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MILK and FOOD TECHNOLOGY

55TH ANNUAL MEETING
August 19, 20, 21, 22, 1968
Chase-Park Plaza Hotel
St. Louis, Missouri
SEE RESERVATION FORM PAGE 1

Official Publication

International Association of Milk, Food and Environmental Sanitarians, Inc.

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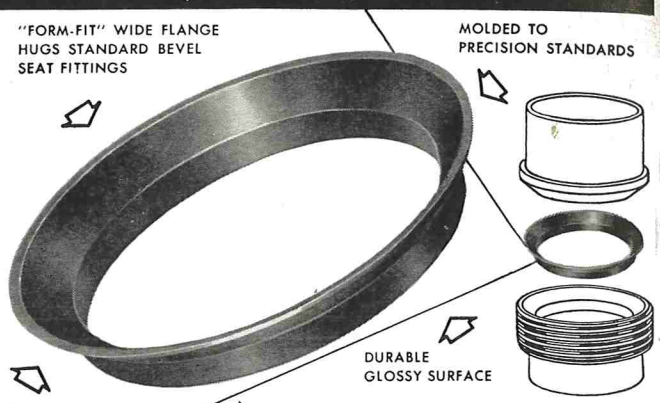

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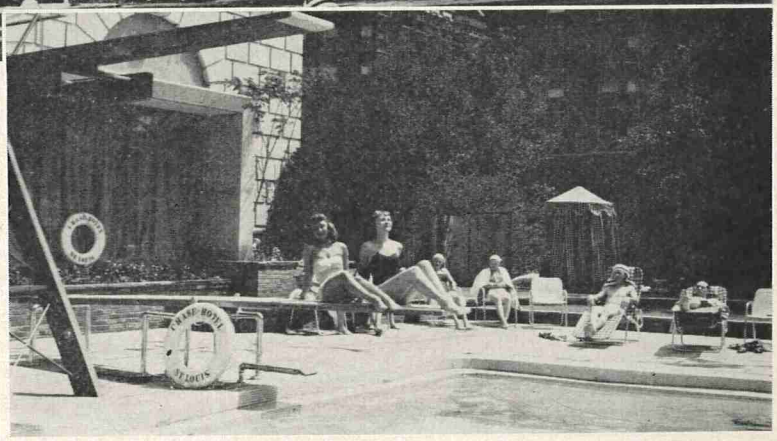

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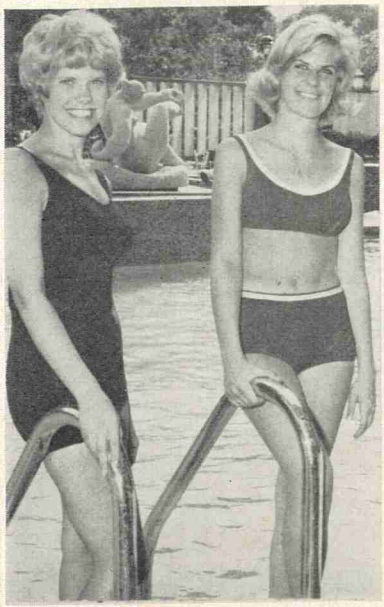
International Association of Milk, Food and Environmental Sanitarians, Inc.

August 19, 20, 21, 22, 1968



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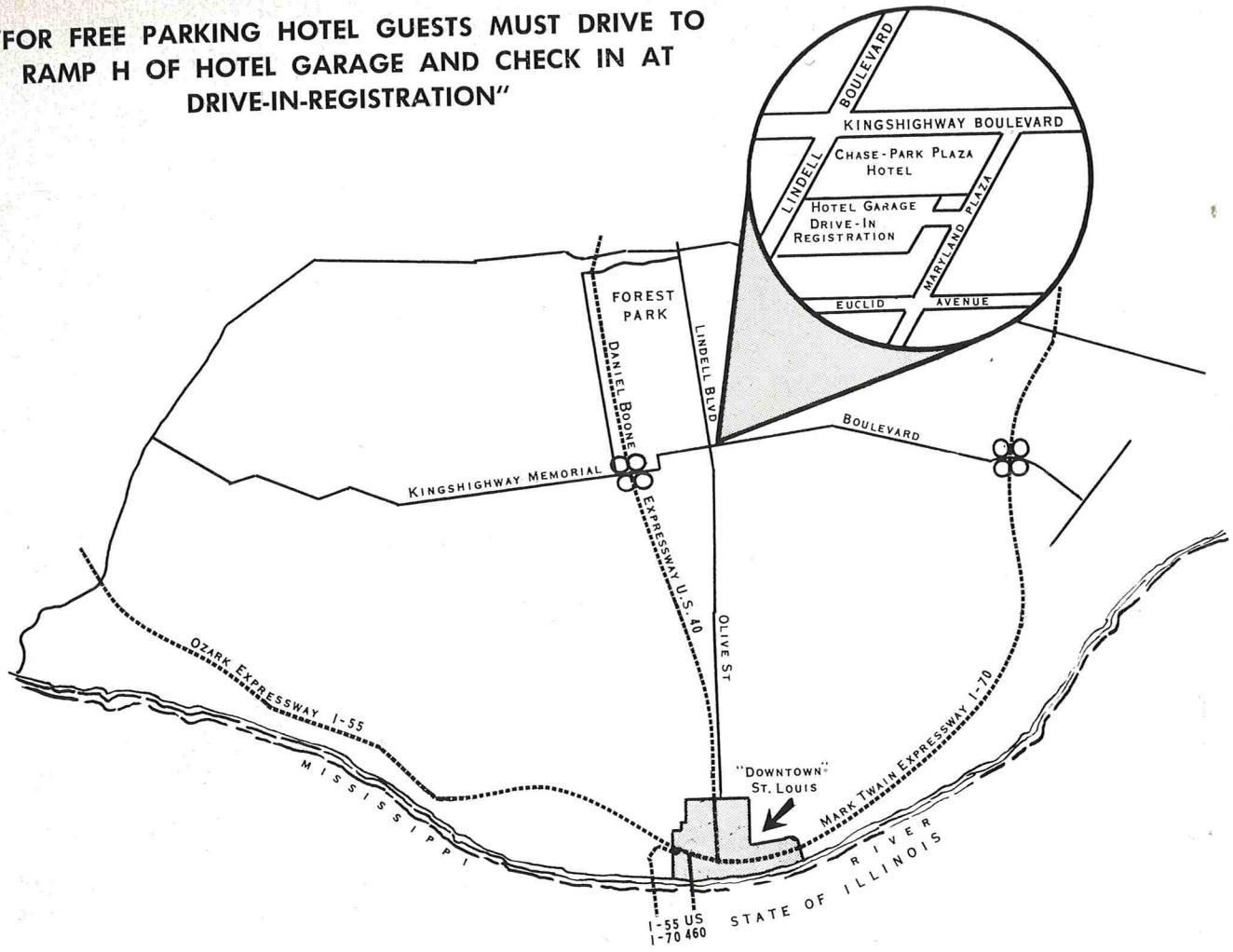

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WHAT THE SANITARIAN SHOULD KNOW ABOUT STAPHYLOCOCCI AND SALMONELLAE IN NON-DAIRY PRODUCTS. II. SALMONELLAE[1]

FRANK L. BRYAN

U. S. Department of Health, Education, and Welfare;
Public Health Service;
Bureau of Disease Prevention and Environmental Control;
National Communicable Disease Center;
Atlanta, Georgia 30333

ABSTRACT

After a review of the nature of salmonellae, including data on the survival of these organisms under both natural and food processing conditions, the epidemiology of salmonellosis is discussed. Salmonellosis caused by salmonellae other than *Salmonella typhi* and *Salmonella paratyphi* A, B, and C are considered zoonoses, diseases or infections transmitted between vertebrate animals and man. This transmission is seldom direct, but usually occurs from the consumption of foods of animal origin. However, other products such as soya milk, yeast, coconut, cottonseed protein, and carmine dye have been incriminated in outbreaks. A number of circumstances must be fulfilled for foodborne salmonellosis outbreaks to occur; these include: a reservoir for the infectious agent, a mode of dissemination of the organism, contamination of a food capable of supporting bacterial growth, enough time at a temperature suitable for bacterial growth, and ingestion of sufficient organisms by susceptible hosts. Control measures must be based on these circumstances. Principles of control, therefore, include limitation of contamination, inhibition of growth, and destruction of the organism. Control of salmonellosis must be aimed at feed ingredient and feed manufacturing plants, farms, hatcheries, animal-transporting and holding facilities, abattoirs, and food processing plants as well as places where food is prepared.

NATURE OF SALMONELLAE

Salmonellae are enteric organisms classified in the family *Enterobacteriaceae*. Other genera in the family are *Escherichia, Shigella, Arizona, Citrobacter* (*Bethesda-Ballerup*), *Klebsiella, Enterobacter* (*Aerobacter*), *Serratia, Proteus Providencia,* and *Edwardsiella.* Salmonellae are gram-negative, asporogenous, motile (by peritrichous flagellae) or nonmotile, rod shaped cells. These organisms have cell wall or somatic (O) antigens and flagellar (H) antigens. A capsular (Vi) antigen is also found in *Salmonella typhi, Salmonella paratyphi* C, and a few other serotypes. Based upon the antigens *Salmonella* possess, approximately 1200 different serotypes have been identified. Endotoxins, polysaccharide-protein-lipid substances, are liberated upon lysis of the cell. These toxins are identical to the somatic antigens.

Salmonellae are aerobic and facultatively anaerobic. They flourish in simple media containing a carbon source, as glucose, and a nitrogen source, as ammonium salt. In foods frequently incriminated as vehicles of foodborne diseases, salmonellae will multiply at temperatures ranging from 44 to 114 F *(6).* They are able to grow rapidly at 59 F on the skin of dressed chickens, even in the presence of rapidly growing spoilage flora *(36).*

McDade and Hall *(58)* showed that salmonellae survive longer in the lower ranges of relative humidity. *Salmonella derby* was not recovered on glass, ceramic tile, polished stainless steel, asphalt tile, and rubber tile after 48 hr exposure to a temperature of 77 F at relative humidities of 53 and 83%, but this organism survived 17 days at the same temperature when the relative humidity was only 11%.

Salmonellae can survive on different surfaces and substrates for varying periods of time: contaminated earth and pasture, over 200 days *(51, 79, 84);* cloth, 228 days *(62);* plastic cover slips, 93 days *(62);* sweeper dust, 10 months *(10);* rodent feces, 148 days *(85);* roach pellets, 199 days *(61);* poultry feces, more than 9 days *(63, 74);* dried cattle feces, over 1000 days *(34);* egg shells, from 21-350 days *(15, 27, 43, 63);* dried whole eggs, over 4 years; meat salad (4 C), 77 days *(72).* In general, longer survival was noted at lower temperatures.

The lowest water activity (a_w) at which salmonellae (16 strains) grew was 0.945 in liquid media and slightly below this figure in foods *(16).* Water activity values for bacteria (except halophilic organisms) usually fall in the range 0.90 to 0.999, but few below 0.965 *(69).* Salmonellae showed no growth in a solution containing 8% sodium chloride; they were destroyed in a week in brine containing 30% NaCl at 68 F; but viable cells were found after 1 month in solutions containing 12% NaCl *(42).* About 20% of a salmonella population survived in ham- or bacon-curing brine after 15 days *(14).*

Salmonellae survived for at least 24 hr at pH 3.95-

[1]Presented, in part, at the 54th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Miami Beach, Florida, August 14-17, 1967.

4.56. All strains tested died within 1 hr at pH 3.4 *(41)*. In another study, salmonellae survived as long as 170 hr in orange juice (pH 3.5) at 37.6 F *(9)*. In lemon juice (pH 2.3) and lime juice (pH 2.5), all of several serotypes tested were destroyed within min. Salmonellae survived in tomato juice (pH 4.3 to 4.4), however, for periods ranging from 10 to 20 days *(7)*. A few serotypes survived for as long as 12 hr in commercially prepared mayonnaise (pH 3.8) and for as long as 6 hr in commercially prepared salad dressing (pH 3.2). The same strains survived for 156 hr in mayonnaise at pH 5 and for 144 hr in salad dressing at pH 4.4 *(86)*. At the alkaline end of the pH scale, no growth of 3 serotypes tested was found in liquid albumen at pH 9 or 10 *(8)*. Growth was observed, however, in the same substrate at pH 6-8 and it was twice as fast at pH 7 as at pH 6.

Salmonellae remain viable in frozen foods for several months *(29, 32, 54, 64, 83, 94)*. Upon freezing, an initial decline in numbers of viable organisms is observed, followed by a leveling-off and slow decline during subsequent frozen storage. Reductions of more than 90% often have been observed, but when the numbers start in the millions, hundreds of thousands survive. There is little destruction at extremely low temperatures (−22 F). *Salmonella typhimurium* survived in distilled water after daily freezing and thawing for as long as 43 days *(84)*.

When ten million *Salmonella senftenberg* 775W cells (most heat resistant strain known) per g were inoculated into custard and chicken a la king, and the foods were rapidly heated to a temperature of 150 F, the organisms were reduced to nondetectable levels within 12 min. Eighty-three min were required for the same level of destruction in these foods at 140 F *(6)*. An earlier study showed similar results, 88.75 min at 140 F (z value 11.95 F at pH 6.1) *(5)*.

EPIDEMIOLOGY

In 1956, only 1700 human *Salmonella* infections were reported in the United States; from 1964 to 1966 more than 20,000 bacteriologically proven *Salmonella* infections were reported each year. Whether or not this is an increase in incidence can only be conjecture; greater interest in salmonellosis, improved surveillance and reporting, and better methods of isolating salmonellae no doubt account in some measure for the change. The recent development of large-volume production of animal feeds, the growing manufacture of processed foods, and the national and international distribution of these items may have also contributed to the increase of salmonellosis.

All members of the genus *Salmonella* are pathogenic for man, for animals, or for both. With the exception of a few serotypes (*S. typhi, S. paratyphi B, Salmonella cholerae suis, Salmonella dublin, Salmonella pullorum, Salmonella gallinarum,* and *Salmonella abortus equi*), salmonellae are not host-adapted and may inhabit the intestine of any warm-blooded animal and many species of cold-blooded animals.

After ingestion of salmonellae by man or animals, three possibilities occur: the organisms cannot be subsequently isolated from feces; an individual remains healthy but excretes the organisms (carrier); or symptoms develop. The alternative depends upon: size of the dose, resistance of the host, and serotype and virulence of the infecting strain. McCullough and Eisele *(55, 56, 57)* reported that large numbers of salmonellae were required to cause illness in healthy adults; individual strains required from 125,000 to 16 billion cells to cause illness. Infants, the elderly, undernourished and debilitated persons, and individuals suffering from concomitant diseases, being more susceptible than the adults studied by McCullough and Eisele, may be made ill by smaller doses and may manifest severe clinical syndromes. For example, capsules containing as few as 15,000 *Salmonella cubana* cells caused salmonellosis in these more susceptible individuals *(44)*.

Salmonellae produce varying clinical manifestations: acute gastroenteritis, typhoidal or septic syndromes, and focal infections. Acute gastroenteritis, caused by nearly all serotypes of *Salmonella*, is the most frequent syndrome encountered. Salmonellae cause inflammation in the small intestine, resulting in a sudden onset of abdominal pain, diarrhea, and frequently headache, nausea, and vomiting. Fever often follows diarrhea. Symptoms appear from 6 to 72 hr after infection, usually from 18 to 48 hr. Severity varies from mild diarrhea to acute dysfunction—rapid dehydration, bloody discharges, tenesmus, and even death. Usual duration of illness is from 1 to 7 days. Salmonellae are often excreted for several weeks after convalescence.

Typhoidal or septic syndromes occur in salmonelloses caused by *S. typhi, S. paratyphi*, A, B, and C, *S. cholerae suis*, but only rarely in salmonelloses caused by other serotypes. Infections by *S. typhi* are characterized by a high, sometimes continuous, fever; severe headache; and enlargement of the spleen. Rose spots may appear on the trunk. Constipation is more common than diarrhea. Blood cultures contain Vi agglutinins in a great many instances. The typhoidal syndrome is sometimes preceded by gastroenteritis by several days.

Focal manifestations caused by salmonellae include appendicitis, cholecystitis, salpingitis, peritonitis, meningitis, pneumonia, pleurisy, osteomyelitis, osteoarthritis, abscesses, urinary tract infections, endocard-

itis, and pericarditis *(11)*. Besides these manifestations, inapparent infections and excretion of salmonellae by carriers are not uncommon. The duration of excretion for carriers is about the same as that of clinical cases.

Salmonelloses in animals are similar to those in man. Both enteric and septicemic syndromes are seen; but manifestations without parallel in human pathology are found in the epidemic abortion in mares caused by S. *abortus equi* and in the ovarial infection of chickens caused by S. *pullorum*.

Salmonelloses caused by salmonellae other than the human host-adapted types (S. *typhi* and S. *paratyphi* A, B, and C) can be considered zoonoses—diseases or infections transmitted between vertebrate animals and man. Such transmission is, however, seldom direct, but usually results from eating food of animal origin.

