions being forced (positive pressure) from the diaphragm pump to the wash tank.

The swabs used for sampling were made by twisting nonabsorbent cotton firmly over one end of a wooden applicator stick six inches long. The swabs were 3/16 of an inch in diameter and 3/4 of an inch long, and were placed in clean, dry, screw cap vials (150 x 16 mm) after which the caps and vials were autoclaved 20 minutes at 121 °C. At the end of 20 minutes, ten inches of vacuum were drawn and maintained for five minutes.

Buffered rinse solution was prepared by adding 1.25 milliliters of stock buffer solution, five milliliters of sodium thiosulfate (10 per cent aqueous), four grams of Asolectin[1] and ten grams of Tween 20[2] to distilled water and making up to one liter(3).

The stock phosphate buffer solution was prepared by dissolving 34 grams of potassium dihydrogen phosphate (KH_2PO_4) into 500 milliliters of distilled water, adjusted to a pH of 7.2 with 1N NaOH solution, and made up to one liter with distilled water. The solution was then filtered and stored in a refrigerator(3).

Buffered rinse solution was distributed into screw cap vials (125 x 20 mm) in amounts so that 20 milliliters remained in each after autoclaving. The vials, with caps loosened, were autoclaved at 121 °C. for 20 minutes.

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1. Associated Concentrates, Inc., Woodside, Long Island, New York.
2. Atlas Powder Co., Wilmington, Delaware.

The swab samples were taken by moistening a sterile swab in a freshly opened vial of buffered rinse solution and pressing the excess solution out, inside of the rinse vial. The swab was rubbed slowly and thoroughly over approximately 26 square inches for the pipe line and drain tees, and approximately six square inches for the milk valves. This was repeated five times over the same 26 or six square inches with rewetting of the swab and pressing out of the excess solution between each swabbing. The swab was then put into the vial of buffered rinse solution in which it had been rinsed and the stick broken off under aseptic conditions below the area which had been contaminated by the fingers. The samples were iced and returned to the laboratory where they were maintained at 34 °F. to 40 °F. until tested.

The media used for the Standard Plate Counts was prepared in the following manner: 18.75 grams of Milk Protein Hydrolysate Glucose Agar were added to 750 milliliters of distilled water. The mixture was heated to boiling to dissolve the medium completely, sterilized 15 minutes at 121 °C., and cooled to 43 °C. to 45 °C. before being used[3].

The media used for the coliform counts was prepared in the following manner: 31.88 grams of Desoxycholate Lactose Agar were added to 750 milliliters of distilled water. The mixture was heated to boiling, to dissolve the medium completely, then sterilized 15 minutes at 121 °C. and cooled to 43 °C. to 45 °C. before being used[3].

The dilution water was prepared by adding 1.25 milliliters of the stock phosphate buffer solution[3] to distilled water and made up to one liter. The phosphate-buffered distilled water was placed in screw cap dilution bottles in amounts so that 99 milliliters remained after autoclaving at 121 °C. for 20 minutes.

Petri dishes and pipettes in canisters were sterilized in a hot air sterilizer for two hours at not less than 170 °C.

Preparations were made for plating the rinse solutions from the swabs by shaking the swab vials rapidly in a four to six inch arc 50 times, striking the palm of the hand at the end of each cycle, and completing the procedure in about ten seconds[3]. Preliminary trials showed that procedures outlined in "Standard Methods for the Examination of Dairy Products"[3] for plating rinse solutions were not practicable with the high bacterial counts that were encountered in the drain tees. The dilutions used for the Standard Plate Count were 1:10, 1:100, and where contamination was expected to be great 1:1000. The dilutions used were run in duplicate. Additional plates were poured for checking the sterility of the glassware, dilution water, agar, and for air contamination.

