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OBSERVATIONS ON THE EFFECTIVENESS OF BACTERICIDAL AGENTS IN RUBBER AND RUBBER-LIKE MATERIALS USED IN MILKING MACHINE INFLATIONS

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The weakest link in the sanitary chain between the cow and the consumer has often been the milking machine. While it is easy to keep the metal parts of a milking machine clean with relatively simple cleaning procedures, it is often difficult to keep the elastic parts in excellent condition because of the mutual solubility of the elastomers with fat. Fat is only one of the deteriorating agents of the elastic parts. Depending upon the formulation of the inflation, it is more or less vulnerable to light, oxygen, ozone, abrasion and other chemical and physical agents.

Inflations and other elastic parts are subject to many kinds of deterioration. Time alone is an important factor, and the relatively slow distribution of parts from manufacturer through distributor and jobber to the farmer, uses up a lot of the potential life of an inflation. To be completely satisfactory, an inflation must have good milking characteristics. It must not be tacky, it must have good resilience. It should be resistant to swelling from water absorption or fat absorption, and it should resist set, the tendency to take on a new shape when held under stress.

The stress of constant flexing should not cause cracking of the inflation, and the surface should not craze like old varnish. Some of the surface characteristics may not be due to the compound and its deterioration but are due to the nature of the surface of the forming mold. If the mold is rough, so is the inflation.

But most serious of all the problems is cracking caused by contact with ozone. Inflations are attacked by ozone and the surface may be permanently destroyed. Ozone is produced by electric motors and is present in small but destructive amounts in the atmosphere of most milk houses.

Surface deterioration of inflations is important because these changes produce conditions which can harbor bacteria. Often the elastomer cannot be properly cleaned. As bacteria grow, they not only help to destroy the inflation but, more important, increased numbers of bacteria are shed into the milk passing through the inflation. High bacterial counts are a principal quality problem of dairy farmers and, in our opinion, deteriorated inflations are a principal cause. Inflations are most often discarded because they are suspected of contributing to the bacterial count.

Previous work done in this laboratory *(3)* has indicated the overall superiority of neoprene inflations over natural rubber. This superiority was shown not only in greatly increased useful life on the farm and in better milking, but also in generally lower bacterial populations because the inflations exhibited surfaces which were much less likely to harbor bacteria mechanically.

In a further effort to reduce the bacterial population of the inflations on milking machines, work was initiated to find suitable bactericidal or bacteriostatic agents which could be incorporated into the elastomer to help control bacteria without impairing the quality of the inflation.

English workers, Cousins *et al. (2)*, have reported a study made with inflations containing tetramethylthiuram disulphide. Their inflations have been found to be mechanically poor and only very limited success with reduced counts could be shown.

Recent advances in bactericidal and bacteriostatic agents led us to believe that it might be possible to incorporate an agent into rubber or synthetic formulations which would prevent bacterial growth. Inflations made of such materials might last longer and be more sanitary during their useful life.

METHODS

A large number of inflations have been studied containing different compounds[1]. It was finally established that the most suitable compounds were of the phenylphenol family, although many other agents were tried. Water soluble types did not prove as successful because they were difficult to distribute uniformly through the neoprene stock and their performance in the field was variable. Oil soluble types proved more satisfactory because of the mutual solubility between the compounds and the ingredients in neoprene. The data given herein are entirely based on inflations containing o-phenylphenol.

A large number of preliminary experiments had to be run to screen the compounds and determine the long-time effectiveness of the materials. For such evaluations the usual procedure was to fill the washed and plugged inflations with bacteriological media inoculated with large populations of the organism under test, or in some cases, a mixture of several organisms. The inflations were left full of this suspension and plated by standard methods at intervals, to determine the bactericidal and static effects.

The phenylphenol compounds were found to be very bactericidal for *Staphlococcus aureus*, pseudomonads, coliforms and mixed raw milk flora. The staphlococci used were food poisoning types or a culture isolated from bovine mastitis. Woodward and Bramble *(4)* have run similar experiments and their results are in agreement.

When satisfactory formulations were found, they were tested by continuous extraction with water to determine the bactericidal life. After two weeks of continuous extraction, no decrease in bactericidal effect could be detected.

We then attempted to place these experimental inflations on operating dairy farms for comparison with regular materials. However, the high cost of this procedure, the attrition of experimental materials through accident, and the lack of uniform predictable handling methods soon made it apparent that the experiments could not be properly performed. For this reason, an experimental apparatus was constructed in the laboratory where milking conditions could be simulated and uniform regular treatment given over long periods of time. This procedure held much less danger of mechanical injury of parts and insured a uniform treatment of all inflations.