Poultry

Salmonellosis in fowl has long been recognized as an important economic burden on the poultry industry. Besides the disease produced, domestic poultry and wild birds carry salmonellae for long periods of time without apparent illness. From 1963-1965, over one-half of salmonella isolations from nonhuman sources reported to the National Communicable Disease Center were from fowl; however, far more cultures were taken from fowl than from other animals because of the national poultry improvement plans for the elimination of pullorum disease and fowl typhoid. As a result of testing programs these 2 fowl diseases have decreased. In 1956, 70.5% of reported isolations from chickens and turkeys were S. *pullorum* and S. *gallinarum;* in 1965, only 6.2% of isolations from chickens and turkeys were these serotypes.

Small numbers of salmonellae can establish infection in chicks *(33)*. Infected poultry have been reported to excrete salmonellae for periods ranging from 15 days to 18 months *(1, 2, 15, 25, 70, 90, 95)*. Sadler et al. *(67)* isolated salmonellae from the intestinal tracts of 2.4% turkeys, 2.6% chicken fryers, and 1.2% chicken hens upon arrival at processing plants. Four years later, by the use of cloacal swabs, Sadler and Corstvet *(66)* found 5.25% turkeys, 1.85% chicken fryers, and 0.54% chicken hens contaminated with salmonellae.

Swine

Salmonellae can be recovered from feces and mesenteric lymph nodes of pigs. Of 2,100 slaughtered pigs in different slaughter houses in Holland, 25% were found to harbor salmonellae in the portal or mesenteric lymph nodes, in the feces, or in both *(39)*. Market pigs show higher rates of infection than do pigs on farms *(24, 31, 40, 45, 59, 71)*. Salmonellae were isolated from 20% and from 50%, respectively, of 2 groups of 50 pigs from Iowa and from 68% of a group of 50 pigs from Arizona *(49)*. Stress factors, including handling, crowding, cold, lack of food or water, and transporting may trigger a mechanism that changes an infected, but non-excreter, pig into an excreter *(89)*.

Cattle

There are many accounts of severe outbreaks of salmonellosis in calves and in adult cattle. Infection is more frequent in calves, and their mortality rates are higher. After cattle are infected with S. *dublin*, they may remain carriers for years, however, the carrier state for other serotypes is usually shorter *(37)*. Salmonella infection rates in calves on farms increased from an average of 0.5% to an average of 35.6% upon slaughter when the calves spent 2-5 days in abattoir pens *(4, 26, 28)*.

Foods

Salmonellae may reach man by direct contact (man-to-man or animal-to-man) and through his foods and water. Of all routes of transmission of salmonellosis, food is undoubtedly the most frequent offender. In the United States, between 1963 and 1966, 85 outbreaks of salmonellosis were traced to foods; in 29 (34.1%), eggs or egg products were responsible; in 18 (21.2%), poultry meat was incriminated; in 9 (10.6%), red meats and their products were the cause; and in the remaining 29, miscellaneous foodstuffs were responsible. In England and Wales, during 1963 and 1964, over one-half of the outbreaks in which the vehicle was determined were associated with processed meat products *(82)*.

Eggs. Eggs have structural and bilogical characteristics that render bacterial invasion difficult but not impossible. Since the ovaries and oviduct are normally sterile, the egg is usually sterile before being laid; but S. *pullorum* and S. *gallinarum* chronically infect the hen's ovarian tissues and may be transmitted directly to the yolk. Salmonellae, however, are more commonly transmited to egg shells during laying, from droppings, or from fecally-contaminated nests. The egg shell is covered with mucin, called the cuticle or bloom. Within the shell is an outer and inner membrane. The bloom may be removed by washing or will disappear during prolonged storage. The pores in the shell are large enough to admit salmonellae, and these organisms can also penetrate the membranes *(30, 88)*. Cracked or checked eggs are even easier to invade. Once in the egg, salmonellae are not markedly affected by inhibitory substances naturally present in eggs *(8, 15, 46, 76)*. High moisture and high temperatures promote penetration.

Cumulative surveys from several countries have

shown that only a fraction of 1% of individual shell eggs were contaminated with salmonellae. Egg products—liquid, dried and frozen whole eggs, yolks, and albumen—however, are frequently contaminated with salmonellae *(75)*. Kampelmacher discussed surveys made in many countries in which 1-16% of egg products were found to be contaminated with salmonellae *(38)*. In England, from 1961-1963, 6.4-20% of imported egg products contained salmonellae *(35)*. In 1963, when only 6.4% of the egg products were positive, 17% of the positive samples were from raw products and only 0.6% from pasteurized products. In the United States, Wilder and MacCready *(87)* reported that 32% of 456 samples of frozen, unpasteurized eggs in the Massachusetts market contained salmonellae. Ager *(3)* reported 24% of 1,758 samples of frozen eggs positive for salmonellae. During the years 1964 to 1966, the U. S. Food and Drug Administration reported 23 to 33% of their samples of egg and egg-containing foods, taken for official and investigational purposes, were found to contain salmonellae. Most of these samples were from unpasteurized sources and many were taken as follow-up of problems and do not represent national averages.

Before egg pasteurization became general, salmonellae were found in many mixes containing eggs such as cake frosting, pancake, waffle, whip-powder, batter piecrust, cookies, biscuit, gingerbread, doughnuts, sponge pudding, coconut dessert, and meringue powder *(78)*.

Poultry meat. Although the number of infected fowl arriving at processing plants is usually low, salmonellae are widely disseminated to poultry meat during processing. During initial stages of processing, in defeathering machines, carcasses are contaminated. A few incoming contaminated birds transfer salmonellae to rubber fingers and other contact-surfaces of the machines, and these surfaces contaminate carcasses that are subsequently processed. In a survey at a turkey processing plant, defeathering equipment was contaminated 76% of the time, and 63% of the carcasses leaving these machines were positive for salmonellae *(13)*. Other pieces of equipment and workers in eviscerating, cooling, and further-processing operations also spread salmonellae *(12, 13, 18, 19, 23)*. Surveys of dressed poultry collected from poultry processing plants or from retail markets have shown varying, but frequently high, levels of contamination; 4.4% *(68)*; 14% *(77)*; 1-10% *(20)*; 17% *(91)*; 27% (93); 27% *(13)*; and 50% *(87)*.

Red meats. An outbreak of human salmonellosis in Sweden—one of the largest outbreaks recorded, resulting in approximately 9,000 cases and 90 deaths—was attributed to meat. Animals held in pens for a long time, over-crowded processing facilities, inadequate cooling, and high temperatures during transport of the meat contributed to the outbreak *(48)*.

Like poultry, red meat becomes contaminated during processing in abattoirs or processing plants. A single infected animal entering an abattoir provides a source of contamination for equipment and workers. Surveys have shown that many items of equipment, such as scald tanks, dehairing machines, and tables, frequently reveal salmonellae *(24, 71)*. Shotts *(71)* isolated salmonellae from 58% of swabs from environmental sources taken after processing, but before cleanup; these organisms were also found in 34% of swabs taken before operations began—indicating inadequate cleanup.

In the United States, Felsenfeld and Young *(20)* found salmonellae in 27% of their samples from uninspected pork, in 14% of those from inspected pork, and in 18% of those from hamburger; Wilson et al. *(91)* found salmonellae in 4% of pork samples, in 3% of lamb samples, and in 1% of beef samples obtained from the Cincinnati market area; Galton et al. *(22)* found salmonellae in 23% of fresh pork sausage samples and in 12.5% of smoked sausage samples. In the United Kingdom, the proportion of samples from imported frozen boneless meat products contaminated with salmonellae were: veal, 18.5%, beef, 15.2%; and mutton, 9.8% *(35)*. In Ireland, salmonellae were isolated from 3% of pork muscle and from 70% of the sausage prepared from the same meat *(60)*. Dixon and Peacock *(17)* found 7.5% of Dutch meat contaminated with salmonellae. In Holland, 6% of pork samples taken from butcher shops were positive for salmonellae *(39)*.

Fish. Smoked white fish has been responsible for outbreaks involving more than 300 cases of human salmonellosis. River water used to wash fish and ice from the river were probable sources of contamination. The smoking process does not destroy salmonellae. Fish taken from open waters are free from salmonellae, but fish caught in sewage-polluted waters have harbored these organisms *(81)*.

Foods of nonanimal origin. Several outbreaks have have been attribued to foods other than those of animal origin. In Sweden, an outbreak of salmonellosis was traced to cereal powder. This powder was ground in a mill where African barley—containing *Salmonella muenchen*—had been processed *(73)*. In a mental institution, a dietary supplement containing dried yeast and cottonseed protein cencentrate was responsible for an outbreak. Many of the same serotypes that had been recovered from the patients, the food supplement, and its raw ingredients, were also isolated from samples taken at the cotton gin and the yeast plant that had produced the ingredients *(53)*.

When coconut from Ceylon was found responsible

for several outbreaks, an investigation disclosed that husked coconut meat was not only air dried for 28 days in the open—where it was subject to contamination by birds, insects, animals, and workers—but after air drying, it was washed with contaminated water before being shredded. Oven drying did not destroy salmonellae already present *(92)*.

Several hospital-associated cases of *S. cubana* infections were attributed to carmine dye, manufactured from female insects of the species *Dactylopius coccus (coccus cacti)*, that was used to investigate gastrointestinal tract illness. Carmine powder from capsules contained 100,000 *S. cubana* cells per g. This red dye is also used in candies, chewing gum, meat coloring and preservatives, seasoning, ice cream, tomato extracts, and many other products *(44)*. Salmonellae recently have been isolated from chocolate candies.

Animal feeds

Mounting evidence points toward rendered animal by-products and feeds as sources of salmonellae for animals. Surveys of over 4,000 samples in England *(37)* revealed salmonellae in 9% of raw ingredients and from 2.8% of finished meals; only 0.27% were found in pelleted feeds. In the United States, in a nationwide survey *(52)*, salmonellae were recovered from 7 to 33% of different feed ingredients and in 6% of processed feeds. Five years later in a more comprehensive survey covering 26 states, similar observations were made *(80)*.

Magwood et al. *(50)* observed that rendered meals were recontaminated after cooking and expelling. Positive samples were found after grinding and from storage room floors and ledges.

Cycle of transmission

From the foregoing discussion, the cycle of transmission of salmonellae is graphically illustrated in Figure 1.

DETECTING AND IDENTIFYING THE ORGANISM

Although the numbers of salmonellae in a sample can be estimated by a most probable number method, qualitative methods are usually used to detect salmonellae in foods. Samples of food (25-30 g) or swabs taken from food surfaces or processing equipment are usually put into 1 of 2 selective enrichment broths, tetrathionate or selenite. Additives, such as brilliant-green and sodium sulfathiazole for tetrathionate and cystine for selenite, enhance their effectiveness. Enrichment incubation time is usually from 24 to 48 hr. In dried or frozen foods where salmonellae are in a state of dormancy or inanition, preenrichment with lactose or nutrient broths may be used to enhance growth prior to enrichment. Preenrichment requires an additional 8-48 hr.

After enrichment, the culture is streaked onto one or more selective, differential media. Bismuth sulfite, SS, or MacConkey agars may be used, but brilliant-green agar (frequently containing sulfadiazine to inhibit pseudomonads) is preferred for isolating salmonellae from foods *(21)*. Incubation requires 24 hr. Suspect colonies (transparent pink to deep fuchsia colonies on the red portions of the plate) are picked and inoculated into a triple-sugar-iron agar slant. After overnight to 24 hr incubation, the slants are checked for typical salmonella reaction: acid (yellow) butt; alkaline (red) slant, gas, and hydrogen sulfide (black deposits).

Growths that show typical salmonella reaction are then subjected to slide agglutination test with common somatic (O) antisera. Cells from slants suspected of containing salmonella are also grown in H broths (or similar media), phenolized, and tested for precipitation with Spicer-Edwards pools containing flagellar (H) antisera. These serological tests disclose group O antigens and one phase of the H antigens. Biochemical tests may be performed for confirmation before sending cultures to a reference laboratory for typing. There, serotyping (determining the exact antigenic composition of the organism) can be acomplished by reversing phases and testing for single antigens.

The time required to determine the presence of salmonellae in a food sample (selective enrichment through slide agglutination) is at least 3 days. Biochemical testing and testing for H antigens requires another day or two. Complete serotyping requires several days after the sample arrives at a reference laboratory. Serotyping is essential for identifying vehicles and sources of the organism responsible for outbreaks. The same serotype should be identified in the patients and in the incriminated food. Strains of a few serotypes *(S. typhi, S. paratyphi* B, and *S. typhimurium)* can be more definitively identified by phage typing.

CONTROL

Control of salmonellae is simple in principle: limitation of contamination, inhibition of growth, and destruction of the organism. The first principle, limitation of contamination, involves: preventing contaminated raw ingredients from contaminating finished products; practicing good personal hygiene and disease control; cleaning spillage immediately; cleaning and disinfecting all product contact surfaces of equipment; designing equipment and plant layout to promote rapid process flow, to prevent contaminating air flow, to avoid producing aerosols or spray, and to facilitate cleaning and disinfecting operations; using

potable process and wash water; and preventing contamination by insects, rodents, birds, sewage backflow and drippage.

The second principle, inhibition of growth, requires that foods be held at 42 F or below or 116 F or above and processed rapidly. Other applications of this principle are removing moisture, lowering pH below 4.5, increasing salt concentration above 8% NaCl, and adding certain chemical substances that inhibit growth of salmonellae but do not interfere with taste, functional properties, or safety of the food.

The third principle, destruction of the organism, can be accomplished by heat, irradiation, low pH, and gaseous disinfection. These procedures require sufficient temperature or concentration for a sufficient time to assure destruction of salmonellae in the particular food or feed substrate in question.

These principles must be applied to a complex of operations carried out in processing feed ingredients, manufacturing animal feeds, raising domestic livestock, hatching poultry, transporting and holding livestock, slaughtering animals, processing foods, and preparing and serving foods. Control measures relating to each area will be briefly discussed.

Feed ingredients

Rendering livestock and poultry by-products requires temperatures sufficiently high to kill salmonellae. Heating and dehydrating in conventional cookers takes 3.5 hr and temperatures up to 230 F are reached; products rendered in continuous cookers reach 200 F; fish are processed at slightly lower temperatures. The essential element for the control of salmonellae is to prevent recontamination. Some problems that contribute to recontamination of rendered feeds are aerosols produced by grinders, common rooms or equipment to handle or convey raw and finished products, and use of river water to pump fish from boats to storage facilities and conveyors. Recontamination can be limited by physically separating processed materials from raw ingredients, by keeping workers employed in incoming raw ingredient areas away from the processing area beyond the cookers, by vector- and bird-proofing plants, and by protecting processed feeds during conveyance and storage. The National Renderers Association sponsors research on the feasibility and development of terminal heat treatment to destroy salmonellae in finished products. Equipment under study in this program uses a temperature of 210 F for 3 to 5 min on ingredients whose moisture content is 8%.

Feeds

Ideally, animal feedstuffs should be made up from ingredients free of salmonellae, but at the present stage of the art of rendering and sampling, it is impossible to guarantee the absence of these organisms. Rossow and Darby *(65)* showed that pelleting will reduce *Salmonella* contamination if the initial level is low. In this process, temperatures reach a range of 160 to 180 F for periods of 2 to 16 sec in ingredients whose moisture is between 11 and 16%. The expansion-extrusion process, attaining temperatures of 200 to 350 F for periods of 45 to 60 sec in ingredients whose moisture content is 30%, is quite effective in reducing *Salmonella* contamination. Some countries require treatment of feeds by procedures such as steam injection or autoclaving for feedstuffs that do not have a history of adequate heating. Irradiation may become a future solution for destroying salmonellae in feedstuffs, but it is still too costly for general use. Unless bagging and storage areas and transportation facilities are free of contamination, the product's freedom from salmonellae will be jeopardized.

Farms

Sanitarians have done much to improve sanitary conditions on dairy farms, but there has been little organized activity useful in the control of salmonellae on other types of farms. Incoming feed, new poults and chicks, or replacement stocks can introduce salmonellae to farms. Once a particular serotype has been introduced to a farm, in many instances, it survives there for months or even years. Feeds should be stored so they will stay dry and safe from contamination by rodents, birds, or domestic livestock and poultry. If feeds containing small quantities of salmonellae are wet by weather or animal excreta, the organisms multiply. Animal drinking water is often overlooked as an important source of salmonellae; troughs become contaminated by animal feces, feed, and barnyard debris. Salmonellae in troughs can survive several days or may even multiply if sufficient organic matter is present and if temperatures are adequate. Environmental sanitation of equipment, barns, broiler and breeder houses, pens, and farmyards aid in Salmonella control. Serological detection of adult carriers in breeder flocks has been used successfully to detect S. *pullorum* and S. *gallinarum*, but these procedures have had only limited application and success for detecting other serotypes. Intestinal carriers of salmonellae may exhibit no serological response. Serological testing of animals is limited to areas where a particular serotype is known to be a problem.

Hatcheries

Formaldehyde fumigation of eggs on farms reduce shell contamination. Frequent collection, segregation of dirty eggs, and storage in a cool place will also help. After eggs arrive at a hatchery, they should

be fumigated with formaldehyde after they have been put into incubators. After each hatch, incubators should be thoroughly cleaned, disinfected, and finally fumigated with formaldehyde (1.2 ml of formalin mixed with 0.6 g potassium permanganate per ft^3 of space for 1 hr).

