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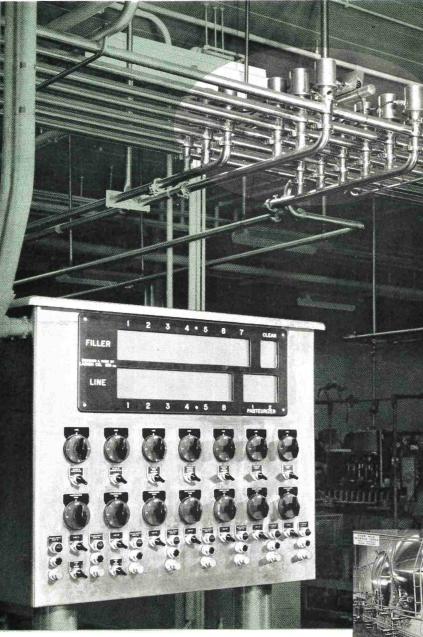
# MILK and FOOD TECHNOLOGY

Official Publication

International Association of Milk and Food Sanitarians, Inc.

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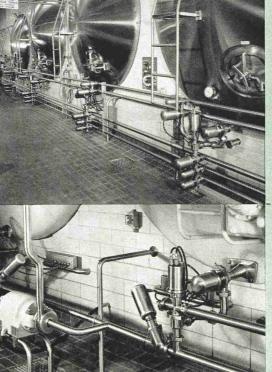
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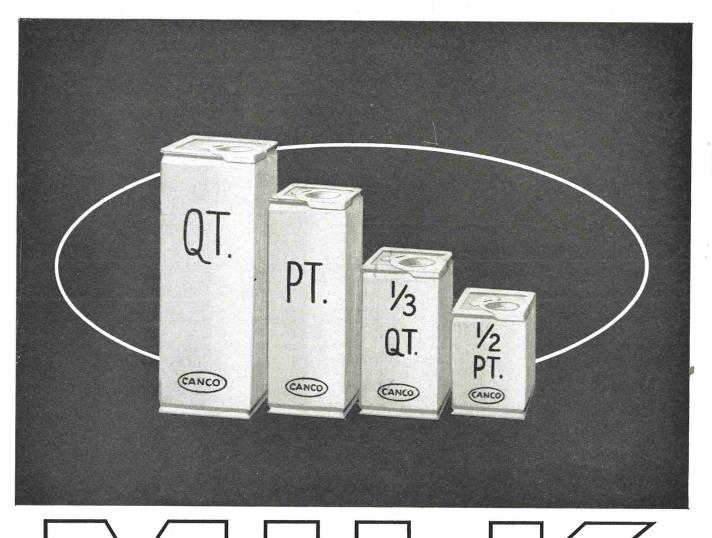
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## EDITORIAL

2

#### Why A Double Sanitation Standard For Milk

Several articles have appeared recently in favor of one sanitation standard for all milk irrespective of usage without explaining the perplexing sanitation and economic problems that such action would create. The time has come to adopt one standard for Grade A milk, according to Dr. J. C. Olson, Jr., in a noteworthy editorial in the August, 1960, issue of this *Journal*. However, until there is one standard only for Grade A, it seems unrealistic to recommend one standard for all milk. Nevertheless it is opportune to discuss the problems involved from the viewpoint of requirements necessary for highest quality and economic production.

Milk of highest quality is necessary for fluid consumption. The assurance of such quality is under the supervision of local health departments and state regulatory agencies from production on farms through processing in plants and receipt by consumers in food stores, homes, and at public eating establishments. If the milk is produced for manufacture its handling may be much more positive in respect to the protection of public health. Thus, evaporated milk is processed and sterilized in hermetically sealed cans the day it is received from producers. Fluid milk products to be used in ice cream are promptly pasteurized at temperatures higher than those used for market milk and they will be pasteurized again in the ice cream mix, then frozen and held frozen as ice cream until consumed. Hence, there is much less need for detailed supervision by sanitation officials for these manufactured products.

Producers located in areas under Grade A regulations are at an economic disadvantage in competing with those farmers producing milk for manufacture where there are no such requirements. Any dairy farmer who has shifted from producing milk for manufacture to making Grade A milk knows something about the extra costs incurred. In a large market milk region such as New York State, where nearly half of the total milk production is utilized for manufactured products, and about 99 per cent of all milk is under the state's Grade A requirements, much of the manufacturing milk is associated with the pricing of Grade A milk through the market pool and is partially subsidized by it.

There is need for specific production requirements for milk for manufacture which are not those for Grade A milk and the question actually is whether or not there can be one standard for all manufacturing milk. Most standards for milk for manufacture give two or more grades of acceptable milk, sometimes based more on the commercial quality of milk than upon public health considerations. Furthermore, differences of opinion exist as to the proper standards for each quality.

Everyone will agree that cows producing milk must be healthy, properly cared for in clean surroundings, and milked in a cleanly manner; and the milk shall be handled in properly sanitized equipment and facilities, must be cooled and delivered with regularity to the receiving plant (or delivered soon after milking in lieu of cooling); and the milk itself must be of good flavor and keeping quality. Clean milk of good quality is the goal irrespective of the usage of the milk and of farm production requirements. It seems reasonable that there ought to be two sanitation and quality standards for milk to be used for manufacture.

The first grade would be that quality of milk which can be made into dairy products of the best commercial quality possible and would have special application for fresh fluid milk products, such as sweet cream. On the basis of current information, it is reasonable that the best grade of raw milk ought to have a bacterial count not exceeding 500,000 (methylene blue reduction time not less than 4% hours or resazurin reduction time not less than 2% hours), sediment less than 2 mg. by off-bottom sampling, and free from off-odors and flavors or pronounced weed or feed odors. Such milk should satisfy both public health and industry ideals for highest quality, in fact, it might be an improvement over some milk now produced as Grade A.

The second grade should represent minimum standards for safe wholesome milk from which a good grade of manufactured product can be made. Any minimum standard must incorporate many compromises. It is reasonable that milk to meet minimum standards of acceptance should not exceed 3,000,000 in bacterial count (methylene blue not less than 2½ hours or resazurin reduction time not less than 1¼ hours); sediment less than 3 mg. by off-bottom sampling, and be free from pronounced off-odors and flavors.

This second grade milk needs discussion to point out an extremely important reason for not establishing lower standards. Recent outbreaks of illness due to consumption of certain dairy products containing toxins produced in the raw milk emphasizes that excessive bacterial counts may indicate a public health danger as well as improper sanitary methods. The problem of whether an acceptable quality product can be made with milk of much larger bacterial counts is incidental to the health problem. The dairy industry and )food officials should recognize that dairy farmers with a few milking cows cannot justify the expense of dairy sanitation facilities which are so inducive to high-quality milk production, but it must be emphasized that a most important factor in producing high-quality wilk is a dairyman with the knowledge and desire to do so. Small producers predominate in many areas now producing milk commonly used for manufacture. There must be a period for education and growth prior to enforcement in such areas.

The control program required to obtain high-quality milk, irrespective of its usage, is tremendous and is essentially an industry problem but one which industry can do successfully only if regulatory officials check their work and take action to assure compliance with the laws. There must be recognized "approved industry fieldmen" acceptable to government officials on the basis of knowledge, personality, and experience in sanitary methods of milk production. These industry men must put into practice on farms the standards for manufacturing milk and regulatory officials can determine compliance through spot-checking.

The dairy industry is not ready for a single standard for milk irrespective of its usage; in fact, a single standard for manufacturing milk cannot be attained now unless incorporated in a federal law with which many unwilling states would be obligated to comply. Our form of government and our type of business competition are not conducive to coercion of this sort.

A. C. DAHLBERG Dept. Dairy Industry Cornell University Ithaca, New York

#### ANTIBIOTICS IN MILK – A REVIEW<sup>1</sup> II. METHODS FOR DETECTION OF ANTIBIOTICS IN MILK

E. H. MARTH

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Methods suggested for use to detect antibiotics in milk have generally been based on: (a) visual detection of antibiotic contamination by incorporating a dye into preparations used for intramammary infusion or (b) changes in bacterial growth. Since most work has been done on the second method, many modifications exist. Information on both general types will be discussed below.

#### ANTIBIOTIC PREPARATIONS WITH ADDED DYES

Suspensions were prepared by addition to penicillin of the dye "Green-S" (monosodium salt of 4, 4-dimethylaminophenyl-2-hydroxy-6, 8-disulphonaphthylcarbinol-anhydride) at the rate of 25 to 75 mg. per 100,000 units (37). After udder infusion, milk produced was colored green for several days; the intensity of color corresponded well with the concentration of penicillin in milk.

Shahani (161) made up aqueous or oil solutions of penicillin to which he added 100 mg. of a greenturquoise dye per 100,000 units of antibiotic. Milk colored green was produced by treated cows for four to six milkings after intramammary infusion. Milk of the first milking after treatment contained sufficient dye to impart color even after it was diluted 500 to 2,000 times the original volume. Color from infused dye-in-oil preparations appeared in milk for a slightly longer time than when dye in an aqueous penicillin solution was used. Udder tissues or milk yield were not affected by the dye. In further work with this dye, Shahani (160) found it was excreted in milk for the same time as the antibiotic when infused with oxytetracycline, chlortetracycline or polymyxin. The dye was secreted longer than the antibiotic when infused with streptomycin. Age or breed of cow and level of milk production did not affect persistence of dye in the udder. Injection of the antibiotic-dye mixture instead of infusion failed to result in production of colored milk.

Four different food dyes were investigated for their suitability as color markers to detect antibiotic-contaminated milk (175). Fat-soluble chlorophyll appeared most promising. The dye was infused into the udder (0.5 to one ml. per quarter) and a vivid green color persisted in milk for five to ten milkings after treatment. Heavily stained particles were found in milk long after general color was depleted.

Hargrove and Plowman (66, 67) studied various compounds for use as indicators of antibiotic-contamination in milk. They found: (a) odorous or flavored compounds unsuitable for use since they were not easily detected, (b) fluorescent compounds superior to others with regard to ease and limit of detection in milk, and (c) of four compounds tested, oil-fluorescein and uranine were not toxic to cattle while 4methyl umbelliferone irritated the udder and methyl anthranilate appeared in urine. Other results obtained indicated: (a) a combination of oil-fluorescein and uranine colored both fat and non-fat portions of milk, (b) a combination of 125 mg. each of oil-fluorescein and uranine correlated better with excretion of penicillin than other test materials (milk was colored for 48 hours but dye could be detected up to 96 hours by ultra-violet light), and (c) retention of dye (and antibiotic) was influenced greatly by level of milk production.

Additional information on this subject was previously reviewed (108).

#### CHANGES IN BACTERIAL GROWTH

Many attempts have been made to employ bacteria for determination of antibiotic residues in milk and milk products. The ability of bacteria to: (a) produce acid, (b) reduce dyes, or (c) grow and produce haziness on agar media have served as the bases for most tests. Those tests dependent on these characteristics and on several others will be discussed below.

#### TESTS BASED ON ACID PRODUCTION

#### Streptococcus agalactiae.

One of the earliest methods for detection of antibiotics (penicillin) in milk, based on acid production, employed a culture of *Streptococcus agalactiae* (190). Test samples of milk were heated at 100°C. for 5 minutes, cooled and serial dilutions of each made in litmus milk. After sterile litmus was added to the original milk, the entire series was inoculated with a sensitive strain of *S. agalactiae*, incubated at 37°C.

<sup>&</sup>lt;sup>1</sup>This is the second of a series of two review papers on this subject. See previous issue of this JOURNAL for Part I. References cited in Part I are included with those listed in Part II.

for three days and checked for color development. A definite pink color was considered evidence of growth and absence of penicillin.

#### Streptococcus lactis.

Several methods which use S. lactis have been suggested. In one (99) a sample of herd milk was divided into four portions. Each portion was inoculated with a different strain of S. lactis, held at 30° C. overnight and observed for coagulation. Strains of S. lactis used were: (a) sensitive to penicillin or streptomycin, (b) resistant to penicillin, (c) resistant to streptomycin and (d) resistant to both. The presence or absence of these antibiotics was indicated by the strain or strains of S. lactis which did or did not coagulate milk.

Another method was based on differences in acid production when *S. lactis* was grown in sterile antibiotic-free milk and in antibiotic-contaminated milk (131). Raw milk samples were used, unheated and heated at 95°C., to distinguish heat stable disinfectants and antibiotics from heat-labile inhibitors such as bacteriophage. Differences of less than 10 per cent in acid production between test milks and controls were considered negative. The quantity of an antibiotic in milk was determined by use of *S. lactis* strains resistant to different concentrations of the antibiotic.

Dopter (41) suggested use of strains of S. lactis resistant to various concentrations of different antibiotics for quantitative detection, in milk, of penicillin, streptomycin or chlortetracycline. Tubes with 10 ml. milk were heated to 90°C., cooled to 30°C., inoculated with 0.25 ml. of different strains of S. lactis, held at 30°C. and checked after 12 hours for coagulation.

Berridge (22) developed a rapid method for detection of antibiotics in milk based on measurement of very small changes in pH brought about by growth of *S. lactis.* An electrometer was developed which detected pH changes of the order of 0.002 unit. The pH was measured of *S. lactis* cultures grown alone and in the presence of penicillin, chlortetracycline, streptomycin and thiomersolate. Thiomersolate halted acid production immediately, streptomycin and chlortetracycline caused slackening-off in acid production after ten minutes and penicillin produced no change during the observation period.

#### Streptococcus thermophilus.

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This bacterium has been suggested for use in antibiotic tests by several authors (21, 23, 34).

Berridge (21, 23) described a method in which a culture of *S. thermophilus* (grown in a milk, yeast extract, glucose and peptone medium containing 0.01 per cent brom-cresol-purple) was maintained in the

log phase. The culture was added in equal volume to the milk sample when the indicator in the culture was an intermediate gray in color. The milk-culture mixture was incubated at  $45^{\circ}$ C. and examined at 30 minute intervals for changes in color. A color change after 30 minutes indicated 0.06 to 0.015 unit penicillin present per ml. while a color change at one hour or later indicated 0.015 unit per ml. or less. A diluted culture was used and the test completed in 2.5 hours (21). Changes in color at 2.5 hours, by the latter method, indicated 0.01 to 0.005 unit penicillin present per ml. While a change after 30 minutes indicated the presence of 0.06 to 0.15 unit penicillin per ml.

Collins (34) suggested addition of one ml. diluted (one part culture plus two parts sterile skim milk) 16 hour-old culture of S. thermophilus to 10-ml. milk which was previously pasteurized at 143°F. for 30 minutes and cooled. The inoculated samples were incubated at 40°C. for 16 hours and checked for coagulation. It was claimed that this method detected the presence per ml. of 0.02 unit penicillin, 0.5  $\mu$ g. chlortetracycline, 6.5  $\mu$ g. streptomycin, 0.7  $\mu$ g. oxytetracycline and 0.9  $\mu$ g. tetracycline.