The plates were poured according to the procedure recommended in "Standard Methods for the Examination of Dairy Products" for milk and cream[3]. After solidification of the medium the plates were inverted and promptly placed in an incubator. The Standard Plate Count plates were incubated for 48 hours at 35 °C. and the coliform plates were incubated for 24 hours at 35 °C. At the end of the incubation period the plates were removed from the incubator and plates were selected and counted according to the procedure recommended for milk and cream[3].

A laboratory evaluation was made of the respective cleaning compounds by a procedure similar to the one used by the New York State Public Health Environmental Sanitation Field Training Center for testing dishwashing detergents[20].

The detergents were evaluated on the basis of tests for the following properties: ease with which the detergents dissolve in the water used, control of water hardness and film deposit, control of foaming, wetting ability, emulsifying ability, and their ability to dissolve and deflocculate proteins. Three solutions of each detergent were required to evaluate the six properties. The solutions were prepared by adding two grams of the detergent to 125 milliliters of the water used for circulation cleaning at 120 °F. A control was run by duplicating the the tests using distilled water. All evaluations were made after the solutions had been shaken for two minutes by a wrist action shaker with the machine set at its maximum level except when evaluations were made of the ability of the detergents to dissolve and deflocculate protein. The ability of the detergents to dissolve and defloccuate protein was evaluated by adding one gram of pure casein to each detergent solution and shaking the solution with a wrist action shaker until the mixture showed complete deflocculation and dissolvement of the casein. The number of minutes of shaking required to accomplish this was used as a relative index of the ability of the detergents to dissolve and deflocculate protein.

The wetting ability of each detergent was evaluated by putting a drop of the detergent solution on commercial waxed paper. The detergents high in wetting ability formed drops that flattened out over the waxed paper. When the drops were allowed to roll off the paper and the tracks were examined it was found that drops of detergents high in wetting ability left a wet track film.

The emulsifying ability was evaluated by adding one milliliter of vegetable oil to each detergent solution, shaking for two minutes with the wrist action shaker, and timing the number of seconds required for the oil to separate out at the surface.

All other properties of the detergents were evaluated by observation of the solutions. Although fine differences between detergents cannot be detected by these simple tests, the detergents can be classified broadly as poor, fair, good, or excellent.

The parts per million of available chlorine in the 225 milliliter detergent solution was determined by the standard thiosulfate method.

Measurement of velocities of various cleaning and sanitizing solutions through a pipe line is not practical when a vacuum system of circulation is used because of the variations in the amounts of air included in the "air brush". Since the velocity of a solution in a vacuum system of circulation is related to the inches of vacuum drawn, vacuum readings were taken with a standard vacuum gage at each milk valve in the installation three times during the course of this study to be used as a relative index of solution velocity.

C. Results

Results of the swab tests made after cleaning the pipe line with a detergent-sanitizer (compound A) and the two detergents containing chlorine (compounds B and C) are summarized in Table 1. Under the conditions of this study, compounds A, B, and C, used without subsequent chlorination, did not yield results which would consistently meet the sanitization standard of 12.5 colonies or less per square inch of swabbed surface in all 15 locations. In all instances, the number of organisms recovered from the drain tees (T_1 - T_4) by the swab procedure did not meet the standard irrespective of the chlorinated cleaner being used for circulation cleaning. Only seven per cent of the samples taken in the milk valves conformed to the standard with compound A, 11 per cent with compound B and 19 per cent with compound C.

From a bacteriological point of view the areas sampled were in an unsatisfactory state of sanitation under all conditions studied with compound A. Since compound A was a detergent-sanitizer it was not recommended that chlorine be circulated through the pipe line before each milking. Therefore, the areas swabbed would have been in an unsatisfactory state when milk was to be transported through the pipe line. The total count in locations T_1 - T_4 ranged from a low of 77 colonies per square inch to a high of 36,000 colonies per square inch during the

Table 1

The Ability of a Detergent-Sanitizer and of Two Detergents Containing Chlorine to Meet the Sanitization Standard without Subsequent Chlorination at 15 Locations in a Pipe Line Milker Installation Cleaned-In-Place

