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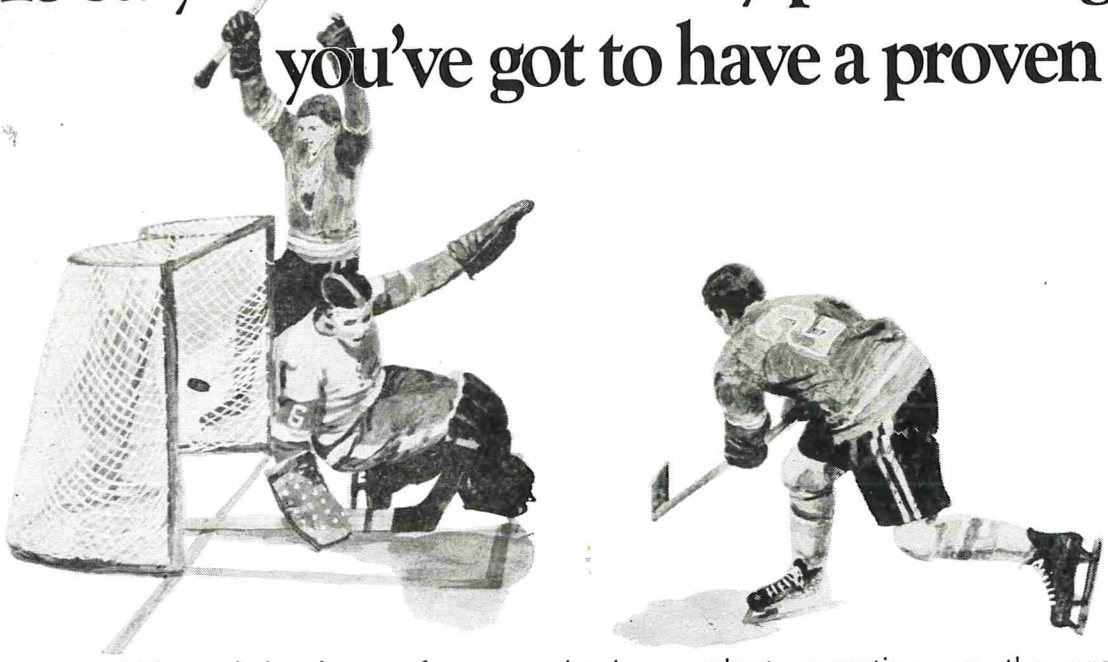

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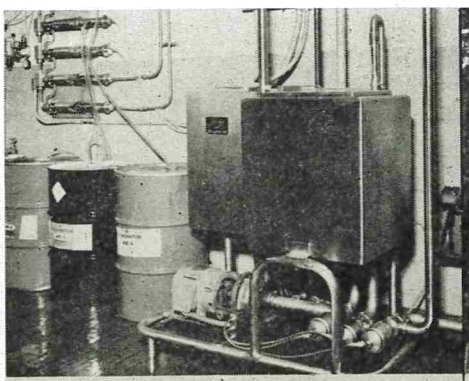

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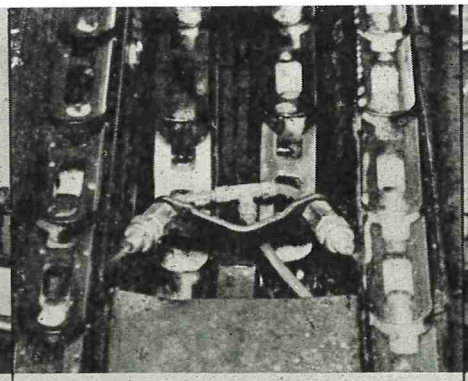

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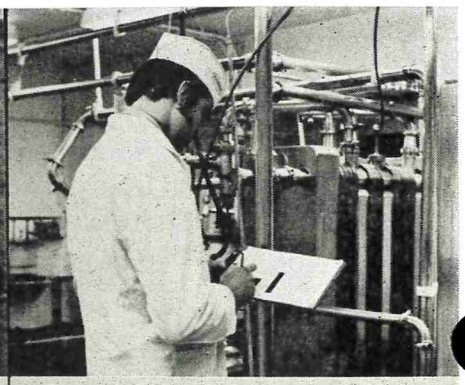

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Vol. 35 December, 1972 No. 12

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COMPILED AND EDITED BY

J. C. FLAKE, A. E. PARKER, J. B. SMATHERS, A. K. SAUNDERS AND E. H. MARTH

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LACTIC ACID BACTERIA IN FOOD AND HEALTH: A REVIEW WITH SPECIAL REFERENCE TO ENTEROPATHOGENIC *ESCHERICHIA COLI* AS WELL AS CERTAIN ENTERIC DISEASES AND THEIR TREATMENT WITH ANTIBIOTICS AND LACTOBACILLI[1]

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(Received for publication May 4, 1972)

Abstract

Recent literature concerning enteropathogenesis and drug resistance transfer factors in *Escherichia coli* are summarized as well as related papers concerning the use of antibiotics in animal feed. *E. coli* infection in swine (colibacillosis) also is considered, especially citations indicating the similarity between the disease in man and animals. The role of intestinal bacteria in human health is reviewed, emphasizing the importance of *(a)* a maintained balance of organisms in the adult, *(b)* breast feeding in infants to establish a large population of bifidobacteria and *(c)* the presence of *Lactobacillus* organisms to maintain healthful conditions in the human vagina. The use of *Lactobacillus* organisms in intestinal and vaginal disease therapy is reviewed as well as the important ecological role that lactic acid bacteria play in the natural scheme where man is concerned.

The lactic acid bacteria consist of rod and spherical-shaped organisms (*Lactobacillus* and *Streptococcus* genera) in the family *Lactobacteriaceae*. They are widely distributed in nature and are easily isolated from mucous surfaces of mammals, green plants, milk, and fermenting foods. In man the lactobacilli are found in the mouth, lower intestine, and vagina. Because lactic acid is a principal end product of their carbohydrate (lactose, glucose, etc.) fermentation, they have been exploited by man for thousands of years in food preservation. This was at first done unknowingly as fermentations were allowed to proceed under the total influence of the natural food flora. More recently, man has learned to select certain species of these bacteria and use them under controlled conditions to produce the desired quality and stability in food products. Today these bacteria are indispensable in the manufacture of many products such as silage, sausage, sauerkraut, sour dough bread, yogurt, sourcream, buttermilk, thousands of cheeses, and other fermented products peculiar to certain countries.

Primitive man was constantly exposed to various lactic acid bacteria from the moment of birth and these microorganisms established themselves to become important members of the indigenous flora. With the coming of the technological revolution in the food industry, however, the types of organisms to which modern man is exposed by eating has changed drastically. Also, the frequent use of antibiotics for a wide variety of diseases and emergence of drug-resistant bacteria have worked to alter the microbial flora colonizing man. With these pressures and resulting microbial ecological changes in mind, the present review article was prepared. Early and recent literature is cited in bringing together the related phases of this subject area.

Enteric Infections Caused by Drug Resistant Escherichia Coli

Severe and highly fatal infantile gastroenteritis is recognized as a serious problem in hospitals today *(59, 101, 166)* and has been the subject of a recent symposium held by the New York Academy of Sciences *(103);* Braun *(13)* has discussed the epidemiology of the disease. Since an effective means of serological typing of *Escherichia coli* was developed *(65)* it has been determined that this disease is caused by certain unusual serotypes of which 14 are particularly important *(32)*. Smith *(149)*, in a study of *E. coli* isolated from diseased humans and domestic animals, observed a high incidence of drug resistance among *E. coli* strains isolated from infants suffering from diarrhea. The strains belonged to serotypes generally accepted as being pathogenic and drug resistance occurred among the infectious type, that is, it was mediated by R factors. It is now known that R factors are genetic elements which transfer multiple drug resistances (R = resistance) to other bacterial cells during physical contact; they are physically composed of deoxyribonucleic acid. R factors were discovered in the late fifties by Japanese workers who observed that multi-drug resistant strains of *Shigella* species were the dominant types appearing as a consequence of using a variety of drugs in the treatment of bacillary dysentery. Akiba et al. *(1)* demonstrated that resistance to several antibiotics could be simultaneously transferred from human-associated strains of *E. coli* to drug sensitive shigellae in physical contact (conjugally mediated

[1]Technical Paper No. 3281. Oregon Agricultural Experiment Station, Corvallis.
[2]Department of Animal Science, Oregon State University.

in mixed culture). That this occurs *in vivo* between members of the enterobacteria (shigellae, salmonellae, *E. coli, Klebsiella, Proteus* and related organisms) inhabiting the intestinal tract of man and animals is now well documented and recognized as a major hazard to public health *(143)*. Also, a number of review articles have been published on infective drug resistance *(3, 90, 92, 129, 130, 176, 177)* with the one by Anderson *(3)* dealing especially with the ecological significance of R factors in enterobacteria.

Growing concern over the magnitude of this problem led to the organization in 1967 by the National Academy of Sciences – National Research Council (in cooperation with the Bureau of Veterinary Medicine, Food and Drug Administration and U. S. Department of Health, Education, and Welfare) of a symposium on the use of drugs in animal feeds. Over 40 papers were presented at the conference, most of which have been published *(98)*. Furthermore, the New York Academy of Sciences *(102)* held a conference last year on the problems of drug-resistant pathogenic bacteria which included several papers on R factors and antibiotics in animal feeds. These and other papers also provide evidence suggesting that infectious drug resistance is an important public health concern.

Smith and Halls *(151)* reported on results of a survey of strains of *E. coli* isolated from man and domestic animals. Their data indicated that infective resistance is probably the most common form of drug resistance in *E. coli* that inhabit the alimentary tract of humans, calves, pigs, and fowls. The disturbing nature of this survey was emphasized in another paper *(149)* where it was pointed out that the enteropathogenic multiple drug-resistant strains frequently cause acute and severe diseases that may terminate in death if drugs used in treatment are not effective. The infective process is so rapid that time is not available to await the results of antibiotic sensitivity tests before commencing treatment.

In 1968, Moorhouse and McKay *(96)* made a hospital study of transferable drug resistance. They isolated *E. coli* strains carrying R factors from numerous patients in a children's hospital in Dublin, Ireland who had been in residence for some time. Furthermore, 15 of 22 infants excreted infectious drug-resistant strains when admitted to the hospital and all 22 children shed the multiple antibiotic resistant strains before leaving. Additional evidence on the wide spread nature of this phenomenon was provided when these authors found that antibiotic resistant enterobacteria carrying R factors (R^+) were isolated from 81 of 100 healthy urban infants. There was no correlation between previous drug therapy and the resistant flora. In a subsequent paper, Moorhouse et al. *(95)* isolated R^+ *E. coli* from several samples of cooked and uncooked sausage. The authors suggested that these strains evolved in the intestines of pigs (R factor transmission between enterobacteria with a selective advantage when antibiotic containing feed is used). They also demonstrated how R factors of animal origin could be transmitted to man, though Smith *(148)* reported recently that animal strains of *E. coli* were less successful than human strains in colonizing the human alimentary tract.

Walton *(174)* in a survey of 400 each of pork and beef carcasses found that the majority yielded drug resistant strains of *E. coli*, 40% of which were R^+. Jarolmen *(60)* and Jarolmen and Kemp *(61)* have presented data suggesting that the *in vivo* transfer of R factors in pigs is rare but Walton *(175)* pointed out that there is a lack of information on the danger to man of transferrable drug resistance in animal bacteria. Furthermore, he indicated that the increase in incidence of bacterial food poisoning in man during the decade that infectious drug resistance has been recognized may be related to changes in microbial populations with emergence of R^+ enterobacteria as a result of careless use of antibiotics and neglect of sanitation in food production. Cohen *(23)* reviewed the ecological consequences of R factors and pointed out the wide spread distribution of R^+ enterobacteria and that as many as 80% of the isolates from infections in man owe their drug resistance to conjugally transmissible R factors. This situation, Cohen further emphasized, has been created by selective population pressures exerted by the use of drugs in therapy or prophylaxis of infection in man and animals and by the use of antibiotics as supplements to animal feeds. Furthermore, clinical evidence was obtained by Aserkoff and Bennett *(5)* that salmonellae from contaminated turkey gained R factors from the antibiotic resistant enteric flora of persons consuming the infected meat.

The emergence of drug resistant bacteria as a result of the use of antimicrobial drugs in animal feeds [more than 2.5 million tons are used per year in the U. S. – *(52)*] has been considered in a recent review article *(145)*. A few other recent articles also are worthy of citation. Smith and Crabb *(150)* studied diarrhea in pigs caused by *E. coli* and found that all outbreaks had one thing in common: the *E. coli* strains implicated were tetracycline-resistant. A similar observation has been made in poultry where the increase in antibiotic-resistant *E. coli* strains causing diarrhea coincided with the progressive increase in use of tetracyclines in broiler feeds *(155)*. The emergence of other drug-resistant bacteria pathogenic for humans (*Staphylococcus aureus, Clostridium perfringens*, salmonellae, streptococci, *Pseudo-*

monas, etc.) has also been pointed out *(143, 146, 147)*.

The increase in magnitude of the animal and human disease problem caused by antibiotic resistant bacteria has led scientists to recommend discriminant use of drugs both as animal feed supplements and in disease therapy *(34, 143, 147)* though at least one author *(63)* has expressed the opinion that the increase in numbers of drug resistant pathogenic bacteria has not seriously hampered disease therapy in man. In England, the addition of antibiotics to feed for cattle and swine is now prohibited *(161)* as a result of the increase in drug resistant salmonellae which gained R factors from *E. coli*. These salmonellae have caused deaths in humans. Recent announcements from the Food and Drug Administration *(35)* indicate the United States is moving in this direction also.

ESCHERICHIA COLI INFECTIONS IN SWINE

Human infections caused by enteropathogenic strains of *E. coli* is paralleled by a strikingly similar disease problem in pigs, recently reviewed by Kohler *(70)* and also Barnum *(6)*. A number of research workers have examined the causes of high piglet mortality and in nearly every instance, β-hemolytic strains of *E. coli* figured predominately in death caused by enteritis and scouring. Kenworthy and Crabb *(66)* noted that hemolytic *E. coli* appeared in the jejunum and ileum of baby pigs at the onset of diarrhea and gastroenteritis. Other bacteria did not increase when this occurred. The intestinal flora associated with enteritis of early-weaned pigs was studied by Chopra et al. *(20)*. These workers found the greatest increase in *E. coli* (coliforms) when diarrhea occurred. They also indicated that there was a balance between lactobacilli and coliform bacteria in nonscouring pigs which became greatly altered when diarrhea was present.

Dillard *(27)* pointed out that colibacillosis (baby pig scouring or diarrhea caused by *E. coli*) is a major cause of economic loss in the swine industry. Even well-managed herds, according to McErlean *(86)*, often are involved and the contents of the small intestine usually provides an apparently pure culture of *E. coli*. Many other reports provide overwhelming evidence that this bacterium is the chief cause of piglet death and impairment of herd performance *(43, 79, 85, 120, 122, 132, 133, 151, 152, 163, 179)*. Recently, Glantz and Kradel *(40)* noted colibacillosis in swine in the United States caused by the "Abbotstown" strain of *E. coli*. This serotype was found by workers in England and Canada to produce a powerful enterotoxin.

A variety of antibiotics have been used to treat colibacillosis in baby pigs. Ampicillin *(21)*; furazolidone *(22, 55)*; neomycin, chloramphenicol, nitrofurazone *(71)*; and oxytetracycline *(8)* among others have saved pigs suffering from the disease. Vaccines prepared from *E. coli* have not been effective *(62)* but immune globulin provided effective postpartum immunity for 5 days when administered orally each day *(72)*. Feeding of *Lactobacillus acidophilus* (*L. bifidus*) also has been reported to be effective *(71, 94)*.

Most strains of *E. coli* causing diarrhea in pigs produce α-hemolysin while few non-pathogenic strains inhabiting the alimentary tract of man and animals have this property *(144)*. Studies by Smith and Hall *(52, 53)*, Kohler *(69)*, and Kohler and Cross *(72)*, however, have shown that enterotoxin present in spent culture medium and in whole cell lysates of enteropathogenic *E. coli* strains would produce positive dilatation reactions in ligated sections of pig intestine as well as diarrhea and frequently death when fed to gnotobiotic pigs. Immunity gained in pigs as a result of feeding or injecting different vaccine preparations also has been studied recently *(72, 159)*; Rejnek et al. *(117)* reported that feeding colostrum or serum from immunized sows would protect gnotobiotic pigs from pathogenic *E. coli*. Svendsen *(159)* and Svendsen et al. *(160)* confirmed these findings and noted further that colostrum or serum from intramammary vaccinated sows appeared to give more protection to newborn pigs than these materials from intramuscular inoculated sows. As a result of these and similar studies, some hope is now held for development of specific strain vaccines which would be used under field conditions to immunize swine; similar studies in humans apparently have not been conducted.

The scouring and diarrhea that occurs in newborn pigs infected with *E. coli* is accompanied by a shift in the proportion of bacteria normally found in the intestinal tract. Kenworthy and Crabb *(66)* found that while the tract is sterile at birth, within 24 hr, lactobacilli, gram-positive cocci, *Clostridium perfringens*, and *E. coli* appear; gram-negative cocci and *Bacteriodes* appeared later. When scouring occurred, hemolytic strains of *E. coli* increased by at least 99% and stress brought on by weaning contributed to the decrease and the shifting balance of organisms. Other workers also have emphasized the importance of a proper microbial balance in the intestinal tract to disease resistance in pigs *(16, 24, 67)*. This naturally has led to studies in which certain bacteria, especially lactobacilli, have been fed to scouring pigs infected with *E. coli* in an attempt to restore the proper balance of bacteria. Before citing work in this area, let us examine the importance of a balanced population of bacteria in the intestinal tract to the health of man.

INTESTINAL MICROORGANISMS AND HUMAN HEALTH

The growing awareness of the role intestinal bacteria play in health and the urgent need for more definitive knowledge on this subject prompted organization of an international symposium on the ecology of the intestinal flora at the University of Missouri on March 30 and 31, 1970. Papers presented at this meeting are published in volume 23 (No. 11) of the *American Journal of Clinical Nutrition*, 1970; No. 12 of this volume contains a collection of papers related to bacteriology and intestinal function in human disease. These and other papers which relate to this will be cited below.

According to Gall *(37)*, "The influence of the balance of the normal flora of the intestinal tract on the health and well-being of the host is well documented. Intestinal bacteria are reported to affect natural resistance *(186)* and to be implicated in metabolism of cancers, serum proteins, cholesterol, hormones, vitamins, and the incidence of caries *(48)*." While a paucity of information is available on the normal bacterial flora in man, Gall *(37)* has found that strictly anaerobic bacteria (not facultative anaerobes) compose over 90% of the total flora and that symbiotic relationships between the different types of organisms present may be important in maintaining the normal balance of microorganisms. Also, Speck et al. *(156)* have shown that the bacterial flora is remarkably stable in the lower intestinal tract of healthy persons and not subject to great changes when the diet is altered. Recently, Gorbach *(41)* reviewed the state of knowledge regarding the intestinal microflora in man.