#### Lactobacillus bulgaricus.

A 2.5 hour test for antibiotics was described which employed a strain of *Lactobacillus bulgaricus* sensitive to 0.01 unit per ml. of penicillin (183). It normally coagulated milk in the alloted time when incubated at 45°C. Samples of milk (two ml.) were heated to 90°C. in 10 minutes, cooled to 45°C., inoculated with one drop of culture and incubated at 45°C. until the control coagulated. A second method was suggested by Ullberg (183) in which three per cent of the culture was added to sterile skim milk plus yeast extract. The diluted culture was mixed with test milk, incubated at 45°C. and milk checked for coagulation after 2.5 to three hours. A test similar to these was described by Trecanni (181).

#### Commercial starter cultures.

Many authors have recommended starter cultures for detecting antibiotics in milk. Ruehe (151) suggested that 10 ml. samples of milk, after heating to  $175^{\circ}F$ . for five minutes and cooling to  $72^{\circ}F$ ., be inoculated with one ml. of a good starter culture. If penicillin was absent, a satisfactory coagulum formed in ten hours or less. Krienke (93, 94) added one to three per cent buttermilk culture to samples of milk previously pasteurized at  $143^{\circ}F$ . for 30 minutes. Inoculated milks were incubated at  $70^{\circ}F$ . or  $95^{\circ}F$ . and checked for acid development after 18 or seven hours depending on incubation temperature. Similar procedures were used by Hansen, *et al.* (63). The addition of starters to diluted cream or skim milk from antibiotic-treated cows and subsequent titration (after incubation for 24 hours at  $22^{\circ}$ C.) with 0.1 N sodium hydroxide was suggested by Jorgensen (84) for detecting antibiotic contamination.

Bertelsen (25) found test milks could be heated to 100°C. instead of 85°C. but amount of starter added had to be increased from one to three drops and incubation temperature could not be less than 20°C. Absence of a coagulum after 24 hours indicated presence of not less than 0.25 unit penicillin per ml.

Yogurt cultures were suggested for use in detecting antibiotics in milk (1, 49). It was claimed the yogurt culture was 10 times as sensitive to penicillin as other starters and could detect 0.005 unit per ml. (1). Another work (49) indicated 0.02 unit per ml. of penicillin could be detected with a yogurt culture. One method (49) suggested the heating of 20 ml. of test milk to boiling, subsequent cooling, inoculation with one ml. of yogurt culture, incubation for 2 hours at  $44^{\circ}$ C. and titration for acid production. A difference between sample and control of five or more degrees in acidity (Soxhlet-Henkel) indicated the presence of penicillin.

#### Tests Based on Reduction of Dyes

This group of tests makes use of the principle that actively growing bacteria reduce certain dyes with resulting color changes. If antibiotics are present in milk, test bacteria will not grow, the dye will not be reduced and the color remains unchanged.

#### Methylene blue.

A method, suggested by Schiffer and Peterson (156) used methylene blue and a culture of *Bacillus cereus* var. *mycoides* to detect chlortetracycline in milk. A 0.5 ml. sample of milk was placed into a Wasserman tube and serial dilutions made with sterile methylene blue milk. The diluted samples were inoculated with 1.5 ml. culture per tube, incubated at 37°C. for four hours and checked for dye reduction. It was claimed that 0.031  $\mu$ g. per ml. of chlortetracycline could be detected by this procedure. The method was modified (157) by use of *Bacillus mesentericus* and was claimed satisfactory for detecting chlortetracycline and oxytetracycline in milk.

Galesloot (55) suggested the following procedure to detect penicillin in milk at levels down to 0.01 unit per ml. Thirty ml. of milk sample were divided equally between two tubes, heated for 10 minutes at  $80^{\circ}$ C. and cooled to  $37^{\circ}$ C. Penicillinase was added to one tube and both tubes were held at  $37^{\circ}$ C. for 30 minutes. One-tenth ml. of a 16-24 hour culture of *S. thermophilus* plus 0.4 ml. methylene blue solution were added to both tubes which were then held at  $45^{\circ}$ C. Reduction times were determined for both samples. A difference was considered as proof for presence of penicillin.

The methylene blue method was also applied to acid dairy products (buttermilk, ripened cream, starters and yogurt) for detection of penicillin (55). The sample of product was neutralized to pH 6.8 with 10 per cent sodium hydroxide and the balance of the test performed as described above (55). A more sensitive method based on an extraction procedure has also been developed for these products (55).

Bertelsen and Mattson (26) reported they were able to detect 0.05 unit penicillin per ml. of milk by addition of methylene blue and a yogurt culture to samples prior to incubation at  $37^{\circ}$ C. or  $45^{\circ}$ C. for two hours. The use of triphenyltetrazolium chloride (test discussed later) permitted detection of 0.025 unit per ml. but this procedure had the disadvantage that dye could not be added with the culture.

#### Resazurin.

A method to detect penicillin in milk with resazurin and S. thermophilus was reported by Hietaranta and Timroth (71). Samples of milk to be tested were divided into two portions, heated at 80°C. for five to 10 minutes and cooled to 38°C. Penicillinase (0.4 unit per ml. milk) was added to one portion and resazurin plus five per cent culture were added to both portions after which they were incubated at 38°C. and examined at 30 minute intervals for color changes. Presence of penicillin was indicated in about 45 minutes.

Slatter (173) found the resazurin method did not distinguish between different levels of penicillin in samples of pasteurized milk. The titratable-acid test permitted limited detection of differences in penicillin levels but longer incubation periods were required.

Resazurin has also been used with a plating procedure (163, 164) which will be discussed later in this review.

#### Triphenyltetrazolium chloride.

This method for detecting inhibitory substances in milk was described by Neal (122) and Neal and Calbert (124, 125). The test is based on conversion of 2, 3, 5-triphenyltetrazolium chloride (TTC) to formazone by actively growing bacterial cells. This conversion is accompanied by a color change from the leucoform to red. Antibiotics inhibit growth of bacteria which in turn eliminates conversion of TTC and the associated color change (124). The procedure suggested follows (125): (a) 10 ml. of test milk, in a sterile screw top test tube, was pasteurized at 80°C. for five minutes and cooled to 37°C.; (b) samples were inoculated with (one ml.) a 1:1 dilution (in sterile antibiotic-free skim milk) of a S. thermophilus culture and incubated at 37°C. for two hours; (c) 0.3 ml. of a 1:25 solution of TTC was added to each sample which then were reincubated at 37°C. for 30 minutes; (d) samples were checked for color changes — red indicated no antibiotic present and white to pink possible antibiotic contamination.

It was claimed (124) the test detected the presense, in milk, of different antibiotics at the following minimum levels: penicillin, 0.04 unit; chlortetracycline, 0.2  $\mu$ g.; oxytetracycline, 0.25  $\mu$ g.; and streptomycin, 4.0  $\mu$ g. per ml. Sanitizing agents have been found to affect the test (125). Slight inhibition of the test organism was produced by presence, in milk, of 10 p.p.m. quaternary ammonium compounds, 30 p.p.m. Iobac (polyethoxy-polypropoxy-ethanol-iodine complex), 8 p.p.m. Iosan (Iobac plus detergent), and 30 p.p.m. chlorine. Strong inhibition was produced by these compounds at levels of 14, 90, 24 and 90 p.p.m. respectively.

Neal and Calbert (126) found 2, 3, 5-triphenyl tetrazolium chloride was more sensitive than neotetrazolium or blue tetrazolium in detection of oxytetracycline or streptomycin in raw milk. The TTC test was applied to herd milks by Neal and Calbert (123). A good correlation was found between lack of color development and subsequent inhibition of acid production in the same milks.

Dragon (42) suggested a modification to the TTC test which enabled one to determine whether inhibition resulted from antibiotics or from sanitizers. Tubes which initially showed inhibition were treated further by addition of one ml. each of a sterile two per cent glucose solution and a suspension of baker's yeast. The mixture was incubated at 30°C. for three to 12 additional hours and checked for gas production. Sanitizers, if present, inhibited yeast and gas was not produced while antibiotics, if present, did not and gas was produced.

The TTC test was used for detection of antibiotics in dried milk by Kotter and Muspack (92). The dried product was reconstituted to its original composition, centrifuged to remove fat and tubed in 10 ml. portions. The regular procedure was then followed except a yogurt culture was substituted for S. thermophilus.

Parks and Doan (129) compared sensitivities of the TTC test and disc-assay method (which will be discussed later) to various antibiotics. The TTC test was about equal to the disc-assay method in detection of penicillin and chlortetracycline but was less sensitive to streptomycin and unsatisfactory for detection of <sup>4</sup> neomycin.

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Recently a more rapid TTC test was suggested by Igarashi, *et al.* (73) which employed *Bacillus stearothermophilus* and an incubation temperature of 61-62°C. The test, completed after about 35 minutes

incubation, detected the presence, in milk, of 0.005 unit per ml. of penicillin and about 0.5  $\mu$ g. per ml. of tetracycline, chlortetracycline or oxytetracycline. The test was less sensitive when used to detect neomycin, polymyxin, bacitracin, streptomycin or dihydrostreptomycin.

#### Tests Based on Plating Procedures

These methods are all based on inhibiting growth of sensitive bacteria by an antibiotic placed in contact with a portion of inoculated agar prior to growth of the culture.

#### Cylinder-plate methods.

Methods to assay penicillin were first developed for evaluation of fermentation liquors and pharmaceutical products. Foster and Woodruff (53) described a method in which 15,000 spores of *Bacillus subtilis* were added per ml. of an agar medium (beef extract, peptone, sodium chloride, agar and water) after which 13 ml. of the seeded agar was added to a petri dish. Small, sterile glass cups (cut from tubing) were warmed and set lightly on the agar. These cups were filled with the sample or a standard solution. Petri plates were than incubated at 30°C. for 12 to 16 hours. The area around the cup in which microbial growth did not occur (zone of inhibition) was measured and that of the sample compared with that of a standard solution.

Schmidt and Mayer (158) suggested Staph. aureus for assaying penicillin by means of the cup (or cylinder)-plate method. They reported the size of zone decreased as depth of agar medium in a petri dish increased. When 25 ml. of medium per plate was used, the edge of the zone was distinct and clear. If only 15 ml. were used, however, the edge of the zone became indistinct. The most clear cut zone was obtained with agar medium at pH 6.0. As pH was increased, the edge of the zone became more indistinct.

The cup-plate method, described above, was modified by Beadle, *et al.* (19) who suggested use of 4.5 by 11.5 inch rectangular glass dishes instead of petri plates. A guide was used to drop cups on inoculated culture plates.

The cylinder-plate method was adapted for use with milk by different workers (14, 75, 174, 176, 180, 182, 191) and is the "official" procedure used by Food and Drug Administration laboratories (62).

The methods of Schmidt and Mayer (158) and Beadle, et al. (19) were applied to milk for detection of penicillin (191). It was noted in these experiments when penicillin was diluted in milk, growth of Staph. aureus (the test organism) was stimulated at the periphery of zones. As a result, standard penicillin solutions were also prepared in sterile skim milk instead of phosphate buffer. This modification was later used in the test for detection of streptomycin (176).

Slight variations of the previously described method were suggested by Thorp, *et al.* (180). They adjusted the pH of milk to 6.5 with a phosphate buffer solution. Petri plates were poured with 22 ml. sterile nutrient agar which was allowed to harden. After hardening, three ml. of nutrient agar seeded with *Staph. aureus* was spread evenly over the surface of each plate. Before inoculated agar hardened, four sterile aluminum cups were placed equidistant on top. The cups were filled with standard or unknown solutions and plates were covered with porcelain tops before incubation at 37°C. Afterward, zone sizes produced by the unknown were compared to those of the standard solution.

The use of the cup-plate method with *Staph. aureus* as test organism to detect penicillin in milk was also suggested by other authors (14, 75, 85, 116, 128).

Nilsson and Nilsson (128) found aluminum cylinders unsuitable for this method since they inhibited growth of *Staph. aureus*. Stainless steel and glass cylinders were satisfactory.

Different factors which affected the cylinder-plate test when applied to milk were studied by Meewes and Milosevic (116). They found zones of inhibition produced by penicillin increased with incubation time and decreased with depth of agar in petri plates. It was further observed that clear cut zones of inhibition were produced if the test organism was spread over the surface of agar medium rather than mixed with it. Raw milk, which contained no penicillin, sometimes inhibited Staph. aureus as did the presence, in skim milk, of Bacillus subtilis, Pseudomonas florescens and Ps. pyocyaneum. The presence of Ps. pyocaneum or Bacterium linens in raw milk also inhibited Staph. aureus.

Other organisms have been suggested for use in cylinder-plate procedures. These include Sarcina lutea for detection of penicillin (85), Bacillus cereus for streptomycin (75), Bacillus cereus var. mycoides for chlortetracycline (75), a group A streptococcus for penicillin (174) and Bacillus subtilis (ATCC 6633) for streptomycin (182).

The use of *B. subtilis* to detect streytomycin in milk (182) was preceded by coagulation of milk through addition of acid, separation, clarification and neutralization of whey. The resultant clear whey served as the test substance.

#### Food and Drug Association Methods.

A brief description of these tests will be included here. The details for preparation of standard solutions, test organisms and media may be obtained from the appropriate reference by interested readers (62). All tests employed stainless steel cylinders to hold standard and test solutions.

#### PENICILLIN

(a) milk sample diluted with antibiotic-free milk, (b) 10 ml. agar medium poured into petri plate, (c) four ml. agar medium seeded' with previously standardized suspension of Sarcina lutea (ATCC 9341) and spread over surface of hardened base layer, (d) cylinders put in place and solutions added, (e) plates incubated at 26°C. for 16 to 18 hours, diameters of zones measured.

#### Streptomycin

(a) milk sample diluted with phosphate buffer at pH 8.0, (b) 10 ml. agar medium (pH 7.8 to 8.0) base layer added to plates, (c) four ml. agar medium seeded with suspension of *Bacillus subtilis* (ATCC 6633) spores, spread over base layer, (d) cylinders put in place and solutions added, (e) plates incubated at 37°C. for 16 to 18 hours, diameters of zones measured.

#### Tetracyclines

(a) milk sample diluted with phosphate buffer at pH 4.5, (b) six ml. agar medium (pH 5.6 to 5.7) base layer added to plates, (c) four ml. agar medium seeded with suspension of *Bacillus cereus* var. *mycoides* (ATCC 9634), spread over base layer, (d) cylinders put in place and solutions added, (e) plates incubated at  $30^{\circ}$ C. for 16 to 18 hours, diameters of zones measured.

