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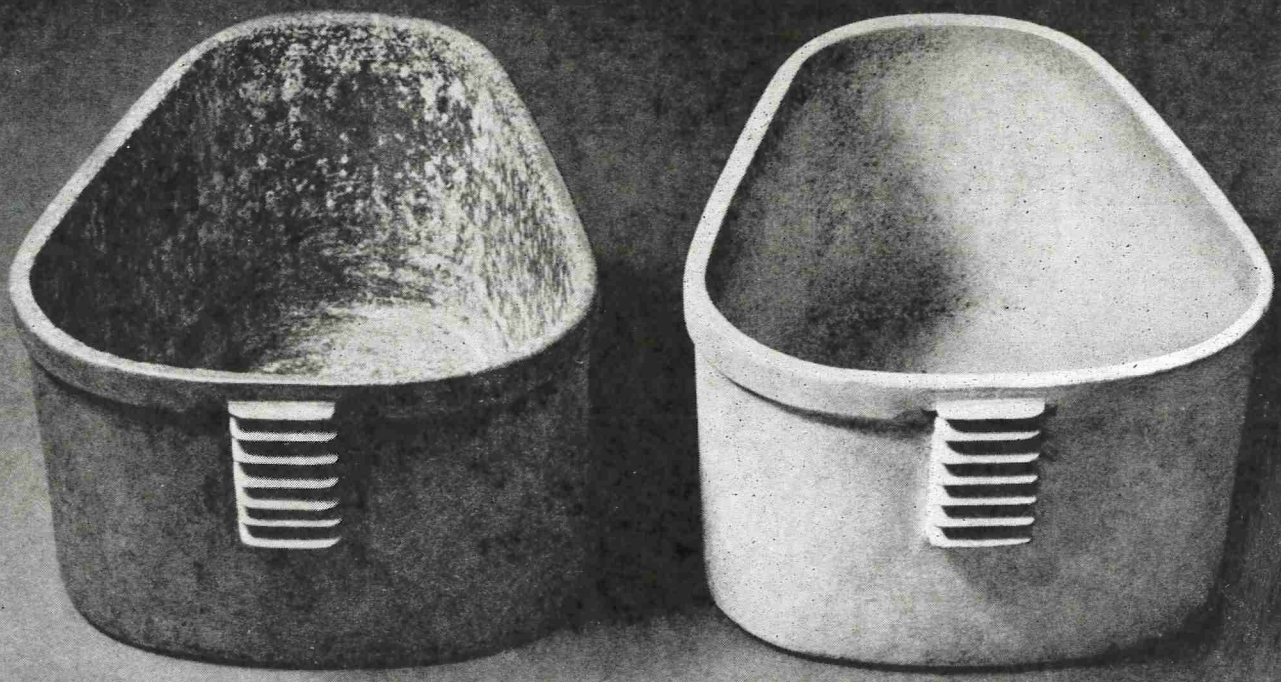

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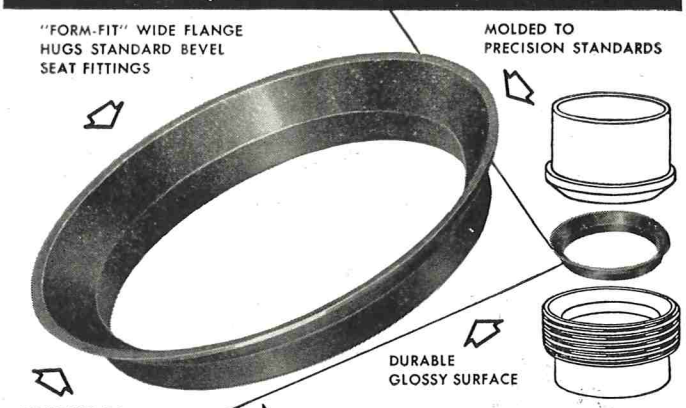

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SURVEILLANCE METHODS FOR VIRUSES IN FOODS[1]

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Abstract

Viruses contaminate foods and sometimes cause illnesses in consumers. Methods have been needed for detection of food-borne viruses both in routine field samples and in samples associated with outbreaks of disease. Viruses are detected by inoculating living hosts such as tissue cultures or laboratory animals. Food samples, made fluid if necessary, can be inoculated directly into the test host. This approach has resulted in several isolations of viruses from field samples of foods. If greater sensitivities are desired, larger samples must be tested. This usually requires that the sample be concentrated before inoculation into the test host, and concentration can be performed only if food solids in the suspension are at a minimum. A family of methods has been developed for extraction and detection of enteroviruses from food samples. More recently, a procedure for dislodging enteroviruses from food surfaces has been devised. These procedures do not possess all of the desired properties of an ideal test method. In particular, they cannot detect all of the viruses known to be transmitted in foods. They do offer the possibility that several samples could be tested per day, with good sensitivity, at a moderate cost per sample. The means for routine surveillance of foods for virus contamination are at hand. Growing points for further development of surveillance methods are discussed.

Viruses are sometimes transmitted by foods *(7)*. Most food-borne disease is preventable with the means at hand, and the same is true of food-borne virus disease. Food-borne diseases occur, however, and surveillance methods are needed to assist in controlling them.

Surveillance of viruses presents some special problems because viruses are submicroscopic in size and are totally incapable of multiplying outside of appropriate living host cells. This means that viruses cannot multiply in foods and are seldom present in the quantities typical of many other food-borne disease agents. Another consequence of these properties is that, for most purposes, virus can only be said to have been detected when it produces a demonstrable infection in some living host.