Swab Location	Detergent		
	A	B	C
	Number with 12.5 or less per sq. in./No. Observations		
Drain Tees			
T_1	0/6	0/5	0/6
T_2	0/6	0/5	0/6
T_3	0/6	0/6	0/6
T_4	0/6	0/5	0/6
Milk Valves			
S_1	0/6	2/5	0/6
S_2	1/6	0/5	0/6
S_3	1/6	1/5	1/6
S_4	0/6	1/5	1/6
S_5	1/6	0/5	2/6
S_6	0/6	1/5	1/6
S_7	0/6	0/5	3/6
Milk House			
M_1	1/6	1/5	2/6
M_2	0/6	2/5	4/6
M_3	0/6	2/5	0/6
M_4	1/6	3/5	1/6

15-day period of circulation cleaning with a temperature range of 140 °F. to 110 °F. During the 15-day period of circulation cleaning with a temperature range of 160 °F. to 140 °F. the counts in locations T_1 - T_4 ranged from a low of 150 colonies per square inch to a high of TNC (too numerous to count). TNC represented some number above 260,000 per square inch because numbers of this size are recorded. Ninety-three per cent of the samples taken in locations S_1 - S_7 during the two 15-day periods would not meet the standard for sanitized equipment. Table 4 shows the actual counts per square inch obtained with compound A.

It can be seen from the data in Table 4 that compound B did not yield results which would meet the standard of 12.5 colonies per square inch of surface when the swab samples were taken before chlorination of the pipe line. The data showed that during a 15-day period with a circulation temperature of 140 °F. to 110 °F. in locations T_1 - T_4 the lowest count obtained was 300 colonies per square inch and the highest was TNC. In the same locations during a 15-day period with a circulation temperature of 160 °F. to 140 °F. the lowest count obtained was 5,400 colonies per square inch and the highest was 65,000 colonies per square inch. Eighty-nine per cent of the samples taken in locations S_1 - S_7 during the two 15-day periods would not meet the standard for sanitized equipment. Information on the sanitary condition of the pipe line after circulation of a chlorine solution was not obtained during this study.

During the 15-day period of circulation with a temperature range of 160 °F. to 140 °F. the drain bushings and stainless steel lines became coated with a reddish brown residue. A sample of the deposit from the stainless steel line was found to contain approximately 60 per cent protein with only a trace of fat. Circulation of an acid cleaner removed the deposit. This condition was never observed again.

In general, the results obtained from swab samples indicated that compound B would not do a satisfactory job; however, the sanitary condition of the pipe line after the circulation of a chlorine solution must be known before a valid statement can be made.

While compounds A and B failed to give consistently satisfactory results, it may be seen from Table 2 that the other compounds tested also could not be depended upon to give counts of 12.5 per square inch of surface in this installation at all times. However, this standard was approached more often and more closely when circulation cleaning was followed by chlorination of the pipe line before milking. Table 2 shows the results obtained with compounds C, D, and F in four locations (T_1 - T_4). Twenty-six square inches were swabbed in each location after

Table 2

Results Obtained With Three Detergents in Four Locations After Circulation Cleaning and Again After Circulation of a 200 Parts Per Million Chlorine Solution

Detergent	No. of Observations	Swab technique plate count/location							
		T_1		T_2		T_3		T_4	
		After washing	After chlorine	After washing	After chlorine	After washing	After chlorine	After washing	After chlorine
		Total bacteria/sq. in.							
C	3	13,000	2,200	1,400	8	270	0	77	0
		5,800	160	680	15	330	0	680	19
		85,000	20	54	4	150	0	96	0
D	2	19,000	0	26,000	4	220	0	9,000	0
		35,000	27	15,000	0	18,000	0	18,000	0
F	1	20,000	1	6,300	3	20,000	1	8,600	2

circulation cleaning and the same 26 square inches were swabbed after rinsing and again after circulation of a chlorine solution. The effect of the previous swabbing on the results of the later swabbings was not determined.