#### BACITRACIN

(a) undiluted milk used, (b) 10 ml. agar medium base layer added to plates, (c) four ml. agar medium seeded with suspension of *Micrococcus flavus* (ATCC 10240), spread over base layer, (d) cylinders put in place and solutions added, (e) plates incubated at  $26^{\circ}$ C. for 16 to 18 hours, diameters of zones measured.

#### Polymyxin

(a) 10 ml. agar medium (pH 7.2 to 7.3) base layer added to plates, (b) four ml. agar medium seeded with suspension of *Brucella bronchiseptica* (ATCC 4617), spread over base layer, (c) cylinders put in place and solutions added, (d) plates incubated at  $37^{\circ}$ C. for 16 to 18 hours, diameters of zones measured.

#### ERYTHROMYCIN

As for penicillin except: (a) different agar media used for base and seed layers, (b) plates incubated at  $32^{\circ}$ C. to  $35^{\circ}$ C. for 16 to 18 hours.

#### MAGNAMYCIN

As for penicillin except: (a) different agar media used for base and seed layers, (b) six ml. of agar medium used for base layer.

#### Disc-plate methods.

One of the earliest disc-plate methods suggested was that of Welsh, *et al.* (192) which employed three ml. agar medium seeded with spores of *B. subtilis* per petri dish, filter paper discs (seven mm. in diameter) to each of which 0.03 ml. test liquid was added, and an incubation temperature of  $39^{\circ}$ C. Results were read after four hours.

Drury (43) described a procedure which was commonly used for many years. This method employed: (a) Difco whey agar seeded with B. subtilis spores so that the final concentration was 250,000 spores per ml. of agar, (b) ten ml. seeded agar per petri plate, (c) 0.25 inch paper discs soaked in milk (absorbed about 0.017 ml. milk) and placed on surface of seeded agar, and (d) incubation at  $37^{\circ}$ C. for four hours. Plates were then checked for zones of inhibition.

This method was studied by Silverman and Kosikowski (168) who used 0.5 inch discs in addition to the 0.25 inch ones. They reported the method ineffective for detection of sulfa drugs or quaternary ammonium compounds.

Modifications of the above described test were introduced by Cerny and Morris (29). They suggested use of: (a) six ml. seeded agar in a flat-bottom petri dish, (b) milk heated to  $180^{\circ}$ F. for five minutes, (c) two 0.5 inch filter paper discs, one superimposed on the other, and (d) incubation of plates at  $37^{\circ}$ C. for eight hours or overnight at  $77^{\circ}$ F. They found use of double discs (0.5 inch) made the test precise to 0.01 unit penicillin per ml. while precision below 0.1 unit per ml. was lost with 0.25 inch discs. Double discs (0.5 inch) absorbed about ten times as much milk as a single 0.25 inch disc. It was claimed the test would also detect low concentrations of chlortetracycline, oxytetracycline, streptomycin, polymyxin-B, neomycin, bacitracin and combinations of sulfa drugs.

Gogas and Bicknell (58) described a disc-plate method to detect penicillin in milk which provided results in two hours or less. The procedure outlined for this test follows: (a) two ml. of a 48 hour broth culture of *B. subtilis* (ATCC 6633) was added to 100 ml. of penassay agar, (b) 15 ml. of inoculated agar added per petri dish and covers of dishes replaced by steri-fized porcelain or glass (with filter paper inner lining) covers, (c) plates were inverted, incubated for 2.5 hours and refrigerated until needed, (d) sterile filter paper discs (12.7 mm. diameter—held 0.023 ml.) were moistened with milk and placed on the surface of

agar, and (e) plates incubated at 37°C. for two hours or less and checked for zone formation.

Johns and Berzins (80) studied the method just described and found zones could be detected in two hours only if fresh plates were used. When previously incubated plates were refrigerated overnight, zones could first be detected after five hours of incubation. It was further observed that larger zones resulted when four instead of 15 ml. of seeded agar was used. Growth on whey agar was slower than on penassay agar and overnight incubation with the latter resulted in overgrowth of zones.

A disc-plate method for detection of chlortetracycline in milk was suggested by Grady and Williams (60). This method was based on one previously used to determine chlortetracycline in feeds. The following was suggested: (a) petri plates were prepared with a base layer of agar medium and a layer seeded with spores of *Bacillus cereus*, (b) serial dilutions of milk and of a known chlortetracycline solution were prepared, (c) 0.1 ml. of all dilutions were pipetted onto separate discs previously placed on the surface of agar, (d) plates were refrigerated for one hour, then incubated 28 to 30° C. for 15 to 18 hours, (e) zones produced by milk samples were compared to those of standard solutions.

Pital, et al. (134) described a method for determining sensitivity of organisms to antibiotics. Discs impregnated with antibiotics were placed on seeded plates and incubated at 37°C. for a short time. The discs were then removed and 0.6 ml. of a 0.05 per cent resazurin solution was poured over the surface of agar and allowed to diffuse for ten minutes. Plates were returned for incubation and zones of inhibition were clearly marked by a color difference. Tests were completed in 4.5 hours for Bacillus anthracis, 5.5 hours for Brucella suis and 2.3 hours for Staph. aureus. Chloramphenicol, chlortetracycline, oxytetracycline and penicillin were tested. This method was later applied to detect antibiotics in milk by Shahani and Badami (163, 164). They suggested the following: (a) 100 ml. of an agar medium was inoculated with four ml. of Staph. aureus (brain-heart infusion agar) or Lactobacillus bulgaricus (whey agar plus ten per cent filtered tomato juice), (b) 20 ml. seeded agar was added per petri dish, (c) 0.5 inch discs were soaked in milk and placed on agar, (d) after incubation at 40°C. for 40 minutes, discs were removed and the surface of agar covered with 0.5 ml. 0.084 per cent resazurin solution, (e) the surface was covered with mineral oil (to prevent reoxidation of dye), plates were reincubated at 40°C. and observed periodically. Within ten to 90 minutes, dye was reduced by organisms growing in agar except where antibiotics from milk had diffused into the agar. After 40 to 150 minutes,

zones were violet or deep red and the balance of the agar surface was light red in color. The test, with *Staph. aureus*, was claimed to detect 0.04 to 0.06 p.p.m. of penicillin, chlortetracycline, oxytetracycline or tetracycline and 0.17 p.p.m. streptomycin in two to three hours. When *L. bulgaricus* was used, similar results were obtained for all antibiotics except streptomycin which was detected at a level of 0.11 ppm The test, however, required only 1.6 to 1.9 hours for completion. Heating of milk to  $170^{\circ}$  F for two minutes eliminated false-positive results from natural inhibitory substances but decreased the sensitivity of the test to 0.1 ppm for penicillin and chlortetracycline and 0.3 ppm for streptomycin and oxytetracycline (163).

Wolin and Kosikowski (196, 197) studied the effect of natural inhibitory substances in milk on disc-assay methods. Dried milk (lyophilized) discs prepared from milks with the substances produced larger zones than milk-soaked paper discs. Bacteria such as *B. subtilis*, S. *faecalis* and *E. coli* were sensitive to these inhibitory substances. Raw milks produced two zones; the first appeared after 12 to 24 hours incubation at 20°C. and was characterized by no growth. The second zone appeared later and was characterized by disappearance of colonies. Incubation at 37°C. for six hours produced zones but better results were obtained at 20°C.

Sensitivity of the disc-plate method as affected by discs was studied by Siino, *et al.* (167). They found the test more sensitive when 0.5 inch discs (single or double) were used instead of 0.25 inch (single or double) ones. The sensitivity of the disc-plate method was also increased by use of freeze-dried discs prepared from antibiotic-contaminated milks (see above) in place of 0.5 inch milk-soaked paper discs (91).

Parks and Doan (129) compared the TTC and discplate (Bacto whey agar, Bacto subtilis spore suspension and 0.5 inch discs) tests for sensitivity to different antibiotics. The disc-plate method detected 0.02 unit penicillin O, 0.013 unit penicillin G, 0.055  $\mu$ g. chlortetracycline and 0.75  $\mu$ g. streptomycin per ml. Seeded agar had to be refrigerated for 48 to 96 hours to detect the low level of chlortetracycline indicated and 250 hours for streptomycin. The TTC test was similar in sensitivity for penicillins and chlortetracycline but less sensitive for streptomycin.

Recently Arret and Kirshbaum (7) reported a discassay method which was said to detect 0.05 unit of penicillin per ml. of milk in 2.5 hours. Procedures were described for preparation of media and of a *Bacillus subtilis* (ATCC 6633) spore suspension (the test organism). Testing methods called for: (*a*) preparation of petri plates with ten ml. of previously

seeded agar (rate of seeding to be determined by trial and error) and storage at  $15^{\circ}$ C. for three to five days before use, (b) 0.25 inch discs dipped in milk samples and placed on surface of seeded agar together with a control disc previously prepared from a 0.05 unit per ml. penicillin solution, (c) plates incubated at 37°C. for 2.5 hours and checked for zone formation. A procedure, based on the use of penicillinase, to determine whether antibiotic activity was caused by penicillin was suggested. Preincubation of seeded plates for 0.5 to 1.0 hour at 37°C reduced sensitivity of the test. The authors later modified the indicated procedure by recommending that seeded agar plates be stored in the refrigerator instead of at 15°C. (8).

Johns (79) studied the Arret and Kirschbaum method and concluded it was less reliable, sensitive or simple than the disc-assay method which used commercially available materials. The test was claimed to be less simple for it required: (a) preparation of spore suspensions, and (b) incubators set at  $15^{\circ}C$  and 37°C, neither of which were usually available in dairy laboratories (the 15°C incubation requirement later was replaced by need for a refrigerator - see above). Johns claimed the method was less reliable for, as described, it failed to detect penicillin. The method was found less sensitive than others for: (a) it suggested use of 0.25 inch discs which hold about one-sixth as much milk as 0.5 inch discs, and (b) required use of ten ml. agar medium per plate when it is generally recognized that a thinner layer provides greater sensitivity.

Results of studies on the F.D.A. method carried out in this (author) laboratory (2) were in substantial agreement with those of Johns (79). A comparison of three media; penassay, brain-heart and whey agar; showed greatest sensitivity was obtained with penassay agar. Studies on spore concentration needed in agar showed that about 8,000,000 per ml. were necessary to obtain sufficiently sensitive results in 2.5 hours. The level of seeded agar needed in petri dishes for best results was checked and five ml. was most suitable for regular detection of 0.05 unit penicillin per ml. of milk. When 10 ml. were used, erratic results with frequent false-negatives were obtained. Flat bottom, regular and plastic petri dishes were all found suitable for use provided they were poured on a flat surface and that bottoms were not excessively scratched. Porcelain covers did not seem to improve the test. Greater sensitivity was obtained when 0.5 inch discs were used instead of recommended ones.

Commercial preparations (Difco and Baltimore Biological Laboratories) are currently available for use in performing the rapid disc-plate test. Spore concentrations have been adjusted appropriately so that results are obtained in 2.5 hours.

Recently Igarashi, et al. (78) suggested a rapid discplate method which employed: (a) trypticase-yeast extract-glucose agar, (b) Bacillus stearothermophilus, (c) 0.5 inch discs and (d) an incubation of  $65^{\circ}$ C. for 1.5 hours. The test detected as little as 0.005 unit penicillin present per ml. of milk.

#### Field test kits.

Kosikowski (89) suggested use of plastic plates seeded with antibiotic sensitive-bacteria and packaged under vacuum or gas in aluminum-polyethylene pouches together with discs and a tweezer as a complete test kit for use in detection of antibiotics in milk. Studies showed that either S. lutea or Staph. aureus remained dormant and survived 12 days at  $34^{\circ}$ C or 21 days at  $37^{\circ}$ C when stored in kits described above. Sensitivity to penicillin was unimpared but growth was less luxuriant after storage. When B. subtilis was handled in a similar fashion, it survived but had lost its sensitivity to penicillin (98).

More recently Kosikowski and Ledford (90) suggested a procedure which eliminated need for anaerobiosis to preserve test materials from time of production until use. A spore suspension of B. subtilis was incorporated into non-nutrient agar (with 0.9 per cent NaCl) which was poured into plastic petri dishes. Discs were prepared by soaking in a nutrient solution after which they were dried. The seeded petri plate, discs and a tweezer were packaged together. When the test was used, nutrient-saturated discs were dipped into milk samples and placed on the surface of agar. Both nutrients and antibiotic diffused into the agar. Growth of bacteria was prevented in a small zone around the disc (into which the antibiotic and nutrient had diffused) but occurred in a circular area further from the disc (into which only the nutrient diffused).

Levowitz (100) described a similar test kit which used *B. subtilis* or *S. lutea*. Plates prepared with *B. subtilis* were read after four to six hours of incubation at 95°F. and were sensitive to 0.05 unit per ml. of penicillin. An incubation period of 16 hours at room temperature was required for plates seeded with *S. lutea*.

#### MISCELLANEOUS TESTS

Earlier work (109) indicated morphology of certain lactic acid bacteria was influenced by antibiotics present in the growth medium. Liska (103) reported penicillin caused a swelling and an elongation of individual cells of S. lactis, S. thermophilus and Leuconostoc dextranicum. Changes in appearance of S. thermophilus cells could be detected after exposure to 0.025 to 0.5 unit per ml. of penicillin for 30 minutes at 37°C. These observations served as bases for

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the direct microscopic test to detect antibiotics in milk as suggested by Liska (101, 102). Samples of milk and an appropriate control were heated to 80°C. for three minutes, cooled to 37°C. and inoculated with a 1:10 dilution (in antibiotic-free skim milk) of a previously coagulated milk culture of S. thermophilus. The inoculated samples were held at 37°C. for 60 to 90 minutes and mixed at 30 minute intervals. Slides were than prepared from the sample, stained with acid-water free methylene blue or the Newman-Lampert method and examined microscopically. The average number of bacterial clumps in five fields was determined and bacterial cells were observed for distortion or enlargement. Results indicated antibiotics were present in any sample which caused cell distortion or enlargement and/or had a clump count per field which was 50 per cent less than the control. It was claimed the method (after 90 minutes of incubation) could detect a minimum of 0.015 unit of penicillin, 0.15  $\mu$ g. of chlortetracycline or oxytetracycline, 0.01  $\mu$ g. of bacitracin or 0.75  $\mu$ g. of chloramphenicol per ml. of milk. The presence in milk of five p.p.m. chlorine or 40 p.p.m. Iodophors gave results similar to those obtained with antibiotics.

A test for detection of penicillin in milk and based on morphological changes of *S. cremoris* was suggested by Whitehead and Cox (193). After inoculated test milks were incubated at  $30^{\circ}$ C. for five hours, the presence of 0.05 unit penicillin per ml. resulted in the formation of swollen cells while 0.1 unit per ml. frequently produced rod-shaped cells.