That stress conditions can alter the microbial flora of the intestinal tract is now well documented. This has come to light especially as the effects of bioisolation on the intestinal flora of astronauts have been noted. Bengson has reviewed this subject *(9)*, which is regarded so serious as to cause Bengt Gustaffson to state at an ONR-NASA conference on human ecology in space flight (1965 – Princeton, New Jersey), " . . . the two most hazardous things an astronaut takes into his capsule on extended flight are his brain and his intestinal flora" [quote from Bengson *(9)*]. The concern is, therefore, that certain bacteria normally suppressed by larger numbers of adventitious organisms, will overgrow and create disease conditions. That this fear is well-founded has been noted by Khazen *(68)* who indicated that Soviet astronauts experienced functional and structural disturbances along with changes in the composition of the intestinal microflora during a 120-day spacecraft cabin test.

The proper balance of organisms in the intestine of infants also is important to their post-natal adjustment. Attention was drawn to this fact as early as 1899 when Tissier *(164)* noted characteristic Y-branched (bifidus) lactic acid bacteria in feces of breast-fed babies to the exclusion of coliform and other bacteria. Snyder *(154)* reviewed the early literature on this subject, himself noting that the bifidus bacterium [*Lactobacillus bifidus*, now called *Actinomyces bifidus* – *(126)*] was present in 20 of 21 breast-fed infants, 12 of 19 on supplemented feedings and in only 12 of 142 weaned infants. Also, it is well known that in breast-fed infants, a stable microflora develops in the colon and feces within 3 or 4 days which usually consists of more than 99% type D IV *Lactobacillus bifidus* found nowhere else in nature *(45, 46, 49)*. That these organisms are beneficial to the health of the infant is concluded from the fact that breast-fed babies have a lower incidence of colic and other digestive disturbances. In this regard, Robinson and Thompson *(123)* have shown that infants partially nursed, even for 2 or 3 days, showed significantly greater weight gains during the first month than infants completely bottle-fed. They also reported that bottle-fed infants on formulas supplemented with *L. acidophilus* showed greater gains in weight than control subjects. Many other references on this subject exist with the most recent being reviewed by Brown and Townsley *(14)*. Of particular interest, however, is a recent study by Mata and Urrutia *(83)* made on the intestinal colonization of breast-fed village children belonging to the Maya-Cakchiquel cultural group in Guatemala. This primitive society has not been influenced to any extent by custom and practices of modern technology. The population of the village is about 1300 and the inhabitants live in one or two room homes and sleep on floor mats. Midwives, who lack knowledge of modern obstetrical practices assist in delivery of the infants in the homes. Mothers use the squatting or kneeling position and defecation is common during delivery. Breast-feeding begins right after delivery by a foster mother and then by the mother as soon as colostrum and milk become available; it continues for up to 4 years of age with small amounts of food being used to supplement the breast milk starting as early as 3 months.

Results of this study showed that for 30 consecutive newborns, half revealed the presence of *E. coli* (10^8 to 10^{11}/g) during the first 24 hr of life and the remaining (10^5 to 10^{11}/g) by the second day of life. Bifidobacteria rarely appeared the first day of life but they increased in proportion to age and by the end of the first week, all infants had these bacteria in high numbers (10^9 to 10^{11}/g). The frequency of bacteria in 12 breast-fed infants was followed throughout the first year of life and *Enterobacteriaceae* as well as other aerobic or facultative bac-

teria were always present in concentrations 2 or 3 logs below the level of bifidobacteria. Also, of 7,792 weekly cultures, only 40 infections with enteropathogenic *E. coli* were found. Infection was highest when weaning was at its peak, suggesting that the stress of diet changes may influence intestinal colonization by pathogenic *E. coli* serotypes. Diarrheal disease caused by *Shigella* was more significant than that caused by *E. coli* but here again, the incidence of these organisms was low during the period of exclusive breast-feeding. These authors suggest that a probable explanation for the resistance of breast-fed infants to intestinal colonization with enteric pathogens is the predominance of the bifidus flora which is stimulated by maternal milk (see Addendum).

An explanation for the predominance of bifid bacteria in the feces of breast-fed babies has been sought. Workers at the University of Pennsylvania identified the bifidus factor present in human milk as a number of oligosaccharides containing glucose, galactose, fucose, and N-acetylgucosamine *(38, 47)*. These findings were confirmed by Lambert and Zilliken *(77)*. According to Rosebury, however, *(121)* it is doubtful that the presence of this bifidus factor in human milk can account for the predominance of *A. bifidus* in the nurseling stool since the bacterium studied by the Pennsylvania group is not the characteristic strain found in the feces. Data of Gyllenberg and Roine *(45)* suggest that the role of human milk is to inhibit growth of other bacteria to allow dominance by *A. bifidus*. More recently, Yoshioka et al. *(185)* observed nutritional differences between strains of bifid bacteria isolated from infants and suggested that carrot extract, used in treatment of infant diarrhea *(75)*, contained growth factors for *A. bifidus* in addition to that reported by Kanao et al. *(64)*. These latter workers identified a new bifidus factor as the coenzyme A precursor, pantetheine phosphate.

The exact source of the bifid bacteria that gain entry into the feces of nurseling infants remains a mystery today *(127, 128)*. Shirota *(140)* has suggested that they are derived from the Döderlein bacillus *(28)*, a beneficial lactic acid bacterium inhabiting the human vagina. This, however has not been established. Nevertheless, the vagina, like the gastrointestinal tract, depends on dominance by these bacteria to suppress undesirable types which may cause vaginitis.

LACTIC ACID BACTERIA AND VAGINITIS

In the early twenties, Schröder *(139)* discovered the clinical importance of Döderlein's bacillus in patients with vaginitis. Vaginal smears are still classified by his method into three types, I: only Döderlein lactobacilli, II: Döderlein lactobacilli plus other bacteria, III: complete absence of Döderlein bacilli. The latter grade showed a preponderance of micrococci, diphtheroids, and streptococci; most of these patients also exhibited an alkaline vaginal discharge with many pus cells. Hunter and Long *(56)* reported that grades I, II, and III were as likely to occur among normal women as among those suffering from vaginitis, which contradicts the report of Lock et al. *(81)*. These latter authors showed Grade I was consistently associated with normalcy of the vagina. Furthermore, pure cultures of Döderlein's *Lactobacillus* was isolated in 49% of unmarried women under 25 and in these cases the pH of the vaginal secretion was always 4.5. More recently, Butler and Beakley *(17)* confirmed these findings and reported the successful reimplantation of the Döderlein organism in women lacking the bacterium. Also, since antibiotics so widely used today destroy the normal vaginal lactic acid bacteria and alter the flora, these workers recommended that balance be restored by vaginal applications of pure cultures of the Döderlein *Lactobacillus*. A lyophilized preparation now is available commercially for this purpose.

These interesting findings make the assumption of Shirota *(140)* that *A. bifidus* and Döderlein's bacillus are related a likely but unproven possibility. It is easy to understand how the infant's intestinal tract would soon become colonized with these organism when close contact through breast-feeding is maintained. Harrison et al. *(50)* observed a sudden marked increase in *A. bifidus* in the vagina of pregnant women immediately before delivery. The organism also colonizes the colostrum and skin of the lactating breast *(18)*.

The confusion on the relationship between Döderlein's bacillus, *L. acidophilus, L. bifidus (A. bifidus)*, and *Lactobacillus bulgaricus* was mentioned by Hunter and Long *(56)*. They examined this point further *(57)* but clarification is still needed. Recently Perez-Miravete *(109)* studied 71 vaginal strains of lactobacilli using the classification system of Rogosa and Sharpe *(124)*; 26 were *L. acidophilus*, 9 *L. delbreuckii*, 5 *L. plantarum*, 2 *L. fermenti*, 2 *L. brevis*, 1 *L. bulgaricus*, 1 *L. lactis*, and 20 unclassified. Rogosa and Sharp *(125)* studied 35 *Lactobacillus* strains isolated from the vagina of normal women; 14 were *L. acidophilus*.

LACTOBACILLI AND INTESTINAL DISEASE THERAPY

Attempts to manage the flora of the human vagina to improve health have been preceded by many efforts to alter or maintain a proper microbial balance

in the intestinal tract for the same reason. Since the early work of Metchnikoff *(87, 88, 89)* and the clarification provided by Herter and Kendell *(53)* and Rahe *(114)* that *L. acidophilus* rather than *L. bulgaricus* implanted in the intestinal tract, lactobacilli have been used extensively in therapy. The older literature regarding its therapeutic value has been documented in one bibliography of abstracts *(36)* and three text books *(73, 118, 119)*.

With the introduction of antibiotics taken orally as therapy against systemic infections of various types, patients began to complain of discomfort in the gastrointestinal tract. Yeast and mold infections were often diagnosed in these cases *(58)* and the standard therapy has been the use of concentrates of *L. acidophilus (30, 42, 113, 182)*. The ability of this organism to produce substances with antibacterial activity also has been noted *(42, 111, 170, 181)*, the most complete being a report by Vincent et al. *(173)*. Furthermore, dried preparations of the organism are available in pharmacies for use in establishment and maintenance of *L. acidophilus* in the intestines; most are ineffective, however, because low numbers of viable organisms are present.

Studies in radiation biology with whole animals also have emphasized the importance of a balanced intestinal microbial flora in healthy animals. In this regard, Vincent et al. *(172)* substantiated earlier reports that lactobacilli constitute the predominant gut flora of small laboratory animals. They went on to show that post-irradiation bacteremia caused by coliform and pseudomonads results when lactobacilli decline in the small intestine of the rat.

Recent reports by Dubos et al. *(29)*, Savage and Dubos *(134)*, Savage et al. *(135)*, and Savage *(137)* have documented the intimate association between anaerobic streptococci, lactobacilli, fusiform bacteria, and yeasts in particular areas of the epithelium of the gastrointestinal tract of mice. These findings again emphasize the importance of a balanced population of microorganisms in healthy animals. That feed plays an important role in this balance was emphasized when these workers found *(134)* that yeasts appear and establish colonies in the mucin of the secreting epithelium only after the animals are weaned. In a subsequent report, Savage *(136)* found that administering penicillin to rats and mice resulted in a replacement of lactobacilli by *Torula* yeasts, a situation which persisted as long as antibiotic was administered. When penicillin treatment was discontinued, indigenous lactobacilli again colonized the epithelium.

Recent literature also attest to the value of using lactobacilli in treatment of intestinal disorders, though one report *(93)* questions their value. Hawley et al. *(51)* have reviewed factors important in successful implantation of lactobacilli in the human. At least two considerations are important: that large numbers of viable cells be fed and that a fermentable carbohydrate be available to the cells in the intestinal tract. Beck and Necheles *(7)* used lyophilized cultures of *L. acidophilus* to treat 59 patients with different types of diarrhea, constipation, abnormal fermentations, and food poisoning. Of these, 22 were cases of diarrhea caused by antibiotic treatment; 20 excellent and 2 good therapeutic results were obtained. In 19 cases of epidemic diarrhea, excellent results were obtained in 17 cases and fair results in 2 cases. Failure occurred in one case of chronic pancreatitis with diarrhea and in one case of colostomy with diarrhea. MacBeth et al. *(84)* used *L. acidophilus* in the treatment of systemic encephalopathy, a toxic condition believed caused by bacterial degradation of nitrogenous substances in the lower intestine and absorption of the toxic materials, especially ammonia and amines. Treatment of this disease presently involves evacuation of the intestinal contents, antibiotic suppression of the flora of the gut, and surgical removal of the colon, all designed to interrupt the activities of bacteria high in urease and amino acid oxidase activity. In their studies, MacBeth and colleagues altered the intestinal flora of two hepatic encephalopathy patients by feeding *L. acidophilus*. Urease and amino acid oxidase activities as well as blood ammonia levels were lowered in both patients when the aerobic microbial flora was suppressed. These findings were generally confirmed by Read et al. *(115)* and Muting et al. *(97)*.

L. acidophilus therapy in man and domestic animals is more widely practised in other countries especially Europe, than in this country *(15, 19, 74, 76, 82, 91, 141, 142, 162, 165, 167, 168, 169, 171)*. It is noteworthy that Tomic-Karovic and Fanjek *(166)* demonstrated the effectiveness of *L. acidophilus* milk in destroying pathogenic *E. coli, in vitro* and *in vivo*. The *in vivo* effect was tested on 20 infants suffering from diarrhea caused by *E. coli* serotype O_{111} B_4. In all cases, the *E. coli*, which was resistant to a number of antibiotics, disappeared from the stools of the infants within 1 to 5 days of starting the therapy with acidophilus milk; the infants made a rapid recovery. Comparable results were obtained by Aritaki and Ishikawa *(4)*, Fedotov et al. *(33)*, and Pene et al. *(108)*. In addition, Vicek and Kneifl *(171)* aided the postnatal adjustment of premature infants by twice daily administration of capsules of "Omniflora," a commercial mixed culture of *L. acidophilus, A. bifidus*, and a non-pathogenic *E. coli*. These organisms became established in the intestines of 20 out of 24 premature infants within one week.

Since the report of Dahlquist and Gryboski *(26)* on the inability of the upper intestinal tract to absorb

or hydrolyze lactulose (1,4,-galactosidofructose) which is degraded by bacteria in the lower tract with reduction of pH, increased use of this compound has been made in intestinal therapy. Bircher et al. *(10)* and Elkington et al. *(31)* successfully treated chronic encephalopathy by feeding lactulose to create in the intestine an environment more suitable for growth of lactobacilli. Conn and Floch, *(25)* however, in a study of the influence of lactulose on the human fecal flora, failed to observe an effect on numbers of bacteria found in either normal or cirrhotic patients. Possible reasons are discussed and these authors emphasize the need for more studies on the ecology of the intestinal flora. Other pertinent references on the benefits of lactulose in intestinal therapy include Hoffman et al. *(54)*, Niemann et al. *(100)*, Schneegans et al. *(138)*, Braun *(12)*, Petuely *(110)*, Ruttlhof et al. *(131)*, Bosma *(11)*, and Gedek *(39)*.

Japanese scientists have been intensely active in studying the relationship between the human intestinal flora and disease, especially since 1935 when Shirota *(140)* isolated the Yakult *Lactobacillus* which would implant in the gastrointestinal tract. A summary of these research reports has recently been prepared in English *(183)*. A large industry with franchised plants in Japan, Taiwan, Hong Kong, and Brazil now provides Yakult as a health food to millions of people daily. In Japan alone over 15 million bottles (65-ml capacity) are consumed each day. The product is a pleasant tasting, slightly sour, thin liquid containing an extract of *Chlorella* algae, added vitamin C, and greater than 10^8 viable cells of the Shirota *Lactobacillus* strain per milliter *(105, 184)*.

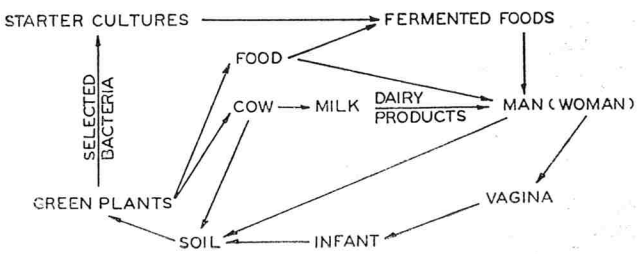
Just as infant coliform diarrhea has a parallel disease in swine (colibacillosis), a disease in pigs corresponding to encephalopathy also is common. This is to be expected since, from the above cited references, it is clear that microbial imbalance in the intestinal tract is the underlying cause in each case. Porter and Kenworthy *(112)* have studied the disease in pigs and cited other pertinent references. In fact, the positive response to antibiotics in swine feed has been suggested *(78)* to be caused by inhibition of amine-forming bacteria such as *E. coli* in the intestinal tract.

Feeding lactic acid bacteria, especially *L. acidophilus*, to swine as therapy by restoring a healthful microbial balance has been the subject of a few reports. Mollgaard *(94)* showed that the presence of lactic acid facilitated absorption of calcium and that pigs fed cultures of lactobacilli had increased amounts of lactic acid in the intestine. These pigs grew better than the control group which had not been given lactobacilli. These effects have been confirmed *(16, 24, 67)* with a suppression of *E. coli* also noted in each case. Also, Leitgeb *(80)* observed that in growing, fattening pigs, the count of *E. coli* in the intestinal tract was inversely related to that of the lactic acid producing bacteria, chiefly, *L. bifidus*. Alexander and Davies *(2)* also showed that the main lactic acid producing bacterium in the large intestine of the pig was *L. bifidus*. Pasicnyj *(106)*, Redmond and Moore *(116)*, and Nedyalkov et al. *(99)* also have noted a beneficial effect on swine in terms of weight gain and reduced enteritis by feeding *L. acidophilus*. In Sweden, a commercial preparation called Majdres is used for prophylaxis and therapy of intestinal disturbances in pigs. Olsson *(104)* fed 50 g of this lyophilized *L. acidophilus* preparation daily (10 days) to counteract diarrhea in weaning pigs. Enteritis disappeared and weights of the animals increased 25 to 40%. Also, at least two patents have been issued *(107, 180)* on methods to prepare lactic acid bacteria to use as animal feed supplements. Furthermore, a product manufactured in France (Biacidol-Labortorie de Biologie Industrielle Appliquee, Saint-Quen) consisting of lyophilized *L. acidophilus* is widely used in France and Germany to treat domestic animals with intestinal diseases.

ECOLOGICAL CONSIDERATION

The ecological impact of the continued use of various drugs in animal feeds and in disease therapy and the uncertainty that shifting microbial populations represent for the future welfare of man demand that research attention be given to means other than routine antibiotic use to maintain health. That drugs are essential in disease therapy is unquestioned and they probably will find ever increasing use in the future. All of us, however, need to be aware of the effects that drug treatments have not only on the troublesome bacteria but on the needed ones as well. Nature has provided a degree of protection for man through the activities of lactic acid bacteria, important members of our indigenous flora. The relationships these organisms have to man in the natural scheme may be represented as follows:

ECOLOGY OF THE LACTIC ACID BACTERIA



Green plants, in particular, serve as a natural reservoir for lactic acid bacteria from where selected strains may be isolated and used to manufacture fermented foods. The organisms also are recycled

through man to the soil and back to plants. Woman plays a special role as a source of lactobacilli which colonize the intestinal tract of the newborn, especially in the breast-fed infant.

ADDENDUM

Since this review article was written, Bullen et al. (*Brit. Med. J.* 1:69-75, 1972) reported on their most recent findings on the resistance of breast-fed infants to *E. coli* infections. In addition to the importance of *L. bifidus* in the feces causing a low pH, these authors found that human milk is unusually rich in iron-binding protein (lactoferrin — 2 to 6 mg/ml). When the iron-binding capacity of the milk was saturated, bacteriostatic activity against enteropathogenic *E. coli* was lost.