It can be seen from the data in Table 2 that in location T_1 with compound C a count of 13,000 colonies per square inch had been reduced to 2,200 colonies per square inch after chlorine circulation. In the same location with compound F a count of 20,000 colonies per square inch had been reduced to 1 colony per square inch after the chlorine circulation. In the first instance, a count of lower magnitude was not reduced to the acceptable count of 12.5 colonies or less per square inch, whereas in the second instance, a count of greater magnitude was reduced to an acceptable count. This same phenomenon can be seen in location T_4 between compounds C and D, and C and F. There was no apparent evidence that the counts after cleaning and the counts after chlorine circulation were correlated.

Based on samples taken following cleaning but before rinsing or chlorination as shown in Table 4, comparison of the total number of organisms left by the same compound at different circulation temperatures was not valid because the types of organisms left by the detergent solution at 160 °F. to 140 °F. may or may not have been the same as the types left by the solution at

140 °F. to 110 °F. However, on the basis of numbers of viable organisms removed, no clean cut advantage was observed for either hot or cold circulation.

Two of the six detergents under investigation in this study had characteristics which caused the termination of their use after a trial period of five days each. Compound E was unsatisfactory because of excessive foaming of the solution when circulated by vacuum. The foam caused the vacuum pump to stop operating several times when the foam backed through the vacuum lines into the pump. The use of compound F was discontinued because it would not dissolve completely under the existing conditions. Granules of the compound were found in the pipe line and in the area between the diaphragm and the casing of the diaphragm pump. From these locations it was possible for the compound to enter the milk.

The data shown in Table 2 indicated that under the conditions of this study compounds C and D could not be depended upon to give satisfactory results in all locations swabbed all of the time even when a chlorine solution was circulated before each milking.

The results of a laboratory evaluation of the physical properties of the six compounds investigated are shown in Table 3. It will be observed that under these conditions the

Table 3

<u>Laboratory Evaluation of Six Detergents Using 2 Grams of Each Detergent in 225 Milliliters of Barn Water at 120 °F.</u>

Detergent Property	Detergent A	B	C	D	E	F
Solubility	1	1	1	1	1	1
Cloudiness	2	1	2	2	2	2
Sediment	1	1	1	1	1	1
Non-foaming	1	1	2	1	4	2
Wetting ability	3	3	3	3	1	2
Emulsification of fat	2	2	3	1	a	2
Deflocculation of protein	2	2	1	1	1	1
Available chlorine p.p.m.	106	248	99	0	0	0

Code: 1 - excellent; 2 - good; 3 - fair; and 4 - poor.

[a] Results obstructed by foam.

compounds varied only slightly in the majority of detergent properties studied. Compound F, which proved to be unsatisfactory under field tests because it would not dissolve completely, was rated excellent on solubility under laboratory conditions. Compound E was rated excellent on wetting ability under laboratory conditions but produced an excessive amount of foam which prevented its use in the pipe line system.

In this experiment there was no apparent effect of differences in vacuum on the number of organisms left in different locations nor an apparent effect of barn temperature on the total number of organisms.

Since drain tees were found to be the chief offender as far as bacterial filth is concerned, at the termination of the study three of the drain tees were replaced with 90° glass ells. Drain tees T_1, T_2, and T_3 were replaced with glass ells and drain tee T_4 was left as a control. Following in-place cleaning with compound A, counts obtained were as follows: elbow one, 17 colonies per square inch; elbow two, 110 colonies per square inch; elbow three, 11 colonies per square inch; and T_4, 35,000 colonies per square inch.

IV. DISCUSSION

In this study the swab contact method was used for determining the residual bacterial contamination of 15 locations in the pipe line after circulation cleaning. The 15 locations selected for sampling were not representative of the conditions throughout the pipe line installation. These locations were selected because it was believed that if any bacterial build-up were to occur it should be more prominent at these points.