The preliminary observations had indicated that it might be desirable to use inflations made from bactericidal elastomer and store them in water solution between milkings, theorizing that enough of the bactericide would be dissolved by the storing water to prevent growth of organisms. Another method of storing between milkings would be to store in lye solution which, of course, would minimize fat absorption. It was finally decided to give these inflations a number of different treatments and compare the results with those obtained using natural rubber without bactericide, buna-N without bactericide, neoprene without bactericide and neoprene containing bactericide. These inflations were then given a replicate of various treatments.

Inflations in metal shells were closed by putting a large rubber stopper in the wide opening. They were then filled with enough raw milk so that when the inflations were in the closed position the capacity was slightly exceeded. The inflations were connected by transparent glass tubing to a pulsating mechanism while they were suspended, wide opening down, in at waterbath of slightly over 100°F. The pulsating milk was in contact with the inflation surfaces and was visible in the glass tube at each stroke of the pulsator. The inflations were thus pulsated for a period of one hour, night and morning, each day during the course of the test. At the end of the milking period, the inflations were given differing treatments. Some were rinsed, and stored in distilled water, some rinsed and stored dry. Some were rinsed, washed, and sanitized, by good hand-washing methods and stored in distilled water. Others similarly cleaned were stored dry between milkings. The tests were run for approximately three months and a careful record was kept of the physical properties and the sanitary conditions as measured by bacterial counts.

The bacterial counts were made by the adaptation of the method of Claydon *(1)*. The inflations were carefully plugged at the large end with sterile stoppers and using aseptic technique, 15 ml, or such amount as to just rise into the glass when flexed, of distilled water was introduced by pipette. The opening was plugged with a sterile pipette and pulsated for 5 minutes on the regular milking unit without immersing in water. Samples of the pulsated water were removed aseptically and plated by standard methods.

RESULTS AND DISCUSSION

At the end of the 12-week period, some of the inflations had set. This was the principal defect

[1]All bactericidal inflations were manufactured and provided by The D. S. Brown Co.

TABLE 1. LOGARITHMIC AVERAGES OF BACTERIAL COUNTS OF INFLATION RINSINGS OBTAINED DURING A 12-WEEK PERIOD

Type of inflation	Methods of cleaning			
	Cleaned, sanitized, wet stored	Rinsed only and wet stored	Cleaned, sanitized, dry stored	Rinsed only and dry stored
	(per ml)	(per ml)	(per ml)	(per ml)
Rubber	1,800,000	16,000,000	2180	2,640,000
Synthetic	410,000	26,000,000	517	14,600
Bactericide neoprene	35,000	1,280,000	9	850

TABLE 2. LOGARITHMIC AVERAGES OF BACTERIAL COUNTS OF INFLATION RINSINGS OBTAINED DURING THE 11TH AND 12TH WEEK OF A 12-WEEK PERIOD

Type of inflation	Methods of cleaning			
	Cleaned, sanitized, wet stored	Rinsed only and wet stored	Cleaned, sanitized, dry stored	Rinsed only and dry stored
	(per ml)	(per ml)	(per ml)	(per ml)
Rubber	4,800,000	120,000,000	4700	2,500,000
Synthetic	13,000,000	discontinued	67	7,600,000
Bactericide neoprene	3,160,000	32,000,000	10	430

found in the neoprene. However, the neoprene surfaces were still good at the test conclusion. The buna-N types showed serious ozone checking and the rubber types were at the end of their useful life.

The bacterial counts varied with the method of cleaning and storing. In Table 1, the logarithmic averages of the bacterial counts over the 12-week period, point up the effectiveness of the bactericide impregnated inflations. Regardless of type of inflation wet storage was unsatisfactory, although the bactericide impregnated inflations had significantly less population even under these conditions.