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MANAGEMENT SEMINAR FOR FOOD PROCESSORS

A "Management Seminar for Food Processors" will be held March 15 & 16, 1973 at the Holiday Inn, Batavia, New York, sponsored by the Institute of Food Science and Marketing, Cornell University, and the Associated New York State Food Processors. The program will cover the broad areas of Personnel Relations, Environmental Quality, and OSHA. For more information, contact D. L. Downing, Department of Food Science and Technology, New York State Agricultural Experiment Station, Geneva, New York 14456.

NEW CONCEPTS FOR DAIRY WASTE MANAGEMENT[1]

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ABSTRACT

An Environmental Protection Agency funded dairy waste management project has been carried out in Southern California. The program is conducted in close collaboration with the State and local health departments, farm advisors and dairy industry. A simple aeration process produces compost at low cost. The product is "pasteurized," weed-seed free, and an attractive soil amendment. A re-cycling system gives promise of housing 200 cows per acre without producing surface or ground water pollution, or odor and fly nuisances.

New and effective concepts for environmental management of high-density housing for hundreds to thousands of milk cows on dairy farms with limited area and located near residential developments is a major challenge. The problem is far more complex than the traditional management of dairy wastes by pasturing cows and use of the manure spreader. Development of new, environmentally acceptable systems is a matter of high priority, which is stimulating much concern, study, and experimentation.

The "environmental-ecology movement" is bringing public demands for fly, odor, and dust control. New laws and policies bring new legal demands to stop polluting surface waters with drainage from corrals and barns; new studies show that pollution from cow wastes penetrates the soil and may contaminate well water supplies beyond the maximum allowable concentrations for nitrates. High labor and land costs must be taken into account in selecting sites, designing facilities, and developing management practices for an environmentally acceptable dairy farm.

Agricultural, health, and milk industry representatives are collaborating to meet the new challenges. Experiences in Southern California with high density cow housing in open earth corrals illustrate some of the problems and attempted solutions. A few decades ago there was a high demand for manure for fertilizing orange groves and crop lands. In dry weather sale of manure paid for corral maintenance. As orange groves were replaced by subdivisions and chemicals replaced manure, the market for manure became uncertain and at times, the supply exceeded the demand. A group of dairymen in and around the city of Dairy Valley (later re-named Cerritos) established the Dairymen's Fertilizer Cooperative for collecting dry manure (under 30 to 40% moisture). This was stockpiled in what became a hugh pile of several hundred thousand cubic yards, for aging for a year to produce an anaerobic form of compost. This was sieved, shredded, and marketed both as a bulk product and in sacks.

A new freeway was routed through the "manure mountain" and necessitated finding a new site which is at the Los Angeles-Orange County line. Even though the processing facility was designed to meet air pollution standards and the site was arranged and located to minimize environmental problems, lawsuits against the plant were brought by neighbors. The question of health hazards from salmonellosis, tetanus, Q-fever, and respiratory problems was argued during the extensive court cases. Although the Cooperative won the court cases, the City Council of Cerritos, which had approved the new site, the Board of the Cooperative, the Health Officers of the two concerned counties, the Farm Advisors, and the State Department of Public Health all joined in developing the demonstration project application which led to this study. All agreed to provide input in terms of manpower, facilities, and in some instances, funding. What is now the Office of Solid Waste Management Programs of the Environmental Protection Agency provided financial support.

The participants agreed that the "health problems" should be resolved by avoiding creation of a huge, central manure storing and processing operation. They decided that if health hazards from manure dust do exist, control should begin on the dairy. They decided that first priority should be given to processing the manure on each dairy by methods which would handle wet manure in wet weather, thereby helping to cope with the worst fly and odor problems which result when the winter's manure accumulations remain on corrals until dry enough for the stockpile.

The City Council and the Cooperative's Board of Directors strongly favored a program to "utilize," rather than "dispose" of the manure. It was decided that the processing, preparing for sale, and marketing might best be accomplished by a central cooperative

[1]Presented at the 58th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, San Diego, California, August 15-19, 1971.

organization which would serve many dairies by providing technical direction, specialized equipment, and marketing service.

The project became a truly cooperative venture with teams covering various special subjects such as "Entomology and Odors," "Neighborhood Attitude Studies," "Veterinarian and Epidemiology," "Farm Advisors," etc. As project Consultant, the Aerojet General Corporation made a marketing analysis which showed that in the six-county marketing area, at present, manure from 60,000 cows is being sold as bulk or sacked "Steer Manure." Their studies included a comparative analysis of costs per cow per year of various possible processing, utilization, and disposal methods. The economic aspects were considered along with the anticipated "environmental effects" of each method. The study included not only earth corrals, but also covered all-paved corrals, both water-flushed and mechanically cleaned. Their cost analysis was summarized as follows:

1. EARTH CORRALS WITH MANURE COMPOSTED ON EACH DAIRY

System	*Cost/Cow/Year*
a. Composted in aeration bins	$28[2]
b. Windrow composting by special machine	$40[2]

2. PAVED CORRALS

System	*Cost/Cow/Year*
a. Liquid flush-irrigate with waste	$21
b. Liquid flush-separate solids-irrigate	$22
c. Mechanically scrape-direct land utilization	$34
d. Scrape and compost by aeration	$28
e. Aerobic, liquid stabilization	$44

3. COLLECTION OF RAW MANURE FOR CENTRALIZED PROCESSING

System	*Cost/Cow/Year*
a. Stockpile to "compost"	$19[2]
b. Aerated central composting	$25[2]
c. Central-mechanically turned windrowing	$29 to $69[2]
d. Heat drying	$49[2]
e. Incineration	$79[2]
f. Pyrolysis	$57[2]
g. Wet oxidation (Zimpro Process)	$48[2]

[2]Includes $7.80 per cow per year for harvesting from corrals and loading onto trucks.

The environmental "raters" were sanitarians, entomologists, farm advisors, and city officials. The rating scale was "0" for no effects to "9" for major effects. They each considered the three prevailing climatic conditions and found the following concerns: *(a)* dry summer—principally dust with lesser concern about flies, odors, and drainage; *(b)* wet winter—the season of least complaints with some concern for drainage; and *(c)* warm spring before winter manure accumulation is dried or removed—major problems are flies (ranked "9"), odor (ranked "8").

Neighborhood opinion sampling was conducted by a separate evaluation system designed by Orange County's Environmental Health Director and his staff, and utilized by both his staff and by personnel of the San Bernadino County Health Department, to measure attitudes of people living within various distances of dairies and in various locations with respect to prevailing winds. Nearly all householders living adjacent to corrals were concerned and dissatisfied. Those 350 ft away were influenced by the quality of dairy design and maintenance; and, those at 700 ft were unaware of the dairy unless it was visible across open fields.

ENVIRONMENTAL AND ECONOMIC ASSESSMENT OF VARIOUS SYSTEMS

Early in the project it was decided that this study would be principally concerned with dairies of limited area in locations which are becoming somewhat residential in character. This would tend to exclude dairies on large farms where raw manure can be applied directly to crop lands. It would also tend to exclude water-flushed systems which may be acceptable on large, irrigated farms where manure can be mixed with irrigation water for direct application on the land.

EARTH CORRALS

The project objectives were significantly changed during the course of the study. The public attitude survey and environmental ranking systems showed that earth corral dairies are basically unacceptable neighbors to residents living within 350 ft. It was also noted that new water pollution control policies may lead to "outlawing" earth corrals where they produce either surface or ground water pollution. A long range prediction, based upon results of the study, is that earth corral dairies are becoming both environmentally and economically unacceptable in areas of mixed residential and agricultural uses and where land costs are too high to permit direct agricultural utilization of solid and liquid wastes. They will also be unacceptable where serious surface of ground water pollution results.

WATER FLUSHED, ALL-CONCRETE HOUSING

Theoretically, the water flushed dairy has many advantages. Some have claimed that the water already used for cooling, washing and cleaning milking parlors is simply re-used for flushing the 15 ft wide, several hundred feet long, sloping (2-3%) area between free-stalls and feeding and watering facilities. Project observations show this system requires up to two and one-half times as much water as would have

to be used for washing cows and milk parlors.

A major problem is how to utilize and dispose of this highly polluted water produced by dairies of limited land area, without producing surface and ground water pollution and nuisances. A secondary question is the somewhat controversial one of whether the wet concrete produces a serious problem of sore cow's feet? The project study indicates that at several dairies where this system is used, sore feet did not cause serious problems. Also, mastitis rates did not go up and milk production rates were maintained at times when production was down for cows housed on wet, muddy earth corrals. On the other hand, mastitis rates did go up among those cows which had to literally wade through mud-manure mixtures.

Among the possible, partial solutions to problems from water-flushed systems are: *(a)* Use of extended aeration to produce an effluent which can be recycled for certain cleaning functions; and which will produce an effluent acceptable for surface channel or ditch disposal during rainy weather. *(b)* Grouping of dairies to make it possible to build a "community" dairy waste sewerage and waste-water treatment system. Special problems may then exist, or extra treatment may be necessary, in situations where phosphates and nitrates may promote eutrophication of lakes and reservoirs, or where nitrates would contaminate water supplies.

Mechanically Cleaned, All-Concrete, Roofed Housing

Project participants and consultants recommend that major emphasis be given to a system which will minimize the quantity and pollutional quality of waste-water and which will permit high-density housing with a minimum of adverse environmental effects. This involved: *(a)* development of economical and non-nuisance producing manure processing methods; *(b)* roofing and paving cow-housing to avoid surface and ground water pollution; and *(c)* distribution of the compost for utilization by plants, lawns, and crops.

Composting Methods

Bench-scale testing in six, 6 yard^3 bins at the site of the Dairymen's Fertilizer Cooperative demonstrated that composting by mechanical turning and agitation is inefficient and involves excessive labor.

On the other hand, efficient and effective composting is accomplished by introducing air into the manure by low-pressure blowers discharging through perforated pipes in the bottom of the material. By controlling the air flow rate the temperature can be maintained between 140 F and 170 F. Tests and observations by the Project's Entomology team and by agricultural specialists of the University of California showed the product did not attract flies nor produce fly larvae, even though subsequently moistened. It was found to be weed-seed free, nearly odorless, and free of pathogens. When screened, it was an attractive soil amendment that was easy-to-apply to lawns and gardens.

Full Scale Demonstration Project

Success with the aerobic composting process in the bench-scale units led to a full-scale demonstration project. This also was supported by the Office of Solid Waste Management Programs. It was administered by the Public Health Foundation of Los Angeles County, and actively participated in by the farm advisors and health agencies of four Southern California counties, the State Department of Public Health, and the University of California's Agricultural Extension Service.

Major input was provided by Alta-Dena Dairies which furnished space, facilities, equipment, manpower, construction and maintenance, and other invaluable participation.

Composting Manure from Earth Corrals

A composting facility for a 400 cow, earth corral dairy was built on a one-half acre, paved area on one of Alta-Dena's two dairies, which houses 2,000 dairy cows and is located in the City of Industry. The basic factors found by the demonstration Project were: *(a)* a moisture content of 40 to 60% was most satisfactory; *(b)* a mixture of 10% of compost, or more, mixed with the raw manure, appeared to improve the end product; *(c)* 4 to 7 days of aerobic composting in each of two bins, plus 30 days storage for "aging," produced a product with the desired qualities; *(d)* air flow rates began at 3 to 5 ft^3 per minute per cubic yard at the start and were gradually reduced, as needed, to maintain temperatures at over 140 F but below 174 F; and *(e)* air pressures averaged 4 inches of water gauge (0.15 p.s.i.) at the start and were decreased to 1 inch water gauge by the end of the aerating period.

The process was successfully used in both hot and dry summer weather, and cool, rainy winter weather. The corrals are well graded (2½-4% slopes), so the manure can be and is harvested within a few days after each rain. This material could be successfully composted.

Aerobic Composting Over Pipes on Open Slabs

The original tests indicated it was necessary to

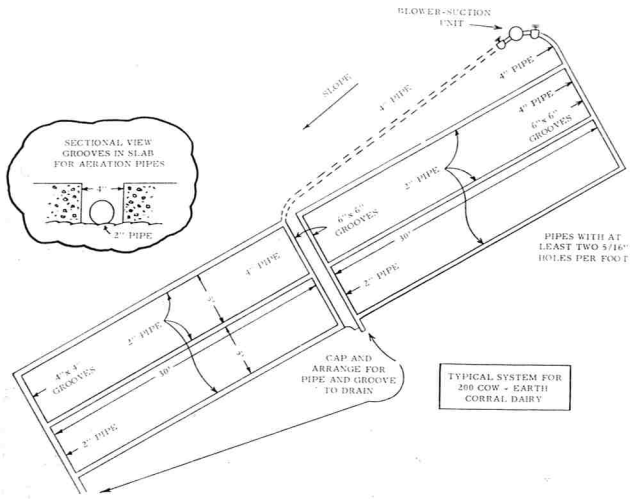

Figure 1. Aeration system for typical 200-cow earth corral dairy.

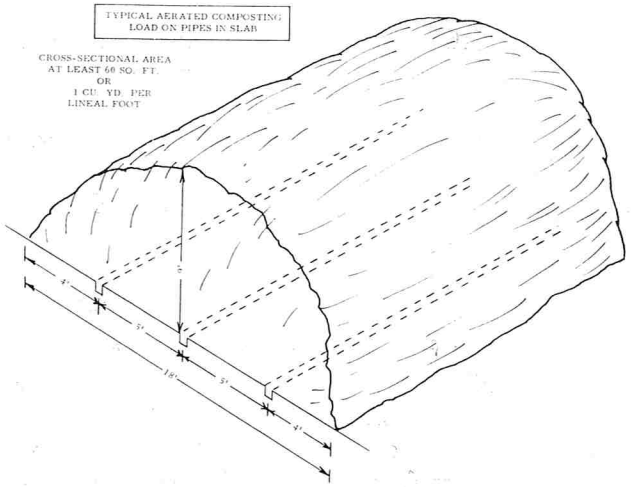

Figure 2. Aerated composting load on pipes in a slab.

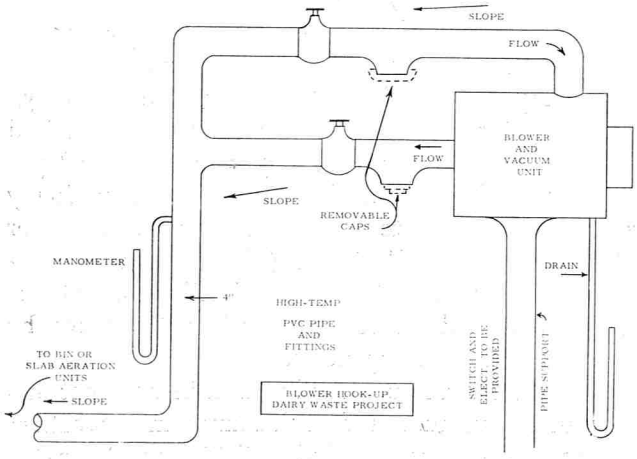

Figure 3. Blower hook-up for aeration of dairy wastes.

confine the material in bins to assure a uniform air flow rate which was considered necessary for maintaining proper temperatures. Subsequent observations at the open ends of the 8 ft deep bins of the full-scale demonstration Project indicates this feature may not be essential. Composting could be accomplished in unconfined piles over perforated pipes. The system (Fig. 1, 2, 3), has the following advantages over the bin system: *(a)* lower construction costs, and *(b)* can process with tractor and truck normally available on the dairy.

Mechanically Cleaned Concrete Corrals

On an experimental basis, Alta-Dena Dairy had, for several years, been using free-stall, mechanically cleaned, paved housing for about 400 of their 2,000 cows at the project site. The stalls are roofed, but the remaining area is not; so, these units could not be used in the wet winter weather. Each four days the paved area was cleaned with a tractor drawn scraper-bucket. The manure-urine mixture, with 87% moisture, was dragged to an earth corral for "drying". However, before it dried it had become heavily infested with fly larvae and had to be hauled for plowing into agricultural land. Various methods were unsuccessfully tried to dry the material, including mixing it with dry compost. Farm Advisor Frank Smith then suggested covering the cleaned concrete corral surface with a layer of dry compost. This would serve the dual purpose of blotting up moisture and acting as a cushion for the cow's feet. This method was successfully used for about five months in one 90 cow-free stall unit. The surplus compost was used as much appreciated cow bedding in all 400 free-stalls and some was distributed and used for evaluating its characteristics as a soil amendment. Among the features of the compost are: *(a)* since it contains nutrients from both manure and urine, it is nearly twice as rich in nitrates and phosphates as manure from earth corrals; *(b)* the material is a rich, golden-brown, and attractive; and *(c)* seven recycling tests of the manure showed there to be no build up in salt content, a good feature, because the material is normally higher in salts like chlorides than compost from earth-corral manure.

The major problem with the recycling system is that four to five volumes of compost are needed for each volume of raw manure, so as to reduce the moisture to 55%, which is optimum for composting. This necessitates handling rather large volumes (a cubic yard per cow per week.)

"Environmental Housing" Recycled Aerated Manure System (RAMS)

The apparent success of the trials led the Alta-Dena Dairy to build two 100 cow, roofed, "loose housing" type units. The stalls were removed. They seemed to be unnecessary because it had been observed that the cows preferred to bed in compost in their feed-

ing and loafing areas, as opposed to use of their individual free-stalls. If successful, this design would eliminate much hard labor required for maintaining clean bedding in free stalls. This phase of the project is an attempt at providing "pollution free" housing, since all manure and urine are "captured", processed, and are then widely distributed where a major portion of what could be "pollutants" becomes valuable plant food.

The present method of operation of the two new roofed "loose housing" units is to remove the wet and soiled manure-urine-compost mixture and add a new layer of dry compost, each week. The removed material is adjusted in moisture content if needed by adding compost to produce a 55% moisture content, is aerobically composted for two weeks, stockpiled for "aging" for a month, and is then ready for reuse in the corrals.

Surplus material is run through a 10 ft × 4 ft shaker screen with ½ inch openings, and is sacked for marketing in 2 ft^3, plastic bags. Screen rejects are mixed with compost for covering the cow housing floors.

Costs-Benefits

The cost of composting, screening, and sacking the manure from earth corrals is about $2.30 per cubic yard, bulk, and $0.35 per 2 ft^3 sack. The material brings $0.89 per sack at retail and over $0.50 wholesale. The Southern California market is now buying about 6 million sacks per year of material which is inferior and much of which is selling at a lower cost.

A cost analysis now being finalized indicates that land developed for earth corrals is costing upwards of $6,000 per acre in Southern California's dairy region. Environmental housing systems, with their higher cow density will cost less per cow per year than the present type earth corrals. In addition, those dairymen with earth corrals on permeable soils over aquifers which are used as domestic water supplies, may find this method a solution to current estimates that a density of over 4 cows per acre will cause excessive nitrate contamination.