Transportation and holding animals

Animals in transport and in holding pens should be neither overcrowded nor held for long periods of time. Feeding facilities, troughs, pens, and trucks should be of sanitary design and kept in a sanitary condition.

Food processing

Animals or products of animal origin may introduce salmonellae into processing plants. Processing, when possible, should be effected within the bacterial lag period (2 to 3 hr) to prevent bacterial multiplication.

In general, to insure destruction of salmonellae, foods must be heated to internal temperatures of 160 or 165 F, but if these temperatures cause undesirable changes in products, lower temperatures for longer intervals can be effective in many instances. Low temperatures, for instance, have been used to pasteurize eggs. The following methods have been approved by the U. S. Department of Agriculture *(47)*:

1) Whole eggs: heat to not less than 140 F for 3.5 min.
2) Egg whites:
   a) neutralize pH, add aluminum to stabilize conalbumin, heat as above.
   b) heat to 125 F for 1 min, add 0.75% hydrogen peroxide, hold for 2.5 min.
   c) heat to 125 F, add 0.1% peroxide, hold for 3.5 min.
   d) adjust pH to 9, heat to 134 F, hold for 3.5 min.
3) Dried egg whites with moisture content greater than 6%: incubate to 125-130 F and hold for 7-10 days.
4) Yolks: heat to 144 F for 3.5 min or to 140 F for 7 min.
5) Sugared or salt yolks: heat to 148 F for 3.5 min or to 144 F for 7 min.

Foods that undergo heat treatment, and that are not ordinarily cooked later, require measures to prevent recontamination. Processed potentially hazardous foods should be chilled as fast as feasible and held at temperatures no higher than 45 F for storage periods not exceeding 2 days, and at 40 F or lower for storage exceeding 3 days.

Laboratory testing for salmonellae in incoming ingredients (or animals), line samples, equipment surfaces, and finished products is necessary to disclose areas needing intensified control efforts.

Food service

One problem of epidemiological significance that is often overlooked in routine inspections is cross-contamination from raw products to finished products. Raw foods of animal origin (cracked eggs, poultry, red meats) are frequently contaminated with salmonellae and can contaminate workers' hands or preparation equipment. This contamination can then be transferred to foods that are not subsequently heat processed.

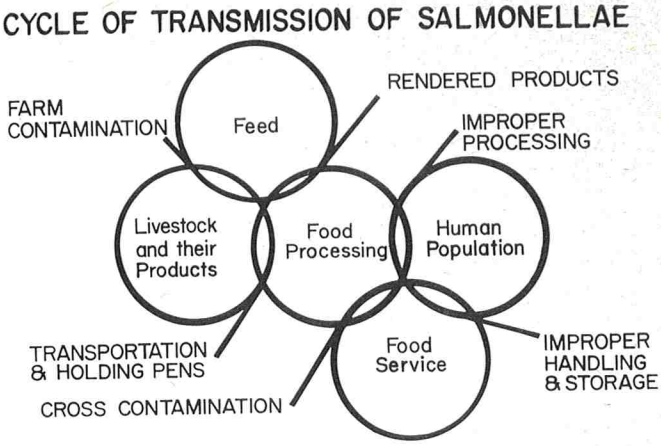

Figure 1. Cycle of transmission of salmonellae.

Unless the treatment will impair quality, foods should be heated to 165 F; if a food cannot be so treated, control must be based on limiting contamination and preventing multiplication. (See Part I of this paper for details.)

CONCLUSION

Effective prevention of staphylococcal intoxications and salmonellosis depends on an awareness of the characteristics of the organisms and the nature of the problem they pose. Surveillance of foods and human illness is a necessary requisite for control. Feed manufacturing, farm environments, and food processing plants, as well as food-service establishments, need emphasis in salmonella control programs. Within these establishments, daily supervision by an informed management is essential for effective control. The misconception that the so-called "ubiquitous" organisms cannot be controlled must be replaced with a feeling of determination to apply the known principles of control (limitation of contamination, inhibition of growth, and destruction of organisms) to feed or food production, processing, or preparation.

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JUNKED AUTOS

A new approach to a familiar eyesore and health hazard—junked autos—is being investigated by E. J. Wren of the Gulf South Research Institute, Baton Rouge, Louisiana. Mr. Wren intends to fill junked automobiles with worn-out tires and squash the cars with special compacting equipment.

The flat steel masses will be interlocked, through a special die developed by the research project, and placed along a river bank to protect the levee slopes and prevent erosion by wave action.

Henry C. Steed, Jr., Chief of the Office of Grants Administration of the Public Health Service's National Center for Urban and Industrial Health in Cincinnati, today announced a $44,374 grant to support the one-year research project.

Under Mr. Wren's plan, the compacting equipment could be installed on a Mississippi River barge. The barge will travel up and down the Mississippi River, stopping at collection points to flatten out the junked cars and tires. The interlocking junk will be concealed from view by willow trees which grow along the levees.

Mr. Wren will consult with the Army's Corps of Engineers on the problems of installing the metal shapes along a river bank. The project, according to Mr. Wren, might well be used on other navigable rivers and lakes.

A RAPID TEST TO FIND "POTENTIALLY" PSYCHROPHILIC ORGANISMS IN PASTEURIZED DAIRY PRODUCTS

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ABSTRACT

The application of the oxidase test to plates prepared for the Standard Plate Count is used as a measure of "potentially" psychrophilic organisms in dairy products. Samples were examined by both the oxidase test and the standard test for psychrophiles, and a statistical analysis shows the tests to be positively correlated. The test is based on the proposition that most problems with psychrophiles can be traced to pseudomonads. These organisms are strongly oxidase-positive and this attribute allows for their differentiation from other bacteria found in food products. The oxidase test consists of flooding the plates used for the total bacterial count with a reagent which causes the strongly oxidase-positive colonies to develop a blue color. This simple test affords a rapid measure of "potentially" psychrophilic spoilage organisms.

The presence in foods of organisms able to grow at refrigeration temperatures is important to the food industry. Many of these organisms (loosely labelled as psychrophiles) growing in a refrigerated food product are able to produce off-flavors and odors in a relatively short period of time and make the product unpalatable. As used in this report, psychrophiles are defined as organisms which can grow at refrigeration temperatures. In the food industry this is usually less than 10 C.

Psychrophiles are found among many bacterial genera. The most important are probably the pseudomonads and most problems in dairy products are caused by this single genus *(11)*. The review by Witter *(11)* gives a good discussion of these organisms in relation to flavor problems. Of particular importance in the dairy industry are *Pseudomonas fragi, Pseudomonas mucidolens, Pseudomonas viscosa, Pseudomonas putrefaciens* and *Pseudomonas fluorescens* and it has been shown also that 93% of the bacteria found on chilled meat are pseudomonads *(4)*.

The determination of psychrophilic organisms in a sample is a lengthy and time-consuming procedure. One of two standard methods is usually employed. The total bacterial count of the sample is determined and a duplicate set of plates prepared at the same time is incubated at a low temperature for 5-7 days and the colonies are then counted. In the other method the sample is refrigerated for 6-8 days and a total count is made before and after this cold incubation. In both methods, if there is a significant increase in numbers after the cold incubation, the sample is assumed to contain psychrophiles. With either of the standard procedures a minimum of 7 days is required before data are available to determine if the sample contains psychrophilic organisms. After 7 days, the product, if heavily contaminated, may be unsavory; the possibility of finding the source of contamination after this delay becomes increasingly difficult, and the data obtained are of only historical significance.

If the psychrophilic count could be found within 1 or 2 days of processing, immediate remedial action could be taken. Such a test may be made on the premise that most of the psychrophiles in food are pseudomonads. Evidence for this in pasteurized dairy products is shown by the data of Baumann and Reinbold *(2)* and in the review by Witter *(11)*. A measure of "potentially" psychrophilic organisms would be obtained if the pseudomonads could be differentiated and enumerated selectively in a sample containing a mixed flora. Correlative data between the per cent of pseudomonads present in the regular plate count and the actual number of psychrophiles determined by the standard psychrophile test would substantiate the validity of such a proposed test.

Pseudomonads are biochemically very active and one of their characteristics is that they are oxidase-positive (i.e. they contain a strong cytochrome *c* oxidase system which, for example, is able to catalyze the coupling of 2 compounds such as *a*-naphthol and *p*-aminodimethylaniline oxalate to form indophenol blue). Most other Gram-positive and Gram-negative organisms (with some exceptions) are not *(10)*. Some qualitative data of this aspect concerning dairy products has been reported by Castell and Garrard *(5)*. They have also reported that in butter found to con-

tain an off-flavor, oxidase-positive organisms were always in abundance. The oxidase characteristics of the pseudomonads is the basis for a rapid test for "potentially" psychrophilic organisms. Samples collected in a local dairy were used to gather the data presented in this report, but it is felt that analogous data could have been collected in any food industry.

METHODS

Samples used. All samples were bottled fluid milk products obtained from daily plant production. Pasteurization was at 171 F for at least 16 sec. Samples, tested on 12 different days over a 3 month period, included fluid milk and cream, chocolate milk, half and half, nonfat milk, and vitamin-mineral fortified milk.

Total counts. Plates (Difco Plate Count agar) were incubated at 32 C for 48 hr as prescribed in *Standard Methods (1).*

Oxidase count. After obtaining the total count, each plate was flooded with about 2 ml of the oxidase reagent described below and allowed to stand for 10 to 15 min in order for the reagent to diffuse through the agar. The plate was then recounted, enumerating only those organisms which turned blue. For better evaluation of the plate it was necessary to diminish the intensity of the light from the colony counter (Quebec or equivalent with blue bulb). This was accomplished by placing a piece of filter or lens paper between the light source and the bottom of the petri dish. Examination of a few plates with different papers should be tried to determine the best visibility of the blue colonies.

Preparation of oxidase reagent. Equal amounts of a 1% solution of α-naphthol in 95% ethanol and a 1% solution of *p*-aminodimethylaniline oxalate (Difco) in water were mixed together. To dissolve oxalate, the solution was heated and then cooled to room temperature in a cold water bath before being mixed with the α-napthol solution. The reagent was subsequently protected from light. Although the reagent should keep for a few days at refrigeration temperatures, experience has shown that a fresh solution works best. **Care should be taken in the use of this reagent; adequate ventilation while counting the plates is required.** The oxidase reagent has been described for use in the identification of *Pseudomonas aeruginosa (7).*

Standard test for psychrophiles. Methods as outlined in *Standard Methods (1)* were used. Samples were incubated for 6 days at 5 C; plated and the plates incubated at 32 C for 48 hr.

Calculations. Determine per cent of total count which is oxidase-positive. Any sample with an oxidase-positive count of 2% or greater of the total count is considered to contain "potentially" psychrophilic organisms.

RESULTS AND DISCUSSION

The rapid test to enumerate "potentially" psychrophilic organisms is based on two points. One is the well known fact that in dairy products pseudomonads are involved in more problems with off-flavors and odors than any other single group of organisms. The second point is that this group of organisms is strongly oxidase-positive while the remainder of the organisms generally found in dairy products are weakly positive or negative. It is this oxidase-positive characteristic of the pseudomonads which makes them amenable to differentiation in a mixed flora.

A test was made of some pseudomonads and other organisms common to the dairy industry as to their reaction in the oxidase test (Table 1). All of the pseudomonad cultures were oxidase-positive. The higher counts on some of the test plates over the standard plate count may be attributed to the size of the colonies. Pseudomonads sometimes form pinpoint colonies which are difficult to see under ordinary counting procedures but when they turn blue in the oxidase test they are easily enumerated. Bacteria other than pseudomonads do respond to the oxidase test but many of these are pathogens and the liklihood of finding them in food is not great. The data of Steele *(10)* are quite important in this respect, since he tested the oxidase potentialities of a large number of bacterial genera. Castell and Garrard *(5)* also made some qualitative determinations on some organisms with a similar oxidase reagent.

Some loss in accuracy must be accepted with the oxidase test in order to gain the advantage of doing a 7-8 day test in 2 days. The incubation temperature of 32 C used for the standard plate count may be too high for certain of the psychrophilic pseudomonads, since 32 C is near the upper limit for growth by some of these organisms. A slightly lower temperature of incubation (28-30 C) could be used without too much loss in incubation time, but then duplicate plates would have to be prepared, thus increasing the work time under routine conditions and detracting from its simplicity. Also, all pseudomon-

TABLE 1. OXIDASE REACTION OF SOME ORGANISMS COMMON TO THE DAIRY INDUSTRY

Organism[a]	Oxidase reaction	Plate count[b]	Oxidase count[c]
Pseudomonas fragi	+	404	419
Pseudomonas fluorescens	+	186	186
Pseudomonas mucidolens	+	119	133
Pseudomonas putrefaciens	+	215	221
Pseudomonas viscosa	+	178	178
Streptococcus lactis	−	55	0
Escherichia coli	−	120	0
Aerobacter aerogenes	−	102	0

[a]A negative oxidase reaction was observed with *Bacillus cereus, Lactobacillus casei, Bacillus subtilis, Bacillus megaterium, Alcaligenes viscolactis,* and *Microbacterium lacticum.* A doubtful reaction was observed with *Alcaligenes metalcaligenes.*

[b]Standard plate count. The dilution is not indicated for any organism. The same plate was used for the SPC and for the oxidase test.

[c]In some cases pseudomonads form pin point colonies and are not enumerated under regular conditions. When they turn blue in the oxidase test they are readily visible.

ads which grow at 32 C may not be psychrophilic. However, all pseudomonads are "potentially" psychrophilic and it is felt that it is better to overestimate some samples (i.e. call them "potentially" psychrophilic when they are not) than to miss them entirely or wait 7 days before having the result. Conversely, not all psychrophiles are pseudomonads. The oxidase test for the detection of potentially psychrophilic samples was compared with presently accepted tests for psychrophilic organisms *(1)*. A study was made on pasteurized bottled dairy products; a total of 133 samples being examined. A standard plate count, an oxidase count on the same plate used for the initial standard plate count, a standard 8-day psychrophilic test and an oxidase count of the 8 day psychrophilic count were made on each sample tested. The criteria for a positive test in any one category were arbitrarily defined as follows. If the psychrophilic count by the 8-day standard test was greater by 10% of the initial total bacterial count of the sample, it was considered to contain psychrophilic organisms. If the oxidase test made on the initial total bacterial count showed that at least 2% of the total was oxidase positive, then the sample was considered to contain "potentially" psychrophilic organisms.

The data obtained and shown in Fig. 1 are in the form of a four square design. The appropriate Chi-square tests were made as suggested by Dr. C. I. Bliss (personal communication) and are outlined in his recent book *(3)*. The data for (a + d)-(b — c) show that the oxidase test is positively correlated with the standard test when applied to the 133 samples examined. The analysis of (b — c) indicates that more samples were negative by the standard test than with the oxidase test. The number scoring the same in both tests exceeded those that scored differently in each test in spite of the difference (b) and (c) in scoring between tests. The test of (a — d) shows that the number of positives in both tests exceeded the number scoring negative in both tests. Until such time as the relative numbers of oxidase-positive organisms required to produce a noticeable flavor defect in a natural sample is ascertained, the ratio of oxidase-positive organisms to the total count as a criterion for samples with "potentially" psychrophilic organisms should be used.

Since pseudomonads are supposedly killed by proper pasteurization, it is surprising that so many of the samples examined contained oxidase-positive organisms. This would tend to indicate post-pasteurization contamination. However, other data (unpublished) indicate the possibility that certain pseudomonads present in dairy products are able to withstand pasteurization. This phenomenon will be studied further in relation to flavor defects in milk. It has been reported that psychrophiles are present in unused paper cartons and thus the packages are a source of contamination of pasteurized milk *(8)*.

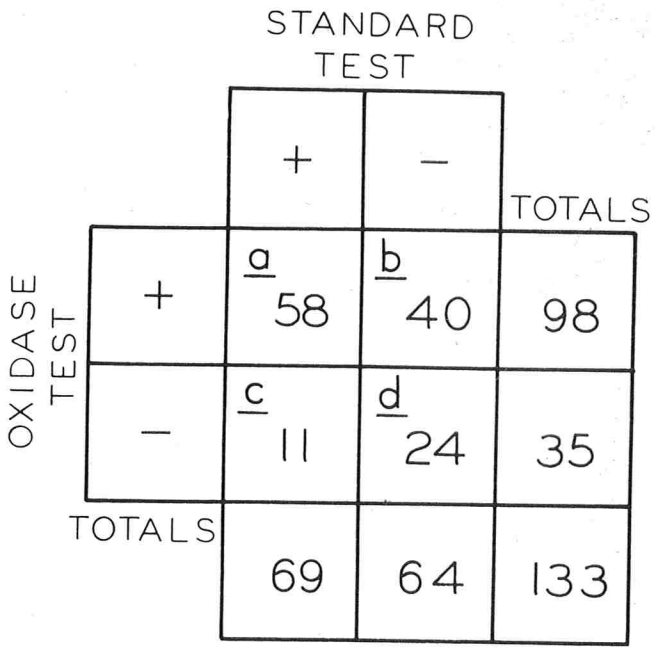

Figure 1. Chi-square analysis between standard test for psychrophiles and oxidase test. + indicates contains psychrophiles and — indicates does not contain psychrophiles. Test of (b — c), $x^2 = 15.37$, $p < 0.001$; Test of (a — d), $x^2 = 13.28$, $p < 0.001$; Test of (a + d) — (b — c), $x^2 = 5.742$, $p < 0.02$.