Mattick (113) and Mattick, et al. (114) reported a method for detection of antibiotics in milk based on inhibiting reduction of nitrate to nitrate by Staph. aureus. This test detected antibiotics at indicated minimum levels: penicillin, 0.1 unit per ml; chlortetracycline, 0.5 ppm; streptomycin, 1.0 ppm; oxytetracycline, 0.9 ppm; and bacitracin, 10 units per ml. The procedure suggested follows: (a) 0.1 ml. of a 20 per cent sodium nitrate solution and five ml. of a 24 hour Staph. aureus culture were added to 10 ml. of milk sample, (b) after incubation at 37°C. for 90 min., one ml. of 15 per cent trichloroacetic acid was added and the mixture filtered, (c) one ml. of sulfanilic acid was added to a mixture of five ml. filtrate and five ml. nitrogen-free water in an ice-bath, (d) one ml. of alphanapthylamine hydrochloride was added, the degree of color was determined by means of a colorimeter and compared to appropriate standards.

Mathews and Hesketh (112) suggested a method for detection of penicillin in milk (down to 0.025 unit per ml.) based on inhibition of *Sarcina lutea*. Equal volumes (two ml.) of test milk and melted, sterile proteose-peptone agar were mixed and slanted. After solidification, slants were streaked with *S. lutea*, incubated at 25 to  $37^{\circ}$  C. for 16 hours and checked for presence or absence of yellow colonies.

The two methods described briefly below have not been applied to milk but are included here because of their possible interest to the reader. Berridge and Barret (24) suggested a turbidimetric method to assay for antibiotics. Serial dilutions of the test antibiotic were added to log-phase cultures of S. agalactiae. After 30 minutes of incubation, turbidity determinations were made and compared to previously established standards. The method was found applicable for assays of penicillin, streptomycin, chlortetracycline and gramicidin. Royce, et al. (150) suggested the use of penicillinase in assays for penicillin. The sample (50 mg.) was added to water (10 ml.) with phenol red (0.2 ml.) and the mixture adjusted to pH 7.5. One ml. of penicillinase (at pH 7.5) was added and allowed to react at room temperature for 30 minutes. The mixture was titrated with 0.01 N sodium hydroxide until the solution color matched a control. The quantity of penicilloic acid present was measured by the titration. Penicillinase catalysed the hydrolysis of penicillin to penicilloic acid. It was claimed that each ml. of 0.01 N sodium hydroxide used in the titration represented the presence in the sample of 6,023 units of penicillin.

#### SUMMARY

Milk supplies, under certain conditions, were contaminated with antibiotics as a result of: (a) treatment of bovine mastitis, (b) injection of dairy cattle, (c) oral ingestion (when used to preserve silage, control bloat or as a feed supplement). Proper use of antibiotics reduced or eliminated contamination.

Constituents of milk partially inactivated different antibiotics. Low temperature storage had no adverse effect on antibiotic activity.

Allergic reactions in certain sensitive individuals have resulted from consumption of penicillin-contaminated dairy products and have been treated with penicillinase.

Common bacteriological tests used to detect antibiotics in milk include those based on: (a) acid production, (b) dye-reduction or (c) growth of test organisms in an agar medium. Several others have been described.

The use of antibiotic-dye mixtures in treatment of mastitis has been suggested as a means for visual detection of contamination in milk.

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## BOVINE MASTITIS: ITS DETECTION AND PREVENTION<sup>1, 2</sup>

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"CORONER ACTS TO CURB TOOL OF PEN-ICILLIN – Cites 3 Deaths, Asks Sensitivity tests." This was an actual newspaper headline (1). It is estimated that five to six per cent of all individuals are sensitive to some form of penicillin, with 3,000 to 4,000 cases annually of anaphylactoid shock and a mortality rate of ten per cent (33). Demonstration of definite penicillin sensitization to humans through contaminated milk has been recorded (34, 32). These incidents have created a greater cognizance of the importance of penicillin and other antibiotics in milk utilized for human consumption with the concomitant emphasis on mastitis prevention and therapy (31).

#### WHAT IS MASTITIS?

Mastitis is an inflammation of any part or all of the mammary gland with the cardinal symptoms of heat, redness, swelling, pain, and the abnormal secretion that usually accompanies inflammation of tissue. The presence of an organism within the mammary gland does not in itself constitute mastitis, as most quarters of nearly all mammary glands contain organisms living as commensal parasites (9, 16, 7). These organisms are all potential mastitis producing organisms, and will become pathogenic if the mammary gland is subjected to stress and its resistance lowered.

Mastitis differs from many of the infectious diseases of cattle such as tuberculosis, brucellosis, and anthrax in that no one specific organism alone is responsible for the initiation and the production of the symptoms of mastitis. Numerous investigations have been concerned with *Streptococcus agalactiae* as a specific causative agent of mastitis. Confusion has resulted in that inhibitory bacteriological media were utilized to exclude all other organisms, or if other organisms were isolated on a non-inhibitory medium, organisms other than *S. agalactiae* often have been designated as contaminants. The theory that mastitis is the presence within a mammary gland of an organism, with total disregard for clinical symptomatology, has

contributed to additional confusion with wide variance in investigational data and conclusions (3, 14, 19, 21).

Clinical mastitis may be classified on the basis of pathology and severity. In mild mastitis a few clots appear in the first milk removed. Sometimes there is a slight swelling of the infected quarter and this quarter may have a temperature above normal.

In acute mastitis many clots are formed and the infected quarter has an extensive abnormal secretion. The quarter is swollen, hard and hot. The body temperature is abnormal and the cow may be off feed.

In peracute mastitis all the symptoms of the acute form are exhibited only in a more intense manner. In addition, the animal is usually completely off feed and very depressed. All cows that have mastitis will give less milk, but in a varying degree.

In chronic mastitis intermittent symptoms of any one of the above types may be present.

#### What are the Causative Agents of Mastitis?

Investigators utilizing non-inhibitory media and isolating organisms from mammary secretions have reported staphylococci as the predominant organism isolated (2, 4, 6, 17, 18, 20, 22). Investigations by Krabbenhoft *et al.* (12) have corroborated these findings in isolations from mammary secretions of clinical mastitic and nonmastitic glands or quarters (Table 1).

In addition, many other microorganisms and viruses have been isolated from the mammary gland and/or designated as the causative agent of mastitis (Table 2). When consideration is given to the

TABLE 1–DISTRIBUTIONS OF MICROORGANISMS (% DISTRIBUTION) FROM MASTITIC AND NON-MASTITIC QUARTERS

Microorganisms Isolated	Mastitic quarters		n-mastitic uarters
Micrococcus -	62	2	76
Escherichia and Aerobacter	10		9
Streptococcus	14		3
Proteus	4		1
Corynebacterium	2		4
Bacillus	2		3
Klebsiella	1		0
Gaffkya	0		3
Sarcina	0		2
Diplococcus	2		0

<sup>&</sup>lt;sup>1</sup>Published with approval of the Director, North Dakota Agricultural Experiment Station.

<sup>&</sup>lt;sup>2</sup>Presented at the 47th Annual Meeting of the INTERNATIONAL Association of Milk and Food Sanitarians, Inc., at Chicago, Illinois, October 26-28, 1960.

Bacteria	Bacteria		
(Gram +) Staphylococcus Streptococcus Clostridium Gaffkya Sarcina Listeria Serratia Mycobacterium Corynebacterium	(Gram –) Pasteurella Brucella Klebsiella Erysipelothrix Escherichia Aerobacter Proteus Pseudomonas Leptospira	Nocardia Candida Cryptococcus Blastomyces Viruses New Castle Disease Vesicular Stomatitis Vaccinia Psittacosis Foot and Mouth Disease Enteric	

TABLE 2-MICROORGANISMS ISOLATED FROM MASTITIC AND/OR INCRIMINATED AS CAUSATIVE AGENTS OF MASTITIS

multitudinous forms of microbiological life existing in the environment of the bovine, and the anatomical arrangement of the mammary gland, it is readily understandable that the ease of mammary invasion provides extensive variations of intra-mammary microbial life. The intramammary flora of clinical mastitis-free mammary glands deserves greater cognizance. Investigations indicate that the chemotherapeutic removal of one genus usually provides a more suitable environment for other existing organisms, or for the intramammary invasion of organisms existing in the immediate environment that previously have not been present in the mammary gland.

Through various organisms apparently live a parasitic (commensal form) life within the mammary gland, they all possess a pathogenic potentiality that is released upon subjection of the mammary gland to stress. Stress may result from trauma or lowered resistance within the mammary gland with resulting inflammation and abnormal secretions.

#### HOW MAY MASTITIS BE DETECTED?

Many tests have been proposed to detect mastitis, through few have withstood the test of time. The strip cup, when used at each milking, has proved one of the most reliable tests available to the dairyman. It provides detection of the early symptoms of mastitis as well as the specific infected quarter. It is a simple, inexpensive, rapid test not requiring the addition of chemicals or stirring.

Laboratory bacteriological examination of milk samples has often been advocated. This method provides information regarding the specific organism present within the mammary gland. If clinical mastitis exists, the time lapse between collection of the milk sample and bacteriological identification is too great to provide information that will contribute to chemotherapy. Thus, by the time identification has been completed, successful therapy will have resulted by "guess" chemotherapy; or the inflammation will have progressed beyond possible successful therapy. When bacteriological examination of milk is utilized in a prevention program, a decision must be made as to which organism will be designated as the causative agent of bovine mastitis. If the designated organism is eliminated, the possibilities of its return or its replacement by even a more virulent type must be considered.

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The Whiteside test has been demonstrated to be of value in detecting the existence of mastitis within a herd when carried out on a sample of herd milk from a bulk tank (10, 26). Its use on an individual cow/or individual quarter basis has indicated a high incidence of mastitis to exist in herds where little or no clinical mastitis has existed over an extended period of time. Recently, a modification of the Whiteside test (California Mastitis Test) has been proposed for the individual cow and herd detection of mastitis. Investigations indicate that this test, as the Whiteside, is extremely sensitive when employed on a per quarter or cow basis (8). Instances of 75% or more of the quarters being positive with no record of clinical mastitis existing for eight months prior to the test and six months following the test have been recorded (25).

Bacterial counts have been employed and advocated as indicators of mastitis, but investigations have demonstrated little correlation between bacterial counts and the incidence of mastitis (30). Relationship between bacterial counts, dairy equipment sanitation and the cooling process of milk have definitely been demonstrated.

#### PREDISPOSING CAUSES OF MASTITIS

Mastitis can be described as a "do-it-yourself disease of dairy cattle." The necessary tools are found in the tool box "of inefficient management." Prevention is nothing more than the exercising of good management procedures and sane dairy practices that are often ignored or inadequately carried out.

#### Milking

Milk "let-down" is one of the paramount essentials of good milking. Cows that have been improperly stimulated will not let down their milk, with resulting crawling of teat cups and mammary gland tissue damage. Washing the udder with warm water and massaging are part of the necessary procedures required to induce milk "let-down." Disinfectants in the wash water have not proved to be of value in the prevention or control of mastitis (5).

The milking machine always should be kept in maximum operating condition (15). The milking machine serviceman can be of inestimable value to the dairyman through routine machine inspection, re-

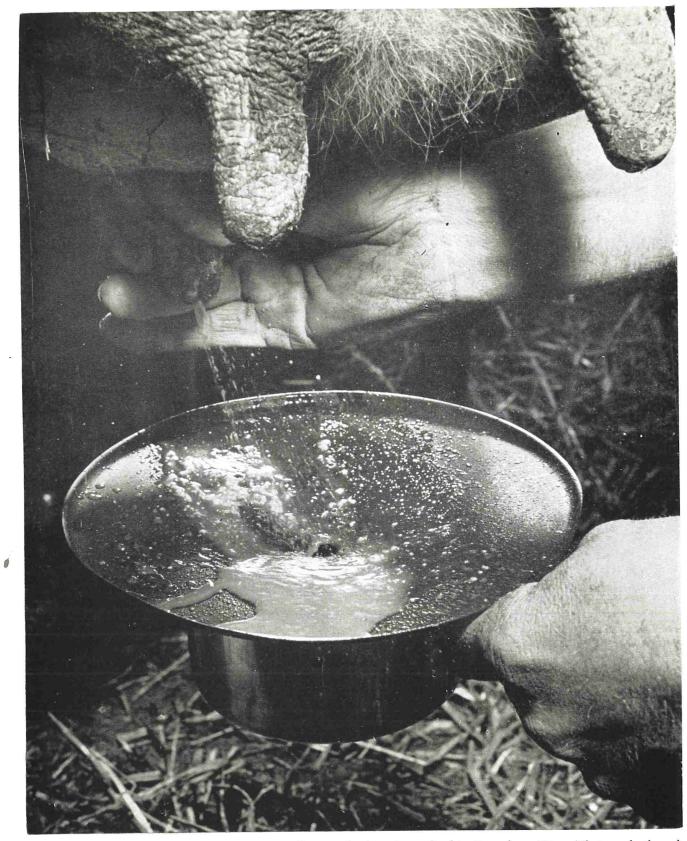


Figure 1. The use of the strip cup at every milking is the best for early detection of mastitis. (Photograph through courtesy of Babson Bros.)

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pair and in training the dairyman to correctly use the milking machine.

Though the milking machine has received much blame as the cause of mastitis, the operator is the predominant influencing factor. If the milking procedure is to be adequately carried out, one man should operate no more than two units at any one time. The predominant oversight on the part of the milker is the failure to remove the teat cup when the quarter is dry. All quarters do not milk out at the same time. A good milker knows the variations in the milk-out time of each of the quarters of each of the cows he milks. When the teat cup is not removed from a milked-out quarter, the intramammary vacuum is increased and trauma results, providing the necessary conditions for the initiation of mastitis. When the teat cup is removed, the vacuum should be released before removal to prevent eversion of the teat canal and irritation to the sensitive lining.

Rubber inflations that are cracked or have lost their elasticity and are caked with milk deposits are another predominant cause of mastitis. Two sets of rubber inflations should be used interchangeably for weekly intervals. While not in use, storage in a 5% lye solution prolongs the life and increases the milking efficiency of rubber inflations (11). All rubber parts of the milking machine should carefully and frequently be inspected for cracks or leaks, as any alteration in the normal recommended vacuum often contributes to injury of the mammary gland. A milking machine should be operated according to the manufacturer's instructions. The pulsation rate and vacuum, when incorrect, may cause mastitis. It is always a good policy to stop milking with machines when production is below five pounds per milking.

#### Housing and the Housing Area

Dampness, cold, and drafts are factors of stress to the mammary gland, with mastitis usually following. Cold concrete floors, lack of bedding, open hay or stray chutes, broken windows or doors, and poor ventilation may cause these stresses. The cow should be provided with stall or laying space of adequate size and maximum comfort if injury and stress are to be avoided. Barn yards and pastures that have barbed wire, sharp rocks, and rubbish laying around may cause injury to the mammary gland often associated with mastitis.