Waste-Water

Waste-water management, and control of pollution from the cow washing, holding, and milking facilities is another element. A first step is more efficient use of water. One item is to avoid the wasteful use of water which is now observed with current cow washing systems.

Shaker screens can successfully remove most of the fibrous material from this waste-water. Preliminary tests at the project indicate that extended aeration of simulated milking parlor and cow washing waste-water may produce an effluent which can be recycled for washing floors, etc. Both the volume and the pollutional strength of this waste are far lower than from waste-water produced by all-concrete, flush-out corral type dairies. The effluent can be used in dry weather for irrigating on the dairy site. It may be sufficiently stabilized so it will not normally cause problems when it becomes mixed with storm run-off.

Conclusions

Aerobic composting, coupled with "environmental housing" and with aerobic treatment of liquid dairy farm wastes, gives promise of providing relatively nuisance-free and pollution free, high density housing for dairy cows. The resultant compost is an attractive soil amendment and a well accepted answer to the current demands for "organic gardening" and for "recycling" rather than "depositing" of our wastes.

Recycling of compost into roofed, paved cow housing gives promise of being a method whereby large numbers of cows per acre (close to 200), can be raised in a relatively nuisance and pollution free system.

Acknowledgments

The author is deeply grateful to: the Office of Solid Wastes Management Programs of the Environmental Protection Agency for financial support and guidance; Alta-Dena Dairy for devoting much effort, knowledge, and expense to the project; the many public agency participants including personnel from the health agencies, Farm Advisor Offices, and agricultural specialists of the University Extension Service; the Public Health Foundation of Los Angeles County for administering the project; and Aerojet-General Corporation for the market analysis, cost analysis, systems approach of the project design and technical guidance during the first two years.

DAIRY WASTE FLOCCULANTS

Dairy Waste Flocculants—Information on the use of chemical flocculant for the removal of suspended and dissolved solids in dairy wastes available from American Collid Company, 5100 Suffield Court, Skokie, Illinois 60076.

A Research Note

IDENTIFICATION OF BACTERIA ISOLATED FROM PASTEURIZED MILK FOLLOWING REFRIGERATED STORAGE[1]

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ABSTRACT

Eighty four percent of the bacteria isolated from commercially pasteurized milk held at 4.5 C for 30 days were identified as belonging to the genus *Bacillus*. Organisms from the genera *Micrococcus, Microbacterium, Achromobacter,* and *Alcaligenes* were also isolated.

Grosskopf and Harper *(3)* first reported the isolation of psychrotrophic sporeforming bacteria from pasteurized milk. Similar organisms have since been isolated by others *(2, 5, 6)*. The percent of raw milk samples containing psychrotrophic sporeformers has been determined and the reported values range from 25 *(3, 5)* to 83.3% *(2)*. Milk pasteurization is not a sterilization procedure and the microflora of freshly pasteurized milk has been reported. Thomas et al. *(8)* found micrococci and microbacteria accounted for 75% of the isolates from freshly laboratory pasteurized milk. Maxcy *(4)* sampled freshly pasteurized packaged milk and identified the microflora as consisting of 40% gram-positive nonsporeforming rods, 32% micrococci, 22% bacilli, 5% streptococci, and 1% coliform organisms.

The purpose of this study was to isolate and identify the bacteria present in commercially pasteurized milk following refrigerated storage for 30 days.

MATERIALS AND METHODS

One-quart cartons of milk from 10 processing plants were obtained on the day of pasteurization. Samples were iced, transported to the laboratory and stored at 4.5 C for 30 days. Following refrigerated storage, cartons were opened aseptically, samples were removed, and decimal dilutions of samples were prepared. Standard plate, yeast and mold, and coliform counts were done according to *Standard Methods for the Examination of Dairy Products (1)*. A spore count was determined by heating milk to 80 C for 10 min and then plating the milk on Trypticase Soy Agar. Isolates were identified according to *Bergey's Manual,* following procedures as outlined in the *Manual of Microbiological Methods (7)*. Identification of isolates was based on gram stain, catalase production, motility, growth and gas production in lactose broth and brilliant green bile broth (BGBB), reaction in litmus milk, colony morphology on plate count agar, violet red bile agar (VRBA), and eosin methylene blue agar, spore formation (staining characteristics and resistance to 80 C for 10 min), heat resistance (63 C for 30 min, 80 C for 5 min, and 80 C for 10 min), citrate utilization, triple sugar iron agar reaction, indole production, methyl red test, aerobic and anaerobic growth, and Voges-Proskauer test. All bacteriological media were from BBL.

Stored samples were evaluated organoleptically by a panel of seven persons and scored on the basis of individual judgment as to unacceptable, acceptable, and good.

RESULTS AND DISCUSSION

Processing times and temperatures for the samples ranged from 164 to 176 F for 16 to 17.6 sec. The quality of the milk and the microbial data obtained following 30 days of storage could not be correlated with processing conditions.

Data obtained from two samples of each plant are summarized in Table 1. Approximately one-half of the samples were still acceptable to the panel after storage. Coliform counts were determined and many atypical colonies appeared on VRBA; however, none of these colonies grew and produced gas when inoculated into BGBB.

Spore counts were consistently lower than the total plate counts for the same samples. Shehata and Collins *(6)* have shown that some psychrotrophic sporeforming bacilli do not sporulate well at low temperatures.

Approximately 100 isolates were obtained from all of the samples. Colonies were picked at random and streaked onto nutrient agar slants. Isolates were identified to genus and numbers expressed as percent of the total isolates examined (Table 2).

The predominance of sporeforming psychrotrophic bacilli is probably attributable to: *(a)* the rather high level of raw milk samples commonly positive for psychrotrophic sporeformers *(2, 3, 5)*, *(b)* the trend toward increasing pasteurization times and temperatures thereby eliminating many nonsporeforming species, and *(c)* conditions caused by extended refrigerated storage which select for a heat resistant psychrotrophic organism such as *Bacillus (6)*.

Psychrotrophic bacilli are a problem in milk stored for a long time *(3)* and they can produce off flavors *(6)*. Results of the present study indicate that psy-

[1]Scientific Article No. A1800. Contribution No. 4592 of the Maryland Agricultural Experiment Station, Department of Dairy Science.

[2]To whom request for reprints should be sent.

TABLE 1. ANALYSIS OF COMMERCIALLY PASTEURIZED MILK FOLLOWING THIRTY DAYS STORAGE AT 4.5 C

Plant	Total plate count/ml	Yeast and mold/ml	Psychrotrophic count/ml	Spore count/ml	Flavor score[a]
1a	3.3×10^{8}[b]	<300	4.1×10^{8}	6.4×10^{4}	1.0
b	2.0×10^{8}	<300	2.5×10^{8}	2.2×10^{4}	1.0
2a	<300	<300	<300	<300	2.2
b	<300	<300	<300	<300	2.4
3a	3.4×10^{8}	<300	2.6×10^{8}	2.1×10^{3}	1.0
b	3.3×10^{8}	<300	2.7×10^{8}	1.3×10^{3}	1.0
4a	<300	<300	<300	<300	1.6
b	<300	<300	<300	<300	1.2
5a	<300	<300	<300	<300	2.8
b	<300	<300	<300	<300	2.8
6a	<300	<300	<300	<300	2.8
b	<300	<300	<300	<300	2.8
7a	<300	<300	<300	<300	2.6
b	<300	<300	<300	<300	2.6
8a	8.8×10^{3}	<300	1.6×10^{4}	1.4×10^{3}	2.8
b	2.6×10^{3}	<300	1.0×10^{4}	2.6×10^{3}	2.3
9a	1.7×10^{8}	<300	1.9×10^{8}	1.9×10^{3}	2.0
b	5.7×10^{7}	<300	1.3×10^{8}	2.0×10^{3}	1.5
10a	2.6×10^{7}	<300	2.6×10^{7}	5.1×10^{3}	1.0
b	3.1×10^{8}	<300	4.9×10^{7}	5.9×10^{4}	1.0

[a]Good 3.0, acceptable 2.0, unacceptable 1.0
[b]All Counts are averages of duplicate plates

TABLE 2. MICROBIAL FLORA OF COMMERCIALLY PASTEURIZED MILK FOLLOWING THIRTY DAYS OF STORAGE AT 4.5 C

Species	Percent of total isolations
Bacillus	84
Microbacterium	9
Micrococcus	2
Achromobacter	2
Alcaligenes	2
Streptococcus	1

chrotrophic bacilli were the major spoilage organisms in the stored commercially pasteurized milk samples analyzed.

ACKNOWLEDGMENT

The authors thank Mr. David Resh for obtaining processing information and samples from the plants.

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FURTHER STUDIES ON THE FLAVOR QUALITY OF RETAIL MILK IN CONNECTICUT

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ABSTRACT

Over 1100 retail milk samples taken during 1970-71 in Connecticut were examined for flavor quality and results compared with those of a similar study made in 1969-70. In the 1970-71 study fewer samples were judged to be cooked but more were criticized as having a feed flavor. The monthly pattern for samples classed as organoleptically unsatisfactory changed from winter to summer. Indications are that causes of off-flavors shifted from those originating at the farm to those developing from processing, or during distribution and storage.

Many consumers relate keeping quality of perishable foods to decline in flavor, odor, and palatability on continued refrigerated storage. Color change may also play a role. What the consumer wants, therefore, is assurance that the product was prepared in a sanitary manner, and that it has a good flavor and will keep a reasonable time under refrigeration before flavor declines to an unacceptable level. In this respect, the flavor score of freshly pasteurized milk, although subjective, is much better than standard microbiological and chemical tests *(1)* as a predictor of keeping quality (i.e., the number of days required to attain an unacceptable flavor score).

Programs such as the Connecticut Milk Flavor Improvement Program in the Connecticut Department of Agriculture have been designed to benefit consumers. Personnel in the Connecticut program advise and help producers, farm cooperatives, processors, consumers, and others (i.e., equipment manufacturers) so that the consumer receives a product of better quality.

We have reported on flavor quality of over 1000 milk samples collected at retail outlets in Connecticut during 1969-70 *(2)*. The study was continued into 1970-71, and this report describes changes that have occurred in milk offered for sale. Present data essentially follows the format of the previous study *(2)*, allowing a direct comparison.

METHODS

During 1970-71, 1180 samples of whole milk were collected at retail outlets in Connecticut at the rate of about 100 per month. These were examined for temperature, flavor score, flavor criticism, Standard Plate Count, oxidase count, and coliform count. Of the 1180 samples, only 825 were also examined for acid degree value (ADV). The method of collection, as well as the details of the tests made, have been described *(2)*.

RESULTS AND DISCUSSION

In Table 1, flavor scores of milks collected in 1970-71 are compared with samples collected in 1969-70. The 14% decline in samples classified as good in 1970-71 can be accounted for by the 10% rise in the fair category and the 5% rise in the unsatisfactory group. Some of this apparent decline in quality is attributed to more stringent scoring of samples during the 1970-71 test period. However, 88% of the samples were acceptable (excellent, good, fair) in 1969-70, whereas those acceptable in 1970-71 totaled 83.3%, a slight overall decline of 4.7%. Some of this decline is concentrated only in certain months and in certain off-flavors. Of greater concern is the increase of 4.7% in samples judged to be unsatisfactory.

Under specific flavor criticisms, the largest changes occurred in those called cooked, a decline of 33% from 1969-70, and an increase of 34% in those judged as having a feed flavor. A partial explanation for

TABLE 1. RANGE OF FLAVOR SCORES OF MILK SAMPLES COLLECTED AT RETAIL OUTLETS IN 1969-70 AND 1970-71

Flavor score and designation		1969-70 (% in each group)		1970-71 (% in each group)	
40	Excellent	1.6		0.1	
39	Good	47.2	68.1	12.8	54.3
38		20.9		41.5	
37	Fair	3.3	18.3	13.7	28.9
36		15.0		15.2	
35	Unsatisfactory	6.6		6.0	
34		1.2		3.6	
33		0.2	12.0	1.3	16.7
32		0.5		2.1	
31		0		0	
30		3.5		3.7	

the rise in feed off-flavor may be the prolonged cold period in the winter of 1970-71. Because of the severe cold, barns were tightly closed, resulting in poorer ventilation and concentration of feed odor in the barn. Although samples criticized as having a feed off-flavor increased, no sample with a feed flavor scored less than 36, while 72% scored 38 or over.

The decline in cooked flavor can probably be accounted for by the change in use of vat pasteurization in favor of high-temperature short-time equipment and thus better control of holding times. Changes in other flavor categories were an increase of 4.5% in oxidized and a decline of 2% in unclean.

The monthly distribution of samples judged as fair and unacceptable was determined. Samples classed as unsatisfactory were distributed more evenly throughout the year in 1970-71 than in 1969-70. Of interest is the finding that in 1969-70 more unsatisfactory samples were found in the winter months while the reverse occurred in 1970-71. This may reflect a shift from off-flavors originating at the farm to those developing from processing, distribution, and storage practices. Samples classified as fair were spread out quite evenly through the year with no months of low incidence noted.

Samples classified as either fair or unsatisfactory and criticized as old or lacking freshness, oxidized, rancid, and unclean were also compared in both studies.

The number of samples judged to be rancid remained low (0.9%) during 1970-71. The range of acid degree values and percentages in each group were essentially the same in both studies. There were about 3% fewer samples in 1970-71 with an ADV greater than 1.2. The number of samples criticized as unclean dropped from 4.6% in 1969-70 to 2.4% in 1970-71. This, we feel, reflects effort in one phase of the Connecticut Milk Flavor Improvement Program. Installation of milk moving equipment(i.e., pipelines, pumps, etc.) at the farm must be approved before use to insure proper movement of milk under sanitary conditions.

Samples called oxidized and also classed as unsatisfactory showed a large increase (from 2.3 to 6.8%) during 1970-71, especially in March and September. This rise was not seen with samples classified as fair. The oxidized flavor appears both at the farm and after bottling of milk (light induced). Oxidized milk traceable to the farm is a gradually increasing problem which is not easily solved. However, at the retail level, oxidized milk can be traced mainly to milk sold in glass bottles or plastic containers. In the 1970-71 study only 4.4% of milks in paper cartons were oxidized, while of those in glass and plastic containers, 31 and 33% were oxidized, respectively.

Samples criticized as old or lacking freshness in the 1970-71 survey show a large rise (20% over the 1969-70 study) in the month of May. This same peak month, although of lower magnitude, was ob-

TABLE 2. PEAK MONTHS OF ACTIVITY IN SEVERAL CATEGORIES COMPARING MILK SAMPLES COLLECTED AT RETAIL OUTLETS DURING 1969-70 AND 1970-71[1]

Category	1969-70	1970-71
Unsatisfactory flavor score	mar, jul, sep	dec/JAN/feb, MAY/JUN
Temperature over 45 F when collected	AUG	AUG
Off-flavors		
Unclean		
samples classed fair	JAN, mar, oct	SEPT
samples classed unsatisfactory	JAN	no peaks
Oxidized		
samples classed fair	FEB	no peaks
samples classed unsatisfactory	dec/jan/FEB	may, sept
Old or lacking freshness		
samples classed fair	rise from may to peak in DEC	apr/MAY/jun
samples classed unsatisfactory	dec/JAN, MAY/JUN, aug	mar/apr, MAY/jun
Unsatisfactory bacteriological count and organoleptically unsatisfactory		
Standard plate count	MAY/jun	MAR/apr/may, jul, sept
Oxidase count	MAY/jun	MAR/apr/may, jul
Coliform count	MAY/jun	MAR/apr, jul, sept

[1]Month in capital letters indicates highest peak and months separated by slashes indicates broad peak over those months.

served in the 1969-70 study. In 1970-71, there was a general rise from May to December (from 2 to 27%) in samples called old or lacking freshness and classified fair.

The range of temperatures of milk sampled at retail outlets remained almost unchanged in 1970-71 over that found the previous year. Most samples (55.3%) were in the 40-45 F range. The number of milks exceeding 45 F increased during the summer months and decreased during the winter months, as was seen in 1969-70. In this unacceptable temperature range (>45 F), the summer months (June, July, August) accounted for 78.2% of the samples. A high of 34% was reached in August, with a low of 6-9% from September to December. In 1969-70 the number of samples over 45 F declined starting in September, but the decrease was not as dramatic as in 1970-71. These data indicate that little added attention was given to the care and refrigeration of milk offered for sale at retail outlets in the second year's study. Based on data of temperatures of milk at retail outlets provided by both the 1969-70 and 1970-71 studies, industry groups instituted educational programs for store managers emphasizing the need for proper refrigeration of perishable products. The effectiveness of this educational program will be determined by monitoring milk temperatures at retail outlets.

In the second year's study, quality was further judged by applying the following bacterial standards. Classed as unsatisfactory were those samples having a Standard Plate Count of 25,000/ml or greater, a coliform count of 5/ml or greater, or an oxidase count of 5,000/ml or greater.

Samples classified by organoleptic analysis as unsatisfactory and also not meeting the above bacterial standards were segregated by month. The main peak occurred in March with secondary peaks in July and September. In the 1969-70 study there was only one peak (May/June). The March peak of bacterial activity coincides only with a peak for oxidized flavor. No other off-flavor peak coincides with the March bacterial peak. The oxidized flavor peak in September also coincides with the bacterial peak for that month. However, flavor, and not bacteriological counts, benefits consumers more directly in their quest for a product of good quality.

What may be concluded from this second year study by the Connecticut Milk Flavor Improvement Program? Some of the data concerning peak months of activity are shown in Table 2: *(a)* fewer samples (33%) were judged cooked, *(b)* more samples (34%) were criticized as having a feed off-flavor, *(c)* there was an increase of 4.5% in samples called oxidized, *(d)* a decline of 2.2% occurred in samples evaluated as unclean, *(e)* other off-flavor criticisms remained essentially unchanged, *(f)* better control of refrigeration temperatures at retail outlets was not noted, *(g)* the monthly pattern for samples classed as having an unsatisfactory flavor changed from winter to summer months, and *(h)* the peak month for samples classed as organoleptically unsatisfactory and also not meeting bacteriological standards shifted to March (in 1970-71) from May (1969-70) with secondary peaks in July and September; the September bacterial peak coincides with peak months for oxidized and unclean flavors; the March peak also coincides with one of the oxidized flavor peaks.

ACKNOWLEDGEMENTS

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ANNOUNCEMENT

A National Symposium on Ultimate Disposal of Wastewaters and Their Residuals will be held in Durham, N. C. on April 26 and 27, 1973. Sponsored by the Research Triangle Universities and several national organizations, the program will include sessions on land disposal, marine disposal, sludge handling, design practice, recovery, and recycling.

For further information, please contact: F. E. McJunkin, Associate Director, Water Resources Research Institute of The University of North Carolina, North Carolina State University, 124 Riddick Building, Raleigh, N. C. 27607.