Examination by the oxidase test of the standard plate count plates prepared after incubating the samples for 6 days at refrigeration temperatures (standard psychrophile test) was made to see if those samples shown to be positive by the standard test actually contained large number of oxidase-positive organisms. Such data (Table 2) indicate that the largest proportion of samples, as detected by the 8-day test, actually contained considerable numbers of oxidase-positive organisms and thus presumably pseudomonads. Over 60% of the samples contained more than 50% of oxidase-positive organisms. Some samples with appreciable numbers of psychrophiles did not contain large numbers of oxidase-positive organisms. This was not unexpected since all psychrophilic problems are not pseudomonad in origin.

The plates prepared for the 8-day test from the 40 samples chosen as being "potentially" psychrophilic by the oxidase test but not by the standard test were examined for oxidase-positive organisms (Table 2). The oxidase test overestimated on about one-half of these samples which further points out that not all of the oxidase-positive organisms seen in pasteurized milk products are psychrophilic. However, if any food product which must be refrigerated contains oxidase-positive organisms, the presence of psychrophiles must be suspected.

TABLE 2. PERCENTAGE OF OXIDASE-POSITIVE ORGANISMS IN (A), 40 SAMPLES CHOSEN AS "POTENTIALLY" PSYCHROPHILIC BY THE OXIDASE TEST (POS. OX. TEST) BUT NOT BY THE STANDARD TEST (NEG. STD. TEST) AND IN (B), 69 SAMPLES FOUND TO CONTAIN PSYCHROPHILES BY THE STANDARD TEST (POS. STD. TEST).

	Number of Samples	
	(A)	(B)
% Oxidase Positive in SPC[a]	Pos. Ox. Test Neg. Std. Test	Pos. Std. Test
0-10	20	14
11-20	6	2
21-30	5	1
31-40	2	6
41-50	2	4
51-60	1	5
61-70	0	4
71-80	1	5
81-90	0	8
91-100	3	20
	40	69

[a]Standard Plate Count.

Eleven samples were picked by the standard test as containing psychrophiles but they were not detected by the oxidase test. Most of these samples contained oxidase-positive organisms when the standard 8-day plates were examined but had very low oxidase counts (close to the 2% minimum) when the test was made on the initial standard plates. A possible explanation is that the temperature of incubation (32 C) was slightly too high for the organisms in these samples and hence, if they did not grow at that temperature, could not be detected by the oxidase test. Five of the 11 samples chanced to occur on the same day. The 23 samples picked as not having psychrophiles by either test should not be overlooked since they are as relevant to the analysis of the data as the positive tests.

We cannot be sure from the initial oxidase count that any one sample will become bad since the number of pseudomonads needed for this phenomenon to occur varies with different species. A noticeable flavor defect usually requires large numbers of organisms, but even with fewer organisms subtle changes take place which are designated as "lack of freshness" or "stale quality". The reader is referred to the work of Elliker et al. *(6)*, Punch et al. *(9)* and Overcast *(8)* in relation to the increase of bacteria and flavor changes in milk samples stored at low temperatures.

The actual counts of oxidase-positive organisms in the pasteurized samples are shown in Table 3. Many samples have low oxidase-positive counts (i.e. under 200 per ml of sample). Some samples contained counts in excess of 500 per ml and in many instances this makes up the largest proportion of the total pasteurized population. Of the 98 samples detected by the oxidase test, those samples with over 500 per ml constitute over 15% of the total. Of the 69 detected by the standard test the ratio was over 10%. Note that 2 samples called positive by the oxidase test and containing over 18,000 oxidase-positive organisms per ml, were not classified as containing psychrophiles by the standard test. The standard test samples were kept under very good refrigeration. It must be considered what might have happened under only fair refrigeration temperatures such as in the home refrigerator. These points have to be evaluated in the interpretation of data obtained by the oxidase test, and it is for this reason that positive samples are not called psychrophilic but are called "potentially" psychrophilic.

The benefits which could accrue from the routine analysis of pasteurized bottled products by the oxidase test are many. Manufacturers, by the use of this test, could weed out those samples containing high oxidase counts and divert their use for short shelf-life purposes. The development of a "potential" psychrophilic problem is known within 2 days instead of the 7-8 days required for the standard test, and immediate remedial action may be taken if deemed advisable. A stricter quality control pro-

TABLE 3. DISTRIBUTION OF OXIDASE-POSITIVE ORGANISMS IN 98 MILK SAMPLES PICKED AS "POTENTIALLY" PSYCHROPHILIC BY THE OXIDASE TEST AND 69 SAMPLES PICKED AS PSYCHROPHILIC BY THE STANDARD TEST.

No. Oxidase positive organisms/ml sample	Samples picked[a] by oxidase test	Samples picked[a] by standard test
100	46	44
100-200	27	14
201-300	4	2
301-400	2	0
401-500	4	2
501-600	4	1
601-700	2	2
701-800	4	3
801-900	0	0
901-1000	2	0
XXXXXXXXXXX	XXXXX	XXXXX
1801-1900	1	1
XXXXXXXXXXX	XXXXX	XXXXX
18001-19000	1	0
XXXXXXXXXXX	XXXXX	XXXXX
29001-30000	1	0
Totals	98	69

[a]Oxidase test made on samples after 48 hr incubation at 32 C on Plate Count Agar.

gram is achieved without materially increasing the work load of the laboratory and additional data can be obtained from tests now routinely being made. The oxidase test affords a rapid index for the quantification of potential psychrophilic spoilage organisms.

Acknowledgments

I would like to thank the following who graciously supplied some of the test organisms used in this study: Dr. M. E. Morgan, University of Connecticut; Dr. W. E. Sandine, Oregon State University; Dr. T. Clayton, Kansas State University; Dr. J. Jezeski, University of Minnesota; Dr. H. C. Olson, Oklahoma State University; and Prof. S. Katz, Quinnipiac College. The help of Dr. C. I. Bliss with the statistical analysis and of Agnes Timpson with technical parts of the experiments is gratefully acknowledged.

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COMPOSTING ANIMAL WASTES

A government-assisted study will evaluate, among other disposal techniques improved sanitary methods of composting manure from cattle corrals and feedlots in Los Angeles and Orange Counties in southern California.

Richard D. Vaughn, Chief of the Solid Wastes Program of the Public Health Service's National Center for Urban and Industrial Health in Cincinnati, Ohio, announced that the program has awarded a $90,000 grant for the study.

The City of Cerritos, which received the award, will provide $45,000 as its share of the total project cost of $135,000. Charles L. Senn of the University of California at Los Angeles, will direct the one-year investigation. The County Health Department and members of the Dairymen's Fertilizer Cooperative will participate.

"The study," said Mr. Vaughn, "will aim at developing new techniques in converting animal wastes into soil conditioners or fertilizers. Currently, untreated manure is hauled from the corral sites and stockpiled near urban areas in the two California counties for as long as a year before it is composted. One stockpile in Cerritos, southeast of the City of Los Angeles, is 30 to 40 feet high and contains over ten million cubic feet of manure. Disease-carrying flies breed prolifically in these environments, seepage from such stockpiles can pollute ground and surface waters, and the air is filled with odor and dust.

"Under the study, the grantee proposes to devise a method that will permit prompt composting on the premises. With the manure piles removed, attendant vermin, odor, and dust would be eliminated as well."

Feedlots for beef cattle and corrals for dairy cattle are becoming common-place adjacent to major centers of population in this country, Mr. Vaughn said. Many of these centers are confronted with sanitation problems similar to those being experienced in Los Angeles and Orange Counties and the entire southern California dairy region.

"Improved composting of animal waste can also assist such large dairy states as Wisconsin, Minnesota, New York, Pennsylvania, Ohio and Michigan," Mr. Vaughan continued. "Other states — Iowa, Nebraska, Illinois, Texas, Calorado, Kansas — that raise beef cattle in concentrated feedlots will also have an interest."

SUPPLEMENT NO. 7 TO
SANITARY STANDARDS FOR FITTINGS USED ON MILK AND MILK PRODUCTS EQUIPMENT AND USED ON SANITARY LINES CONDUCTING MILK AND MILK PRODUCTS

Serial #0808

Formulated by
International Association of Milk, Food and Environmental Sanitarians
United States Public Health Service
The Dairy Industry Committee

This supplement incorporates the fitting (valve) described below into 3-A "Sanitary Standards for Fittings Used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Milk and Milk Products," dated March 29, 1950, Serial #0800, as amended.

DIAPHRAGM-TYPE VALVES (Reference: 3-A Drawing No. 3A-100-26)

(1) The valve assembly shall consist of a bonnet assembly [handwheel or power (air) operated] a diaphragm, and a suitable body. The bonnet shall be secured to the body with a minimum number of clamps or nuts. The diaphragm shall separate the product from the working assembly in the bonnet. The valve shall not have a stuffing box.

(2) All product contact surfaces and surfaces which may become product contact surfaces if the diaphragm fails in service shall be readily accessible for cleaning and inspection.

(3) The valve shall be of such design as to be self-draining in its installed position. Diaphragm valves shall be permanently marked with a descriptive stainless steel mounting instruction plate on the bonnet, to show and describe positively the self-draining angle, when the valve is placed in service. The mounting instruction plate shall also have a statement that only food grade lubricant shall be used on the valve stem.

(4) The chamber on the exterior side of the diaphragm (herein defined as the bonnet) shall have one or more 3/32 inch holes just above the bonnet flange in a suitable bossed area(s) located so that one hole will be at the lowest point in the installed position for the detection of leakage.

(5) All non-product contact surfaces shall be of corrosion-resistant material or material that is rendered corrosion-resistant. If painted the paint used shall adhere. All non-product contact surfaces shall be relatively non-absorbent, durable and cleanable. Parts removable for cleaning having both product contact and non-product contact surfaces shall not be painted. Non-product contact surfaces to be painted shall be effectively prepared for painting.

(6) The valve bonnet, if not made of corrosion-resistant material, shall have the interior surface (1) plated with a corrosion-resistant, non-toxic, non-absorbent metal or (2) covered with a plastic material conforming to (10) herein. The plastic coating shall be bonded in such a manner that the bond is continous and mechanically sound so that in normal service the plastic material does not separate from the base metal. The interior of the valve bonnet shall be at least as smooth as ACI Surface Indicator Scale SIS-3[1].

(7) Retaining grooves for the positioning of removable rubber or rubber-like parts and/or plastic parts shall be readily cleanable.

(8) All internal angles except those connecting removable parts shall have radii of not less than 1/16 of an inch.

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[1]Specifications for Visual Inspection of Cast Surface Finish as well as the Surface Indicator Scale may be obtained from the Alloy Casting Institute, 300 Madison Ave., New York, N. Y. 10017.

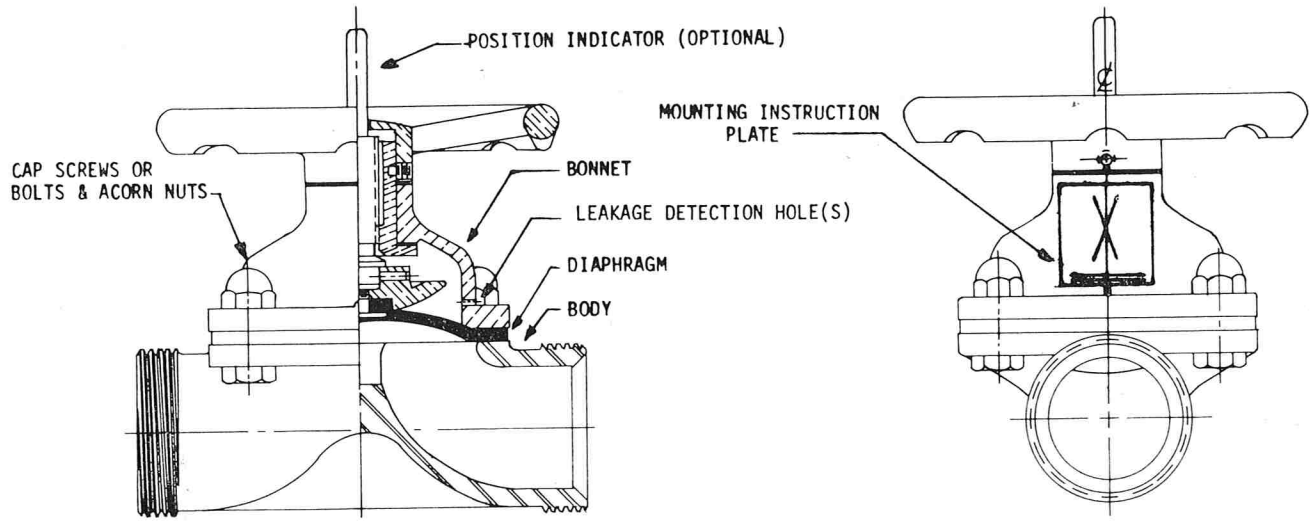
(9) Rubber and rubber-like materials may be used for diaphragms and sealing surfaces. These materials shall comply with the applicable provisions of the "3-A Sanitary Standards for Multiple-Use Rubber and Rubber-Like Materials Used as Product Contact Surfaces in Dairy Equipment, Serial #1800."

(10) Plastic materials used for diaphragms, valve bonnet coatings and sealing surfaces shall comply with the applicable provisions of the "3-A Sanitary Standards for Multiple-Use Materials Used as Product Contact Surfaces for Dairy Equipment, Serial #2000", as amended.

(11) The valve bonnet and all bonnet parts shall be readily demountable.

This supplement shall become effective September 14, 1968.

DIAPHRAGM-TYPE VALVE



The internal design shown is intended to demonstrate general principles only, and is not intended to limit individual ingenuity. The design used shall conform with the general sanitary requirements set forth in this 3-A Sanitary Standard and specific requirements for diaphragm-type valves.

The connections may be threaded or be of a design to be used with non-threaded sanitary connections (flanged, clamp type, etc.) or may have plain ends for use in welded sanitary pipelines. Valves made with threaded connections shall conform with the dimensions for (1) threads and (2) those at the connections (including the inside diameter) shown on the drawings for other fittings included in this 3-A Sanitary Standard. Center to face dimensions will vary with valves made by different manufacturers.

The mounting instruction plate is to (1) show and describe positively the self draining angle when the valve is put in service and (2) have a statement that only food grade lubricant shall be used on the valve stem.

3A STANDARD
SANITARY FITTINGS
3A-100-26

THE NATIONAL LABELING COMMITTEE'S NEW LABELING GUIDELINES[1]

JOHN F. SPEER

International Association of Ice Cream Manufacturers
Milk Industry Foundation
Washington, D. C.

The historians of tomorrow will look back at the '60's as one of the more exciting, constantly changing, dramatic, and potentially dangerous decades. While 3 yr. remain, it seems certain that many food processors will refer to it as the "Labeling Era." Never before has so much interest been accorded the labeling of food products. Hardly a day goes by but that either at the state or Federal level a proposed regulation or law is introduced which, if promulgated, would require relabeling of consumer packages. Keeping abreast of these laws and regulations requires considerable effort by food manufacturers to insure that their products are correctly labeled.

The dairy processor or manufacturer is faced with an additional burden in that not only must he comply with new labeling requirements but, in nearly all instances, he must carry multiple inventories of containers, thereby making his cost of compliance greater than that of his competitor in other food products who is able to carry fewer carton inventories.

Looking back several decades and analyzing the local characteristics of the dairy industry, we do not find that these multiple labeling requirements placed an undue burden upon the processor or manufacturer. His market was limited; his routes extended to the city limits and requirements in the next valley concerned him not. Today's situation is vastly different. It is commonplace to see plants shipping dairy products across state lines; possibly into 20, 30 or more different political subdivisions. The horse-drawn wagon of yesterday has been replaced by the modern over-the-road refrigerated diesel tractors operating around the clock, and truck-like containerized units which can be transported thousands of miles by rail.

The dairy industry, and reference is made primarily to fluid milk and fluid milk products, has kept pace with other segments of the food industry in efficient production methods, and the growth of specialized plants capable of high volume processing of a limited line of dairy products. However, the labeling regulations of the industry as a whole have been painfully different from those governing other foods. In fact, it is fair to say that if other foods which are distributed nationally were subjected to the labeling straightjacket in which the dairy industry finds itself, there is a question whether the multitude of products available on the retail shelf would ever have seen the light of day. In all probability many products would have been adandoned by the manufacturer and the consumer denied the opportunity to purchase those wholesome and economical foods.

FOUNDING OF NATIONAL LABELING COMMITTEE

The National Labeling Committee was founded in 1962 by a group of regulatory officials and industry representatives whose intent was the establishment of uniform labeling regulations to keep pace with industrial growth. The primary function of this Committee then, as it is now, was elimination of unnecessary and costly proliferation of labeling requirements which impeded efficient distribution of milk and its products and required maintenance of burdensome multiple inventories.