Stagnant pools of water frequently contain mastitisproducing organisms and it is not unusual to observe cows standing udder-deep in such pools during hot weather. These pools will not only provide contact with mastitis-producing organisms but cause chapped and irritated teats. The resulting pain during the milking process will cause failure of milk letdown, trauma and mastitis.

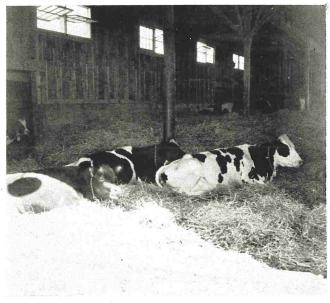


Figure 2. Adequate housing space that is dry, well bedded, and free of drafts is essential in preventing mastitis.

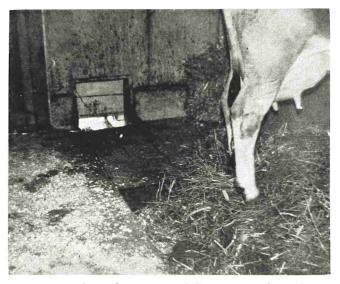


Figure 3. The outlet opening of the automatic barn cleaner may serve as the source of cold draft on udders of stanchioned cows.

Approaches to the milking parlor or the barn should be of cement roughened enough to prevent slipping and designed to provide maximum drainage and to assure the absense of any protruding material that might cause injury to the mammary gland.

Investigations have demonstrated that a simple, sane, well-presented mastitis prevention program (see below) will greatly aid the dairy farmer in preventing mastitis and lower the antibiotic content of milk supplied to the market (23) (Table 3). This can be TABLE 3-FIGURES REPRESENT PERCENTAGE OF HERDS HAVING CLINICAL MASTITIS, AND PERCENTAGE OF BULK MILK SAMPLES CONTAINING ANTIBIOTICS BEFORE AND FOLLOWING PRESENTATION OF MASTITIS PREVENTION PROGRAM OUTLINED IN TEXT

Clinical mastitis	Antiibotics in bulk samples
31.1%	8.1
15.0%	
34.4	5.4
43.5	13.5
Mastitis meeting with producers prevention program presented	
7.3	0.0
7.3	0.0
5.1	0.0
	31.1% 15.0% 34.4 43.5 Mastitis meeting with producers prevention program presented 7.3 7.3

achieved only when maximum cooperation is obtained by all persons associated with the dairy industry, including the veterinarian, the milk inspector, the milk plant fieldmen, the dairy farmer, the milking machine serviceman, and the dairy extension service.

#### Inheritance

The possibility of inherited resistance has often been suggested (13). Investigations indicate that the most important aspect of the inheritance to mastitis resistance is udder attachment and teat anatomy and placement. Teats from which it is difficult to remove milk or that are attached so as to tend to become horizontal often are on quarters that prove to have mastitis. An extremely large udder with a weak attachment will become pendulous with age and is subject to consistent trauma and/or injury.

#### VACCINATION

Vaccination with mixed bacterins and toxoids have,



Figure 4. Cows with strongly attached udders usually are less susceptible to udder injury and mastitis.

at various times, received much attention as a means of mastitis prevention. Though vaccination may be of some benefit, it cannot replace good management and sane dairy practices. A possible reason that vaccines have failed over a period of time has been the attempt by the dairy farmer to replace good management and sane dairy practices with vaccination.

#### TREATMENT

The dairyman who devotes full time to mastitis prevention and relies on a competent veterinarian for chemotherapy, when it is required, is usually one who has few veterinary costs and few problems with mastitis.

When mastitis is detected, stimulate the infected quarter for milk let-down and completely milk it out. Repeat this process at hourly intervals at least four to five times. If the inflammation does not appear to be subsiding, a dairyman has but one recourse, and that is to obtain competent veterinary service immediately.

Avoid the use of the highly advertised intramammary medicants. The antibiotic content of these preparations is seldom of sufficient strength to counteract the infection if it were all released from the vehicle and uniformly dispersed throughout the infected quarter. Most of the vehicles of these preparations fail to penetrate the involved mammary area and remain in the lower third of the mammary gland (24). Antibiotics remain bound to the vehicle and are not released for distribution throughout the gland, but are milked out in subsequent milking (28, 29).

All milk from treated quarters should be discarded for at least 72 hours following administration. Written instructions regarding discarding of milk following treatment should be obtained from the attending veterinarian.

Field investigations have demonstrated that the above mentioned approaches to mastitis are efficacious (27).

A Suggested Mastitis Prevention Program

Inheritance

Select cows with well attached udders. Select rapid, easy milkers. Avoid excessively large uddered cows. Raise your own replacements. Housing Provide adequate stall or pen space. Provide adequate quality bedding.

Avoid: Dampness, cold, and drafts. Open hay or straw chutes. Broken windows and doors.

Prevent udder trauma by avoiding: High door sills. Rubbish in yards and pasture.

Slippery floors and entrance aprons.

Corn stubble or brush pasture.

Hard driving with dogs.

Flies, mosquitoes.

Dehorn all animals.

Employ correct milking procedures and good milking equipment.

Prevent cow pox.

Prevent sunburned, chapped and/or frozen teats.

Avoid use of irritating disinfectants on cow teats.

Milking

Stimulate milk let-down one minute before attaching machine:

Wash and massage udder with clean warm water. Use strip cup.

Remove teat cup when milk ceases to flow.

Never milk with more than two units per operator.

Always break vacuum before removing teat cups from teat. Keep milking machine in maximum operating condition:

Have milking machine checked annually by recognized serviceman.

Learn to recognize defective machine operation and to correct or repair it.

Follow manufacturer's operating instructions for: Correct pulsation rate.

Recognized vacuum level.

Keep vacuum line clean.

Check and eliminate any vacuum leaks.

Have vacuum regulator and gauges checked annually for proper operation.

Keep rubber inflations clean and replace after 1000 milkings.

Use two sets of rubber inflations – alternate each week.

Obtain competent veterinary service upon detecting first signs of mastitis.

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#### FOOD SANITATION AND QUALITY CONTROL – FACTS AND FALLACIES

#### PAUL E. LAUGHLIN

National Biscuit Company, New York

Is the cleanliness of an American food processing plant to be taken for granted because the quality of its products meets a standard or because there is an official inspector present? To what extent are the terms "sanitation" and "quality control" synonymous? In our efforts to capsule a description for these aspects of food processing in a few neat words, have we lost sight of their distinctions and differing functions within the food processing operation? Are we being deceived by forms of official approval for plants and products which may imply that all is perfect as to sanitation and quality? How can we distinguish between sanitation fact and the camouflage substituted for organized and managed sanitary maintenance? To answer these questions, it is necessary first of all to clarify the terms "sanitation" and "quality control" as applied to food processing operations.

#### DISTINGUISHING THE DIFFERENCES

Quality control requires the application of all the physical sciences (and some which are not physical) to assure the desired flavor, consistency, appearance and tenderness of foods during and after processing. It employs mathematics, chemistry, physics, bacteriology, biology and microbiology in the manufacture, storage, distribution and merchandising of foods, including the control of raw materials and the minimizing of change in foods during processing and marketing. Quality control evaluates and applies desired standards to products; it is concerned primarily with things rather than people. It has only incidental interest in the total plant environment and then only as it may interfere with end-product objectives. It is, therefore, appropriate for all phases of quality control to operate as a laboratory function, wherein research activities may also be conducted since product development frequently is a customary part of quality control. These laboratory and quality control functions are essential to modern food production and are, therefore, a proper adjunct of the production management structure. On the other hand, industrial sanitation as applied to food processing, does not belong within this organizational pattern. The placing of the sanitation responsibility under

quality control and its laboratory service, an unfortunately common practice in many food firms, is neither logical organization nor effective in terms of efficient results.

To appreciate the distinctions between these two functions and to place industrial sanitation in its true perspective within the food manufacturing environment will require a broader concept of this term than many of you may have been willing to accept. While sanitation and quality control are interwoven in relation to the product, they are distinctly different in other respects. As we see it, there are just three basic elements in any manufacturing operation: the first being production; the second, engineering or mechanical maintenance; and the third, the maintenance of the physical environment in which the first two are carried on - and properly called industrial sanitation. Production and mechanical maintenance, being highly organized and well understood, need no explanation. Industrial sanitation is simply care of the industrial setting and includes the varied array of tasks to be done in and around a manufacturing plant that are neither production nor mechanical maintenance. Obviously, care of the work environment requires sizeable expenditures proportionate to those of production and mechanical maintenance and, therefore, justifies recognition by management even though it may not always get its just share of understanding and support.

#### Logical Approach

Regrettably, sanitation has remained unorganized, divided, and subordinate to other activities in far too many food plants. The result has usually been duplication of efforts, inconsistent and haphazard work performance, and a mediocre to poor level of cleanliness, with costs much higher than they should be. For maximum efficiency, sanitation must be organized as a separate and distinct function, properly managed and competently supervised. Decentralized sanitary maintenance is wasteful and inefficient. When sanitation is properly organized and managed, the benefits in terms of dollars and level of cleanliness are quite apparent. Such an approach to this essential function permits sanitation effort to deliver more value for the money allocated to it because its labor needs and costs can be determined and analyzed. Less money plus efficient supervision will provide a higher sanitation level than more money

<sup>&</sup>lt;sup>1</sup>Presented at the 47th Annual Meeting of the INTERNATIONAL ASSOCIATION OF MILK AND FOOD SANITARIANS, INC., Chicago, October 26-28, 1960. Mr. Laughlin is Sanitarian in the Sanitation Department of National Biscuit Company.

and poor supervision.

Included in our broad concept of sanitation are duties which rarely have the coordinated supervision and control necessary for maximum efficiency such as grounds maintenance, plant security, floor maintenance, structural cleaning, pest control, and cleaning of machinery and equipment. The selection, testing and approval of tools and materials for sanitary maintenance are necessarily a part of this function and include everything from mechanical devices to detergent-sanitizers and toilet tissues. I know of one food firm that saved 6,000 dollars in detergent purchases alone the first year a competent sanitarian was employed. Effective short interval scheduling for maximum utilization of sanitation labor becomes an essential sanitation supervisory function, along with continuous study of time factors, methods and materials to improve the technique of performing each task. Planned use of sanitation labor is essential because sanitation is a service function, manually performed for the most part. Much of management has yet to realize that the recent and tremendous automation of production has not increased the productivity of sanitation labor, but has further burdened it with complex tasks. Where new automatic equipment reduces production labor, it usually increases the work of sanitation and mechanical maintenance because every highly mechanized production unit requires more precise cleaning and adjustment; this can rarely be done by pushing a button.

#### The Food Technologist

Sanitation in a very narrow sense may be concerned with insect fragments in the product or the development of mold on processing equipment, which may require laboratory assistance for identification or isolation of such foreign material, but these and other similar joint interests are certainly not valid reasons for placing sanitation within or subordinate to the quality control function. Do you know of a food technologist or quality control supervisor that can tell you the procedures, materials, techniques and costs of the cleaning requirements within a sizeable operation; or who can supervise the labor necessary to perform this work and, at the same time, do the routine analytical work and interpret data essential to quality determinations? There are some but they are rare, indeed. My remarks are in no way intended to take anything from food technologists or to under-value their abilities and professional competence. My only purpose is to clarify the distinctions between sanitation and quality control functions. A food technologist is not necessarily equipped or capable of developing and administering

a sanitation program appropriate to a food plant by virtue either of his academic background or his laboratory experience. Furthermore, the demands and routines placed upon the usual quality control program are not conducive to its effective extension to the organization, planning and direct supervision of sanitary maintenance. These two functions may be combined under single supervision in a small plant for reasons of economy, but balanced handling is rarely the result. Almost inevitably, one becomes subordinate to the other and lapses into varying degrees of neglect.

Food technologists and food scientists have indeed made great contributions to the food industry and have played an important part in raising our standards of living. It is a fact that one-third of all food items now on supermarket shelves did not exist ten years ago and probably half of the food products which will be in supermarkets in 1970 are now in developmental stages. These remarkable accomplishments are worthy of commendation and as one writer has said of food researchers and technologists -"they have permitted the harvest of the field, the fruit of the orchard and the catch of the sea to be brought beyond the temporal confines of season and the limits of distance, to the table of even the poorest." Recognizing the importance of quality control and the professional stature of those who man it, may I suggest that these food technologists responsible for food quality are too busy in the thousands of laboratories, seeking better methods of preservation, freezing, dehydrating, processing, packaging - too busy developing new products and attempting to fiimprove a host of existing ones - to devote the time and acquire the skills necessary to manage an industrial sanitation program.

#### SANITATION PERSPECTIVE

There are many distressing instances of industrial sanitation being crippled by being subordinate to other functions in the food industry, and there are many executives who cling stubbornly to their old beliefs only because they have not objectively studied the sanitation function in the same way that they have studied other plant operations. One fallacy in the quality control approach to sanitation is dependence on microbial indices. The only index of filth in some food plants has been bacteriological culturing. A low count within an established purity tolerance may be taken to mean all is well, even though employees may wade in dirt and debris up to their ankles. Many adherents of the quality control approach to sanitation relegate to "housekeeping" this incidental soil and its removal. This term "housekeeping" is usually a down-grading reference to a general catch-all of seemingly unimportant tasks for janitors and porters. I can assure you that the proper maintenance of a variety of floors, the control of infestations, the cleaning of processing machinery and the effective handling of labor require as much or more skill than many of the tasks performed by technicians in a laboratory. Let me say that in the Nabisco approach to sanitation, we avoid the use of the term "janitor" and belittle no work involved in maintaining the work environment. We are indeed proud of our company's unique and distinguished position in the food industry and of the contributions made thereto by our sanitation function because it is rationally organized and is productive of the desired results.

There is no necessarily direct relationship between quality control and plant sanitation. In fact, a product may be considered to be of high quality as to color, texture, taste and appearance, and yet be dirty. It is a fallacy to gear all sanitation to microbial indices or to relate all factors of quality and sanitation to the product. Certainly, an unclean food cannot be considered high in quality nor should a food product processed in a dirty plant bear a seal or certificate indicative of purity and cleanliness. The confusion has led many people to believe that as long as the product is reasonably clean and of acceptable quality the rest doesn't matter. Honest and sincere food processors, as well as the federal Food and Drug Administration, feel that both the plant and product should actually and consistently be clean and sound. There is a clear distinction between *clean* and *cleaned* foods and just because garbage can be sterilized does not make it acceptable to the American consumer. In this day of miracle drugs and easy remedies, the tendency is toward corrective rather than preventive action. To rely on chemicals, or other short-cuts to sanitary maintenance can prove damaging. An example of how such complacency can backfire is the problem of antibiotic-resistant staphylococci in hospitals where cleanliness had been relaxed in favor of the prophylactics. Many serious infections have stimulated investigations to determine the real nature of the problem, and our hospitals are now undertaking to restore aseptic objectives in their sanitation maintenance practices regardless of how many wonder drugs there are to kill the organisms responsible for specific infirmities. Accepting the modern approach to sanitary maintenance in combating this problem, many hospitals now have established distinct sanitation programs headed by sanitarians. Hospitals are learning, as the food industry should have by this time, that the best results in maintaining a clean institutional or industrial environment cannot be ob-

tained by dividing sanitation responsibilities among nurses, housekeepers, dieticians, building maintenance supervisors, purchasing agents, administrators, and committees.