HOLDING TIMES OF RAW MILK DILUTIONS: A REASSESSMENT[1]

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ABSTRACT

It has been suggested recently that the maximum holding time of dilutions of raw milk to be used for the standard plate count be reduced to 5 min. The data upon which this suggestion was based are reassessed by normalizing each sample to equal weight before summary computations are made. Results indicate that the average increases of counts did not exceed 10% during holding times of up to 20 min.

Huhtanen, et al. *(3)* reported that colony counts of raw milk samples diluted in buffered water increased 4.1, 27.7, 17.2, and 21.6% when the dilution blanks were held for 5, 10, 15, and 20 min, respectively. They concluded that, since most of the increase in counts, "appeared at 10 min holding time, . . . it is suggested that the holding time of dilutions to be used for the standard plate count be no longer than 5 min." Their results and conclusion were contrary to what were obtained on another food *(2)*. We rechecked, therefore, their data (Table 1 of reference 3) and noticed only the following minor errors: the average for F, 20 min, should be 8.53 (not 8.58); the average for G, 0 min, should be 41.5 (not 44.3); and the over-all averages should be 55.65, 58.09, 72.98, 67.35, and 69.32 (not 56.05, 58.36, 71.60, 65.69, and 68.19). These corrections did not affect appreciably the over-all results obtained or conclusion reached.

Closer scrutiny revealed that the data and computations were essentially correct but that the computational procedures used could be questioned. We offer two alternative treatments of the data (Table 1 of reference 3) and a modified conclusion.

In the first series of calculations, an average was made of each pair of counts (Table 1 of reference 3). The highest average (regardless of holding time) was set at 100%, and the counts obtained at the other holding times for that milk sample were calculated in relative percentages. Milk sample no. 1, for example, had average counts ($\times 10^3$) of 4.85, 4.70, 4.55, 4.60, and 4.60, respectively, at holding times of 0, 5, 10, 15, and 20 min; the calculated percentages were 100.00, 96.91, 93.81, 94.85, and 94.85, respectively. What this treatment does is to normalize each set of counts to a definite base, rather than to use the counts themselves. Although Huhtanen, et al. *(3)* stated that, "statistical procedures forced us to use the actual numbers," we believe that this rigid attention to statistics led to domination of the results by a few high-count samples. Some of these samples were far out of the range for adequate precision of plate counts. (See, for example, investigator C, samples 13-15). With our treatment of the data, which involves calculations of percentages *before* comparison of different samples, each sample carries equal weight.

We calculated the "percentage of highest count" for each of the 63 sets of counts in Table 1 of reference 3. These percentages were then averaged according to time of holding. The over-all average percentages were: 0 min, 81.89; 5 min, 85.13; 10 min, 88.28; 15 min, 88.57; and 20 min, 89.80. The average percentage increases at holding times of 5, 10, 15, and 20 min, therefore, were 4.0, 7.8, 8.2, and 9.7, respectively (Table 1).

TABLE 1. PER CENT INCREASE IN PLATE COUNT AS CALCULATED BY THREE DIFFERENT METHODS.

	Holding time (min)				
Method of calculation	0	5	10	15	20
Arithmetic means *(3)*	—	4.1	27.7	17.2	21.6
Percentage	—	4.0	7.8	8.2	9.7
Geometric means	—	5.2	7.2	9.6	9.9

When we submitted the present manuscript for review, a referee suggested that a second method could be used to reevaluate the data of Huhtanen, et al. *(3)*. This method, which probably is preferable to our "Percentage" method, involves calculation of the geometric means of the counts at each holding time. The geometric mean is the *n*th root of the product of *n* items. Results of calculations using geometric means (Table 1) showed that the increases in plate count with increased holding time were similar to those that were obtained when the "Percentage" method was used.

In the light of these reassessments of holding times of raw milk dilutions, we should like to paraphrase the final paragraph of reference 3. The 12th edition of *Standard Methods (1)* specifies that not more than 20 min elapse between diluting and pouring of the plates. According to the work reported here, such an indeterminate interval would not lead to excessive

[1]Journal Paper No. J-7276 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No. 1763.

differences in results. For instance, the counts after 5 min would be only 4 to 5% higher; after 10 min, they would be 7 to 8% higher; and at 20 min, they would be 8 to 10% higher. We suggest that a 20-min holding time is appropriate, if it is convenient.

One final point should be made about the wording of section 4.062 of *Standard Methods (1)*. Part of that section reads, "Select number of samples to be plated in any one series so that not more than 20 min elapse between diluting first sample and pouring last plate in series. (Should a continuous plating operation be conducted by a team, plan the work so that the time between the initial measurement of a test portion into diluent or directly into a dish and the pouring of the last plate for that sample is not more than 20 min.)" What is not clearly spelled out in this statement is that two variables are involved: *(a)* the time that the diluted sample is in the dilution bottle *before pipetting*; and *(b)* the time that the pipetted sample is in the petri dish *before pouring*. Our report pertains only to the first of these two variables. The second variable, the time that the sample rests in the plate before agar is added, may well be the most important of the two variables. When samples are held in plates for extended periods before agar is added, bacteria may adhere to the plate; this can lead to difficulties in counting and lowered counts.

Because reassessment of the data of Huhtanen, et al. *(3)* led to a conclusion different from theirs, we also re-examined the data in their other recent paper *(4)*. The latter study *(4)* is a comparison of 2- and 3-days of incubation of plates for enumerating bacteria in samples of raw milk. A 5% increase in counts was obtained at 3 days when compared with the counts obtained after incubation for only 2 days. It was concluded that, "The 5% difference in counts at 3 days would ordinarily be well within the limits of experimental error" *(4)*. When the counts were normalized on a percentage basis and the average percentage increase was calculated, we obtained an increase of 8%. This value is about 40% higher than the value obtained by averaging actual counts; it would not, however, alter the conclusions made by Huhtanen, et al. *(4)*.

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DAIRY HOUSING TO BE FOCUS OF NATIONAL MEETING

One of the first meetings of its kind will be held February 6, 7, and 8 as the National Dairy Housing Conference convenes at the Kellogg Center on the Michigan State University campus, East Lansing. Some 50 speakers will cover all aspects of dairy cattle housing and facilities during the three-day, information-packed program.

The brain child of Structures and Environment Committee 403 (Dairy Housing), the conference is being sponsored by ASAE. However, it will be an industry-wide effort as indicated by the 14 industry, professional, and governmental groups which are cooperating. They include: The American Dairy Science Association, American Society of Farm Managers and Rural Appraisers, American Veterinary Medical Association, American Association of Bovine Practitioners, Canadian Society of Agricultural Engineering, International Commission of Agricultural Engineering, Farmstead Equipment Association, International Association of Milk, Food, and Environmental Sanitarians, Milking Machine Manufacturers' Council, National Milk Producers Federation, Northeast Dairy Practices Committee, Agricultural Research Service, Extension Service, and the U. S. Public Health Service.

A registration fee of $45 will be charged for the conference. This covers the cost of three luncheons and a banquet which will be held on February 7. The banquet speaker will be The Reverend Keith L. Hayes, who is known for his entertaining messages. Each registrant will receive a copy of the conference papers, which includes a complete copy of each author's presentation.

THE ENVIRONMENTAL PROTECTION AGENCY[1]

ROBERT FRI

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ABSTRACT

Each of us shares a concern for the deterioration of our environment, its abuse, and misuse. And I know that we share a sense of urgency about the need to halt this deterioration and win the struggle for a clean and wholesome relationship between man and his environment. This concern for the condition of the physical world is not a special interest, but cuts across social and economic strata and across generations. It is the product of a nearly universal understanding of the problem and of the deadly consequences of inaction. There is a growing realization among all of us that pollution control must become a way of life in this nation, and that it must remain a way of life. We know now that in exploiting our resources we have been exploiting ourselves.

The Environmental Protection Agency, (EPA), was born of public demand for a cohesive, national effort to defend and enhance the environment. Establishment of EPA as an independent agency, with Administrator William Ruckelshaus reporting directly to the President, has impressively heightened the efficiency and effectiveness of our national effort and insured the independent advocacy of our programs. The programs of EPA, present and projected, are action-oriented; we are moving quickly to solve the problems we can solve, and have taken steps to learn how to solve others we currently understand less well.

Each of us shares a concern for the deterioration of our environment, its abuse and misuse. And I know that we share a sense of urgency about the need to halt this deterioration and win the struggle for a clean and wholesome relationship between man and his environment. This concern for the condition of the physical world is not a special interest, but cuts across social and economic strata and across generations. It is the product of a nearly universal understanding of the problem and of the deadly consequences of inaction.

The International Association of Milk, Food, and Environmental Sanitarians has an especially wide-ranging concern for the wanton exploitation of the gifts of nature, for its members are dedicated to protecting the public health and enhancing the public welfare through sanitation. After all, the essence of sanitation is the absence of pollutants of all kinds.

Now we are all too familiar with the dreary list of gross pollution incidents and present threats of *irreversible environmental damage.* Hence, I want to concentrate on the *prevention and cure* of the disease rather than on its symptoms. To that end, I want to discuss what the Environmental Protection Agency is, I want to report to you on some of the steps we are taking in the fight against pollution, and I want to enlist your aid and support. For I believe that there is a growing realization among all of us that pollution control must become a way of life in this nation, and that it must remain a way of life. We know that in exploiting our resources we have been exploiting ourselves.

ENVIRONMENTAL PROTECTION AGENCY

First, let me discuss the Environmental Protection Agency (EPA). EPA was born of public demand for a cohesive, national effort to defend and enhance the environment. Heretofore, the Federal pollution control effort had been fragmented and uncoordinated in its approach and execution. For example, two Federal departments were responsible for determining the adverse effects of pesticides, and a third exercised legal responsibility for controlling introduction of pesticides into the environment. Air and water pollution control authority was vested in two separate departments. Control of radiation hazards was split between the Public Health Service and the Atomic Energy Commisstion.

Establishment of EPA as an independent agency, with Administrator Bill Ruckelshaus reporting directly to the President, has impressively heightened the efficiency and effectiveness of our national effort and insured the independent advocacy of our programs. EPA provides government with the machinery to launch a coordinated attack on pollution problems, and consolidate its thrust. Programs have been drawn together in recognition of the interrelationship of forces which combine in the natural environment; thus environmental control is being approached in its totality.

I'll give you three examples of this total approach. *First,* anti-pollution programs in seven primary environmental areas—water, air, solid wastes, water supply, pesticides, radiation, and noise—have been centered under EPA's organizational roof. We have established a broad organizational framework at EPA headquarters in Washington, but the programs are not going to be tightly held and implemented there.

[1]Presented at the 58th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, San Diego, California, August 15-19, 1971.

And that is my *second* point—the action is in the field.

Ten EPA regions, matching the boundaries of the 10 standard Federal regions, have been established. It is our steadfast conviction that a successful and coherent national program of environmental control is dependent upon a powerful regional organization which will improve the quality and promptness of our assistance to State and local agencies and facilitate our communication with industry and organizations. Hence, the focal point EPA operational programs will be in the field, and strong decision-making powers are being delegated to the regional administrators. We are adding more people to our regional offices and are presently seeking highly qualified regional directors who will be fully responsive to State and local problems and requirements. I might note here that as of June 30th, our headquarters staff totaled 1,840 people compared with 4,119 in our field offices. Our policy is *decentralization*, and the *action is in the regions.*

Finally, we must study the environment as a whole.

So EPA is establishing three National Environmental Research Centers—at Cincinnati for process technology; at Research Triangle Park, North Carolina for studying the effects of environmental problems upon man and animals; and at Corvallis, Oregon for ecological evaluation. We believe that while confronting today's problems, we must also be developing the knowledge and mechanisms to prevent pollution tomorrow.

This forward looking, total approach is in sharp contrast to EPA when it began to transact business last December (1970) 2nd. We were confronted by a profusion of pollution problems clamoring for immediate attention and solution in addition to the normal problems involved in starting a new organization. EPA was pressed for immediate action, and was initially a captive of short-range proposals and short-range responses.

Recent months, however, have given us greater opportunity to look ahead and to forge the long range policies needed for truly effective results. The programs of EPA, present and projected, are *action-oriented;* we are moving quickly to solve the problems we can solve, and have taken steps to learn how to solve others we currently understand less well. Let me therefore tell you about our programs—first, two that concern you the most: water and pesticides.

WATER AND PESTICIDES

As you are well aware, pollution has contaminated a substantial part of our nation's waters, and its major sources are municipalities, industry, and agriculture.

Water pollution

The more than 300,000 water-using industrial plants in the nation generate the largest volume and the most toxic pollutants. And this volume of industrial wastes is growing several times as fast as that of sanitary sewage.

Last (1970) December 23rd, President Nixon announced a new program to control water pollution from industrial sources through the permit authority in the Refuse Act of 1899, which outlaws discharges and deposits into navigable waters without a permit from the Army Corps of Engineers. Issuance of these permits is subject to review by EPA, and our primary concern, in this instance, is the enhancement of water quality. We will establish these effluent limits on the basis of the best information available to us in each instance, including industrial studies when this is possible.

EPA is prepared to use the courts to obtain these applications from those who have not yet filed them. Some weeks ago Bill Ruckelshaus told his 10 regional offices to begin working with the Corps of Engineers to notify industries which had not yet filed applications for permits, to submit within 30 days or face possibility of legal action for failure to comply with the permit program.

Thus, the permit plan will give the Federal government direct control over much of the industrial water pollution. In addition, knowledge gained through the program should enable many States with weak or unclear water quality standards to strengthen them for waterways which are not part of the Federal permit process.

Another major polluter of our nation's waters is agriculture and here we have one of the most difficult sources of wastes to control simply because, in most instances, the pollution does not come out of pipes. Admittedly, the Federal government has done very little work on agricultural pollution problems to date, but this is clearly one of our present "growth areas." We are placing particular emphasis on problems stemming from it, both through the permit program and through further research.

Application of the permit program stem from the fact that beef cattle, poultry, and swine feeding operations, along with dairy farms, are large producers of wastes. The volume of wastes from livestock and poultry production is estimated at 1.7 billion tons annually, about one-half of this amount being produced by animals in concentrated production systems.

Output of animal products has been increasing rapidly and the technology of this expanding production requires that animals be confined in a minimum space and fed a concentrated ration, both of which

magnify the pollution potential of animal wastes.

To give you some idea of the dimensions of the livestock pollution problem, the average population increase in the United States is about 2.5 million people per year. At 1966 consumption rates, each additional million people will require another 172,000 beef cattle, 24,500 dairy cattle, and 433,000 hogs—to say nothing of additional poultry or sheep.

Consequently, EPA and the Corps of Engineers are bringing the largest feedlots—about 3400 of them—into the permit program. This will insure that the discharges from these "super" feedlots will meet applicable water quality standards immediately or through acceptable implementation schedules.

But we also know that the permit program would not be an effective approach to solution of problems of agricultural runoff or irrigation return flow. Present technology is insufficient to provide short-term remedial measures for either of these sources of pollution.

Thus, EPA and the Department of Agriculture are now conducting research and development projects to characterize and quantify agricultural pollutants in water, to develop treatment methods for removal of these pollutants, and to investigate control of pollutants by methods other than treatment.

All these activities are having a significant impact on the control of agricultural pollution. But much remains to be done in terms of developing and implementing the technology to solve these problems. To provide a guide for future Federal action in the agricultural pollution area, the Council on Environmental Quality has announced that it will conduct a study in conjunction with EPA, the Department of Agriculture, and other agencies. The study will analyze major pollution problems resulting from agricultural activities, consider alternative solutions to such problems and recommend measures the Federal Government might adopt to reduce or eliminate these difficulties.

Pesticide pollution

EPA is also at work on the pesticide problem, a delicate one which by its very nature is critical to sanitarians.

Pesticides have provided important benefits by protecting man from disease and increasing his ability to produce food and fiber. But the need for continuing production achievement does not constitute a license to misuse pesticides, a misuse that has become one of the major concerns of all who are interested in a better environment.

Our record is good on this point. EPA, through notices of cancellation, has instituted the administrative review process with respect to two related pesticides: aldrin and dieldrin, and a third pesticide, mirex. We are continuing cancellation proceedings concerning two other pesticides, DDT and 2, 4, 5-T.

What is more important, we are hearing all opinions on the specific pesticide questions, and our decisions are being ventilated to public view. Mr. Ruckelshaus announced recently that a public hearing will be held in the fall (1971) to obtain additional facts on 2, 4, 5-T while the cancellation order continues on use of the herbicide on food crops grown for human consumption. In making the announcement, the Administrator stated: "Courts frown on decisions made behind closed doors, and the EPA shares the judicial attitude that hearings are desirable to bring the public into the decision-making process." For this reason, SAC reports are being distributed.

On a second front, we now recognize that policy regarding use of pesticides in food-handling establishments have experienced a great reduction in the number of substances available to them to control pests. If this unsystematic elimination process were to continue, the food industry could find itself deprived of any chemical remedy for pests.

Thus, the EPA has recently asked its Hazardous Substances Advisory Committee, chaired by Dr. Emil Mrak, to recommend policy guidelines for registering pesticides for use in food handling establishments. Pending receipt of this report, the EPA and the FDA (which shares responsibility for registration with EPA) will neither establish nor cancel pesticide registrations or tolerances relating to food-handling establishments unless an imminent hazard or other serious problem demands such an action. During this period, currently registered pesticides will be used in accordance with instructions on the label.

It is hoped that such a policy review will result in a systematic and intelligent approach to use of pesticides in food handling will prevent the frightening prospect of a choice between cockroaches or poisons in food we consume.

We have also submitted legislation to Congress which would give us control over ultimate use of pesticides—a control which we do not now possess. The legislation is vital to our mission and will permit us to control the benefits of pesticides and greatly minimize or eliminate their environmental risks.

TOXIC SUBSTANCES AND AIR QUALITY

There are two other major programs at EPA today which I will review quickly—the Toxic Substances Control Act and the air quality standards.

Control of toxic substances

The Toxic Substances Control Act, now before Congress, is an example of our efforts to *prevent*

serious pollution problems from arising. The legislation would authorize EPA to restrict or prohibit the use or distribution of a chemical substance if necessary to protect health and the environment. We would also be authorized to prescribe standards for tests, and for the test results which must be met before a manufacturer could market a new product.

The Act would establish as national policy that new chemical substances should be adequately tested. This proposed legislation, I believe, reflects the growing uneasiness in our society about our present deficiencies in dealing with chemicals in the environment, and a genuine national commitment to a new policy of *thinking* before *using*.

Air quality

In the air program, EPA has proposed primary and secondary air quality standards under the 1970 amendments to the Clean Air Act for six major air pollutants. These are tough standards with a real bite to them. Yet, the cost of air pollution in terms of the Gross National Product has been estimated to be in the magnitude of $16 billion a year, while the estimated cost of ending it as only about $4 billion a year. This is an impressive cost/benefit ratio. We intend to see that these standards are met.