The swab contact method was chosen for determining the residual bacterial contamination of the locations to be sampled because it was the only practical method available which could be used in the type of installation utilized in the study. Since all bacteria on surfaces are not removed by swab techniques the results obtained tend to be conservative.

The rinse solution used for rinsing the swabs contained a wetting agent for the more effective removal of organisms from the surface swabbed and the subsequent recovery of the organisms from the swab. The wetting agent in the rinse solution proved to be a handicap in that it was very difficult to pipette accurate amounts of the sample for plating due to the foam produced when the samples were shaken.

The standard accepted count for the swab test is 100 colonies per eight square inches or 12.5 colonies per square inch. Although this is the standard for sanitized equipment it was used in this study as the standard after washing and rinsing. A cleaner, which was used in conjunction with a chlorine sanitizer, cannot be rated inferior if it did not meet this standard when swab samples were taken after circulation cleaning, but before chlorination of the areas swabbed. The data obtained in this study indicated that the performance of a cleaning compound must be judged on the end result after chlorination and its relative contribution to the end result, namely the sanitary condition of the pipe line when used for transporting milk. If the areas swabbed did not meet the standard after the proper circulation of a chlorine solution this is an indication that the cleaner is unacceptable for use under the conditions of this investigation.

It is evident that compound A did not meet the standard and should not be used as a detergent-sanitizer under the conditions established for this study. The ability of compound A to meet the standard, if used in conjunction with the circulation of a chlorine solution immediately before milking, was not determined. Compounds B and C contained chlorine, but were not designated as detergent-sanitizers. It was shown by the results that any attempt to use compounds B and C, without subsequent chlorination of the pipe line, would not give results in this physical plant which will meet the standard for sanitized equipment. The ability of compound B to meet the standard after the circulation of a chlorine solution was not determined. The data obtained indicated that compound C would meet the standard some of the time but could not be depended upon to give satisfactory results in all locations swabbed all of the time.

The respective detergent manufacturers recommended that when using compounds B, C, and D that a 200 parts per million chlorine solution be circulated through the pipe line immediately before milking. Unlike compounds B and C, compound D contained no available chlorine. Results of swab samples taken after chlorine circulation indicated that generally satisfactory results could be obtained with compound D but it would not give satisfactory results in all locations swabbed all of the time.

Data on the ability of compound E to meet the sanitation standard, before chlorine circulation, was obtained one time in the 15 different locations before use of the compound was discontinued due to excessive foaming of the solution under vacuum. Drain tees, T_2 and T_4, swabbed before circulation of the chlorine solution, met the standard of 12.5 colonies per square inch of surface. Ninety-six observations were made in the drain tees after circulation cleaning with all compounds studied and these two were the only ones meeting the standard. However, the foaming factor definitely eliminates compound E as a detergent for cleaning this pipe line installation.

The results of one observation made in locations T_1 - T_4, before use of compound F was discontinued, showed that swab samples taken after circulation of a chlorine solution met the standard in all four locations. Its use was discontinued to eliminate the possibility of any undissolved particles of the compound getting into the milk supply.

An assumption made at the beginning of this study that the number of organisms left by a cleaning compound would be a good indication or measure of the cleaning ability of that compound appears to be incorrect. Swab samples taken before and after circulation of chlorine showed that the number of organisms left by each cleaner before chlorine circulation was not a good index of the effectiveness of that compound as a cleaner. The data indicated that the ability of chlorine to control or remove the residual organisms depended upon the compound and not the number of organisms.

Compounds A and B were studied for one 15-day period each with a circulation temperature range of 160 °F. to 140 °F. and for one 15-day period each with a circulation temperature range of 140 °F. to 110 °F. On the basis of numbers of viable organisms removed, no clean cut advantage was observed for either circulation range. For the study of the two temperature ranges cited above to be valid, the sanitary condition of the pipe line after chlorine circulation was necessary.