Sanitation cannot be taken for granted nor can it be made the responsibility of everyone simply by endeavoring to so motivate all supervision and employees, as some would like to think. To instill sanitation consciousness in all employees in a food plant becomes a part of any good sanitation program, but it is to be effected chiefly through supervision, with the continuing stimulus coming from those with the full-time sanitation responsibility. Sanitation is a constantly fluctuating relative condition requiring hour-to-hour, day-to-day attention, supervision and control. To establish and schedule the frequency for cleaning the complex of machinery, equipment and structural surfaces in a food plant becomes a sizeable task in itself. Frequency and degree of cleanliness depend on many factors relating to soil load and they vary considerably with food products and plant loca-These factors account for the emphasis on tions. bacterial control in an industry such as milk, while dust control becomes more important in industries such as milling and film processing. Therefore, it makes good sense to recognize and approach sanitation from its total environmental situation rather than from the narrow perspective of one or two scientific disciplines.

#### **GOVERNMENT INSPECTION SERVICES**

What about grade standards, official inspections and seals of approval as they relate to industrial sanitation? One might wonder just what the designation "packed under continuous inspection" really means. The distinct differences between sanitation and quality control as discussed thus far should not be confused with official inspection or the actions of official agencies which provide neither of these essential food plant requirements.

The production, processing and marketing of our foods are regulated and controlled by various federal, state and local laws. Important among these are the federal Food, Drug, and Cosmetic Act, the Meat Inspection Act, and the Poultry Products Inspection Act. These laws contain all the provisions necessary for enforcing good sanitation and quality, leaving no doubt as to the objectives that plants operating under them shall be maintained in a sanitary condition. The sanitation sections are replete with words such as "sanitary," "abundant," "sufficient" and "adequate," which terms are of little help in indicating just what degree of anything is expected. Our questions are what constitutes a sanitary condition, what is required to maintain this condition, and to what extent are food plants actually maintaining such conditions, with or without the aid of an official inspector? It will serve no purpose here for us to embark upon a discussion of "how clean is clean?" in an effort to answer this question, but it is important to keep in mind that desirable sanitation is not accomplished by the mere passage of a law, regardless of its detail as to design, construction, or operating conditions.

The Food and Drug Administration (FDA), Department of Health, Education and Welfare, has jurisdiction over all food plants shipping in interstate commerce except those processing meat and poultry. The Meat Inspection Division, Agricultural Research Service, U. S. Department of Agriculture (USDA), inspects, grades and certifies meat and meat products, and the Agricultural Marketing Service, USDA, performs the same function for poultry products. In addition, there are federal marketing orders which establish minimum grade standards for various agricultural These marketing orders are instrucommodities. ments to facilitate the distribution of the product; they are not essentially for purposes of quality control as might be implied. Product inspection is required under a federal marketing order and while it is not mandatory for the USDA to act as the inspection agency, it is common for the commodity under a Federal Marketing Order to accept USDA inspection. Trade associations also have acted as a third party inspection agency and in my experience this arrangement has been superior to government inspection. The USDA offers (a) continuous inspection, in which an inspector is present at all times while the food is processed, (b) plant inspection, wherein the plant is checked and product certified but an inspector is not present at all times, and (c) lot inspections, in which a single lot is sampled, tested, and recorded.

It is not uncommon for food processors to rely on one or more of these official inspection agencies for their sanitation counsel and control. Some plant operators seem to derive immense satisfaction from declaring to all interested parties that their plants and products are under official inspection and, therefore, must be pure and above question. Some owners have even gone so far as to request and obtain testimonial letters from the USDA inspection service to support their claims of purity, as being a certain consequence of the continuous inspection contract with the USDA. With regard to plant sanitation, this is essentially ridiculous. There were recently 84,000 plants under FDA regulation and the present inspection force, taking each plant in turn until all have been visited, could inspect each plant about once every five and a half years. This cannot be considered a sanitation service even if constructive sugges-

tions are made with each visit. The FDA, by far the superior government inspection agency, is not intended to be a handmaid for reluctant units of industry, nor does it pretend to take the place of fulltime organized and supervised sanitation within each plant, or to offer sanitation consulting service. Its job is to protect the American consumer by finding, abating, and preventing violations of the Food, Drug and Cosmetic Act.

#### Some Discrepancies

The USDA appears to offer a complete sanitation service under its "continuous inspection contract," for which a food processor pays a fee; usually no more than a cent or two per case. These plants contracting for this service can display the official shield designating, "Packed Under Continuous Inspection of the U. S. Dept. of Agriculture." Mr. F. L. Southerland, Chief, Fruit and Vegetable Division, Agricultural Marketing Service, Processed Products Standardization and Inspection Branch of USDA, has said (Agriculturing Marketing, July 1958), "Processed fruits and vegetables that wear this shield give consumers the assurance that they are getting products that are clean and wholesome ..... the shield means that the processor has a plant, equipment and operating methods which meet USDA requirements and that he is packing a good quality product . . . . . . to qualify for continuous inspection the plant meets rigid requirements for construction, equipment and sanitation." I challenge these statements and the pseudosanitation service offered by USDA as being misleading and not entirely in accordance with the facts.

A plant under such continuous inspection has one or more inspectors assigned to it on a full-time basis. These inspectors are not routinely rotated but remain in the same plant indefinitely, being normally present whenever the plant operates. In addition to product grading, they are officially obliged to make a daily sanitary inspection of the plant before operaations begin. This inspection is usually made using a check list on which items are marked as either "Satisfactory" or "Needs Attention." In theory, if a plant fails to receive approval in all departments its product cannot receive the USDA grade certification, but strangely enough, the instances of such failure or interruption of production are so rare as to invalidate the law of probabilities. USDA inspectors have actually reported that, even if several insanitary conditions are found, no action is taken other than to report the condition to the plant management. A USDA inspector may get little backing from his superiors and will be inclined to avoid situations which would cause disagreement with plant management. I am sure you will agree that one representative being in one plant for a long period breeds a familiarity which is not altogether favorable to good, unbiased and objective inspection by an outside agency.

We might expect the sanitation surveillance to be poor and the reports to be less than accurate when amateurs attempt to engage in a function with which they are largely unfamiliar, whereas application of grade standards precisely written into the regulations could be expected to be well done. In my judgment, such grading can be subject to wide variations. The constant pressure to get along with plant management and to be a "good guy" make it difficult for an inspector to determine grades accurately and to judge plant conditions objectively, assuming he is qualified to do so. The complaints of plant management can make life very unpleasant for a USDA inspector who is not a "good guy." Let me relate several actual examples which have come under my observation. A frozen food processor was packing lima beans and, due to unseasonable rains, there was excessive mold so extensive it was not economically feasible for this plant to put up a Grade A pack. The USDA inspector, in accordance with grade standards, recorded a Grade C on this pack. The plant complained to the inspector's supervisor who in turn is reported to have told the inspector "he knew the beans were not Grade A but it was a tough year and he should ease up a little." Since it was the "politic" thing to do, the inspector complied, thus putting a sizeable tonnage of lima beans on the market with an incorrect grade. Another instance involved a cauliflower freezer. Adverse growing conditions resulted in more head mold than was allowed in the standards for a Grade A pack. The inspector was reported to have been instructed to overlook this condition under the pretense that maybe it wasn't mold. One other prime example of such grading variation deserves mention, not because of its being a more flagrant violation but because there are documented proofs of the results. In the raisin industry, the USDA provides both incoming and outgoing inspection. Recently, rain during the drying season caused a great deal of mold which was an inspection problem. An inspector grading incoming raisins accepted a lot as meeting the standards. The raisins were subsequently packed and given a G.N.C. (grade not certified) classification by the USDA processed-fruit inspector. The processed-fruit inspector was told the lot had been accepted by his agency and it would not look right if they were now graded as being high in mold. The inspector refused to comply with his supervisor's request to change the grade and was subsequently relieved of his duties. The pity of such a situation is not that incoming raisins might have been improperly graded but that representa-

tives of an official agency would rather have misrepresented raisins placed on the market than to admit an error in grading.

Such discrepancies in grading are detrimental to the processor, consumer, and the inspection agency. To give you an idea of the far reaching ramifications of such inspection laxity, I refer to an item and editorial comment published in the "California Fruit News," June 27 and August 1, 1959. By letter to the editor, a prominent exporter raised strong criticism of the inspection laxity on export shipments, making reference to the very inferior quality of Natural Thompson Seedless raisins which had been shipped abroad. He said that while his firm appreciated the difficulties encountered in a bad crop year, they nevertheless felt there should have been an even more stringent quality control in a year when quality was so diverse in order that the normal high reputation of California raisins should be preserved in the foreign market, even if some sacrifices were necessary to achieve this. It seems that this export firm had 50 tons of such raisins rejected at dockside by one customer because of poor quality and could not dispose of them at a price well below These raisins were delivered replacement level. with a USDA quality certificate that insulates the packer against any quality claim whatsoever, whereas the exporter could not contract with his customers in a similar fashion. It was further stated by this exporter that this shipment of raisins would not have passed our own FDA if they were to be shipped back to this country. My sentiments are with the editor who concluded his comment by stating that, "A quality certificate issued by an agency of the U. S. Government should be as dependable as the one dollar bill is of value."

#### GRADE VERSUS BRAND

The American consumer has been urged for years to purchase by grade with the implication that a Grade A product of one brand selling for a few pennies less than a Grade A name brand results in money saved, while the products are exactly the same in quality. Considering the lima bean, cauliflower and raisin incidents, it is possible that USDA grades are not always as represented to the consumer. The fact remains that grade determinations may vary by inspectors and as the result of crop and seasonal conditions. A marketing order is intended primarily to distribute the product and not necessarily to improve its quality. In surplus crop years, the grades are likely to be high, in short crop years, the inferior products become passable. Buying by brand is to be considered a more reliable guide to quality than grade because a processor of quality brand products is not likely to permit significant fluctuations in quality which could destroy the hard-earned public acceptance of quality brand food products. In lean years, rather than jeopardize the brand, the prudent processor will refrain from packing under the quality brand label when a product of nature fails to meet his quality specifications.

The USDA Fruit and Vegetable Division publishes a booklet entitled "Plants Approved to Pack Processed Fruits and Vegetables Under Continuous Inspection," which lists the names, addresses, and products handled by these plants so honored. For anyone familiar with the environment, physical facilities, and operational levels within some of these approved establishments, it becomes obvious that the claims regarding requirements for approval by USDA are not always met either physically or operationally. Among the approved are plants known in the trade and by regulatory agencies to be inferior as to plant sanitation, fluctuating in quality, and involved in questionable practices. Conspicuously absent from this list are plants known to have the best physical facilities, well organized in-plant sanitation, and high quality products marketed under quality brand names. A comparison of these USDA-approved plants with the Notices of Judgment listing violators of the Food, Drug, and Cosmetic Act makes very interesting read-Some approved plants have appeared more ing. than once for violations due both to adulteration of product (Section 402, a, 3) and insanitary plant conditions, (Section 402, a, 4) of the Act. It, therefore, seems possible for plants listed as meeting all USDA sanitary requirements and having continuous inspection to be prosecuted for insanitary conditions and adulterated products, whereas many plants not listed as approved have operated for years without being subjected to FDA action. In view of these circumstances, it would be extremely interesting if the FDA were routinely to inspect both meat and poultry plants under somewhat similar USDA inspection.

Federal Food and Drug inspectors are required by the Administration agreement with USDA to identify themselves to USDA inspectors when visiting plants under continuous inspection. The USDA man is then invited to accompany the FDA inspector while he is in the plant. In theory, such a practice should enable the USDA to rate a plant as satisfactory or actionable on the basis of the FDA inspector's findings and to take the necessary follow-up action. In practice it does not appear to work that way. An FDA inspector may discover insect or rodent evidence in storage areas or mold and slime on processing lines which are violative conditions. Contrary to what we would expect, the USDA inspector may continue to certify the products and disregard the insanitary conditions called to his attention by the FDA inspector. A specific example known to me is the following case: A small raisin packer recently complained that because he couldn't afford the advertising costs of a name brand he has relied on the USDA stamp to sell his products and has had continuous inspection for several years. Recently, the FDA sampled shipments of USDA-certified raisins from this plant and subsequently seized them. One regretfully concludes, therefore, that the continuous inspection program is not all that it is represented to be and that variable grading occurs, while assurance is given to subscribers and consumers alike that this program satisfies all food laws and regulations.

#### NO SUBSTITUTES FOR PLANT-ORGANIZED SANITATION

Let none of us be deceived by these claims of "continuous inspection." With all due respect to this official arrangement, under a federal marketing order or otherwise, the assigned personnel are not necessarily qualified nor do they in fact perform the function of an industrial sanitarian. In contrast to the sanitary engineers and sanitarians who long have been practitioners in environmental sanitation, acquiring this status by meeting academic and professional requirements plus considerable experience, there is this group of heterogeneous individuals recruited at random, exposed to short courses on how to count raisin stems and read moisture machines, given a USDA stamp, and then presumed to be able to find, evaluate, correct and prevent recurrance of complex sanitary deficiencies within a food processing operation. It is indeed a fallacy for such "quality control" inspectors to be represented as dealing competently with plant sanitation.

Sanitation and quality control are not synonymous; they do represent distinct and separate functions and should be so organized in food plant operation. Quality control is essential to production with regard to the product, and industrial sanitation is essential to the entire operation with regard to maintenance of the total work environment. The cleanliness of a plant, its premises and personnel facilities, and the aesthetic factors conducive to a good operation, including employee welfare, comfort and appearance, all reflect the degree of organization for sanitation. These desirable objectives hardly can be realized by any number of "Sanitary Sams," outside consulting services, official inspections, plaques, awards, or seals of approval, official or otherwise. A high level of industrial food sanitation can only be achieved by establishing sanitation in each individual plant and firm as a managed function, organized and supervised at a management-supported level comparable to the other basic elements of manufacturing.

#### STAINLESS STEEL IN MODERN DAIRY EQUIPMENT – ITS CARE AND MAINTENANCE<sup>1</sup>

#### R. B. GUNIA

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Historically, the stainless steels are late arrivals on the scene when compared with other metals which have been in use for many years. No one knows precisely who should receive credit for the development of the stainless steels, but it is rather widely accepted that the earliest research work on these alloys occurred during the early 19th Century. Usually credit is given to the English and Germans who developed alloys, in the period from 1910 to 1915, which were very similar to those in use today. Comparatively few uses for these alloys developed until the mid 1920's when their commercial production began in this country. It is significant that most observers report that the first commercial sales in this country involved the application of stainless to dairy equipment. Indeed, several American stainless steel producers point with pride to dairy equipment that has been in continuous service for about 35 years.