ALL ASPECTS OF THE ENVIRONMENT

The charter of the Environmental Protection Agency is to look broadly at environmental conditions, and to keep in mind the *whole* problem as we deal with each of its parts, to exercise leadership, to inform and guide as well as serve the people of this nation. In turn, we at the Environmental Protection Agency will often need information and guidance from individuals and groups, such as IAMFES, who have strong background and deep interest in a particular area, as we work together to develop realistic solutions to our environmental problems. The role of advocate for a better environment has become a common task. The effort of everyone is needed to insure that the world our children inherit will be cleaner and healthier than the one we know.

IAMFES COMMITTEE ON FOOD PROTECTION (CORRECTION)

(Expire 1974)

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QUALITY CONTROL IN THE BREWING INDUSTRY[1]

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ABSTRACT

The history of brewing and of brewing quality control technology is reviewed. Emphasis is placed on progress made in microbiological control, cereal development, technical knowledge of brewing chemistry, and packaging improvements. The current industry trend toward lighter brewing is related to flavor technology and product stability. Modern processing equipment and increased knowledge in the field of sanitation microbiology has resulted in sensitive quality control parameters. Included in sanitation consideration is the impact of the good manufacturing practices section of the food and drug regulations. The quality control of brewing is a dynamic, well-organized technology.

HISTORY OF BREWING QUALITY CONTROL

Brewing has interested civilized man for early 7000 years. Historians and archaeologists agree that people living in the Mediterranean area about 5000 B.C. used barley to prepare a fermented beverage. The predominant cereal grain, among others, in England of 3000 B.C., is reported to have been barley *(29)*. The Government of China in 1116 B.C. published a book that discussed fermented beverages *(1)*. The Magna Carta, signed by Charlemagne in 1267 A.D. set forth price regulations for ale and provided penalties for watering the product *(29)*. In Bavaria, 1516, King Wilhelm IV specified that beer will be brewed of barley, hops, yeast, water, and nothing else. This *Reinheits-gebot* is still in effect for beer to be consumed in West Germany *(21)*. Our American history books tell us the Pilgrims landed at Plymouth Rock instead of Jamestown because they ran short of provisions, especially their beer.

This chronology serves as a reminder that beer and ale have been with us for many years, and we should add that no scientific control of brewing was successfully practiced until the late nineteenth century. Louis Pasteur, in the 1870's, brought research and industry together in an exhibition of international cooperation when he visited and worked with several London breweries. He proposed that "every alteration in the quality of the beer coincides with the development of the microorganisms foreign to the nature of the true beer yeast" *(35)*. At this same time, Emil Christian Hansen was working at the Carlsberg Laboratory in Copenhagen, Denmark. He developed a single-cell culture method for brewers' yeast to eliminate "wild yeasts" *(25)*. In 1881, Alfred Jorgensen established his laboratory of fermentology in Copenhagen and using Hansen's technique, in 1884 he introduced pure culture yeast into the Tuborg breweries.

Beer, at this time, was unlike the beer we know today. These historic beers and ales were fermented without refrigeration, contained less carbon dioxide and were consumed fresh—before they spoiled. The alcoholic content was considerably higher and the drink we now call the beverage of moderation, was not so moderate. Monks and inn keepers in Western Europe made beer during the cool months of the year and stored it in caves and hillsides. While in storage, the beer clarified itself by sedimentation. Addition of hops in the brewing process served a dual purpose. It imparted a pleasant bitter flavor and provided a natural germicidal barrier to some spoilage microorganisms. We now refer to this type of beverage as lager beer. Yeast from lager beer fermentation settles to the bottom of the fermenting vat and is harvested for reuse after decantation of the beer. Ale yeast rises to the surface after fermentation and is skimmed for reuse. This provides a very distinct and practical classification of brewers yeast —bottom or lager yeast (*Saccharomyces carlsbergensis*), and top or ale yeast (*Saccharomyces cerevisiae*).

During the early twentieth century, the industrial revolution had begun, breweries in Europe prospered, and almost every sizable city in the northern and central portion of the United States had its own brewery, or two. As the malting barley fields moved west from New England to the Great Lakes, through the Dakotas and finally to California, so did the brewer *(34)*. Immigrants from Germany were numerous during this period. The many brewers who were among these people brought their knowledge and skill to the areas, among others, of New York, Philadelphia, Cincinnati, Cleveland, Chicago, Milwaukee, Detroit, St. Louis, Denver, San Antonio, San Francisco—an industry was formed. Centers of technology were founded, including brewers schools and independent laboratories such as Wallerstein Laboratories, the Siebel Institute, Wahl-Henius Institute,

[1]Presented at the 59th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Milwaukee, Wisconsin, August 21-24, 1972.

and the United States Brewery Academy. The Master Brewers Association of America held its first meeting in 1888. The science of brewing in America was dormant from 1917 through 1933 as the result of the "noble experiment"—prohibition. But beer came back and the dormancy was broken. The American Society of Brewing Chemists was organized in 1934; the Malting Barley Improvement Association began its work 10 years later. In 1952, five industry groups participated in founding the Brewing Industries Research Institute to engage in cooperative scientific research for the general benefit of the industry. This work was carried on until 1969 when the Institute was dissolved *(27)*.

Brewing laboratories today, as well as in the past, have significantly contributed to the world of science. Is there need to remind you that, in addition to Pasteur, Lavoisier, Priestly, Scheele, Sorensen, Kjeldahl and Büchner had direct contact with breweries or worked in brewing laboratories *(3)*? Brewing science and research is being conducted in a very formidable manner by industrial laboratories and institutes in the United Kingdom, Belgium, Germany, Ireland, Japan, Mexico, Canada, France, and Spain and is reported in their literature *(22)*. Breweries in the United States and their supporting industries constantly contribute to the growth of brewing technology and control.

Significant Areas of Quality Control

Let us define beer. It is the resultant liquid from a fermentation, by yeast, of a boiled and cooled solution containing the sugars from malted barley plus cereal adjuncts, flavored with hops. Quality control of brewing necessarily begins with knowledge and specifications of its primary ingredient.

Malting barley has been the subject of industry research for many years. Farmers wanted disease resistance, firm straw, plump kernels, and a high yield per acre. Maltsters and brewers sought good germination, thin, firm husk, modifiable endosperm, sufficient diastatic power, controlled protein content, and a high yield of fermentable extract. Through the efforts of the Malting Barley Improvement Association, the USDA, and midwestern and western universities continued progress in malting barley development has occurred *(10, 11, 12, 13)*. Hannchen, Traill, Larker, Trophy, Dickson—these are names given to genetic variations and hybrids of barley used during the past few years. A most recent and significant work of the Brewing Industry Research Foundation in Great Britain indicates that malt can be produced without embryo growth in less than one-half the time taken for conventional malting *(14)*. Quality parameters of brewers' malt are detailed in Table 1.

In addition to these physical and chemical analyses, the brewer and the FDA is interested in insect and rodent infestation, insecticide residual, and mycotoxins. Methods used for these determinations are found in publications of the American Association of Cereal Chemists and the Association of Official Analytical Chemists *(15, 16)*.

Several major changes in production methods affecting beer quality have occurred during this century. The first of these was "chillproofing." The colloidal protein in beer coagulates to form haze at low temperatures. In 1911, Leo Wallerstein patented a method to treat beer with the proteolytic enzyme papain *(36)*. This treatment, now in universal use, gives beer protection against chill haze. The enzyme preparation is added after the primary filtration and

Table 1. Summary of Analysis of Midwestern Type Malts[1]

Physical characteristics	Average	Chemical analysis	Average
Bushel weight, Lb.	41	Moisture %	4.4
1000 Kernel weight, g, as Is	30.5	Extract, fine grind, as is %	74.9
1000 Kernel weight, g, dry Basis	29.2	Extract, fine grind, dry basis %	78.3
Foreign seeds %	0.2	Extract, coarse grind, as is %	73.1
Broken kernals %	0.3	Extract, coarse grind, dry basis %	76.5
Growth: 0 - 1/4 %	1	F-C difference %	1.8
1/4 - 1/2 %	2	Color, lab. wort, °SRM	1.46
1/2 - 3/4 %	6	Diastatic power, degrees	132
3/4 - 1 %	90	Total protein, as is %	11.95
Overgrown %	1	Total protein, dry basis %	12.5
Mealiness: Mealy %	97	Soluble protein, as is %	4.83
Half Mealy %	3	Soluble protein, dry basis	5.05
Glassy %	0	S/T Ratio	40.4
Assortment: On 7/64 Screen %	26.1		
On 6/64 Screen %	51.9		
On 5/64 Screen %	20.5		
Thru %	1.5		

[1]Reprinted, with permission, *Brewers Digest,* Vol. 47, No. 4, p. 76.

is allowed storage time to react. The beer is then polish-filtered before packaging. Haze formation can also result from the combination of beer colloids with trace metals, tannins, polyphenols, and polypeptides.

The interest in haze formation led to the study of beer oxidation. Volumes could be compiled with the literature concerning the causes, analyses, and preventive measures of oxidation. Air is injected into wort to provide oxygen for yeast reproduction. Any air absorbed during storage, filtration, or packaging has a detrimental effect on the beer. Until 1968, the analytical method was a measurement of the volume of air mixed with carbon dioxide that could be shaken out of a beer sample *(16)*. It is now possible to measure dissolved oxygen in beer using a portable or in-line analyzer *(23)*.

Beer filtration is an interesting and important quality area. During fermentation, the beer is very turbid. It contains millions of yeast cells per milliliter, protein precipitates referred to as trub, hop resins, and carbohydrate gums. Following fermentation and yeast separation, the natural sedimentation aids in clarification of the beer. But, to produce a brilliant, clear product, filtration is required. Use of diatomaceous earth has just about replaced the pulp filter *(11, 30)*. Quality considerations during this processing are carbon dioxide retention, air or dissolved oxygen content, turbidity levels, and sanitation.

Pasteur focused attention on the microbiological causes of beer spoilage. It was not until 1950 that a practical method for yeast suppression in bacteriological plate cultures of beer samples was discovered *(17)*. The antibiotic cyclohexamide ("Actidione", The Upjohn Co.) is added to the agar plating medium to supress yeast colony development while bacterial growth is not affected. The microscope always had been the basic tool for bacteriological examination of yeast slurry and other non-filtered beer samples, but the use of "Actidione" immediately increased the sensitivity of control. Plating techniques were developed to give results with repeatable accuracy. Washing with ammonium persulfate-phosphoric acid was often used to purify yeast slurry on a regular basis because of the lack of sensitivity and accuracy of the microscopic estimation of contaminant levels *(5)*. It is now possible to specify bacteriological control limits for all operations from wort processing to packaging.

Change has also taken place in beer packaging. Bottles and cans have replaced kegs as the major containers for beer. The returnable bottle has given way to the twist-off non-returnable and the "tin" can is now made of tin-free steel with a pop open, aluminum end. The all aluminum can has found its way to the market and research is underway to use containers other than metal. The quality of incoming materials has received emphasis. Packaging machinery has been improved resulting in high-speed operation and very low oxygen addition.

TABLE 2. SUMMARY OF ANALYSIS OF AMERICAN BEERS[1]

	1951	1971
Apparent extract, %	2.89	2.51
Real extract, %	4.56	4.16
Original extract, °Plato	11.50	11.17
Degree of attenuation, %	60.3	62.8
Alcohol by weight, %	3.55	3.61
Reducing sugars (Maltose), %	1.18	1.08
Acidity (Lactic acid), %	0.14	0.13
pH	4.25	4.22
Protein (N × 6.25), %	0.33	0.33
Color °SRM	3.0	2.8
Bitterness units	—	15.8[2]
Air content, ml	2.3	1.4
Gas volumes (Air corrected)	2.57	2.67

[1]Reprinted, with permission, *Brewers Digest*, Vol. 26, No. 10, p. T140, Vol. 46, No. 11, p. 84.
[2]Average, 1964, 18.8, *Brewers Digest*, Vol. 39, No. 8, p. 65.

THE TREND TO LIGHTER BEER

The industry trend during the past 20 years has been toward the production of a light beer; see Table 2. The definition for "light" is less satiating, less color, and mild flavor. Although individuals have their own definitions for flavor, we must agree that beer is no longer a robust, hearty, strong-flavored beverage. Most American beer is now refreshing and pleasant tasting. To achieve this change, brewers have gradually reduced the specific gravity of the wort using new varieties of malting barley and by varying the malt/adjunct ratio. Hop flavor has also been reduced. The traditional method of hops utilization was the addition of dried hop flowers or cones to the boiling wort in the kettle. Hop extracts are now in common use. Although patents for hop extraction have been recorded since 1869, the general use of extract did not begin until 1964. Consumption of commercial hop extract has risen from 1000 lb. in 1963 to over 3,000,000 lb. in 1970 *(15)*. The control of hop addition and subsequent flavor effect was in the hands of the brewer. No standard laboratory technique for hop flavor analysis or bitterness value was available until 1964 when after nine years of work, the European Brewing Congress and the American Society of Brewing Chemists, in joint action, published a method for this measurement *(4)*. Among the advantages listed for the use of hop extracts are the significant reduction in storage space required, the stability of hop quality in extract form, and the ease of maintaining a standard of bitterness in beer *(14)*.

As a direct result of the change to a lighter, milder brew, the quality of flavor has been a paramount objective. The darker, strong flavored beers of the past tended to mask nuances of flavor caused by wooden vessels, oxidation, process variations, etc. This masking effect has now been removed and flavors contributed by very low levels of alcohols, aldehydes, ketones, mercaptans, phenols, fusel oils, etc. are discernible to the taste. Brewing and flavor chemists, aided by modern laboratory techniques, have published generously on beer flavor and its control. A most comprehensive review of this vast subject has been compiled by Rosculet, listing over 1500 references *(32, 33)*. Some of the methods used to measure flavor characteristics include headspace sampling, direct injection, gas entrainment, liquid-liquid extraction, and liquid-solid extraction, all for subsequent gas chromatographic analysis *(28)*.

Beer Stability

Of concern to the brewer and beer drinker is the stability of beer or shelf life. Stability can be classified into two main categories, chemical and biological. Chemical stability is achieved through the proper balance of colloidal systems and their reaction with trace elements *(20)*. Biological stability is the result of good process sanitation plus pasteurization of the packaged beer or the aseptic filling after either bulk pasteurization or micro-filtration. A high degree of sanitation control is required to consistently package beer aseptically *(24, 26)*. This sanitation control begins with wort processing and carries through fermentation, storage, prefiltration, and final filtration. Aseptic conditions are achieved as a result of the combined efforts of the master brewers, engineers, brewery workers, and quality control technicians. The ultimate test is the bacteriological condition of the product after processing. Certain lactic acid bacteria and species of wild yeast present a potential spoilage situation when their concentration is <10 viable microorganisms in 12 oz. of packaged beer *(6, 7, 18)*.

The classical method for beer preservation has been the tunnel pasteurizer. Bottled or canned beer is conveyed through a series of heated water sprays that gradually increase the beer temperature to 60 C. This heat is maintained for several minutes and the temperature is reduced. After a study of thermal death times of spoilage organisms, the concept of pasteurization units was used *(19)*. One pasteurization unit represents exposure to 60 C for 1 min. During the past few years, along with the trend toward lighter beer, the number of pasteurization units used to preserve beer has been gradually reduced. The reason is two-fold. Sanitary conditions of processing and filling have been improved presenting fewer organisms to be pasteurized. Over-pasteurization has an unfavorable effect on flavor. With these reasons in mind, along with economical factors, bulk pasteurization or microfiltration are used by some brewers.

Sanitation in the Brewery

Twenty years ago many breweries used an open wort cooler of the Baudelot type. Hot wort flowed over an arrangement of pipes that carried a circulating refrigerant. The cooled wort collected in a trough below the pipes and was then pumped to the yeast starters, which in many instances were also open vessels. The potential for wort contamination with this system was very high. Another factor that influenced this potential was manual cleaning of wort process equipment. Modern breweries are now equipped with closed coolers and closed yeast starters. Carefully designed clean-in-place (CIP) systems remove residue and sanitize the equipment with improved quality and efficiency. Wort contamination is almost a thing of the past. The use of wood for fermenting or storage tanks has disappeared along with inherent problems. Another term, now common to design engineers and operating personnel, is sanitary valves, many of which are a part of automated transfer systems. Beer meters, used to tally tax totals, were piston operated and difficult to sanitize. New models are electronic sensing devices that measure volume flow through a beer transfer line with no sanitation problem. Another tradition in processing, the rubber beer hose, is gradually being replaced by stationary, stainless steel transfer lines. The quality of beer process sanitation has improved to the point that, in many instances, 100-ml samples for bacteriological examination have replaced 1-ml samples.

An advantage for brewers in the area of microbiology is the limiting nature of the product. Few organisms other than yeast, and lactic acid and acetic acid bacteria can survive in beer. No pathogenic bacteria are able to use beer as a growth medium *(19)*. The low pH, absence of oxygen, presence of hops, alcohol, and high carbon dioxide tension combine to create this unfavorable condition for disease bacteria. The organisms usually associated with beer spoilage are facultative anaerobic lactic acid bacteria that produce haze and diacetyl. Wild yeast that ferment dextrins and other polysaccharides are also able to cause spoilage in unpasteurized beer. *Acetobacter* will spoil beer if the oxygen content is unusually high.

The most significant microorganism of concern to brewers and to the quality of the beer is the culture yeast. Brief mention has been made as to the nature of beer and ale yeast. In either instance, the culture

yeast is the heart of the fermentation. It must be propagated under sterile conditions and kept sanitary through its generations of use by rigorous attention to the cleaning and handling of process equipment. A diseased yeast culture would have catastrophic effects on the quality of beer. Most breweries have some form of pure culture propagation equipment to insure a regular supply of high quality yeast. The number of generations that a yeast is used depends upon the overall sanitation program in the yeast handling and fermenting areas, the physiological condition of the yeast cells, and the experienced judgement of the master brewer.

Since the time of Pasteur, brewers have practiced sanitation with all the tools and material available. As knowledge of bacteriology, chemistry, and sanitary engineering increased, so did the efficiency of the cleaning methods. Certainly the rudiments of good manufacturing practice had been in effect. Many directives contained in the Federal legislation that became effective in 1969 have been standard operating procedure for some time in breweries. Pest control has been given serious attention for many years. A large midwest grain processor has played a significant role in the application of the Good Manufacturing Practices (GMPs) to brewery operations by providing seminars for managers and supervisors *(2)*. This training has led to self-compliance programs in an effort to cooperate with the FDA. Continued progress is still the watch word in sanitation of beer processing.

We can conclude from this brief review of brewing quality control, its history and progress, that the ancient art of brewing is now a dynamic, well organized combination of science and experience. Change, for the sake of improved methods, better quality, and profit oriented efficiency is a basic part of the brewing industry. Technology is shared through the workings and publications of the Master Brewers Association of America (MBAA), the American Society of Brewing Chemists (ASBC), the Malting Barley Improvement Association (MBIA), the United States Brewers Association (USBA), and the European Brewing Congress (EBC). Research and application of new instrumental techniques will continue to provide the brewers and their customers with a beverage of ever-increasing quality.