As originally conceived, the National Labeling Committee (NLC) was composed of 3 sections: a *Regulatory Section,* made up of state regulatory officials; an *Industry Section,* comprised of industry and trade association personnel, and an *Advisory Section* consisting of Federal representatives from the U. S. Public Health Service, Food and Drug Administration and the U. S. Department of Agriculture. Officers elected in 1962 and who still serve are Chairman, Jeff Jefferson, Virginia Department of Agriculture, and Vice Chairman, Shelby Johnson, Kentucky Department of Health who is also the official representative of IAMFES.

The first secretary of the NLC was Harold Barnum, who concurrently served as Secretary of the Dairy Products Improvement Institute. Later the secretaryship was transferred to this Association and "Red" Thomasson served as Executive Secretary when Mr. Barnum returned to his post as milk sanitarian in Denver. In the latter part of 1964, the Office of the Secretary was again moved—this time to the headquarters of the milk and ice cream associations. One of the primary reasons for moving the office to the product association headquarters was the decision

[1]Presented at the 54th Annual Meeting of the International Association of Milk, Food and Environmental Sanitarians, Inc., Miami Beach, Florida, August 14-17, 1967.

of the Executive Committee that all future drafts of NLC proposals should originate with the industry section.

It was evident from the outset that the attention and efforts of the NLC would primarily be directed to fresh milk products. This emphasis on the fluid milk industry was largely because the great bulk of other dairy products had been defined by FDA and standards of identity, including labeling provisions, were currently in existence.

Objectives of NLC

The major objective of the NLC has been the preparation of a uniform labeling regulation for fluid milk products—but before this is discussed the accomplishments of this voluntary organization will be reviewed. The first undertaking by the Committee was the preparation and distribution of a National Uniform Coding System, in which containers could be identified by the name and place of business of the company's main office and a code number either printed or embossed on the container at the time of filling. The use of this system allowed dairy operations with multiple plants to purchase cartons in larger quantities. Prior to the existence of this program, which has now been adopted in 34 states, it was necessary to purchase cartons separately for each individual plant in order that the name and address of the filling plant could appear on the package. This coding system has saved countless thousands of dollars and at the same time provided health officials necessary information as to the origin of the individual package.

A little known activity of the NLC was its successful effort in developing meaningful labeling requirements for lowfat milk, which in its infancy was sold under a multitude of names and varied widely in composition. The several states which embraced this product were surveyed in 1963 and, based on this information, the Committee recommended to USPHS the inclusion of low-fat milk in the 1965 Pasteurized Milk Ordinance.

A Model Labeling Regulation

The problem of drafting a model labeling regulation can be briefly stated. Basically the task consists of setting forth the information required by law to appear on the label of each product and the placement and conspicuousness of such information on the label.

The framework within which the Committee has operated in devising practical labeling requirements has 4 fairly rigid boundaries: *(1)* the Federal Food, Drug, and Cosmetic Act and its regulations; *(2)* the Fair Packaging and Labeling Act and its regulations; *(3)* the USPHS Grade A Pasteurized Milk Ordinance; and *(4)* the vast body of diversified state laws and regulations now on the books.

Dealing, as the Committee must, with a host of containers ranging from small tetra-paks to gallon jugs, and a diversity of items from plain milk to products which are fortified, recombined, cultured, flavored, sour, concentrated, homogenized, acidified, etc., it is apparent that the job is a mammoth undertaking.

Immediately preceeding this convention (54th annual IAMFES meeting), a meeting of the National Labeling Committee was held to review the sixth working draft of the model regulation. After 2 days of concentrated review and incorporation of certain amendments, agreement was reached on a final document. Two steps remain before the cooperative work of the NLC is made available to the states. The first is a review of the model regulation by the Federal Food and Drug Administration in order to make certain that the several requirements are in compliance with the Federal Act and its regulations. The second matter which is holding up publication is the Fair Packaging and Labeling Act (FPLA) of 1966 and the subsequent regulations published by FDA. As provided for in Section 5 of the Act, the Milk Industry Foundation (MIF) has requested an exemption from the dual declaration requirement; the limitation of terms for declaring the net contents (e.g. the terms "one half pint" and "half gallon" would be prohibited) and the location requirement for the quantity of contents statement on glass and plastic fluid milk containers. In the NLC document these 3 FPLA requirements are not included so it can be readily seen that until FDA has acted on the MIF exemption request, the model standard could not be forwarded to the states.

Until FDA has acted on the "dairy exemption petition," no company should change or alter its existing labels. Making container labeling changes prematurely based on anticipating what the final regulations will actually be, can be a costly gamble on the part of dairy companies.

Returning to the NLC document, some of the substantive provisions are briefly highlighted below. Divided into sections, the requirements are as follows:

Section 1: is devoted to defining such terms as "label," "package," "Principal Display Panel," "Product Identification Panel," "Required Information Panel," etc.

Section 2: defines in specific terms that part or those parts of the several styles of dairy containers which constitute the "Principal Display Panel" (PDP). On this panel the name of the product and the quantity of contents *must* appear. Several pages in this section are devoted to graphic mock-ups of cartons showing the location of the PDP.

Additionally, the portion(s) of the container which must contain *all* the mandatory information [Required Information Panel(s)] and those panels which must bear the name of the product [Production Information Panel(s)] are also illustrated.

Section 3: sets forth detailed requirements for the labeling of the quantity of contents statement. These provisions closely follow the proposed Fair Packaging and Labeling Regulations, except as previously discussed.

Section 4: deals with the labeling of added ingredients such as vitamins, minerals, nonfat dry milk, artificial sweeteners, stabilizers, distillates and other optional ingredients.

Section 5: defines what is prominent and conspicuous labeling for the mandatory information appearing on the "Required Information Panel." Minimum type sizes for declaring some of the information are required. Additionally, the order in which the information must appear is prescribed. For example, on a rectangular "gable top" fluid milk carton, the mandatory information must appear in the following sequence: Grade A Pasteurized (if applicable), Modifying Terms, Name of Product, Ingredient Statement (if applicable), Name and Place of Business, and Quantity of Contents.

Section 6: deals with a host of miscellaneous provisions such as the use of pictorials or other promotional communications which are not misleading; the placement of the NLC plant identification code number; the continued use of returnable glass and plastic milk containers and other labeling criteria.

Section 7: expresses the intent that when these regulations are adopted by a state, either by law or regulation, all local and municipal labeling requirements are superseded.

While it is impossible to completely and thoroughly review in detail the recommendations of the National Labeling Committee's Model Regulation, it is hoped that an idea of the tremendous effort which has gone into this undertaking has been conveyed by this discussion. Many persons have labored hard over a period of several years to make this document a reality. Existing state laws have been studied and reviewed. Regional conferences were held and the regulatory community and industry representatives have set forth what they believe are meaningful and basic labeling requirements for the fluid milk industry.

The beliefs of many who have worked diligently in this endeavor can best be summarized from a portion of the NLC forword:

"This document in its final form will represent the views and comments of both the dairy industry and the regulatory community. In addition to providing prominent and conspicuous labeling standards, one of the fundamental purposes of this model regulation is to eliminate the necessity of milk processors using several different cartons—all of which contain the identical product—but which are eventually distributed in several different political subdivisions, each with the authority to promulgate its own labeling regulation. This is a wasteful and costly luxury which industry cannot afford."

BOOTLEGGING OF RAW SHELLFISH PUBLIC HEALTH HAZARD

Public Health Service scientists of the National Center for Urban and Industrial Health have found oysters and hardshell clams contaminated with viruses in polluted waters in an east coast inlet where shellfiesh harvesting is prohibited.

"Thus," they say, "bootlegging of raw shellfiesh from prohibited areas is definitely a public health hazard and should be carefully controlled by the state authorities."

The findings were contained in a paper prepared for presentation at the annual meeting of the American Society for Microbiology in Detroit. The authors are Dr. O. C. Liu, Associate Director of the Northeast Marine Health Sciences Laboratory at Narragansett, Rhode Island, and four other members of the staff there. They are H. R. Seraichekas, D. A. Brashear, W. P. Hefferman, and V. J. Cabelli.

In the past 10 years, the paper says, there have been about 1,700 reported cases of infectious hepatitis, an inflammation of the liver, related to the consumption of raw shellfish. The two major outbreaks included one in Sweden, involving 629 cases, and the other in New Jersey, involving 49 cases.

Because of this problem, the authors conducted a study of hardshell clams and oysters taken during July through September, 1967, from an inlet in Rhode Island in places one to three miles from a sewage treatment plant. The water was heavily polluted with human waste, and shellfish harvesting is prohibited there by the state.

"Shellfish growing in polluted areas were found to contain human enteric (intestinal) viruses," the authors state. "Although no technique is available to detect the hepatitis virus, the detection of other enteric viruses in shellfish suggests that shellfish could be polluted with hepatitis virus, if the virus were present in the environment in sufficient quantity."

The authors also say that further studies should be made to determine the extent to which other diseases—including certain heart ailments—can be attributed to the consumption of shellfish aken from polluted waters. They listed such heart diseases as chronic endocraditis, valvulitis and idiopathic myocarditis.

INDIANA FARM METHODS COMMITTEE 1966-67 REPORT ON MASTITIS ELIMINATION

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The timing of this study seemed appropriate because of the increased attention devoted to abnormal milk in the U. S. Public Health Service Grade A Pasteurized Milk Ordinance, the requirements outlined in the Interstate Milk Shippers Agreement, and the procedures outlined by the U.S. Public Health Service for conducting milk sanitation surveys. The study was made by conducting dairy farm milking time inspections, to determine methods and procedures to employ in a voluntary educational program directed to help the farmer with a condition in his dairy herd called "mastitis." While almost all interested persons are in accord that this should be done through voluntary education, there are various ideas as to how this is best accomplished. Some of the questions which arise are:

1. What is the most effective way to do this? If meetings are conducted, the farmer that needs this information many times does not attend. Information on mastitis that is mailed to him may be misunderstood and either discarded or filed away to be forgotten.

2. Can we as sanitarians and fieldmen be of service and helpful to the farmer by conducting milking time inspections? Did the farmer want this visit or inspection conducted during the milking time operation?

3. What is the most effective and accepted manner of exchanging information and communicating with a farmer about improving and including better milking methods in his dairy farm management?

4. Should a milking time inspection form be devised for general use or is the present Grade A farm inspection form sufficient?

5. What equipment should a sanitarian and fieldman employ for a milking time inspection that is directed to pinpoint the causes of possible injury to the cow's udder?

6. Who should explain to the farmer the purpose of the study and solicit his complete, voluntary cooperation? How were the farms for this study to be selected? Who is the key person in lowering the leucocyte counts in milk on dairy farms?

This appeared to be an opportunity to determine if we as sanitarians and fieldmen could develop an educational plan that would have the support of everyone concerned and a method that would be practical and employed by a sanitarian or a fieldman to be of service in lowering the leucocyte counts in milk.

SETUP OF THE STUDY

The State of Indiana was divided into geographical areas. Indiana State Board of Health personnel in the respective areas worked jointly with dairy plant fieldmen and dairy sanitarians. John M. Schlegel, Indiana State Board of Health, and Kenneth Weinland, D.V.M., and Willard Dillon, Ph.D. of Purdue University served as information centers and worked with committee members on problem farms.

A milking time inspection form was revised by the committee. This detailed form was used for the collection of data to motivate more thorough inspections and to provide more uniform information. This report provided space to report milk tests of individual quarter samples from each cow. The California Milk Quality Test and the strip cup test were conducted on the foremilk from each individual quarter. This was done to show the practical application of the California Milk Quality Test as a rapid cow-side test and to compare the results with the strip cup test.

Originally two milking time demonstrations for committee participants, using the milking time inspection form and conducting the California Milk Quality Test and strip cup test, were conducted by Mr. Schlegel, and Drs. Dillon and Weinland. Following the completion of the demonstrations the participants discussed the items of the milking time inspection report form.

Each committee member selected at least one dairy farm under his jurisdiction for study that had repeated high leucocyte counts on his bulk tank milk. The dairy plant fieldman or sanitarian contacted the farmer. He explained to the farmer in detail the purpose of the study and solicited his voluntary and complete cooperation. It was stressed there would be no regulatory enforcement. The main purpose was to be of service to the dairy farmer in locating the possible causes of mastitis, to encourage him to eliminate poor practices, and to include accepted methods in his milking management.

A prearranged date for the milking inspection was established that was satisfactory to the farmer. The inspection form was filled-in at the farm. The results

of the cow-side tests on the milk for the California Milk Quality Test and the strip cup test were reported and the respective cow identified and recorded. Recommendations were written on this form listed in accordance with their individual importance.

A convenient arrangement was made with the farmer to provide a copy of the report and to personally discuss the findings and recommendations. This was to be done in such a manner that it would encourage and motivate the farmer's desire to include the recommendations in his daily milking management system. On several farms the farmer's wife was in on the discussions. We informed the farmer that within two or three weeks the sanitarian and/or fieldman would call on him for a short visit and inquire if there were any questions. He was further advised that within a reasonable length of time (four or five months) a follow-up milking time inspection and cow-side milk tests would be conducted and the findings and test results recorded.

A copy of the follow-up inspection was provided and on each occasion the inspection report was discussed personally with the farmer at a time that was convenient to him. This was done to promote a better understanding, communication, a better exchange of information and actually a better compliance and working relationship with that farmer. On each report our appreciation was expressed for his courtesy extended during the farm inspection.

Equipment used on the inspection included the California Milk Quality tester, a strip cup tester, vacuum gauge with small hose attachment, milking time inspection form that was devised by the committee and a stop watch.

Arrival at the dairy farm was one-half hour prior to milking time. At this time through observation and discussion the detailed inspection form was completed as much as possible. During the milking operation the methods employed were noted and recorded. The vacuum reserve, vacuum fluctuation at teat end, and pulsation ratio and milking time per cow were recorded. The appearance of any "flaking" on the strip cup test on the milk from each quarter was recorded. The milk from each cow's quarter was tested by the use of the California Milk Quality tester and reported for each identified cow.

SUMMARY

Data collected on the dairy farms was tabulated and analyzed by the members of the committee at Purdue University on September 7 and 8, 1967. A total of 17 farms were completed on this study that included a total of 965 cows. There were 3,837 quarter milk samples tested by the California Milk Quality Test and 3,837 quarter samples of milk were examined by the strip cup test. There were 35 milking time inspections.

Following is a comparison of the final inspection with the initial inspection on the total farms:

	Initial Inspection	Follow-up Inspection
Overmilking	41%	23%
Abnormal Milk, C.M.T.	21.3%	16.7%
Abnormal Milk, Strip Cup	2.8%	1.4%
Leucocyte Count	1,155,300	760,900
Milk Production Per Cow	34.8 lbs/day	36.6 lbs/day

On farms not cooperating with recommendations the following data was collected:

	Initial Inspection	Follow-up Inspection
Leucocyte Count	1,600,000	1,340,000
Milk Production Per Cow	33.1 lbs/day	33.3 lbs/day

Altogether 12% of farms changed from large bore to small bore inflations, 18% of farms changed cow housing and environment, and 29% of farms changed their milking methods. There were 53% of the milking machines not designed to provide visibility for the milker to determine when milk flow stops from each quarter of the cow's udder.

RECOMMENDATIONS

The recommendations, comments and considerations of the committee participants and farmers can be summarized as follows:

1. The present milking time inspection form should be revised and simplified.
2. The milking time inspection form should be used by the sanitarian or fieldman on a regular basis inspecting the dairy farms that are experiencing high leucocyte counts.
3. We in our milk programs devote a lot of attention to the items of sanitation that are stressed by the State Milk Sanitation Rating Officer. We believe that it will motivate milking time inspections if the Rating Officer during the periodic survey will inspect the sanitarian's and/or fieldman's records for milking time inspection reports.
4. The lowering of leucocyte counts is possible through milking time inspection reports if the complete cooperation of the farmer is obtained. The farmer should be given assistance with this problem. The recommendations should be personally discussed with the farmer to promote a better understanding and generally results in better compliance. There are various ways to submit recommendations to a

farmer but there is no substitute for person-to-person communication.

5. Education on milking methods should coincide with regulatory requirements on mastitis.

6. The milking time inspections should be prearranged including the person-to-person discussions relating to recommendations. "After a farmer has worked in the field all day, conducted his milking chores, and has not eaten his evening meal, it is not the time to discuss recommendations."

7. One accurate uniform mastitis screening test should be considered.

8. We in milk sanitation work should be familiar with the methods of conducting milking time inspections. We should be able to train and/or work jointly with the dairy plant fieldman on the mastitis problem. We should be able to "pinpoint" the causes for high leucocyte counts on a dairy farm and to personally discuss with a farmer in such a manner that it will motivate him to include the changes in his milking management system. We should have the necessary equipment available for this work.

9. For many years the strip cup has been widely recommended as an essential test for the detection of mastitis in the milk from each cow's quarter at milking time. On the contrary, this study revealed the test to be unreliable for the detection of mastitis. This study included the strip cup test on the milk from 3,837 quarters. On the initial inspection of these farms 21.3% of the cow's quarters showed abnormal milk by the California Milk Quality Test and only 2.8% of the milk from the same quarters showed abnormal milk by the strip cup test on the same quarter milk. It is a means of detecting visibly abnormal milk, however, and should be used for this purpose until a better test is developed.