The lowered bacterial counts obtained when 90° glass elbows replaced the drain tees support the hypothesis that the drain tees required to remove residual cleaning and rinsing solutions were a major cause of contamination in this particular pipe line milker installation. Results of one observation indicated that the contamination may be materially reduced by replacing drain tees with elbows. Sponges were pulled through the line by vacuum to overcome poor draining with either system, but this practice is discouraged by leading public health authorities because it may lead to recontamination of the pipe line.

Bacterial counts of the magnitude observed with compound A (detergent-sanitizer) were high when compared to a standard of 12.5 colonies per square inch for sanitized equipment. However, reference to Table 7 in the appendix shows that the Standard Plate Count of the milk which had passed through the pipe line during the course of this study met the Virginia Grade A raw milk standard. The validity of using 12.5 colonies per square inch of surface as a standard after the circulation of chlorine in connection with pipe line milkers is questionable. The types of organisms present must be taken into consideration as well as the total number.

V. SUMMARY AND CONCLUSIONS

The data obtained in this study on the cleaning and sanitizing ability of six cleaning compounds used in the vacuum circulation cleaning of a pipe line milker in a stanchion dairy barn indicated:

1. The use of the detergent-sanitizer without the circulation of a chlorine solution prior to milking will not yield results which will meet the standard for sanitized equipment.

2. The number of organisms remaining on the equipment following cleaning is an unsatisfactory measure of the ability of a cleaning compound to contribute to the sanitary condition of the equipment following chlorination, since the bacteria left by one compound are more susceptible to removal or killing by a chlorine solution than the bacteria remaining after cleaning with another compound.

3. Under these conditions there was: first, no obvious advantage of a circulation temperature range of 160 °F. to 140 °F. over a circulation temperature range of 140 °F. to 110 °F. in number of organisms left before chlorine circulation; secondly, that there was no obvious effect of differences in vacuum on the number of organisms remaining in different drain tees; and finally, that there was no apparent effect of barn temperature on the number of organisms present prior to chlorine circulation.

4. It was observed that the drain tees were very difficult to clean and that this difficulty may be partially overcome by replacing them with 90° glass elbows.

5. None of the cleaning procedures tested could be relied upon to consistently give acceptable counts in this physical plant with vacuum in-place cleaning; however, some methods were far superior to others.

6. Little added information could be obtained from the use of coliform counts in the sanitary condition of the pipe line.

7. The Standard Plate Count of the milk which had passed through the pipe line milker installation during the course of this study met the Virginia Grade A raw milk standards.

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IX. APPENDIX

Table 4

Swab Contact Method (Total colonies/sq. in.) on Compounds A and B

Swab Location	Compound A						Compound B				
	Circulation Temperature Range						Circulation Temperature Range				
	140 °F.-110 °F.			160 °F.-140 °F.			140 °F.-110 °F.			160 °F.-140 °F.	
	I	II	III	I	II	III	I	II	III	I	II
Drain Tees											
T_1	6,900	36,000	77	21,000	TNC[a]	260,000	13,000	TNC[a]	TNC[a]	36,000	35,000
T_2	620	12,000	1,000	22,000	150	1,300	1,900	28,000	300	5,400	51,000
T_3	690	2,400	77	3,000	1,900	1,900	4,200	38,000	26,000	27,000	65,000
T_4	920	77	120	6,300	8,800	19,000	13,000	78,000	12,000	22,000	63,000
Milk Valves											
S_1	2,100	500	33	---[c]	19,000	670	17	0	LA[b]	17	0
S_2	0	420	400	---[c]	29,000	1,200	17	270	120	130	17
S_3	33	100	100	480	330	0	0	100	33	67	34
S_4	17	200	50	2,000	170	670	0	33	LA[b]	400	83
S_5	270	0	150	670	170	1,000	50	67	67	320	130
S_6	17	44,000	67	TNC[a]	17	1,200	120	50	LA[b]	130	920
S_7	83	67	280	700	6,300	53,000	100	100	500	1,900	170
Milk House											
M_1	12	110	21,000	---[c]	18,000	39	28,000	4	2,600	150	130
M_2	19	410	15	15	7,200	77	15	4	0	15	180
M_3	380	1,100	31	---[c]	54,000	650	11	4	2,200	2,700	250
M_4	130	39	0	5,300	4,800	92,000	11	0	4	73	200
M_5	50	200	380	1,500	330	500	50	33	120	320	1,600