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INTERSTATE MILK SHIPMENTS GROUP HOLDS NATIONAL CONFERENCE IN MAY

The recently reorganized National Conference on Interstate Milk Shipments will hold its 1973 meeting in Des Moines, Iowa, May 20-24. Details were announced by Conference Chairman John C. Schilling, who is assistant health commissioner, St. Louis (Mo.) Health Division.

At the Des Moines meeting, attendees will consider proposals to improve sanitation and reciprocity agreements for the movement of fluid milk and milk products among states. The Conference, organized in 1950, holds this national meeting every two years.

Attendance at the Conference sessions is open to any interested person. Individuals in government, private industry or otherwise interested in the work of the Conference are encouraged to submit proposed subjects for discussion at the Conference and to personally attend and participate. All inquiries and suggestions should be directed to the NCIMS Conference Program Committee, Suite 1105, 910 17th Street, N.W., Washington, D.C. 20006.

In its recent reorganization, the Conference discontinued the use of task forces to study various problems, and replaced them with three separate operating councils. The new councils are one on Laws and Regulations, chaired by Dudley Conner, Kentucky Dept. of Health, Frankfort; one on Responsibilities of Conference Participants, the chairman of which is Jay B. Boosinger, Florida Dept. of Agriculture, Tallahassee; and one on Application of Conference Agreements, chaired by Milton Scherpf, Hawthorn Mellody, Inc., Chicago.

Conference chairman Schilling has contacted all participants in the most recent conference meeting, requesting they submit subjects for 1973 Conference discussion to the program committee.

Mr. Schilling, a graduate of the University of Missouri, has been with the St. Louis Health Division since 1946. He served as chairman of the Sanitation Section of the Missouri Public Health Association and he is presently on the board of directors of the Missouri Mastitis Council.

Council Responsibilities

The council on Laws and Regulations is concerned with the various sanitation requirements, the control of milk supplies and other legitimate provisions that are part of the Conference Agreement. Chairman of this council is Dudley J. Connor, Director of the Grade A Milk Program in the Division of Environmental Service, Kentucky Dept. of Health, Frankfort. Connor previously served with the Kentucky Dept. of Health as supervisor in the Milk Control Program and as a milk survey officer and inspector.

The council on Responsibilities of Conference Participants is concerned with matters which relate to all conference participants—federal, state, and local governmental associations, and educational and industry representatives. Chairman of this council is Jay B. Boosinger, assistant director of dairy industry, Florida Dept. of Agriculture and Consumer Service, Tallahassee. He previously served as a dairy specialist with the Dept., and prior to that was a graduate assistant in the dairy science department of the University of Florida.

The council on Application of Conference Agreements deals with problems of reciprocity and with other conference agreements. Chairman Milton Scherpf is vice president, quality assurance for Hawthorn Mellody, Chicago. Previously he served as assistant vice president, manufacturing, and director of technical services of his company.

MODEL OF SLUG FLOW HOLDER FOR COMMERCIAL EGG PASTEURIZATION

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ABSTRACT

Problems caused by types of flow in holding tubes of commercial egg pasteurizers are discussed. A model of a slug flow holder which may eliminate or greatly reduce these problems has been built and tested in the laboratory. It is operated by product flow and maintains a front to each slug from which there is no forward mixing of product with the preceding slug. It works equally well with water or a carboxymethyl-cellulose solution with the same viscosity as salt yolk.

Commercial pasteurization of egg products is accomplished by heating and holding for specified times and temperatures *(1)*. In continuous operations, after the product is raised to the desired temperature, the required holding time is attained as the product flows through a series of straight lengths of tubing connected by return bends. This method of holding has various weaknesses including the following:

(a) Non-uniform holding times occur in any flow stream. The flow for the various products may be turbulent, laminar, or partially laminar depending on the viscosity of the product, tube diameter, and mass flow rate *(2)*. In laminar flow the fluid that flows in the center of the tubes has the highest velocity and will go through the holding tube in about one-half the time for the average. The fluid next to the wall has zero velocity. There is a continuing velocity change across the entire section of the tubing so there is a wide range of holding times being experienced by the fluid depending on the position in a laminar flow stream. The material held for a short time limits the effectiveness of pasteurization. The material held a long time may have been heat damaged so that the functional properties of the product are reduced.

In one series of tests covering the various egg products the ratios of minimum holding time to average holding time range from 57 to 81% *(3)*. In some tests in commercial pasteurizers the corresponding figures ranged from 61 to 81% *(2)*.

(b) Buildup of product either as a foam or as a solid may occur in the holding tube. This decreases the effective holding volume and the efficiency of pasteurization. In one instance, a holding tube processing salt yolk had so much foam in it that the ratio of minimum retention time to the anticipated average holding time was <40%. Formation of the foam could be prevented by keeping a pressure of 20 psi or more in the holding tube at all times so that gas dissolved in the product was not liberated. Maintenance of the pressure at all times is uncertain in the present two pump systems, as it depends on use of reliable pressure gages and proper attention from the pasteurizer operator.

A buildup of a coagulated foam product has been observed in a holding tube processing whites. The material was 1/2 to 1 inch thick along the top of a 3-inch diameter tube.

(c) A non-uniform temperature distribution may exist in the holding tube. A difference of 4.8 F between the top and bottom at the exit of the holding tube (1.5 inch diameter) has been reported for salt yolk *(4)* in an experimental unit. Some tests on a commercial unit with 2-inch tubing showed over 1° variation across the tube.

(d) After a diversion has been caused by a low temperature in the product leaving the holding tube there is no definite way to determine when adequate pasteurizing conditions are again attained after the product temperature is raised to the correct value. It has been proposed and is now included in the *Inspection Service Manual* that the product in the pasteurizer be replaced with water before the temperature is raised to the required level.

(e) When a fluid in the pasteurizer is replaced by pumping another fluid, a mixing of the two occur. This mixing of fluids may occur at the start when water is replaced with product, at the end of pasteurizing operations when product is replaced with water, and during operations when products to be pasteurized are changed. Mixing of water and products can cause appreciable losses, especially at the end of operations. Commonly, pasteurizers will process salt yolk, which is a high viscosity product, last. At the end of the operation when water is pumped through the unit to displace the salt yolk, the water goes down the center of the flow channels leaving much of the salt yoke behind. It is possible that over one-half of the solids contained in the system at the time the water flow is started are mixed with water in increasing amounts as

pumping continues.

The amount of product that is diluted with water and wasted to the sewer depends on how much water is acceptable in the final product. In a plant processing 6000 lb./hr there is at least 350 lb. in the holding tube. Assuming laminar flow, one-half of this will have some water in it. For salt yolk worth 50 cents per pound and a cut-off point such that one-fourth is lost, the value lost is over $40 each time salt yolk is processed. For many plants there is also a disposal problem and associated costs for solids that get into the plant sewer system.

The advantages that might be gained in both laboratory and commercial operations by eliminating the poor flow characteristics in tubing has been realized for several years, but no method of continuous operation that avoided these flow problems was developed until recently. A two-phase slug flow unit and its use on milk products in the laboratory has been described *(5)*. Similar use of this type of unit on egg products has also been successful *(6)*. A holding tube based on the principle of a parastaltic pump but with multiple tubing loops and rollers was built and tested. It did not offer significant advantages over the two-phase type of holding tube and the parastaltic action was not extended to the heat exchangers that were needed to heat and cool the product.

Design of Slug Flow Holder

Neither of the above designs can be scaled up for commercial operations. The unit described in this paper is intended for that use. The particular design is one of several that were considered for getting slug flow with a holding capacity suitable for commercial egg pasteurizers. It was chosen over other designs for one or both of the following reasons: *(a)* it minimizes external piping, and *(b)* its actions automatically match the flow rates in the pasteurizer. Most of the designs required a drive system that had to be adjusted for the flow rates.

Figure 1 shows the design. The unit is driven by the flow of product, and consists of four parts: a valve system, a drive, and two holders.

The valve system has two 3-way valves, an actuator, and a switch. The drive is a cylinder and piston with an O-ring seal. The two holders are identical and consist of a cylinder with multiple separators and pistons. The separators are fixed in position, thus dividing the holding cylinders into sections. As shown in the drawing, the product held in the unit may be considered to be separated into seven slugs, one in each of the sections and one in the drive cylinder. The product may be divided into a greater or lesser number of slugs by changing the number of separators and pistons contained in the holders. The holding capacity can be changed by changing the lengths of the holders and the diameter of the unit.

A continuous drive rod runs the length of the unit. The pistons in the holders and in the drive are fastened to this rod so that all the pistons in the holders move with the drive piston. Adjustable stops on the drive rod actuate the arm of the valve switch. O-Ring seals for the drive rod are provided in the two end plates and two center plates. Neither the separators nor the pistons in the holders are sealed to the walls of the holding cylinders, the pistons having enough clearance to move freely. Each separator and piston has an open hole that permits product flow.

In operation the product flow stream enters between the two 3-way valves. As shown in the top drawing of Fig. 1, the right valve is closed to the incoming stream, the left valve is open, and the flow goes through the latter into the drive cylinder. The drive piston is forced to the right and all the pistons in the holders move to the right at the same speed as the drive piston. All of the liquid to the right of the drive piston in the drive cylinder and in the right holder moves to the right at the same speed as the pistons. The slug of product to the right of the drive piston is forced through the right valve into the space created behind the first piston as it moves to the right. The slug of product to the right of the first piston is forced through the hole in the first separator into the space behind the second piston as it moves to the right. Similar action occurs with the slug of product in the second section. The product in the third section is forced out of the unit. There is no flow past the pistons in the right holder on this half of the stroke.

In the left holder there is no flow of slugs from one section to another because the left valve blocks flow from coming into that holder. The pistons in that holder move to the right forcing the product to go through the holes in the pistons.

As the pistons arrive at the right end of the stroke, the stop on the drive rod trips the valve switch, sending a signal to the valve actuator. The valves are moved to the right as shown in the lower drawing, closing the left valve and opening the right valve for flow of incoming product. This flow forces the drive piston to the left. The actions in the two holders are now interchanged, with product flowing out the left holder and the product in the right holder remaining in each section. As the pistons arrive at the left end of the stroke a stop on the drive rod trips the valve switch and the valves are returned to the original position for starting the next stroke.

The speed of the unit is controlled entirely by the volume of product flowing into the unit. The pressure drop in the product stream required to operate the unit depends on the flow resistance in the valving and in the holes in the pistons, separators, and end plates, and on the friction of the O-ring seals on the drive piston and drive rod.

Theoretical timing

Regulations are usually based on an average hold time of not less than 3.5 min. If the unit as shown in Fig. 1 is operated at one complete stroke per minute and there is no mixing between slugs, the theoretical time pattern is given in Table 1. The average hold time is 3.5 min, with none of the slug leaving the holder before 3.0 min and all of it out in 4.0 min. This gives a ratio of minimum holding time to average holding time of 86%. In actual practice there will be hold-up of product in the valves and piping and in the clearance between the pistons and separators at each end of a stroke. If there is no forward mixing from a slug the minimum hold time will still be 3 min but the average holding time will be increased slightly. The hold-up does cause a backward mixing from a slug. The maximum holding time becomes unknown but the quantity held more than the theoretical value of 4 min may be quite small.

Experimental Unit

A laboratory model has been built and connected to a small, 1750 rpm, centrifugal pump and two 5-gal supply tanks for

testing (Fig. 2). Specifications are given in Table 2. In this test model the left holder has been omitted. The flow from the left side of the drive piston that would go to this holder is run directly back to the supply tanks after going through the left valve. The drive cylinder and holding cylinder are made from 6-inch o.d. transparent acrylic resin tubing. The pistons and separators are cut from 1/2-inch aluminum plate. The seal for the drive piston required the use of a 5/16-inch hollow O-ring, as the acrylic tubing did not have a uniform diameter. The clearance between the separators and the walls of the holding cylinder is such that the cylinder will just slide over the separators and is not tight to the passage of air bubbles or liquids. The pistons clear the walls of the holding cylinder adequately to prevent significant rubbing at any point. The separators are held in position by two 1/4-inch diameter internal tie rods that rest tightly in sockets in the end plate and center plate. The pistons slide along the tie rods in loose-fitting holes. Each separator and piston in the holder has a 1/2-inch diameter hole for product flow. When the unit was built it was anticipated that valves might be needed in these holes to prevent backward flow, but tests have not shown any need. Product movement throughout the unit is controlled by the two 3-way valves.

The valves are piston type in a 3/4-inch diameter copper tube. This design, while giving satisfactory valve action, imparts a small surge in the flow stream each time valve position is changed. It is believed this surge would be reduced or eliminated by use of a spherical seating design like that found in some air operated sanitary valves and shown in Fig. 1. The valves are actuated by two 15-lb. single-action solenoids receiving current from a microswitch that is tripped by the stops on the drive rod.

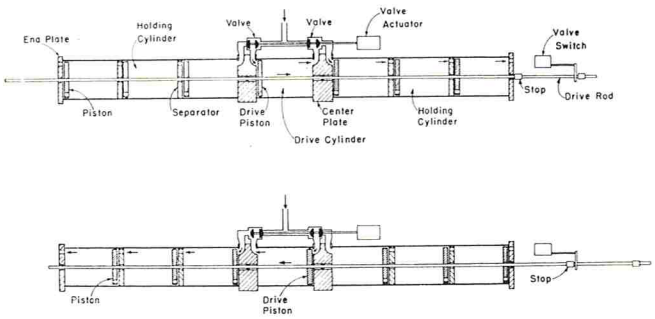

Figure 1. Design for slug flow holder.

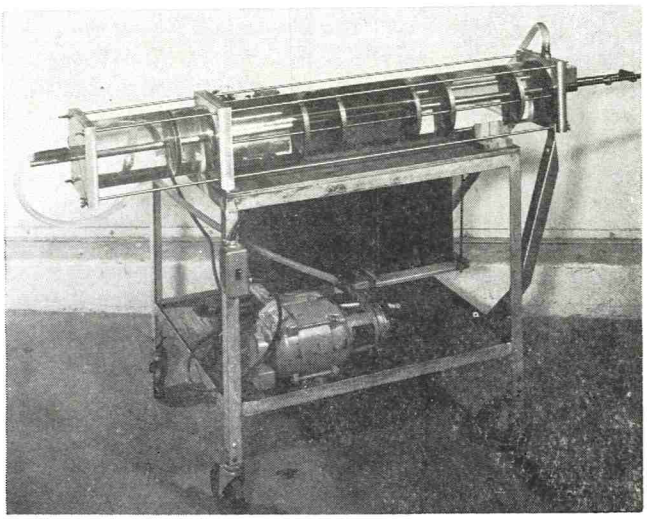

Figure 2. Laboratory model of slug flow holder with pump and supply tanks below. On the left there is clear water in the drive cylinder and between the center plate and the first piston in the holding cylinder. A slug of colored CMC solution extends from the first piston to the second piston with the first separator showing between the two pistons. From the second piston past the second separator and third piston is clear water.

LABORATORY TESTS

The first tests were done with the unit in a vertical position with the holder above the drive so that the flow was upward through the unit. This position permits air and foam to move upward and out of the unit readily. It was quickly concluded that flow in a holder cannot be downward as large air bubbles do not stay with the liquid in a vertically mounted holder. Regardless of the movement of the pistons, they quickly go through the flow holes and wall clearances of the separators and pistons to the top of the holder.

When it is desirable to operate a complete unit of this design in a vertical position, the second holder will have to be mounted separately with the flow also upward. The drive rod in this holder can be operated by a rocker arm or other mechanical linkage from the main drive rod so that the movements of the two drive rods are in opposite directions. It is also possible to design a unit that handles the flow from both sides of the drive cylinder with a single holding cylinder and external piping that is the equivalent of the design shown in Fig. 1. It should operate satisfactorily in a vertical position.

The unit was also operated in an upward inclined position (3/4-inch per foot) and in a horizontal position. After some minor changes the unit performed essentially the same in the three positions except: *(a)* Large air bubbles were slower to clear when the unit was in the horizontal position. Most of the air moved with the liquid slug with which it entered but some was slower in getting through the holder. *(b)* When the unit is vertical and a liquid is followed by a lower density liquid there is some tendency for the latter to float upward in the heavier liquid, thus getting ahead of the slug in which it entered.

Most of the tests were conducted with either water or a carboxymethyl-cellulose (CMC) solution having a viscosity of about 200 centipoises at room temperature. This viscosity is within the range for salt yolk at its pasteurizing temperature. In one of the supply tanks a red dye was added so that slugs of colored fluid could be alternated with clear slugs. Flow within the unit could be closely followed by observing the amount of mixing between the clear and colored solutions and movement of fine air bubbles in the CMC solutions.

TABLE 1. THEORETICAL TIMING FOR MOVEMENT OF SLUG IN SLUG FLOW HOLDER

	Minutes for:	
	Longest held material	Shortest held material
End of filling drive cylinder moving from drive cylinder to 1st section	.5	0
	1.0	.5
no flow in 1st section moving from 1st section to 2nd section	1.5	1.0
	2.0	1.5
no flow in 2nd section moving from 2nd section to 3rd section	2.5	2.0
	3.0	2.5
no flow in 3rd section	3.5	3.0
Start of flow from 3rd section	3.5	3.0
End of flow from 3rd section	4.0	

TABLE 2. SPECIFICATIONS FOR LABORATORY MODEL

Inside diameter of holding cylinder and drive cylinder	5.5	inches
Length of stroke	10.5	inches
Volume per slug in holder	1.07	gallon
Total volume including drive cylinder *and the 2nd holder*	7.5	gallons
Permissible flow rates for 3.5 min average holding time and density of 8.3 lb./gal	1066	lb./hr

Pressures to operate the unit ranged from 3 to 9 psi with momentary surges as high as 15 psi when the valves changed position. The required pressures other than from the surge seemed to be primarily for overcoming the friction of the O-ring seal on the drive piston and the 3 O-ring seals on the drive rod. There were no significant increases in pressure when using the viscous CMC solution. This would indicate that the flow resistances within the unit are from orifice-like restrictions. Pressure drops through orifices are not affected greatly by the viscosity of the fluid.

In some of the early tests in which dyed slugs were interposed between clear slugs, a small amount of the dyed fluid was advancing into the clear slug ahead as the fluid moved up the holder. The tie rods that hold the separators in position take up space in the holding cylinder and there are no tie rods in the drive cylinder. The drive piston moves a volume slightly greater than the space in a section of the holder so some liquid is forced to go to the preceding section. To eliminate this excess a bleeder valve was added so that the flow coming from the drive cylinder to the holder could be reduced. The volume of the two tie rods in a section is about 1 inch^3 or 17 ml. This volume could be compensated for by having a drive cylinder about 0.01 inch smaller in diameter than the holding cylinder. When the unit was being built, it was not anticipated that its actions would be so precise that such small differences in volume would be noticeable. Variations in the diameter of acrylic tube used to make the holding and drive cylinders are much greater than this. For future design it may be desirable to make the drive cylinder slightly undersize.