In the best American tradition this is a fascinating story of ingenuity and inventiveness. From those small beginnings in about 1924 the United States stainless steel production has steadily increased until it now exceeds a million ingot tons annually. And this is only the opening chapter.

#### WHAT IS STAINLESS STEEL?

The American Iron and Steel Institute has defined stainless steels rather broadly as alloys of iron con-

<sup>1</sup>Presented before the St. Louis District Chapter of the Institute of Sanitation Management, St. Louis, Missouri, October 27, 1960. taining chromium above 4% and sometimes including other elements such as nickel, titanium, columbium or molybdenum. About 40 standard compositions comprise the steels to which this name is given. Metallurgically, these alloys can be divided into three broad classes: (a) the martensitic group which includes the alloys which are hardenable by heat treatment, (b) the ferritic group which are not hardenable by heat treatment, and (c) the austenitic group which are hardenable only by cold working. In the dairy industry the ferritic and austenitic steels are of the most interest (see Table 1).

Although these steels as a class are called "stainless" it is important to explain that this name must be taken only in a relative sense. The addition of about 10 to 12% of chromium to iron confers to these alloys a remarkable resistance to chemical attack. Nickel and molybdenum additions further improve the corrosion resistance. Many corrosive agents which readily damage ordinary steels have little or no effect on these alloys. Thus, it would be preferable perhaps to refer to these steels as "corrosion resistant" steels rather than as stainless steels.

What makes stainless steel stainless? A number of theories have been advanced to explain the superior corrosion resistance of these steels. The most generally accepted theory states that the development of a thin, transparent, impervious, surface film of oxide occurs almost instantaneously upon exposure of these alloys to oxidizing environments and is responsible for this remarkable behavior. This film is quite tenacious and has the marvelous ability to heal itself

AISI Type No.	С	Mn	Р	s	Si	Ni	Cr	Others
201	.15	5.50/7.50	.080	.630	1.00	3.50/5.50	16.00/18.00	N .025
	max.		- 14 	101700 001				max.
202 '	.15	7.50/10.00	.060	.030	1.00	4.00/6.00	17.00/19.00	N .02
	max.				max.			max.
302	.15	2.00	.045	.030	1.00	8.00/10.00	17.00/19.00	
	max.	3			max.			
304	.08	2.00	.045	.030	1.00	8.00/12.00	18.00/20.00	
	max.				max.			
303	.15	2.00	0.20	0.15	1.00	8.00/10.00	17.00/19.00	Mo. 6
	max.		max.	min.	max.			max.
								Zr. 6
								max
430	.12	1.00	.040	.030	1.00		14.00/18.00	
	max.				max.			
	Type No. 201 202 302 304	Type No.         C           201         .15           max.         202 '           202 '         .15           max.         302           304         .08           max.         303           303         .15           max.           303         .15           max.           303         .15           max.           303         .15           max.         .12	Type No.         C         Mn           201         .15         5.50/7.50           max.         .15         7.50/10.00           max.         .15         2.00           302         .15         2.00           max.         .304         .08         2.00           max.         .303         .15         2.00           max.         .303         .15         2.00           430         .12         1.00	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$				

TABLE 1-STANDARD AISI COMPOSITIONS OF STAINLESS STEELS USED IN THE DAIRY INDUSTRY

under proper oxidizing conditions. Incidentally, the air we breathe is a most powerful oxidizer.

These steels have a high degree of resistance to the oxidizing acids such as nitric acid. However, the so-called reducing acids, such as hydrochloric, dilute sulphuric, or hydrofluoric will attack stainless readily except under the most carefully controlled conditions.

#### MANUFACTURE

The manufacture of stainless steel begins with the careful selection of the raw materials required. These are melted in electric arc furnaces under precisely controlled conditions. The molten metal is cast into ingot molds and allowed to cool. Then the ingots are reheated and rolled in heavy rolling mills to slab or billet form depending on the desired end product. The slabs, or billets, are permitted to cool and are then conditioned by grinding with rough abrasive wheels to remove certain surface imperfections that occur in these early stages of manufacture.

To produce sheet and strip, the slab, about 3" to 4" thick and 15' to 18' long, is again reheated and rolled on a hot strip mill into a coil about .150" thick and several hundred feet in length. On a continuous annealing and pickling line the hot rolled strip is annealed and pickled. This operation puts the metal in the proper internal metallurgical condition for further processing and removes the heavy surface scale and oxide which develop during hot rolling and annealing. Next the steel is cold rolled, often in stages, to the desired thickness. After cold rolling the coil is given a final anneal and pickle to impart desired mechanical properties and surface qualities. Finally, the coil is cut to length, inspected, packaged, and shipped.

In our illustration we have simplified the actual operations for clarity. At every stage of processing, extreme care is taken and careful inspection is provided to make certain that the desired quality is truly "built-in" in the finished product.

Sheets processed as we have outlined are known in the trade as 2D finish sheets. A 2B finish is produced by subjecting the 2D finish to a "temper" pass on a cold mill. This light cold pass adds some surface smoothness and luster. Such sheets can be polished mechanically with abrasive belts to a variety of finishes such as No. 3 or No. 4 finish. Much of the sheet product employed by the dairy industry is used in the polished finishes.

In discussing the manufacture of stainless steel a typical "cold reduction" process has been used for illustration. Other methods are also used in the stainless industry and each will result in a product equally acceptable to the dairy industry.

#### TERMINOLOGY

As stated earlier, the ferritic and austenitic stainless steels are most widely used in dairy equipment. These are known as USS-17, Type 430 and USS-18-8, Type 302. These alloys are known more familiarly as "straight chromium" and "18-8," respectively. The familiar names derive from their chemical compositions which in the case of USS-17 contains 17% chrominimum and in the USS-18-8 a chromium content of 18% and a nickel content of 8%. In machined parts such as valves, Type 303, an 18-8 especially modified to improve its machinability, is used.

Some quantities of AISI Types 201 and 202 were used successfully in the dairy industry when nickel supplies were critically short during the Korean War. Type 201 has a nominal composition of 17% Cr, 6% Mn, 4% Ni; Type 202 has a nominal composition of 18% Cr, 8% Mn and 5% Ni. Both alloys also contain about 0.15% nitrogen which in combination with the higher manganese replaces some of the nickel.

#### CHARACTERISTICS AND USES

All of these types of stainless lend themselves readily to bending, drawing, spinning and welding. Thus, they can be easily fabricated into tanks, vats, vessels, pipe and coils.

The 17% chromium alloy is seldom, if ever, used in contact with milk or milk products. It is used principally as trim or for external vessel walls.

Milking machines, pails, cream separators, bulk milk tanks, trailer truck tanks, pasteurizers, milk coolers, and various other kinds of dairy equipment are available in stainless steel from leading manufacturers.

Stainless steel possesses many attributes that make it so widely used in the modern dairy industry. For many reasons stainless is the most nearly perfect material yet discovered for handling milk and milk products. It does not impair the flavor of the milk or dairy products with which it comes in contact. Its high polishability gives it a very sanitary surface which is relatively easy to clean and keep clean. It will not break or shatter. It requires no protective coating. It will not chip. It is tough and will stand up under the roughest usage on the farm and in the processing plant.

Even with all these excellent characteristics stainless does have some limitations as do all other products. Stainless is not corrosion-proof, but it is corrosion resistant. Under certain conditions it will corrode. The principal maintenance job with stainless steel is to see that environments that favor corrosion are avoided.

#### Care of Stainless Steel Equipment

From this brief review it becomes clear that stainless steel can and does play a very important role in the modern dairy industry. And it is equally clear that the benefits to be reaped from the use of this marvelous metal can only be realized when such equipment is accorded proper care and maintenance. Fortunately, the maintenance and care of stainless steel is relatively simple. On the other hand, neglect can be disastrous. Manufacturers of dairy equipment continually emphasize that many years of profitable service can be expected of such equipment only if the ultimate user will faithfully follow the maintenance procedures that have been carefully developed by the equipment manufacturers in conjunction with the various dairy associations and steel producers. In this regard, we can quote with feeling the old adage, "an ounce of prevention is worth a pound of cure," we could also add that the penalty of neglect might well be the failure of some fine and costly equipment.

We know of no rigid Ten Commandments for the maintenance of stainless dairy equipment, but we would emphasize that the first and greatest commandment is "Follow Directions and Keep it Clean!". Most equipment manufacturers believe that there are two principal causes for corrosion, (a) allowing chlorine solutions to remain on the surface too long and, (b)allowing other objects to come in contact with the surface too long. The problem with chlorine is always present because most cleaning compounds and germicides contain chlorine or its compounds. The contact corrosion is always present too because rubber hose, tools and other articles used on the farm and in the plant have the odd habit of being forgotten in the most unlikely places and all too frequently it happens that the bulk milk tank or the milk cooler provide convenient shelf space.

Equipment can be kept in top condition by following these recommendations:

(a) Immediately after each use, rinse equipment thoroughly with clean water to remove milk before it dries.

(b) Follow this rinsing, clean with hot water and a commercial dairy cleaner. Use the cleaner in strict accordance with the manufacturer's recommendations. The cleaning solution should be brushed over all surfaces.

(c) When detected, accumulations of milkstone or soil deposits should be removed immediately. Commercial milkstone removers used in accordance with manufacturer's recommendations are usually suitable for this purpose. Stubborn deposits may re-

quire the use of a *stainless steel* sponge. It is important to use only stainless steel sponge, ordinary steel wool may leave deposits of steel that will form nuclei for corrosive attack.

(d) After cleaning, rinse equipment thoroughly with hot water and allow it to dry. Some manufacturers and sanitarians recommend that equipment be sanitized with a chlorine solution after washing. If this practice is used or required, it is most important that the equipment be rinsed to remove this chlorine solution. Prolonged contact of chlorine will cause pitting and corrode the stainless surface.

(e) Other manufacturers and sanitarians recommend that stainless equipment be sanitized just prior to use. This is the most favorable procedure to avoid corrosion. The use of chlorine sanitizing solutions prior to the addition of the milk has been satisfactory and is quite safe when proper care is taken.

Often the question has been asked "What is the relative cleanability of the various finishes applied to stainless steel in dairy equipment?" Does number 7 finish possess greater cleanability than number 4, for example? Until recently the answers were usually based on personal opinion. Work just completed at the Michigan State University, Department of Microbiology and Public Health by Kaufmann and his associates (1) in this area is enlightening.

After exhaustive tests, Kaufmann and his coworkers, concluded that there is no significant differerence in the cleanability of No. 2B, No. 3, No. 4 or No. 7 finishes. These are the finishes that have been generally specified and most widely used in the dairy industry. The 2B finish is bright cold rolled, the No. 3 is finished on belts 80-100 grit, the No. 4 is finished on belts 120-150 grit and the No. 7 finish is applied using 325 grit belts plus buffing.

The Michigan State study showed that significant differences were observed only when the direct agar contact test was used, and the opinion was expressed that these differences might be attributable to the inefficiency of the test itself. Equally good cleanability of all finishes were observed in the direct surface agar plate test and the swab test, including in the latter the Duncan multiple range test.

Thus this study indicates that the cleaning operations listed above will provide surfaces on equipment in the four finishes that will meet current standards for cleanliness required by the American Public Health Association.

#### References

1. Kaufmann, O. W., Hedrick, T. I., Pflug, I. J., Pheil, C. G. Relative Cleanability of Various Finishes of Stainless Steel in a Farm Bulk Tank. J. Milk and Food Technol., **23**: 377. 1960.

## NEWS AND EVENTS

#### DAIRY PRODUCTS IMPROVEMENT INSTITUTE HOLDS ANNUAL MEETING

Public Health regulations designed to insure sanitary milk supplies are being used in some areas as trade barriers, stated Dr. Russell Teague, Commissioner of Public Health for the Commonwealth of Kentucky at the annual meeting of the Dairy Products Improvement Institute held at the Hotel Governor Clinton in New York City on February 16th. He stated that such use of public health regulations to prevent the free movement of products in commerce was contrary to the official action of the National Association of State and Territorial Health Officers. Dr. Teague was introduced by Mr. E. J. Roberts, President of the Institute, who presided over the afternoon meeting which was attended by 250 regulatory officials, educators, and industry representatives.

Dr. W. F. Shipe, Professor of Dairy Industry at Cornell University emphasized that the freezing point of milk is the least variable property of milk and therefore is the best index of the chemical purity of milk. An understanding of the published facts on freezing points and a knowledge of the history of a milk sample are necessary for a precise interpretation of a freezing point value, stated Dr. Shipe.

The programs to eradicate tuberculosis and brucellosis are generally recognized as the greatest attempt to eliminate animal disease ever undertaken in history, according to Dr. E. E. Saulmon, Assistant Director of the Animal Disease Eradication Division, of the U. S. Department of Agriculture. Dr. Saulmon stated that the tuberculosis program began in 1917 and in 1952 only 11 animals in each 10,000 tested were found to be reactors and because complacency had developed the reactors were 15 per 10,000 last year, thus emphasizing the need for continued testing to completely eradicate this disease.

Mastitis, stated Dr. R. W. Metzger, Director of Quality Control of the Dairymen's League Cooperative Association, is undoubtedly the most prevalent and costly disease of dairy cattle. Dr. Metzger outlined a national project to control the disease through the efforts of the dairy industry and regulatory agencies.

Mr. Robert H. North, Executive Secretary of the International Association of Ice Cream Manufacturers discussed up-to-the-present-time developments in the Food and Drug Administration's Federal definitions, standards, and labeling requirements for ice cream and related products.

The Institute's business meeting was held in the forenoon. Elected to the Board of Directors to serve until 1964 were: Irving J. Kern, Middletown Milk and Cream Co., Yonkers, N. Y.; Dr. William C. Welden, H. P. Hood & Sons, Boston, Mass.; A. J. Nixon, Penn Dairies, Inc., York, Pa.; and J. Russell Meyers, Abbotts Dairies, Inc., Philadelphia, Pa.

The Board of Directors also met in the forenoon and reelected all officers as follows: E. J. Roberts, Crowley's Milk Company, Binghampton, N. Y., President; A. J. Claxton, Beatrice Foods Co., Pinehurst, No. Carolina, Vice President; Robert H. North, International Association of Ice Cream Manufacturers, Washington, D. C., Treasurer; and Dr. A. C. Dahlberg, Cornell University, Ithaca, N. Y., Secretary and Advisor to the Board.