General Comments

A high percentage of the farmers on this study appreciated this service. In one area the farmer would tell another dairy farmer of the service that was provided through milking time inspections and there were requests submitted for this assistance. Even the farmers that made no changes in their equipment or methods from the initial to the final inspection were courteous. Several of the farmers on this study have requested a return milking time inspection during the coming winter season to show improvements in cow housing. The work on this study provided a lot of personal satisfaction when a high percentage of the farmers expressed their appreciation for this service.

Many farmers were employing poor milking methods. This committee worked with Purdue University and the Indiana Mastitis Council in the preparation of a minimum list of "Rules for Managed Milking." This information will be on an 8" x 10" placard. Consideration should be given to the distribution of this form during the sanitarian's regular farm inspection and to discussing it with the farmer. It will be of little value if it is mailed to the farmer and not discussed with him.

This study revealed the importance of people trained in various professions working together, having an information center, and well trained fieldmen and sanitarians, veterinarians, equipment servicemen, and above all the willingness of the farmer to cooperate. If complete coperation of the farmer is not obtained, there is very little accomplished. The person to obtain this cooperation is the fieldman and local sanitarian.

Because the number of dairy farms in Indiana is less than a year ago, too many people think that dairy farming is a declining industry. Instead, the type of dairy farmers remaining in this work are strengthening the bright, constructive future of this industry.

RULES FOR MANAGED MILKING

1. Know your cows and give each individual attention.
2. Use no more than 2 units per man when carrying the milk or 3 units with a pipeline.
3. Wash and massage the udder with a warm solution until it is clean and milk let-down occurs.
4. Dry the udder.
5. Check the first squirt of milk from each quarter and keep visibly abnormal milk out of the bulk tank.
6. Apply milking machine within 1 minute after washing.
7. Remove the milking machine inflation from *each quarter* as soon as milk flow stops.
8. Rinse teat cups in sanitizing solution between cows.
9. Subject teat ends to a sanitizing solution after milking.
10. Use properly maintained milking equipment and keep cows clean by providing clean housing and a paved cow yard.

Plant fieldmen, health sanitarians, veterinarians, equipment servicemen, and extension personnel are available to work with you.

Farm Methods Committee
Indiana Association of Sanitarians, Inc.
Indiana Mastitis Committee
Cooperative Extension Service

INCUBATION TEMPERATURES AND RAW MILK BACTERIAL COUNTS

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Abstract

Raw milk samples were plated and incubated at seven temperatures (2, 10, 20, 27, 30, 33 and 37 C). Lowest counts were obtained at 37 C. Plates at 33 C showed higher counts than at 37 C but were significantly lower than the other temperatures. The 10, 20, 27 and 30 C incubated plates were not statistically different in counts. When 32 C was substituted for 33 C, there was a significant difference (lower) only in the 37 C count. Colonies from 2, 20, 27, 33 and 37 C plates were picked and stabbed into plates subsequently incubated at these temperatures. All colonies developed best at 20 and 27 C. Evidence is presented that psychrophilic counts can be indirectly determined as the difference between counts at 27 C and 37 C.

The effect of plating temperatures on recoverable numbers of raw milk bacteria has been studied by a number of workers *(1, 3, 5, 6, 7)*. Nelson and Baker *(5)* studied the effect of incubation time and temperature on market milk. The ideal temperature should be one that permits the growth of the largest numbers of bacteria in a reasonable amount of time. Temperatures of 32 and 35 C were approved by the 11th Edition of *Standard Methods for the Examination of Dairy Products* (American Public Health Association, Inc., New York, N. Y. 10019, 1960).

Materials and Methods

Procedures outlined in the 11th Edition of *Standard Methods for the Examination of Dairy Products* were followed except for the variable of incubation temperature. Incubation times were 10 days at 2 C, 7 days at 10 C, 5 days at 20 C and 2 days at 27, 30, 32, 33 and 37 C. Colonies picked for determination of temperature requirements for growth were representative of the types observed (pin point, surface, pigmented, lenticular, etc.). Each colony was picked once with a needle and stabbed into cooled petri dishes containing 25-30 ml Plate Count agar. Sixteen such stabs representing 16 different colonies were made per plate, the same needle being used to serially stab a total of 5 plates. Preliminary results indicated that up to 100 stabs could be made serially with the bacteria picked up on 1 needle. The stabs showing any signs of growth, as observed in a Quebec colony counter, were considered positive. Raw milk samples were obtained from the bulk tank delivered milk of a local dairy. Regular bacterial counts of colonies were made, also with a Quebec colony counter.

The incubators used were BOD types for 2 C and 27 C.

[1]Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

Table 1. Effect of Temperature on Recoveries of Raw Milk Bacteria[1]

Sample Date	Incubation Temperature (C) 2	10	20	27	30	33	37
5/5/66	11.5	7.1	14.0	18.7	16.0	19.2	
	8.0	5.0	13.2	19.5	18.2	16.1	
5/9	60	130	250	210	210	190	
	120	150	260	280	200	150	
5/11	1880	2150	1500	1600	1370	1050	
	2000	2250	2000	2400	1650	1100	
5/17	115	135	155	135	140	25	
	122	117	143	175	145	32	
5/19	182	182	165	140	101	100	
	135	171	155	182	66	98	
5/25	31	23	27	68	32	26	10
	25	33	47	51	48	24	12
5/27	205	196	210	197	160	68	33
	195	220	260	220	189	69	33
6/10	440	330	460	480	290	340	100
	450	380	470	460	370	310	95
6/14	67	75	86	55	45	37	7
	70	63	80	66	43	40	15
6/20	39	87	360	320	330	300	320
	34	94	300	350	380	340	270
6/27	29	35	60	67	87	57	16
	33	32	70	70	77	75	16
7/6	1350	2240	2800	3000	2900	1800	210
	1150	2640	2600	3100	3000	1750	150
7/12	850	850	770	1030	830	750	33
	800	1050	900	870	900	830	55
7/18	2250	1750	1900	2050	2100	2000	95
		1820	1800	2060	2400	2100	125
8/15	59	77	101	90	106	60	13
	52	90	95	95	110	75	19
8/22	950	1500	1320	1500	1360	920	65
	1100	1400	1460	1500	1180	1100	90
8/29	324	310	290	310	330	150	35
	360	280	320	350	260	210	36
9/12	36	60	105	92	85	70	16
	33	75	86	95	70	75	29
9/19	60	130	130	180	178	130	53
	57	135	145	180	150	125	51
9/26	50	58	102	71	85	85	21
	60	76	85	90	100	80	27
10/11	72	140	135	152	115	105	65
	100	137	170	175	105	90	30
10/18	150	205	195	169	143	170	16
	240	210	205	197	170	140	14
11/2	2500	2900	2300	2500	2400	2450	58
	2100	2200	2500	2550	2200	2700	57
11/14	85	102	118	100	110	115	34
	88	96	110	115	105	100	23
12/1	1450	1150	1150	1200	1400	1250	26
	1250	1350	1750	1450	1300	1400	37
12/12 A	18	36	50	62	91	87	38
	17	28	52	82	79	97	45
B	15	72	78	65	90	70	12
	18	66	81	85	120	51	9
1/13/67	310	390	370	530	410	360	36
	300	260	480	360	320	380	40
1/17 A	2600	2500	2800	2850	2800	280	12
	2700	2700	2300	2900	3000	260	11
B	210	200	200	235	215	120	16.5
	175	200	245	260	225	120	17
1/30	104	81	125	140	137	90	18
	125	115	135	150	140	105	24
2/13	25	45	55	58	85	53	10
	40	54	70	72	66	61	10.5
2/28	2650	2400	2400	2140	2400	2720	9.2
	2400	2200	2500	2500	2300	2250	5.7
Averages[2]	556[a]	608[ab]	629[b]	668[b]	629[b]	479[c]	46[3]

[1]Total counts—Multiply above by 1000.

[2]Figures superscripted with like letters are not significantly different.

[3]The 37 C results were intuitively significantly lower and were not analyzed statistically.

TABLE 2. ANALYSIS OF VARIANCE OF DATA IN TABLE 1

	Sum of Squares (ss)	Degrees of Freedom (df)	Mean Square (ms)	F	Significant Differences
Sample date (Rows)	272,440,474.25	33	8,254,559.82	161.28	+
Incubation temperature (Columns)	1,552,250.37	5	310,450.07	6.06	+
Error	18,936,523.96	370	51,179.79		
Total	292,889,248.58	408			

TABLE 4. ANALYSIS OF VARIANCE OF DATA IN TABLE 3

	ss	df	ms	F	Significant Differences
Sample date (Rows)	77,805,717.91	6	12,967,619.25	51.32	+
Incubation temperature (Columns)	6,445,126.89	6	1,074,187.81	4.25	+
Error	19,474,640.25	85	252,642.82		
Total	105,725,485.04	97			

These had temperature variations of ± 0.5 degree. The 30, 32 and 33 C incubators were forced draft type modified by adding a copper coil in front of the air stream. Cold tap water (10 C) circulated through these coils at 12 psi. No noticeable temperature variation occurred except under rare conditions when the ambient temperature fluctuated greatly. Under these conditions, a maximum variation of ± 0.5 degree was observed. The 10 and 20 C incubators were controlled temperature storage rooms with variations less than ± 0.5 degree. The 37 C incubator was a forced draft type with ± 0.5 degree variation. Temperatures were recorded using a thermometer immersed in a sealed flask of water.

TABLE 3. EFFECT OF TEMPERATURE ON RECOVERIES OF RAW MILK BACTERIA[1]

Sample Date	Incubation Temperature (C) 2	10	20	27	30	32	37
3/13/67	46	60	77	84	78	75	20
	38	66	84	74	88	80	20
3/21	2700	3800	4000	3300	2000	1950	16
	3500	4000	3600	3500	1900	1750	14
4/11	46	76	40	61	39	18	12.5
	64	71	32	52	39	25	12
4/28	11	16	49	37	31	37	2.2
	7	10	32	24	32	27	2.6
5/10	35	39	54	38	32	25	8.5
	24	39	64	25	28	32	10.1
5/17	68	69	92	62	65	43	6.5
	71	78	90	54	35	59	8.3
6/28	1200	1450	760	1520	810	740	26
	2150	1150	1100	1200	800	700	37
Averages[2]	711[a]	780[a]	720[a]	716[a]	427[a]	397[a]	14[b]

[1]Total counts—Multiply above by 1000.
[2]Figures superscripted with like letters are not significantly different.

RESULTS

Table 1 shows the numbers of bacteria recovered from incubation at 2, 10, 20, 27, 30, 33 and 37 C. Analysis of variance showed very significant F values (for this analysis the data obtained at 37 C were not included since these were obviously lower than all the rest). The Duncan Multiple Range Test *(2)* showed the plates incubated at 33 C to have significantly fewer colonies than the others analyzed. Plates incubated at 2 and 10 C were not significantly different from each other; but those held at 2 C were significantly lower in numbers than plates at 10, 20, 27 or 30 C. Hence, plates held at 10, 20, 27 and 30 C could be considered to have the same numbers of colonies while the results from incubation at 2 and 33 C might be different.

Table 3 was compiled from data using 32 C instead of 33 C. In this instance only the plates at 37 C had fewer bacterial colonies while those at 2, 10, 20, 27, 30 and 32 C were similar. The counts at 30 and 32 were lower, but analysis by the Duncan

TABLE 5. RELATIONSHIP OF COUNTS OBTAINED AT 27 C INCUBATION TO THOSE FROM 2 AND 37 C COMBINED[1] [2]

Date	Average Counts From 2 C	37 C	27 C	2 + 37-27 = d
5/25/66	28	11	59.5	−20.5
5/27	200	33	208.5	24.5
6/10	445	97.5	470	72.5
6/14	68.5	11	60.5	19.0
6/20	36.5	295	335	−3.5
6/27	31.0	16	68.5	−21.5
7/6	1250	180	3050	−1620.0
7/12	825	29	950	−96.0
8/15	55.5	16	92.5	−21.0
8/22	1025	77.5	1500	−397.5
8/29	342	35.5	330	47.5
9/12	34.5	22.5	93.5	−36.5
9/12 repeat	27.5	29.0	70.0	−13.5
9/19	58.5	52.0	180	−69.5
9/26	57.5	24.0	80.5	+1.0
10/11	86.0	47.5	163.5	−30.0
10/18	195	15.0	183	27.0
11/2	2300	57.5	2525	−167.5
11/14	86.5	28.5	107.5	7.5
12/1	1350	31.5	1325	56.5
12/2 A	17.5	41.5	72	−13.0
B	16.5	10.5	75	−48.0
1/13/67	305	38.0	445	−102.0
1/17 A	2650	11.5	2875	−213.5
B	192.5	16.75	247.5	−38.25
1/30	114.5	21.0	145	−9.5
2/13	32.5	10.25	65	−22.25
2/28	2525	7.45	2320	+212.45
	14,355.5	1265.95	18,097.00	−2475.55
	15,622			

[1]Student's t = 1.47 • critical t value = 1.703 at .10 level of significance.

[2]d^2 = 2,946,353.8775.

Multiple Range Test did not permit assigning significance to these differences. It is interesting to note in these 2 tables that the sum of the total counts of the plates grown at 2 and 37 C nearly equaled the count at 27 C. This suggests that the 27 C counts were composed of the high and low temperature bacteria. This sum also approached the counts at 10, 20 and 30 C.

These counts were analyzed more carefully with the results shown in Table 5. The hypothesis that the counts at 27 C were composed of the 37 C and 2 C counts was set up with the difference (d) being the sum of the 2 and 37 C counts minus the 27 C count. The true difference (μ_d) should be 0. The mean of the differences ($\bar{d}$) was calculated from the relationship $\bar{d} = \frac{\Sigma d}{n} = \frac{-2475.55}{28}$ $= -88.41250$. The $Sd^2 = \frac{n\Sigma d^2 - (\Sigma d)^2}{n(n-1)} = \frac{28(2{,}946{,}353{,}8775) - (2475.55)^2}{28.27} =$ 101,793.753 and $S\bar{d} = \sqrt{\frac{Sd^2}{n}} = 60.29$. The Student t value, $t = \frac{\bar{d} - u}{S\bar{d}} = \frac{-88.4125 - 0}{60.29} = -1.47$ while the critical t value for 27 degrees of freedom at a 0.10 level of significance was ± 1.703. In other words, the difference observed here was not great enough to negate the hypothesis that the counts at 27 C were composed of the combined counts at 2 and 37 C.

Bacteria usually grow over wide ranges of temperature with much overlapping. This is shown in Tables 6 and 8. When colonies picked from plates incubated at 2 C were stabbed into petri dishes, a few stabs developed at 37 C; a greater number at 33 C; but most at 2, 20 and 27 C (Table 6).

Significantly fewer colonies developed at 33 and 37 C. Colonies from plates held at 20 C showed equal numbers at 20 and 27 C which were statistically higher than those at the other temperatures. The stabbed plates incubated at 37 C showed lowest numbers of colonies developing. Statistically there were no differences between the numbers developing at 2 and 33 C. The results of the stabbed plates, inoculated with colonies from the plates incubated at 27 or 20 C were similar.

The stabbed colonies from the plates held at 33 C grew best at 20, 27 and 33 C with no significant differences. Fewer colonies developed at 2 and 37 C. Colonies picked from the plates held at 37 C showed equally good growth at 20, 27, 33 and 37 C but fewer colonies developed at 2 C.

Table 8 shows similar results when 32 C was substituted for 33 C. In all instances the 20 and 27 C incubation temperatures showed equal numbers of developing colonies and were as high as the plate from which the picked colony had been incubated. Tables 6 and 8 also show that the intermediate temperature counts (20, 27, 32 and 33 C) can be approximated by combining the counts of colonies developing at 2 and 37 C. The results obtained when colonies were picked from plates incubated at 27 C were also subjected to statistical evaluation by setting up the same hypothesis as previously discussed. These results are shown in Table 10. An extremely low Student's t value of 0.06 was obtained indicating that the combined colonies developing at 2 and 37 C were indeed very nearly equal to those developing at 27 C.