The length of the piston strokes is adjusted by moving the stops that trip the valve switch. It was found feasible to end the strokes with clearances between the separators and pistons as small as 1/16 to 1/8 inch. With less clearance pressure surges and the possibility of jet flows causing fluid to get into the slug ahead were increased. With these clearances 98% of the volume of a 10.5-inches stroke is being used. Within this clearance space there is good turbulence at the end of each stroke that moves out much of the residual fluid from the previous stroke.

The present unit was built to establish the slug flow characteristics of the design. It has not been tested as part of an egg pasteurizing system. For such use it would be necessary to modify the design so it would be able to withstand the pressures and so it could be cleaned with the usual caustic compounds. A stainless steel unit of sanitary design and of a capacity to match a commercial operation is the next step for further tests.

RESULTS AND CONCLUSIONS

Tests with clear and dyed slugs of water and CMC solutions indicate that this unit may be quite superior to tubing to provide holding time. The weaknesses previously cited for tube holders are greatly reduced. *(a)* There is no forward mixing at the front of a slug. In a pasteurization operation no part of the product could have a short holding time. *(b)* There are no places where appreciable buildup of either foams or cooked materials can occur. *(c)* The temperature within a slug should be uniform because of the mixing that occurs as the slug moves through the valving going to and from the drive cylinder and through the flow holes in the separators and pistons in the holders. *(d)* After a diversion has occurred and proper temperatures are again reached, the uniformity of product treatment gives a basis for deciding the timing for return to forward flow. *(e)* Mixing of products during changes and mixing of water with product when starting and ending operations would be minimized. In the present pasteurizing systems there is mixing throughout the entire flow system, but most of it is done in the long holding tubes. In the experimental slug flow holder slugs of water and CMC were used in either order with no forward mixing and only slight backward mixing.

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1973 NATIONAL EXPOSITION FOR FOOD PROCESSORS

San Francisco, Jan. 25-28

The Food Processing Machinery and Supplies Association has published a floor plan and list of exhibitors for the 1973 National Exposition for Food Processors (NEFP) which indicates the Industry show will be the largest ever held in San Francisco. Traditionally, the west coast location attracts the greatest number of Food Processing Industry members to the NEFP. It is the largest annual show in the Food Processing Industry.

According to FPM&SA, sponsors of the Exposition, the combined 124,000 square feet of space in Brooks Hall and in the Civic Auditorium is being used to accommodate the exhibitors' displays of equipment, machinery, supplies and services. Industry members attending the four-day Exposition represent every segment of food processing, and account for more than 65 percent of the processed food production in the United States. In addition, many non-food manufacturers attend the show to see new containers, materials handling machinery and other equipment and supplies which they use in their industries. All food processors, and food manufacturers using equipment and services on display may attend the NEFP without charge.

The National Canners Association, which conducts its annual Canners Convention during the Exposition, is again participating with FPM&SA and is sponsoring a number of technical sessions during the week. In addition, workshop sessions on food production, preparation, processing and packaging are planned.

Information on hotel accommodations in San Francisco have been mailed to the Industry by FPM&SA. Requests for hotel reservations for the NEFP should be sent to FPM&SA. Complete details on the 1973 NEFP are available from FPM&SA, 7758 Wisconsin Avenue, Washington, D. C. 20014.

INSTITUTE OF ENVIRONMENTAL SCIENCES 19th ANNUAL TECHNICAL/TUTORIAL MEETING AND EQUIPMENT EXPOSITION REALISM In Environmental Testing and Control

The Institute of Environmental Sciences will hold its 19th Annual Technical/Tutorial Meeting and Equipment Exposition at Disneyland Hotel, Anaheim, California, April 1-5, 1973. The theme will be "REALISM in Environmental Testing and Control". The purpose of the meeting is to provide a forum for exchange between educational, industrial, and governmental activities and to impact the nation's environmental programs with REALISM in relationship to goals, testing, measurement, and control of the environments.

A great effort is being made by the government, universities, industries, and general population to understand the earth's environment, to effectively allocate the resources it provides for generations to come. With each new understanding another complexity and inter-relationship is identified requiring further understanding. In addition, furor over the condition of our natural environment has brought about new rules and the establishment of new regulatory agencies intent upon providing a cleaner and more habitable place to work and play.

It is time that these ideals be evaluated in light of how they may be accomplished. The solutions involve political, social, and economic ramifications as well as a practical application of all environmental sciences. Furthermore, these solutions involve the government, industry, academe, and the individual citizen. All must work collectively to implement programs in a realistic manner.

The Technical Sessions of the program will be chaired by Charles Duncan, NASA Goddard, Physical Sciences Section; Dr. Henry Wohlers, Consultant, Ecological Sciences Section; Dr. R. R. Bouche, Endevco Corp, Government Standards and Practices Section; and Park W. Espenschade, Manager, Education and Training Division, the Tutorial Program.

For further information write Institute of Environmental Sciences, 940 East Northwest Highway, Mt. Prospect, Ill. 60056.

A COMPARATIVE EVALUATION OF OFFICIAL METHODS FOR THE STANDARD PLATE COUNT OF CREAM

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(Received for publication December 9, 1971)

Abstract

Three official methods to measure cream samples for Standard Plate Counts (SPC) were reviewed. A statistical analysis was made of results from 135 analysts over a 2-year period. Results demonstrated that: *(a)* pipetting samples is the least precise method to prepare cream samples for the SPC, *(b)* when compared to the SPC variance of 0.012, variation among analysts is significant for the procedure that weighs 11 g of cream, while the among-analyst variance is not significant when the 1-g weighing procedure is employed, and *(c)* weighing 1 g of cream is the most precise method to prepare cream samples for SPC determinations.

A program of splitting dairy product samples for examination by Standard Plate Count (SPC) and other methods has been conducted since 1957 by the Food and Drug Administration, Division of Microbiology, with analysts in State Department of Health and/or Agriculture central milk-grading laboratories participating in the Cooperative State-Public Health Service Program For Certification of Interstate Milk Shippers. This program has resulted in development of methods to detect outliers and to judge performance of the individual analyst, based on the extent of variation of his results from the logarithmic mean.

The 12th edition of *Standard Methods for the Examination of Dairy Products (1)* (SMEDP) recommends three methods to measure the Standard Plate Count of cream products: *(a)* weigh 1 g, *(b)* weigh 11 g, or *(c)* pipette 11 ml of cream into a 99-ml dilution blank at 20 to 25 C or 35 to 40 C.

Before 1968, SPC results for cream samples were not subjected to statistical analysis because the samples could not be classified as "normal" milk samples *(3, 4)*. For usage in split samples a "normal sample" is defined as any fluid milk or milk product (raw or pasteurized) which has not been heated in the laboratory and to which nothing has been added in the laboratory other than raw milk or bacteria normally present in milk (added to produce a desired range of counts). In 1968, 56 SPC results *(5)* from analysts for the three "normal" cream samples examined were outside acceptable statistical limits *(3, 4)*. Results *(6)* from analysts for the three "normal" cream samples examined in 1969 revealed 70 SPC results outside acceptable limits. A variance of 0.012 using $\log_{10}$ counts was the basis *(2, 3)* for determining acceptable agreement of the SPC results among analysts examining the cream samples and the value of 0.005 *(2)* for the limit of variation among replicates. To determine whether the large number of analyst results being out of limits resulted from method of sample preparation before plating, a comparative analysis was performed on results of the three official procedures for preparation of cream samples for the years 1968 and 1969.

Materials and Methods

Split cream samples were prepared as part of the annual FDA Proficiency Testing Program as described in the Public Health Service Publication *Evaluation of Milk Laboratories (4)*. These samples were shipped to 128 and 143 state central laboratory analysts in 1968 and 1969, respectively. The three cream split samples shipped for each year consisted of one sample in blind duplicate and a single sample. Analysts were instructed to measure the SPC of the cream samples using one of the three recommended procedures *(1)*.

All data submitted were statistically analyzed to determine: *(a)* if the average recovery between duplicate samples differed significantly when compared to the among-replicate variance of 0.005, *(b)* if the variability among analysts for the three methods was significantly different, and *(c)* if the variability among analysts for the three methods differed significantly when compared to the accepted variance of 0.012. All tests were performed at the $\alpha = 0.01$ level.

Results and Discussion

The SPC results for the cream samples by method of preparation, in terms of geometric mean counts for each of the 2 years, are given in Table 1. These results show that bacterial counts using 11-ml measurements with a pipette were lower on 5 of the 6 samples than the counts with either of the weighing methods. None of the tests (Table 1) for differences among duplicates compared to the variance 0.005 was observed to be significant.

The pooled among analyst sample variance of the three recommended procedures for 1968 and 1969 was 0.042. This is more than three times the accepted variance for the SPC of milk, which is 0.012. If data for pipetted samples are deleted, the total pooled variance for the 2 years recomputed is 0.029, which is considerably less than the three-procedure variance of 0.042 but still more than twice the accepted variance of 0.012 for the SPC of milk.

The six pooled variances (Table 2) were examined

TABLE 1. GEOMETRIC MEAN COUNTS PER GRAM (OR MILLILITER) OBSERVED FOR CREAM IN TWO SPLIT SAMPLE STUDIES

Year and method	Sample no.			Test between means of duplicates[c]
	Duplicates			$t = \frac{(\overline{X}_1 - \overline{X}_2)}{\sqrt{\frac{2(0.005)}{n}}}$
	1	2	3	
1968				
	(14)[a]	(14)	(14)	
11-ml pipette	59,000	57,000	58,000	0.56[b]
	(85)	(85)	(85)	
11-g weigh	110,000	110,000	72,000	0.00
	(29)	(29)	(29)	
1-g weigh	94,000	91,000	87,000	0.76
1969				
	(18)	(18)	(18)	1.68
11-ml pipette	230,000	210,000	46,000	
	(95)	(95)	(96)	
11-g weigh	270,000	270,000	43,000	0.00
	(29)	(29)	(29)	
1-g weigh	250,000	240,000	43,000	0.95

[a]Number of observations for the mean.
[b]None of the six tests exceeded the critical value at the $\alpha = 0.01$ level.
[c]The value of 0.005 was used as the limit of variation among replicates of the same sample.

TABLE 2. AMONG ANALYST VARIANCE FOR THE MEASUREMENT PROCEDURES

Year and method	Sample no.			S^2_i Pooled variances	$F_{\nu\ \infty} = \frac{S^2_i}{0.012}$
	Duplicates				
	1	2	3		
1968					
	(13)[a]	(13)	(13)	(39)	
11-ml pipette	0.16640	0.18968	0.17634	0.17747	14.79[b]
	(84)	(84)	(84)	(252)	
11-g weigh	0.07518	0.06424	0.01962	0.05301	4.42[b]
	(28)	(28)	(28)	(84)	
1-g weigh	0.00414	0.00456	0.03988	0.01619	1.35
1969	(17)	(17)	(17)	(51)	
11-ml pipette	0.17174	0.14089	0.04483	0.11915	9.93[b]
	(94)	(95)	(95)	(284)	
11-g weigh	0.02431	0.02576	0.00992	0.01998	1.67[b]
	(28)	(28)	(28)	(84)	
1-g weigh	0.00919	0.00891	0.00684	0.00831	0.69

[a]Degrees of freedom (ν).
[b]Significant at $\alpha = 0.01$ level.

using Bartlett's Test for homogeneity of variance. A $x^2_9 = 276.53$ was computed and is significant at the $\alpha = 0.01$ level. The six pooled variances representing the three methods over the 2 years were then compared to 0.012. The F Tests are shown in Table 2 and indicate that only the variance among analysts using the 1-g weighing procedure did not differ significantly from the standard.

The pooled method variances (Table 2) during the 2-year study demonstrate: *(a)* that pipetting samples is the least precise method of preparing cream samples for the SPC, *(b)* the variation among analysts is significant for the procedure that weighs 11 g of cream compared to the standard of 0.012, while the among-analyst variation is not significant for the 1 g weighing procedure, and *(c)* weighing of 1 g of cream is the most precise method.

The sample SPC variance for all measurement procedures (Table 2) decreased in 1969 from 1968. Despite the decrease in variance of all methods, the pipetting procedure variance is still excessive, indicating that the methods in which either 1-g or 11-g portions are weighed are preferable for obtaining the SPC of cream samples.

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The Modern HAYNES-SPRAY Method of Lubrication Conforms with the Milk Ordinance and Code Recommended by the U. S. Public Health Service

The Haynes-Spray eliminates the danger of contamination which is possible by old fashioned lubricating methods. Spreading lubricants by the use of the finger method may entirely destroy previous bactericidal treatment of equipment.

PACKED 6-12 oz. CANS PER CARTON SHIPPING WEIGHT—7 LBS.

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LOW COST...RE-USABLE

LEAK-PREVENTING

NEOPRENE GASKET for Sanitary Fittings

Check these SNAP-TITE *Advantages*

Tight joints, no leaks, no shrinkage
Sanitary, unaffected by heat or fats
Non-porous, no seams or crevices
Odorless, polished surfaces, easily cleaned
Withstand sterilization
Time-saving, easy to assemble
Self-centering
No sticking to fittings
Eliminate line blocks
Help overcome line vibrations
Long life, use over and over

Available for 1", 1½", 2", 2½" and 3" fittings.
Packed 100 to the box. Order through your dairy supply house.

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HAYNES SELF-CENTERING SNAP-TITE® *Gaskets*

SIZES 1" - 1½"
2" - 2½" - 3"

*MADE FROM
TEFLON®

"The Sophisticated Gasket"

THE IDEAL UNION SEAL FOR BOTH VACUUM AND PRESSURE LINES

Gasket Color . . . slightly off-white

SNAP-TITE self-centering gaskets of TEFLON are designed for all standard bevel seat sanitary fittings. They SNAP into place providing self-alignment and ease of assembly and disassembly.
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FOR A FITTING GASKET THAT WILL OUT-PERFORM ALL OTHERS...

Specify . . . HAYNES SNAP-TITES of TEFLON

- *TEFLON ACCEPTED SAFE FOR USE ON FOOD & PROCESSING EQUIPMENT BY U. S. FOOD AND DRUG ADMINISTRATION*

*Gaskets made of DuPont TEFLON® TFE-FLUOROCARBON RESINS

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All Lubri-Film ingredients are approved additives and can be safely utilized as a lubricant for food processing equipment when used in compliance with existing food additive regulations.

ESPECIALLY DEVELOPED FOR LUBRICATION OF FOOD PROCESSING AND PACKAGING EQUIPMENT

For Use in Dairies — Ice Cream Plants — Breweries — Beverage Plants — Bakeries — Canneries — Packing Plants

SANITARY • NON TOXIC • ODORLESS • TASTELESS

SPRAY — PACKED 6 — 16 OZ. CANS PER CARTON
TUBES — PACKED 12 — 4 OZ. TUBES PER CARTON

THE HAYNES MANUFACTURING CO.
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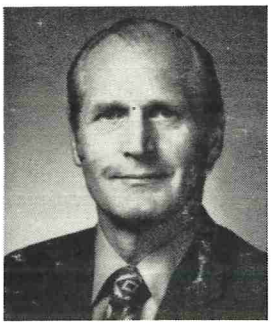
Dairy authorities speak out on better cow milking.



Better cow milking and your dairy income

Donald E. Jasper, DVM, University of California, Davis

DOES IMPROPER MILKING COST YOU $64 PER COW PER YEAR? The answer may well be Yes—and it may be twice that. Furthermore, most of this cost or reduced income comes entirely from the dairyman's profits. In contrast, good cow milking and a sound mastitis control program have a relatively small cost.

There is no substitute for good cow milking. After the expense and effort of careful breeding and feeding, it just makes good sense to get all the milk the cow produces. Good cow milking includes proper pre-milk stimulation, establishing a favorable routine which makes milking a pleasant experience for the cow and gets her full cooperation. Treat each quarter as an individual and guard against over-milking. Besides the benefit of increased production, good cow milking is also the best defense against the dairyman's worst enemy, mastitis.

A sound mastitis detection and control program makes you money in many ways. It can save on total treatment costs, lets you sell all your milk, helps increase production per cow, produces a higher quality product, and extends the milking life of cows in the herd. The table illustrates the value of the savings which would come by reducing the CMT score on bulk tank milk from 2 to trace. This would mean reducing the leukocyte count from over 2 million to less than 500,000. Although most dairies are now consistently below 1,500,000 on their cell count some are still pushing the limit and bounce into the 2 million cell range from time to time. On the other hand, our best dairies have bulk tank CMT scores of negative to trace and cell counts in the 300,000 to 500,000 range.

The average dairy may have tank milk CMT scores of 1 and cell counts of about 900,000. If such dairies can change the CMT scores to trace and their cell counts to about 400,000 their increased income may be about one half that shown in the table, or $40 to $70 per cow, depending upon per cow capability and other factors.

These figures on loss, or potential profits, still do not include several important economic aspects of mastitis control. For example, labor costs are reduced and less culling permits a better opportunity to select replacement heifers and permits more unneeded heifers to be sold. Cows are permitted to stay in the herd longer and to reach their greatest production potential, further increasing the herd average. Each owner can add such values to those listed in the table.

PROFITABLE DAIRYING MEANS DOING EVERYTHING RIGHT. It is like a relay race. If one runner drops the baton, the others can seldom run fast enough to win the race. It begins with understanding of the cow. Gentle treatment and calm surroundings permit successful completion of all the important steps of good milking.

Begin with observation of milking procedures and a thorough check of the milking system. Sometimes substantial changes are necessary to bring older installations up to modern standards. All milking equipment needs regular maintenance and every dairyman should arrange for a competent person to regularly check his equipment and to make repairs promptly. Preventive maintenance is inexpensive insurance to protect the dairyman's investment and income.

Mastitis control requires management and treatment of the infection problem. Lactation treatment, dry period treatment and dipping of teats after milking all play important roles in mastitis control. Optimum application of these tools will vary depending upon herd conditions and the infections involved. It is here that the veterinarian competent in mastitis control is so valuable to the dairyman.

QUALITY MILK. Many high bacterial counts are due to mastitis organisms and high leukocyte counts are a direct result of mastitis. Control is essential to good milk quality. It protects the dairyman's investment and can substantially enhance profit margins. Mastitis control is possible, and not particularly difficult. *It starts with good cow milking and requires that everything is done right.*

DOLLARS RETURNED BY MASTITIS CONTROL

Potential Production pounds/year	Dollars Received per Cow from			Increased Income	
	Increased Production[1]	Reduced Treatment[2]	Reduced Replacement[3]	Dollars per cow	Dollars per 100 cows
8,000	48	15	15	78	7,800
10,000	60	15	17	92	9,200
12,000	72	15	20	107	10,700
14,000	84	15	22	121	12,100
16,000	96	15	25	136	13,600

[1]By reducing the bulk tank CMT from 2 to trace.
[2]By reducing treatments from 2 cases per week to 1 case per 2 months.
[3]By reducing replacement of low producing cows from 10 to 5 cows per year.