#### FDA PROPOSES STANDARD OF IDENTITY FOR FATTY FOOD PRESERVATIVES

The Food and Drug Administration today announced a proposal to establish a standard of identity for chemical preservatives used in food fats and fatty foods.

Such preservatives are used to retard the development of rancidity. The proposed standard would require that the label of the preservative itself declare the specific chemical ingredient or ingredients from which it is made. The labeling of fabricated foods in which the preservative is used, however, may declare the presence of the preservative by its standardized name without listing the specific chemical name.

An acceptable label statement for a fabricated food containing the added preservative would be "fat preservative added" or "fat antioxidant present to retard rancidity."

Commissioner George P. Larrick said that one of the major purposes of the proposed order is to require on preserved fats and foods containing such fats, labeling that will be informative to the consumer. There has been much consumer confusion and misunderstanding because of the declaration by chemical name of the presence of such preservatives. Adoption of the standard of identity will clarify consumer understanding in this area and will promote honesty and fair dealing, Commissioner Larrick stated.

#### NEW DEVICE TELLS TIME-TEMPERATURE RELATIONSHIP FOR FROZEN FOODS

A new device is about ready to be marketed which will indicate, through visible color change, whether frozen foods have been properly held at a predetermined temperature in storage, transit or at the retail level. Food sanitarians, the frozen food industry, transport companies and warehouse operators have been seeking a means to determine the extent of temperature changes in frozen products. The device in question appears to offer a good solution.

An earlier suggestion included packing a cube of ice with a dye in a small sealed plastic bag and if the temperature rose above 32 degrees F the ice would melt, putting the dye into solution. This, however, disclosed only rises in temperature above freezing and even more important gave no indication of how long the food remained above freezing. Such a plan has not met acceptance and the current development offers a much more accurate criteria.

The new method employs the principle of the bimetallic thermo couple plus a change in OH ions and a subsequent change in the color of a built in indicator paper. Prior to use, a small capillary tube inside a plastic covering contains indicator paper, yellow in color. Using pliers, the plastic outside covering is depressed, breaking the glass capillary. This permits a salt solution to wet the paper. If the food is at 0 degrees F - or at some other pre-determined temperature – the paper turns green. No color change will take place so long as the foods remains at 0 degrees F. If the temperature remains constant this color will persist for a number of months.

Conversely, if the temperature rises to 15 degrees F, for example, a color migration begins and the green indicator paper now turns to a red. It may take several days for a complete change to red, or it may be found that not all of the indicator has changed from green to red. However, the color change is indicative that temperature limits have been exceeded. It is a warning that the food should be used relatively soon and that its shelf life is definitely limited.

It is expected the device in question can be marketed at a low price. And as control officials further encourage the holding of frozen foods at acceptable temperatures, this will be a positive method of identifying temperature variations to which the food has been subjected.

It is pertinent to point out that the most palatable, nutritious and desirable frozen foods are those which have *not* been subject to wide temperature fluctuations. The frozen food industry should welcome this development as a step toward insuring high customer acceptance of quality frozen foods.

#### RICHARD W. FUNK APPOINTED LEGISLATIVE COUNSEL FOR VENDING GROUP

Richard W. Funk has been appointed legislative counsel for the National Automatic Merchandising Association effective February 20, 1961, according to Thomas B. Hungerford, executive director.

Funk succeeds Herbert M. Beitel, a member of the N.A.M.A. staff for the past five years who is leaving the Association to join a private business enterprise in which he owns an interest.

A graduate of the University of Chicago in 1950 and Yale University Law School in 1954, Funk practiced law for six years in Chicago. In addition, he is an active member of both the Chicago and Illinois Bar Associations and has worked with the profession in various legislative areas.

Funk has been with N.A.M.A. on a full-time basis since February 6 and has been working with Beitel since that time in order to become familiar with vending's current legislative problems and activities.

Funk, 34, is a native of Des Plaines, Illinois, a suburb of Chicago.

#### INSTITUTE OF SANITATION MANAGEMENT TO HOLD REGIONAL CONFERENCE

The Institute of Sanitation Management has announced the holding of its 1961 Western Regional Conference in Pasadena, California, April 20-21, at the Huntington-Sheraton Hotel.

The Western meeting will feature six formal sessions in the following areas of activity:

**Public Buildings** 

Hospitals and Institutions

Establishing Cleaning and Sanitation Standards

Flour Mills and Bakeries

Food Processing Equipment and Design

Laws and Regulations

Top national officers of the Institute will speak at the meeting in Pasadena, including ISM President Raymond Q. Duke, Detroit Edison Company, Detroit, Michigan; Vice President George B. Wagner, Pillsbury Mills, Minneapolis, Minnesota; and Gerard J. Riley, Executive Secretary, New York, New York.

General Chairman of the Western Conference is Gordon Miller, California and Hawaiian Sugar Refining Corporation, Los Angeles, and Sidney J. Coyne, Coyne Chemical Company, Los Angeles, is Program Chairman.

The Southern California Chapter of the Institute will be host to the Western Regional Conference. President of the Chapter is Clayton Baldwin, Sunkist Growers, Inc., Corona, California. Program details will be announced as they are confirmed.

#### **OBITUARIES**

Leo Bievers, a member of the Michigan City, Indiana Department of Public Health passed away in February. Mr. Bievers was City Food Sanitarian, a position he had held for some twelve years. He was a member of the Indiana Association of Sanitarians and active in the affairs of the Association.

The Indiana Association and International convey to his associates, many friends and family, sincere sympathy on his passing.

Oscar V. Cooper died at the age of 64 at his home in Phoenix, Arizona on February 2, 1961. He was a member of the Arizona and International Association for many years. In his position with the State Health Department as Chief Milk Control Supervisor much progress was made in the quality of milk and dairy products sold in the area of his jurisdiction.

To his many friends, associates and immediate family, International extends heartfelt sympathy.

#### PAPERS PRESENTED AT AFFILIATE ASSOCIATION MEETINGS

\_\_Editorial\_Note:\_\_The following is a listing of subjects presented at recent meetings of Affiliate Associations. Copies of papers presented may be available through the Secretary of the respective Affiliate Association.

#### Conference of Fieldmen and Sanitarians

February 21 & 22, 1961 – Lexington, Kentucky Secretary, Louis E. Smith, 2776 Field Avenue, Louisville 6, Kentucky.

Current Dairy Farm Water Supply Situation. Problem Presentation. H. L. DeLozier Construction of Pond for Potable Supply. Robert C. Green Making Pond Water Safe. Woodrow Smithers Equipment for Processing Surface Water Supplies. Paul F. Baugh

The Mastitis Program – Its Extent and Economic Importance. Problem Introduction. R. W. Hammermeister

Mastitis Problem and Its Relation to Public Health. J. Skaggs Relation of Management to Mastitis. Robert Mather Organizing a Control Program. Durward Olds

Plans and Programs of the Kentucky State Department of Agriculture for esticide Uses. Coburn Gayle

Tips for Maintaining and Improving Quality of Milk. T. R. Freeman

Bulk Tank Sediment Testing. J. C. Sweet & H. B. Anderson, Jr.

The Duties of Fieldmen in Milk Procurement. H. L. Hunt

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#### **QUESTIONS AND ANSWERS**

Note: Questions of technical nature may be submitted to the Editorial Office of the Journal. A question in your mind may be in the minds of many others. Send in your questions and we will attempt to answer them.

#### QUESTION:

Is the Direct Microscopic Count as reliable as the Standard Plate Count for assessing the bacteriological quality of raw milk?

#### ANSWER:

The D.M.C. has been found to grade milk more leniently than the S.P.C., using 200,000 per ml as the limit for acceptable milk; with higher count milks it is more favorably regarded. For reliable results the D.M.C. must be carried out by trained, conscientious personnel who closely follow Standard Methods. Poor illumination of the microscope is often responsible for unsatisfactory results.

#### QUESTION:

What are the relative merits of the various tests for detecting antibiotic residues in milk?

#### ANSWER:

A dye reduction test, such as that developed by Neal and Calbert, allows the screening of a large number of samples with the minimum expenditure of time and materials. However, a positive result may be due to an antibiotic, a sanitizing agent or even a high leucocyte count.

A disc assay procedure is more specific for antibiotics, provided the milk is first heated to 180°F. for 2 minutes to destroy natural inhibitory substances. The common procedure employs a spore suspension of *Bacillus subtilis*; this organism is most sensitive to penicillin, less so to the other antibiotics. By using penicillinase-treated discs, the presence of penicillin can be definitely established. The procedure described by Arret and Kirshbaum of F.D.A. appears to have no advantages but has several disadvantages when compared with the official methods in the new (11th) edition of Standard Methods. The reverse-phase disc assay developed by Kosikowski can be applied in the field without elaborate equipment.

#### QUESTION:

Why does Standard Methods still allow incubation at either 32° or 35°C. for plate counts?

#### ANSWER:

Dairy bacteriologists have, almost universally favored the lower temperature. In Europe, the International Dairy Federation favors 30°C for 2 days for raw milk, for 3 days for pasteurized milk. Psychrophilic bacteria rarely grow at 35°C. and these are becoming increasingly important. The reason offered for retaining the 35°C. incubation is that this has been necessary in smaller public health laboratories which have to use this temperature for water analyses (plate counts and coliform determinations) as well as for coliform tests on milk. Now that 32°C. is officially recognized, along with 35°C., for coliform tests on milk (though not yet on water), consideration should be given to pressing for the elimination of 35°C, incubation in milk analysis.

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#### HELPFUL INFORMATION

Editorial Note: Listed below are sources of information on a variety of subjects. Requests for any of the material listed should be sent by letter or postcard to the source indicated.

- Quality Studies on Milk Cooled in Farm Bulk Tanks. Bul. 610. Vermont Agric. Expt. Station, Burlington, Vt.
- Moving Milk by Conveyor System. Spec. Circular 48. College of Agric., Bulletin Dept., University Park, Pa.
- The Farm Bulk Milk Hauler. College of Agric., Bulletin Dept., University Park, Pa.
- Effects of Bulk Milk Assembly on Hauling Costs. Bulletin 641. College of Agric., Bulletin Dept., University Park, Pa.
- Care and Management of Dairy Cows. Revised, 1959. Supt. of Doc., Gov't. Printing Office, Washington, D. C. 20c.
- The Way Cows will be Milked on Your Farm Tomorrow. Circular. Babson Bros. Co., 2843 W. 19th St., Chicago 23, Ill.
- Insect Control in Flour Mills. Agric'l. Handbook 153. U. S. Dept. Agric., Supt. of Documents, Gov't. Printing Office, Washington, D. C. 15c.
- A Study of Egg Handling in Wisconsin. Agric. Economics Bul. 28. College of Agric., University of Wisconsin, Madison, Wisc.
- Antibiotics in Milk. Circular, March, 1959. Bul. Dept., College of Agric., Univ. of Wisconsin, Madison, Wisc.
- Food-Packaging Materials Their Composition and Uses. A report of the Food Protection Committee, Food & Nutrition Board. Pub. No. 645. National Research Council, 2101 Constitution Ave., Washington, D. C.
- Sanitation: Use More Chemical Sanitizers. J. G. Schahfer. Seco Chemicals, Inc., Detroit, Mich. Sept. 1958.
- Floating Curd Defect in Cottage Cheese. Food & Dairy Dept., Oregon State College, Corvallis, Ore. Sept. 1958.
- Methods of Analysis for Cottage Cheese. Tech. Bul. t-71, H. C. Olsen & M. D. Bonner, Dairy Dept., Oklahoma State Univ., Stillwater, Okla.
- Bulk Handling of Milk on Texas Dairy Farms. Bul. 894. Texas Agric. Exp. Station, College Station, Texas.
- The Chemical Analysis of Foods and Food Products. 3rd ed. M. B. Jacobs. D. Van Nostrand Co., Inc., 250 Fourth Ave., New York 10, N. Y. 970 pages, \$13.75.
- Insects That Carry Disease. Supt. of Documents, Govt' Printing Office, Washington, D. C. 10c.
- Do Blend Temperatures in Bulk Milk Tanks Affect Milk Quality? R. P. March and P. J. Vandemark. Farm Research 24, No. 4, Dec. 1958. Cornell Agric. Exp. Station, Ithaca, N. Y.
- Grading and Inspection of Eggs and Egg Products. Supt. of Documents, Gov't Printing Office, Washington, D. C. 15c.
- Should I Install a Bulk Milk Tank? Bul. 706. Missouri Agric. Exp. Station, Columbia, Mo.
- Costs, Savings and Financing Bulk Milk Tanks on Texas Dairy Farms. Bul. 904. Texas Agric. Exp. Station, College Station, Texas.
- Proceedings of the Tenth Industrial Waste Conference. Eng. Extension Dept., Purdue Univ., Lafayette, Ind. 1956. \$2.50.
- Dictionary of Poisons. Ibert & Eleanor Mellan. Philosophical Library, Inc., 15 E. 40th St., New York 16, N. Y. 150 pages. \$4.75.
- "The Rival World" A Color Movie. How science is combatting insects. 16 MM, 27 min. Shell Oil Co. 50 W. 50th Street, New York 20, N. Y.

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APHA Standard Methods for Examination of Dairy Products XI 1960

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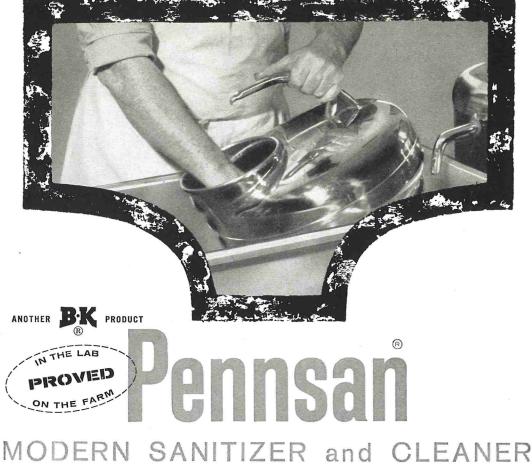
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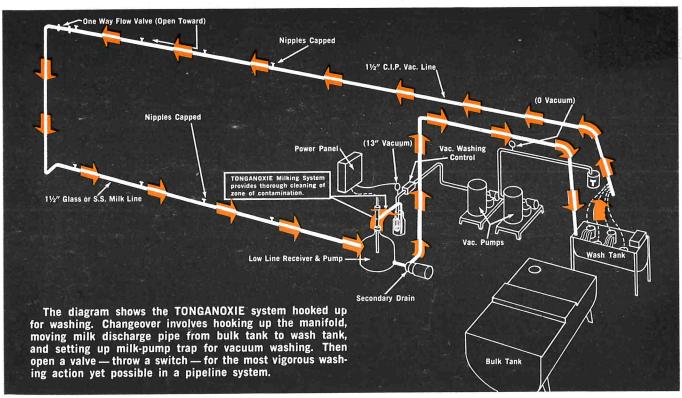
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