TABLE 6. GROWTH OF PICKED COLONIES[1]

Sample Date	2	20	27	33	37	2	20	27	33	37	2	20	27	33	37	2	20	27	33	37	2	20	27	33	37
	Incubation Temperature of plate from which colony was picked (C)																								
	2					20					27					33					37				
	Incubation temperature of picked colony (C)																								
5/10/66	16	16	16	14	2	16	16	16	14	4	16	16	16	12	7	13	14	14	15	6	11	16	16	16	16
6/20	14	16	16	5	2	2	16	15	13	12	0	15	15	15	13	6	15	15	15	15	5	16	16	16	16
7/6	16	15	15	8	8	16	16	16	13	0	10	16	16	15	4	6	16	15	15	7	6	16	16	16	16
8/15	16	16	16	7	0	10	16	15	13	6	7	16	16	16	9	9	16	16	16	10	1	16	16	16	16
8/22	16	16	16	12	0	16	16	16	10	1	15	16	16	12	5	14	16	16	16	3	4	16	16	16	16
8/29	16	16	16	14	0	15	16	16	12	1	12	16	16	9	2	14	16	16	16	4	0	16	16	16	16
9/12	9	16	15	11	0	5	16	16	14	7	7	16	16	14	4	6	16	16	16	10	2	16	16	16	16
9/19	16	16	16	14	1	13	16	16	13	6	3	16	16	13	11	8	16	16	16	11	3	16	16	16	16
9/26	14	15	16	14	1	6	15	15	15	10	12	16	16	14	4	5	14	14	14	8	2	16	16	16	16
10/11	16	16	16	7	2	7	16	16	10	5	2	16	16	10	6	7	16	16	16	6	2	16	16	16	16
10/18	16	16	15	14	0	11	16	16	12	1	14	16	16	16	1	13	16	16	16	3	1	16	16	16	16
11/2	16	16	16	14	2	15	16	16	14	2	16	16	16	16	1	15	16	16	16	0	1	16	16	16	16
11/14	16	16	16	16	0	12	16	16	16	4	15	16	16	14	4	8	16	16	16	9	6	16	16	16	16
12/1	16	16	16	14	0	16	16	16	16	0	16	16	16	15	0	16	16	16	16	0	4	13	14	15	16
12/12 A	16	16	16	16	0	11	16	16	15	4	9	13	15	14	6	9	14	16	16	8	1	16	16	16	15
12/12 B	16	16	16	14	2	15	16	16	12	2	13	16	16	13	3	12	16	16	16	7	6	16	16	16	14
1/13/67	16	16	16	16	0	13	16	16	15	4	16	16	16	16	0	13	16	16	16	3	1	16	16	16	16
1/17 A	16	16	16	3	0	16	16	16	3	2	16	16	16	0	0	16	16	16	16	0	2	15	16	16	16
1/17 B	16	16	16	7	2	14	16	16	8	3	14	16	16	10	2	15	16	16	16	1	2	12	14	16	16
1/30	16	16	16	12	0	14	16	16	16	4	14	16	16	14	4	11	16	16	16	5	1	14	15	15	15
2/13	16	14	15	14	0	13	16	16	13	3	11	16	16	15	4	12	16	16	16	4	0	15	16	16	13
Ave.[2]	15.5	15.8	15.8	11.7	1.05	12.2	16.0	15.9	12.7	3.9	11.3	15.8	15.9	13.0	4.3	10.9	15.7	15.7	15.8	5.7	2.9	15.5	15.6	15.8	15.9

[1]Figures in table represent numbers of colonies developing out of a possible maximum of sixteen.
[2]Figures connected by lines were not significantly different at .05 level of significance.

TABLE 7. ANALYSES OF VARIANCES OF DATA IN TABLE 6

Temperature of Picked Colonies		ss	df	ms	F	Significant Differences
2 C	Sample date (Rows)	74.51	20	3.72	0.18	−
	Inc. temp. (Columns)	3383.96	4	845.99	40.88	+
	Error	1655.44	80	20.69		
	Total	5113.91	104			
20 C	Rows	53.03	20	2.65	0.43	−
	Columns	2043.01	4	510.75	83.12	+
	Error	491.59	80	6.14		
	Total	2587.63	104			
27 C	Rows	96.93	20	4.85	0.42	−
	Columns	1904.53	4	476.13	41.72	+
	Error	913.07	80	11.41		
	Total	2914.53	104			
33 C	Rows	31.26	20	1.56	0.21	−
	Columns	1688.44	4	417.11	57.10	+
	Error	584.36	80	7.30		
	Total	2284.06	104			
37 C	Rows	53.26	20	2.66	1.54	−
	Columns	2753.52	4	688.38	398.80	+
	Error	138.08	80	1.72		
	Total	2944.86	104			

TABLE 8. GROWTH OF PICKED COLONIES[1]

Sample Date	2	20	27	32	37	2	20	27	32	37	2	20	27	32	37	2	20	27	32	37	2	20	27	32	37
	Incubation Temperature of plate from which colony was picked (C)																								
	2					20					27					32					37				
	Incubation temperature of picked colony (C)																								
3/13/67	16	16	16	15	0	11	16	16	16	5	11	16	16	16	5	8	15	16	16	8	0	11	16	16	16
3/21	16	16	16	10	1	16	16	16	10	0	16	16	16	9	0	16	16	16	16	0	3	16	16	15	15
4/11	16	16	16	8	0	9	16	16	11	7	14	16	16	7	1	4	16	15	16	8	0	16	16	14	16
4/28	16	16	16	5	1	8	16	16	11	3	4	15	9	7	12	4	16	10	16	12	0	16	9	15	14
5/10	16	16	13	5	0	14	16	16	9	4	9	16	16	13	6	10	16	16	16	5	0	16	16	16	16
5/17	16	16	14	11	0	16	16	16	5	0	16	16	16	13	1	15	16	16	16	1	0	16	16	16	16
6/28	16	16	16	8	0	16	16	16	13	1	15	16	16	15	0	13	16	16	16	2	0	16	16	16	16
Ave.[2]	16.0	16.0	15.3	8.8	0.3	12.8	16.0	16.0	10.7	2.9	12.2	15.8	15.0	11.4	3.6	10.0	15.9	15.0	16.0	5.2	0.4	15.3	15.0	15.4	15.6

[1]Figures in table represent numbers of colonies developing out of a possible maximum of sixteen.
[2]Figures connected by lines were not significantly different at .05 level of significance.

TABLE 9. ANALYSES OF VARIANCES OF DATA IN TABLE 8

Temperature of Picked Colonies			ss	df	ms	F	Significant Differences
2 C	Rows		19.54	6	3.26	1.18	–
	Columns		1311.43	4	327.86	118.90	+
		Error	66.17	24	2.76		
		Total	1397.14	34			
20 C	Rows		18.74	6	3.12	1.13	–
	Columns		822.40	4	205.60	74.31	+
		Error	66.40	24	2.77		
		Total	907.54	34			
27 C	Rows		42.00	6	7.00	1.75	–
	Columns		661.26	4	165.31	41.27	+
		Error	96.14	24	4.01		
		Total	799.40	34			
32 C	Rows		7.20	6	1.20	0.20	–
	Columns		630.68	4	157.67	26.55	+
		Error	142.51	24	5.94		
		Total	780.40	34			
37 C	Rows		18.68	6	3.11	1.47	–
	Columns		1243.31	4	310.83	146.60	+
		Error	50.88	24	2.12		
		Total	1312.88	34			

TABLE 10. RELATIONSHIP OF COLONIES DEVELOPING AT 27 C TO THOSE AT 2 AND 37 C COMBINED[1] [2]

Date	No. of colonies developing at 2 C	37 C	27 C	2 + 37 − 27 = d
6/10/66	16	7	16	7
6/20	0	13	15	−2
7/6	10	4	16	−2
8/15	7	9	16	0
8/22	15	5	16	4
8/29	12	2	16	−2
9/12	7	4	16	−5
9/19	3	11	16	−2
9/26	12	4	16	0
10/11	2	6	16	−8
10/18	14	1	16	−1
11/2	16	1	16	1
11/14	15	4	16	3
12/1	16	0	16	0
12/12 A	9	6	15	0
12/12 B	13	3	16	0
1/13/67	16	0	16	0
1/17 A	16	0	16	0
1/17 B	14	2	16	0
1/30	14	4	16	2
2/13	11	4	16	−1
3/13	11	5	16	0
3/21	16	0	16	0
4/11	14	1	16	−1
4/28	4	12	9	7
5/10	9	6	16	−1
5/17	16	1	16	1
6/28	15	0	16	−1
	323	115	439	−1
	438			

[1]Student's t = 0.06 critical t 1.703 at .10 level of significance
[2]d^2 = 239

DISCUSSION

The assessment of the bacteriological quality of raw milk destined to be pasteurized presents several difficulties. A milk high in bacterial count may be more desirable than one with relatively fewer numbers of organisms if the thermal resistance of the two be considered. Psychrophiles are unusually sensitive to pasteurization temperatures while the mesophiles and thermophiles often survive these conditions. The temperature of incubation of the plates will therefore determine to a very large extent the efficiency of pasteurization which in turn determines the safety of milk for public consumption. If one incubated the plates at 2 C, the pasteurization efficiency would, on the surface, be very commendable since these organisms have a low heat tolerance. However, the data reported here indicated that very few of the mesophilic organisms, originally isolated from the higher temperature 37 C, could grow at 2 C; therefore the pasteurization efficiency would be misleading as far as the mesophilic organisms were concerned. Most of the organisms of public health significance grow well at 37 C (enterococci, *Enterobacteriaceae*, staphylococci, etc.).

The experiments reported here indicate that both the psychrophiles and mesophiles grow with ease in the temperature range 20-27 C. The 20 C temperature of incubation requires more time for colonial development than 27 C, and there were no significant differences in counts. The counts obtained at 33 C were lower than those noted at the lower temperatures while the counts at 32 C also may have been lower. It appears that these temperatures are perilously close to the maximum for the psychrophilic group. An incubation temperature of 27 C for 2 days should be in a safe range to permit all types to flourish. The results reported here show that this temperature permits growth of bacteria isolated from plates incubated at 2 and 37 C. Ingraham *(4)* showed that a mesophile (*Escherichia coli*) and a psychrophilic pseudomonad had nearly identical generation times at a temperature around 28 C, again indicative of the suitability of this temperature range for bacterial multiplication.

These results point up the possibility of determining numbers of psychrophilic bacteria in raw milk as the difference obtained from plates incubated at a moderate temperature (such as 27 C) from those at a more elevated temperature (37 C). These analyses could be made in 2 days instead of the present 7-10 days required for the standard psychrophilic counts. In addition the counts at 37 C would be of importance since this is a favorable temperature for human pathogens.

ACKNOWLEDGMENT

Appreciation is expressed to Dr. W. C. Stewart of Temple University for statistical evaluation of these results.

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ASSOCIATION AFFAIRS

REPORT OF THE 3-A SANITARY STANDARDS SYMBOL ADMINISTRATIVE COUNCIL AUGUST 1, 1966 TO JULY 31, 1967

C. A. Abele, *Chairman*

Having been in operation for more than eleven years, the administration of the use of the 3-A symbol to identify dairy equipment which conforms to 3-A Sanitary Standards now runs so smoothly that Annual Reports can be quite brief, except for the tabulation of Authorizations in effect.

During the twelve months covered by this Report rosters of Holders of 3-A Symbol Council Authorizations, as of August 20, 1966 and as of February 20, 1967, were published in the September, 1966 and March, 1967 numbers of the Journal.

The Dairy Equipment Fabricating Industry is noted for its ingenuity and progressiveness. Advances in equipment design are commonplace. In some instances, such advances extend somewhat beyond the range fixed by customary interpretations of the provisions of pertinent 3-A Sanitary Standards. Consequently, the 3-A Symbol Council finds itself becoming the agency through which inconsistencies or gaps in 3-A Sanitary Standards, or of the need for clarification of provisions of those in effect or of additional provisions, become evident and eventually result in the adoption of amendments.

Several such matters, including the question of the coverage of multicompartment design of automotive milk transportation tanks by the 3-A Sanitary Standards for Stainless Steel Automotive Milk Transportation Tanks and the efficiency of leak-detector plug valves when installed in horizontal position on the outlets of conical-bottom batch pasteurizers (or the need for specific design requirements for horizontally installed leak-detector valves) have been or are being submitted for consideration by the 3-A Sanitary Standards Committees at the next meeting.

The rescinding of the 3-A Sanitary Standards for Manually-Operated Bulk Milk and Fluid Milk Products Dispensers, authorized at the 1966 meeting of the 3-A Sanitary Standards Committees, became effective on May 20, 1967, when sanitary standards of the National Sanitation Foundation became effective. However, most of the manufacturers had inventories of dispensers conforming to 3-A Sanitary Standards but not to those of the N.S.F. and requested the extension of their authorizations for a period of six months. Consequently, the tabulation of holders of authorizations on July 31, which will appear with this Report when published, indicates no reduction in the number of authorizations covering dispensers.

Mr. James A. Meany, Acting Chief Sanitarian of the Chicago Board of Health, was appointed by President Elliker to succeed the late Dr. M. R. Fisher as a Trustee of the Council.

Because six authorizations covering bulk milk dispensers remain temporarily in effect, the number of authorizations in effect on July 31, 1967, is 149 — 8 more than 12 months earlier. This gain resulted from the issuance of 10 new authorizations and the relinquishment of two.

3-A SYMBOL COUNCIL AUTHORIZATIONS IN EFFECT
23 Types of Equipment

Standards Serial No.	Type of Equipment	July 31, 1966	July 31, 1967
0102	Storage Tanks	17	17
0204	Pumps	14	13
0300	Weigh Cans	0	0
0402	Homogenizers	3	3
0506	Automotive Tanks	17	13
0600	Electric Motors	—	—
0700	Can-Tye Strainers	0	0
0807	Piping Fittings	12	14
0902	Thermometer Fittings	1	1
1002	In-Line Filters	1	1
1102	Plate-Type Heat Exchangers	7	7
1202	Tubular Heat Exchangers	3	3
1303	Farm Milk Cooling Tanks	25	24
1400	Leak-Detector Plug Valves	4	4
1500	Bulk Milk Dispensers	4	5
1602	Evaporators	6	7
1702	Fillers and Sealers	5	6
1902	Freezers	2	2
2200	Silo-Type Storage Tanks	7	7
2300	Cottage Cheese Packagers	2	3
2400	Batch Pasteurizers	4	5
2500	Batch Processors	3	4
2600	Dry Milk Sifters	4	5
		141	149

THE NOBILITY

Oh, they are unsung heroes all
Those Dairy Service Men
They, like the clergy, have a call
Those Dairy Service Men
They must be tactful in the field
When they go to check a patron's yield
If the cows are clean and the barn is neat
They check each item on the sheet

They check all types of sanitation
Each milk pail, hose and each inflation
They check the ceilings, walls and floor
The screens, the flies, the milk house door
They must enforce each rigid rule
That they did learn in Dairy School
They must be firm, polite and neat
As they stand with dung upon their feet
It's hard to show such savoir-faire
With wisps of barn dung in their hair
It takes great poise to sound so pure
When they always smell like fresh manure

—TOM KENNEDY, Audit Dept.
Dairyman's League

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.

CONNECTICUT ASSOCIATION OF DAIRY AND FOOD SANITARIANS

42nd Annual Meeting
Wethersfield, Connecticut
January 17, 1968

(*Secretary,* R. M. Parry, Dept. of Agriculture and Natural Resources, State Office Bldg., Hartford, Conn.)

Salmonella—Detection, Plant Inspection, Self-Certification—*V. D. Ludington*
Vegetable Protein Hydrolysates—Background, Manufacture, Uses—*W. F. Zick*
Role of Dairy Substitutes—*D. E. Miller*

ONTARIO MILK SANITARIANS ASSOCIATION

10th Annual Meeting
Etobicoke, Ontario
January 31, 1968

(*Secretary,* Tom Dickison, 57 Aldershot Crescent, Willowdale, Ontario.)

Bulk Milk Tank Trucks—1967. The Provincial Study & Survey—*Bruce Hayden*
Application of the 3-A Standards to Bulk Milk Tank Trucks and Milk Tanker Cleaning—*C. A. Abele*
The Milk Transporter and His Viewpoint—*H. B. Strachan*

KENTUCKY FIELDMEN AND SANITARIANS CONFERENCE

1968 Annual Meeting
Mammoth Cave, Kentucky
February 27 and 28, 1968

(Sponsored jointly by Dept. of Animal Sciences, Univ. of Kentucky, Kentucky Assoc. of Milk, Food and Environmental Sanitarians and Dairy Products Assoc. of Kentucky.)

(*Secretary,* Leon Townsend, 2205 Brent Drive, Madisonville, Ky.)

Latest Procedures for Cleaning and Sanitizing Milk Equipment Used on the Farm—*M. E. David*
Efficient Forage Production and Utilization—*Warren Thompson*
Current Nutrition Concepts—*Darwin Braund*
Potentials of Freeze Drying—*William Fleig, Jr.*
Food and Feed Tolerance Levels for Pesticides—*R. A. Scheibner*
Practical Problems Facing the Manufacturing Milk Industry—*A. A. Lyle*
Practical Problems Facing the Grade A Milk Industry—*William Corum*
Economical Facilities for Milk Production—*George Turner*
Control of Mastitic Reinfections within the Herd—*D. J. Wood*
Current Status of the Milko-Tester; Locally and Nationally—*D. R. Lambert* and *Mr. Rutgerson*
Problems and Potentials of Bulk Tanks—*Robert Haygood*
The Grade A Fieldman's and Sanitarian's Image—*James McDowell*
The Manufacturing Milk Fieldman's and Sanitarian's Image—*Hewel Blair*

VIRGINIA ASSOCIATION OF SANITARIANS AND DAIRY FIELDMENS ASSOCIATION OF VIRGINIA

Joint Meeting
Roanoke, Virginia
March 6, 7 and 8, 1968

(*Secretary,* Wm. H. Gill, 6702 VanBuren Ave., Richmond, Va. 23226)

The Advantages of Organization—*J. R. Nichols*
Consumer Expectations for Dairy and Food Quality—*Mrs. Joyce Short*
Substitute Products and Problems—*W. R. Mabe*
Dairy Cattle Feeding—*Ray Murley*
Automated Cattle Housing and Handling—*Dan Kite*
Food Packaging Materials—*Ed Martin*
Automatic Merchandising—*D. E. Hartley*

MISSOURI ASSOCIATION OF MILK AND FOOD SANITARIANS

36th Annual Conference
Columbia, Missouri
April 8, 9 & 10, 1968

(Sponsored jointly with Dept. of Food Science and Nutrition, Univ. of Missouri and the Missouri State Division of Health)

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DOOR PRIZES AT 1968 ANNUAL MEETING

Secretary-Treasurer Karl K. Jones has issued the call for door-prizes to be awarded at individual sessions at our 55th Annual Meeting at St. Louis, Mis-

souri, August 19 to 22, 1968. Each year a group of affiliate associations are invited to donate a prize and usually the gift is representative of the products of the particular state.