[a] Too numerous to count

[b] Laboratory accident

[c] Included after start of experiment

Table 5

Swab Contact Method (Total colonies/sq. in.) on Compounds C and D

Swab Location	Compound C — Circulation Temperature Range 140 °F.-110 °F.						Compound D — Circulation Temperature Range 140 °F.-110 °F.				
	I	II	III	IV	V	VI	I	II	III	IV	V
Drain Tees											
T_1	44,000	13,000	TNC[a]	13,000	5,800	85,000	450,000	210,000	230,000	19,000	35,000
T_2	4,500	37,000	15,000	1,400	680	54	1,100	17,000	63,000	26,000	15,000
T_3	41,000	88,000	TNC[a]	270	330	150	18,000	4,700	12,000	220	18,000
T_4	210	52,000	3,100	77	680	96	26,000	104,000	63,000	9,000	18,000
Milk Valves											
S_1	50	50	17	17	930	220	83	17	100	67	17
S_2	1,100	83	130	250	50	180	9,700	120	330	200	33
S_3	650	17	130	0	50	33	7,400	100	200	430	0
S_4	1,600	130	50	120	34	0	67	130	130	170	170
S_5	8,700	0	32,000	34	17	0	6,100	50	180	67	17
S_6	2,000	0	50	50	17	50	1,100	120	420	83	0
S_7	100	0	800	0	17	0	350	50	17	2,000	17
Milk House											
M_1	7,900	1,800	0	650	8	360	46	54	130	50	15
M_2	31	0	4	80	8	0	4	770	120	2,900	100
M_3	240	2,200	1,300	1,700	150	1,600	11,000	1,500	1,800	640	550
M_4	54	5,800	2,100	8	150	2,900	170	82	0	4,200	7,400
M_5	0	50	1,200	17	34	0	130	50	0	820	50
Post Treatment - after rinsing											
T_1				270	1,300	2,600				1,100	8,700
T_2				0	680	23				170	1,200
T_3				31	4	65				50	630
T_4				100	27	27				170	150
Post Treatment - after chlorination											
T_1				2,200	160	20				0	27
T_2				8	15	4				4	0
T_3				0	0	0				0	0
T_4				0	19	0				0	0

[a] Too numerous to count

Table 6

Swab Contact Method (Total colonies/sq. in.) on Compounds E and F

Swab Location	Compound E Circulation temperature range 140 °F.-110 °F.	Compound F Circulation temperature range 140 °F.-110 °F.
Drain Tees		
T_1	890	20,000
T_2	0	6,300
T_3	77	20,000
T_4	0	8,600
Milk Valves		
S_1	300	35
S_2	280	2,900
S_3	33	170
S_4	66	87
S_5	1,500	68
S_6	370	120
S_7	6,200	110
Milk House		
M_1	2,400	1,600
M_2	31	240
M_3	130	2,200
M_4	69	18
M_5	570	19
Post Treatment - after rinsing		
T_1		8,800
T_2		230
T_3		460
T_4		390
Post Treatment - after chlorination		
T_1		1
T_2		3
T_3		1
T_4		2

Table 7

Standard Plate Count/ml. of the Milk Transported Through the Experimental Pipe Line at Different Stages of the Investigation

Date	Standard Plate Count/ml.
9/8/54	5,500
9/14/54	23,000
9/17/54	12,000
9/28/54	3,800
10/5/54	11,000
11/16/54	2,800
12/7/54	600
1/11/55	13,000
1/25/55	7,000
2/8/55	15,000
2/22/55	3,300