When inflations were dry stored, the impregnated inflations remained nearly sterile during the course of the experiment even though some inflations were allowed to dry for 30 minutes before rinsing and were not scrubbed. If the inflations were thoroughly scrubbed and sanitized, the rubber and buna-N inflations had reasanably low counts. The advantage of the bactericide impregnated inflations became apparent when the standarized cleaning procedure was reduced to a cursory treatment. It is evident that the presence of milk solids in trace amounts on the surfaces supported bacterial growth on all but the impregnated inflations. This is evident from Table 2. Here logarithmic average bacterial counts during the 11th and 12th weeks of the study (the last two weeks) are presented. These data emphasize the effect of age on the inflations. The rubber and buna-N inflations had deteriorated rapidly as was borne out by physical examination. If properly washed, deterioration was not very apparent from the counts but the poorly washed inflations were no longer bacteriologically satisfactory. Neoprene milking machine inflations formulated with a bactericidal agent resisted deterioration as was shown by physical examination. Bacterial growth was not supported even when washing methods were not perfect.

Good washing procedures must be recommended but the use of impregnated inflations would be a safeguard against marginal treatment of equipment.

As these experimental data were collected it became necessary to show that the use of these inflations would not result in contamination of the milk by the sanitizing compound or the elastomer itself. Accordingly, exhaustive tests have been run on the compound using the Federal Food and Drug Administration recommended procedures. Extractions with n-hexane, distilled water, 3% sodium chloride, and 3% sodium bicarbonate have been performed. The resulting data were submitted to the FDA. Subsequently, the USDA granted a registration number for the compound. This registration permits the labeling of this compound as a "self-sanitizing rubber." It would seem desirable that all elastomers used in contact with milk and food be examined by this rigorous examination.

The product can be produced in light colors, even white, which will "show the dirt." Unlike buna-N, this compound is made without heavy filling with materials such as carbon black. Many CIP systems on farms and in plants which have carbon-carrying rubber components show a dark deposit when the stainless steel surfaces are swabbed. This compound is carbon black, ostensibly coming from the elastomers deteriorating in contact with chlorinated alkaline cleaners. The use of noncarbon-carrying elastomers might eliminate this "black deposit" problem since no carbon black filler is needed in the neoprene compounds.

These compounds hold promise for use in air and milk tubing on milking machines and for valves and gaskets in pipelines. Its possible uses in milk and food equipment are unlimited.

These inflations make better milk possible. They will save the plant money by reducing field calls; they will last longer and perform better. They will carry less bacteria from cow to cow. They represent a significant advance in the field of sanitation.

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A SCREENING TEST FOR DETERMINING THE SANITARY QUALITY OF PROCESSED POULTRY

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To show that a correlation exists between numbers of bacteria on processed poultry and the resazurin reduction test, a field study was made in hopes that a screening test could be used by the industry and health agencies to help determine the sanitary quality of processed poultry. The study was prompted by the findings of Walker, Coffin and Ayers *(3)*.

METHODS

The work done in this study was carried out by swabbing a series of different carcasses from various age groups. The age groups used were as follows: Fresh, 1 day, 2 days, 7 days. Fresh carcasses were swabbed shortly after killing and while they were still on the lines. All stored carcasses had been put into a chlorine ice slush for 24 hours for chilling and then packed in ice. The carcasses were sampled by swabbing an area 10 cm² *(3)*. To increase accuracy of the sample, a sheet metal strip, with a handle, was measured and cut to 2 cm². Using this metal strip as a guide, five different areas were swabbed. The areas swabbed were: the left and right rib cage, left and right thighs, and the lower back just above the tail.

Materials used and prepared were as follows:

1. Cotton swabs, three inches, sterilized in a screw cap vial by autoclaving at 121°C for 30 min.
2. Sheet metal strip with handle, measured area 2 cm², dipped in 95% alcohol and flamed before use.
3. Peptone, 10 ml of 0.02% solution at pH 7.0 ± 0.1., sterilized in 6-in screw cap vials at 121°C for 15 min.
4. Alcohol, 95% for sanitizing sheet metal strip.
5. Metal container suitable for sanitizing sheet metal strip between carcasses.
6. Sample case.
7. Recombined skimmilk, 5g/100 ml of distilled water sterilized at 115°C for 10 min to prevent "caramelization."
8. Trypticase soy broth.
9. Reduction incubator, preset at 30°C *(3)*.
10. Resazurin, certified for use in testing reduction in milk, prepared according to Standard Methods *(2)*.
11. Nutrient agar pH 7.0 ± 0.1 (Difco)
12. Buffered distilled water *(2)*.

Sampling was done as follows: the sterilized cotton swab was submerged in the sterile 0.02% peptone solution and pressed against the inside of the vial to expel excess solution. The five areas of the carcass previously described were swabbed. After each area was swabbed, the swab was rinsed in the peptone solution. After all areas had been swabbed, the swab was placed in a screw capped vial containing the peptone solution. The vials containing