This year invitations to send in prizes were issued to IAMFES affiliates in the following states: Arizona, California, Florida, Indiana, Kansas, Michigan, Mississippi, Missouri, Oregon, Rhode Island, South Dakota, Virginia, Wisconsin and the province of Ontario, Canada.

NEWS AND EVENTS

FAIR PACKAGING AND LABELING[1] REGULATIONS IN PAMPHLET FORM

The National Labeling Committee has published in pamphlet form the Fair Packaging and Labeling Regulations for Fluid Milk and Fluid Milk Products. This model regulation is the outgrowth of a long-felt need to work toward uniform labeling in the milk industry.

The suggested model represents two years of consideration of the many problems involved. Seven drafts of the document have preceded this final recommendation, one of which was sent to state regulatory officials for comment. Many fine suggestions were received and have been incorporated in this document.

The National Labeling Committee was founded in 1962 by a group of regulatory officials and industry representatives whose purpose was the drafting and implementation of uniform labeling regulations to keep pace with the growth of the industry. The primary function of this Committee then, as it is now, was the elimination of unnecessary and costly proliferation of labeling requirements which impeded efficient distribution of milk and its products and required the carrying of burdensome multiple inventories. This is a wasteful and cost-increasing practice which the dairy industry can no longer afford.

The Committee in its deliberations necessarily had to take into account the provisions of the Federal Food, Drug, and Cosmetic Act, the Fair Packaging and Labeling Act of 1966, the provisions of the 1965 U. S. Public Health Service Pasteurized Milk Ordinance, and existing state laws and regulations bearing on standards and labeling. At the same time, both industry and regulatory representatives recognized the importance of framing the regulations in such a way as not to unneccessarily detract from the esthetic image of the container as a prime mover in getting milk and milk products consumed.

The project has been carried out under the direction of National Labeling Committee Chairman, Mr. M. W. Jefferson, Virginia State Department of Agriculture, and Vice Chairman, Mr. Shelby Johnson, Kentucky Department of Health. Serving on the Committee have been representatives of state and local boards of health and state boards of agriculture, milk industry representatives designated by the Milk Industry Foundation, and representatives of the suppliers of containers to the milk industry.

In addition to Mr. Jefferson and Mr. Johnson, following are the members serving on the Committee: J. F. Speer, Jr., Milk Industry Foundation; F. W. Barber, National Dairy Products Corp.; H. J. Barnum, Denver Dept. of Health & Hospitals; Don Campbell, Dean Milk Co.; L. L. Clough, New York Dept. of Agriculture and Markets; J. H. Erb, The Borden Co.; F. E. Fenton, U. S. Dept. of Agriculture; Harold Findlay, Carnation Co.; C. M. Fistere, Milk Industry Foundation; W. V. Hickey, Plate, Cup and Container Inst.; C. R. Jones, Arkansas State Board of Health; Paul Kennedy, International Paper Co.; Ben Luce, Washington Dept. of Agriculture; H. E. Thompson, U.S.P.H.S.; Max Weimer, Ohio Dept. of Agriculture; and Evan Wright, Kansas State Dept. of Health.

Copies of the pamphlet may be obtained at no charge by contacting the Office of the Secretary, National Labeling Committee, 910 Seventeenth Street, N.W., Washington, D. C. 20006.

[1]See more detailed article by John F. Speer, page 148.

SUMMARY OF FOODBORNE DISEASE OUTBREAKS IN 1967

The weekly Morbidity and Mortality Report for the week ending April 20, 1968, published by the National Communicable Disease Center, Public Health Service, summarizes the outbreaks of foodborne diseases reported to the Center during 1967. Although the data did not include every foodborne outbreak in the United States, various trends and the predominance of certain etiologic agents became apparent.

The total number of people affected in the 273 reported foodborne outbreaks in 1967 were 22,171. There were 15 associated deaths and 118 secondary cases. The etiology was confirmed in 160 of the 273 outbreaks. Salmonella was the cause of most illness and accounted for 12,836 cases in 35 outbreaks.

Beef, turkey, eggs and egg products, and milk were the vehicles most frequently responsible for salmonella outbreaks. *Clostridium perfringens* caused illness in 3,493 people in 29 outbreaks. Beef was the most common vehicle in outbreaks caused by this organism. Staphylococcal food poisoning accounted for illness in 1,914 persons in 55 outbreaks in which beef, pork, fish, and vegetables were the most common vehicles.

When the data were studied to determine the locations of outbreaks, it was found that the largest number of outbreaks, 94, occurred at home, but the number of people involved were only 323. In contrast, outbreaks following banquets accounted for more than 50 percent of all reported illness with 11,373 people affected in 25 outbreaks. In 35 outbreaks 4,129 persons became ill after ingesting contaminated food served in schools. Food served at restaurants was responsible for 69 outbreaks in which 1,386 persons became ill.

LEFT-OVER PESTICIDES

David Shriver, Department of Entomology, University of Maryland, has compiled interesting information on the use of left-over pesticides. In an article in the April, 1968, issue of the Maryland Processor's Report, a publication of the University of Maryland, Mr. Shriver states that "This is the time of year when we usually find all of those left-over pesticides from last year. Don't take a chance with them if there is any doubt about their effectiveness."

The storage or shelf life of most pesticides is of such a variable nature that no general statement can be made for each type. However, the following may be of value in evaluating the condition of a pesticide in question:

Emulsifiable Concentrates—Ineffective if milky formation does not occur with addition of water.

Oil Sprays—Ineffective if milky formation does not occur with addtion of water.

Wettable Powders—Ineffective if lumping occurs, and the powder will not suspend in water.

Dusts—Ineffective if excessive lumping occurs.

Granulars—Ineffective if excessive lumping occurs.

Aerosols—Generally effective until the opening of aerosol can becomes obstructed and will no longer spray. This usually is a sign that either the active ingredients have corroded the can or excessive moisture entered the container during manufacture and has caused rust particles which obstruct the opening.

These are only general symptoms of ineffectiveness. If there is any doubt about the effectiveness of any pesticides, it is wise and sometimes more thrifty to dispose of them and purchase new supplies. The use of a pesticide which has lost its effectiveness (and does not show the typical symptoms) may allow some insect or disease to do more costly damage than the savings of using the old pesticide.

PROFESSOR B. L. HERRINGTON RETIRES

Prof. Barbour L. Herrington has retired after 34 years as dairy scientist at the N. Y. State College of Agriculture, Cornell University. He was known as both an outstanding teacher and a skillful investigator in the field of dairy chemistry.

For many years Herrington taught the introductory course in dairy industry as well as courses for upperclassmen in analytical methods. Also, he was faculty adviser for students majoring in dairy science and was later placed in charge of a curriculum dealing with the food industry and served as adviser to students in food processing.

In his research on milk, he showed that lipase, a fat-splitting enzyme, is active in all samples of raw milk. He introduced a method for studying it and for measuring quantitatively the effects of its action in milk. He is also a world authority on the chemistry of milk sugar.

He is author of the text, "Introductory Dairy Science," and of many articles dealing with the results of his research.

Letter To The Editor

Dear Editor:

A couple of questions regarding the Wisconsin Mastitis Test (WMT) have been raised recently that may be of interest to readers of the Journal.

The first is in regard to the origin and validity of the table on p. 291 of the 12th edition of *Standard Methods for the Examination of Dairy Products.* This table shows the relationship of total cell counts to WMT values which permits the use of a rule calibrated to estimate cell counts directly. Data for this table were obtained from the regression line published in the original manuscript, (*Journal of Milk and Food Technology,* September, 1964, Vol. 27, No. 9, p. 271-275). This relationship was based on bulk tank samples and it has held up very well in the writer's laboratory since the original paper was published. This has been supported by collaboration on split-samples with workers in several states. Further collaborative studies as suggested by the sub-committee on screening tests of the National Mastitis Council should be made to determine the accuracy of various tests for estimating somatic cell counts.

Our experience in evaluating laboratories performing the WMT has shown the care of the sample to be the most important single factor in obtaining accurate results. Samples should be held at 32-40 F and tested promptly after removal from refrigeration the first time. Samples that have warmed to above 40 F, replaced in the refrigerator and used later for the WMT will not give reliable results. Also, samples should

be tested within 30 hr after collection from the farm bulk tank.

Another question is in regard to reagents for the WMT. A recent study in Louisiana disclosed a significant difference in reagent made from a CMT solution as compared with reagent prepared from a standardized WMT concentrate. Apparently changes have been made in the composition of the detergent used in some CMT solutions. This would indicate that only reagents that have been standardized with the reference standard should be used for the WMT.

D. I. THOMPSON

PHS ISSUES STUDY GRANTS IN ENVIRONMENTAL SANITATION

The Public Health Service's National Center for Urban and Industrial Health in Cincinnati has announced that three research grants have been awarded since December 1, 1967, in the field of Environmental Sanitation. The Environmental Sanitation Program, an activity of the National Center, is responsible for setting standards in food handling, milk processing, and for certifying the quality of food and water carried on trains, ships, airplanes and buses. The Program is also concerned with health and sanitation problems in urban and recreational areas.

Under the first award allotted to the Massachusetts Institute of Technology the basic problem under study is finding materials which make the best containers for the storage and preservation of dehydrated and moisture-sensitive food and at the same time prevent food-borne illnesses. The investigators will determine pertinent package properties such as strength, permeability, light transmission, thermal conductivity, porosity, reflectivity, and penetrability necessary for certain classes of foods. They will then attempt to develop protective packaging methods for use by the food industry which will not cause direct or indirect illness.

The University of California at Davis gets the second grant to study humans and animals who can become ill or die from eating arrowgrass, chokecherry, larkspur, locoweed, lupine, water hemlock, and many other alkaloid-containing plants. In addition, the meat from livestock which have eaten these plants can be toxic for humans. In an attempt to reduce these poisonings the investigators will determine relationships between alkaloids and cases of acute toxicity. They also will determine how various factors of growth, age, environment, and particularly nutrition affect the amounts and distribution of alkaloids in the plant.

The objective of the third study under a grant to the Kennedy School of Government, Harvard University, is the importance of natural light to health and welfare. Also it is a factor to be considered in the planning of urban communities, housing developments and working environments. Just as too much noise can aggravate existing mental disorders, so can insufficient natural light be a source of stress. The investigator will study and evaluate how four urban governments, New York, Boston, London, and Liverpool, have used different techniques to influence directly the availability of natural light. One such technique is the use of zoning ordinances.

Among other things, the study should help in the understanding of: the relationship of public health and welfare standards to architecture and city planning, the relationship of technology to legal and political institutions and processes, and the development of common law in response to urbanization.

NEW USES FOR CHEESE AND BUTTER SAUCES IN CANNED VEGETABLES

New formulas and processes for making cheese and butter sauces for use in canned vegetables have been developed at the University of Wisconsin. These cheese and butter sauce-making processes are designed for production methods now in use in the vegetable canning industry.

Dr. K. G. Weckel, Food Researcher of Wisconsin, has pointed out that at present only a limited amount of butter sauce is used in the commercial production of canned vegetables. "Greater use of cheese and butter sauces in canned vegetables can significantly increase the demand for dairy products," he added.

About 139 million cases of canned peas, corn, green snap beans, lima beans and carrots are produced yearly. If only one out of every 20 cases had butter or cheese sauce in it, about 5 million pounds of butter and 15 million pounds of cheese will be needed each year, Weckel estimates. "We've developed butter and cheese sauce formulas for peas, corn, lima beans, green beans, carrots, and potatoes," he said.

The sauces are made of either butter or cheese, a stabilizer, salt, sugar, water, and coloring. Butter constitutes 10 to 14 per cent of the butter sauce, depending on the type of vegetable. The cheese sauce is 21 to 25 per cent cheese.

It is necessary to adjust the ratio in the amount of vegetables to sauce for each kind of vegetable. This is to adjust for the change in composition which occurs when vegetables are heat processed, Weckel explained. For example, carrots yield moisture which is likely to make the sauce thin during the cooking process, while lima beans yield free starch which makes the sauce thicker.

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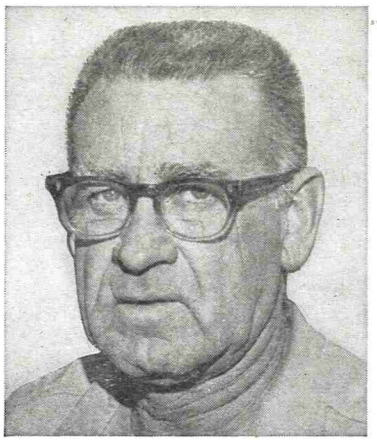

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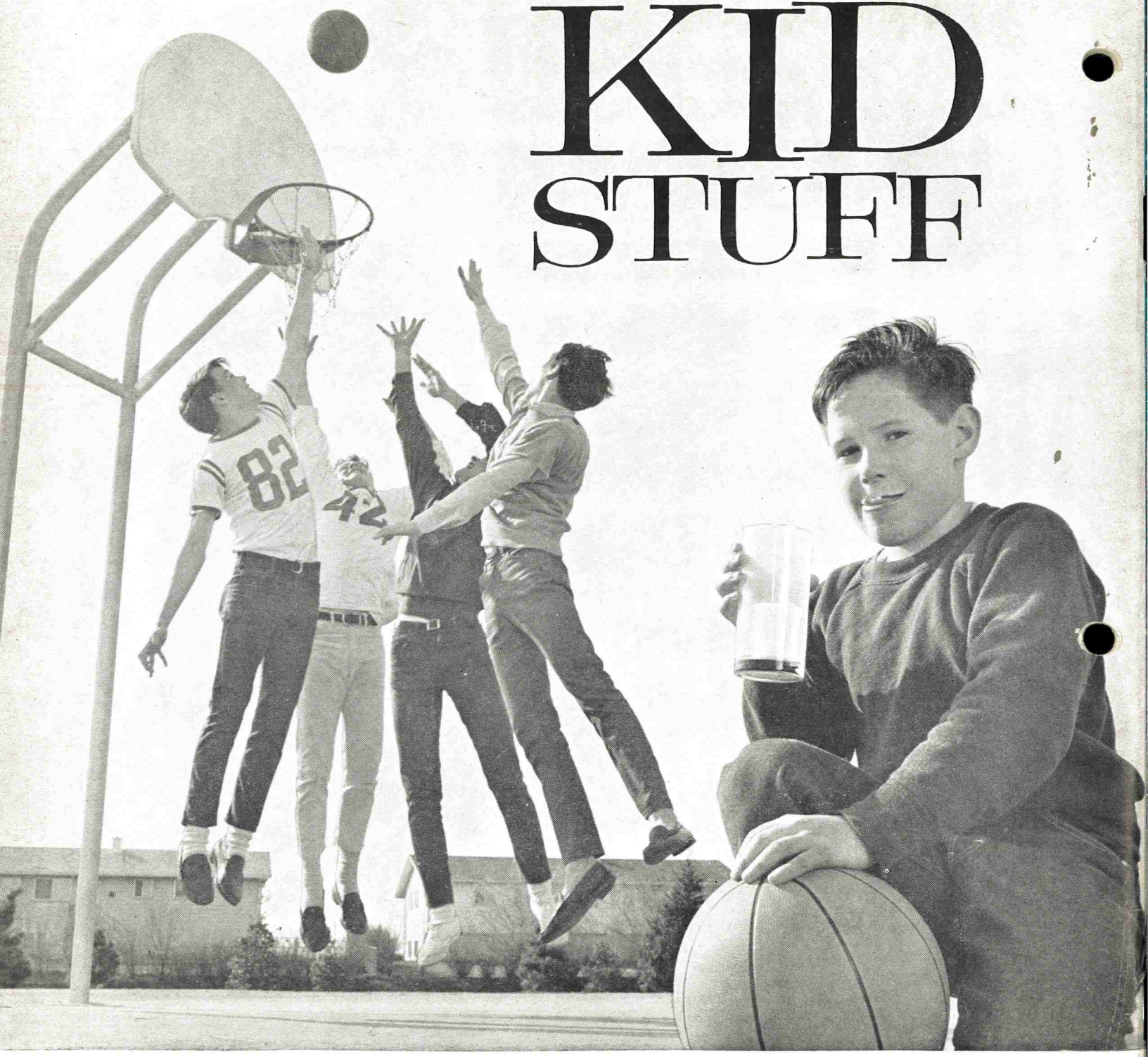

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