Depending upon the virus sought, a suitable living host might be a human, a laboratory animal, or a tissue culture. Tissue cultures have been used exclusively in the authors' laboratory because of their convenience, uniformity, and relatively low cost *(8)*. All living hosts are expensive, however, and none is ideal in other respects. Humans have varying immunological experience, and no laboratory host is susceptible to all of the viruses which infect man.

There are two situations in which foods might have to be tested. These have been designated "field sampling" and "epidemiologic investigations," depending principally upon whether or not people have been made ill by the foods. Properties to which to aspire in developing tests for each of these situations are shown in Table 1. Two of the entries need qualification. First, the 50% end-point of a test procedure is defined as the least quantity of virus which, if present in a sample, is at least 50% likely to be detected. Second, the "possible" virus types are those which might have caused the symptoms seen in an outbreak or which might occur in the food under scrutiny. Enteric viruses (those most common to the human intestinal tract) have usually been emphasized in developing detection methods for food-borne viruses, for several reasons.

Direct Testing

Foods to be tested may be categorized as fluids, surface-contaminated solids, and permeable solids or semisolids likely to be contaminated in depth. In any event, the sample must be fluid before it can be inoculated into a test host. A laboratory diluent may be used as added fluid *(3)*, or liquid from the food itself (e.g., shell liquor from oysters; *22*), may be employed. Foods contaminated throughout may simply be homogenized with the diluent, or attempts may be made to extract the virus into the diluent *(2, 27)*, leaving the food solids behind. As will be discussed below, surface-contaminated solid foods can probably best be sampled by dislodging the contaminant into the fluid phase with as little of the food substance as possible.

The fluid suspension thus obtained might be inoculated directly into the test host *(13)*. If it is toxic or shows a high level of microbial contamination, further treatment may be necessary before inoculation. This might include centrifugation *(20)*, filtration, addition of antibiotics *(26)*, or treatment with an organic solvent *(10)*. The last of these may not be applicable to certain situations in which lipid-containing viruses are sought, for organic solvents inactivate such viruses. However, the enteric viruses

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are not inactivated by most of the organic solvents.

The approach which has been described may be called direct testing. The food sample receives a minimum of treatment and is then inoculated directly into a suitable living host. It works: several isolations of viruses and rickettsia from field samples of food have been achieved in this way. Viruses of the tick-borne encephalitis complex have been isolated from the milk of naturally infected goats in Russia *(25)* and Czechoslovakia *(12)* by mouse inoculations. The rickettsia of Q fever *(Coxiella burnetii)* has been demonstrated in samples of raw cow's milk and cream and, in one instance, a pasteurized mixture of the two by inoculation of guinea pigs *(4, 11)*. The bovine strain (SF-4) of parainfluenza virus type 3 has been detected in the milk of at least 14 cows by tissue culture inoculation *(17)*. Direct tissue culture inoculation has resulted in isolations of enteroviruses from market samples of mussels *(3)* and of ground beef *(27)*, from vegetables from a sewage-irrigated field *(1)*, and from foods and food-contact surfaces in an outbreak of illness at a children's day-care center *(23)*.

Direct testing does place some practical limits upon the sensitivity which can be attained, but this may not be important. Unfortunately, there is no completely objective way of deciding how sensitive a test procedure for food-borne virus must be. One almost never knows how much of a virus is required to produce an infection by the oral route. Even if this information were available, it would seem unrealistic to say that a food was "unsafe" if it caused illness in everyone that ate it, but was "safe" if it infected only one of every 10 consumers. There are no enrichment techniques for viruses, so greater sensitivity can be achieved only by testing larger samples. This may not be possible if larger quantities of the food are not available; almost all of these are destructive tests. If larger samples of food can be obtained, one must then consider the quantities of tissue cultures or other laboratory hosts which would be required to test them by the direct method.

TESTING AFTER CONCENTRATION

It seemed unlikely that the level of sensitivity specified in Table 1 could be achieved by direct testing because of costs *(15)*. The most attractive alternative approach was to concentrate the food suspension before inoculating it into tissue cultures. Such concentration simply reduces the volume of sample to be tested. Many concentration methods have been examined and ultracentrifugation *(9)* and dialysis against hydrophilic substances *(5)* appear to be the most reliable. Each has been used successfully by others to detect viruses in field samples of shellfish *(18, 21)*. The two have now been combined. A 25-g food sample is concentrated first by dialysis against polyethylene glycol 20,000 and then by ultracentrifugation so that the final volume (0.5 ml) can be tested in a single tissue culture *(15)*. Most of the food solids must be eliminated from a sample suspension if this great a concentration is to be performed.

TABLE 1. DETECTION PROCEDURES FOR FOOD-BORNE VIRUSES: DESIDERATA

Property	Routine field sampling	Epidemiologic investgations
Sample size	10 - 100g	100 - 1,000g
Sample number	10-50/operator-day	4-10/operator-day
Direct cost/sample	$5	$ 25
Sensitivity	50% end-point = 1 infectious unit/sample	
Spectrum	100% of possible virus types	
Elapsed time	≤7 days	

Foods contaminated in depth

Several solid and semisolid foods were inoculated with laboratory strains of enteroviruses. The virus was assumed to be within the food, rather than on its surface. Some foods gave the best separations and virus recoveries if treated with trichlorotrifluoroethane (Freon TF, DuPont) and bentonite, while others (usually those low in protein) were better treated with Freon and agamma serum (Fig. 1). Using the method found to be best for each of the foods, the 50% end-point for detection of three model enteroviruses was in the range of 2 to 4 infectious units per sample *(16)*. It was also determined that neutralized enteroviruses, such as might be shed during the convalescent phase of an infection, were reactivated by the Freon treatment.

These methods are complex but are not difficult to learn. It is estimated that an experienced operator, with proper equipment, could test 12 to 24 samples per day at a direct cost of $4 to $8 per sample. These

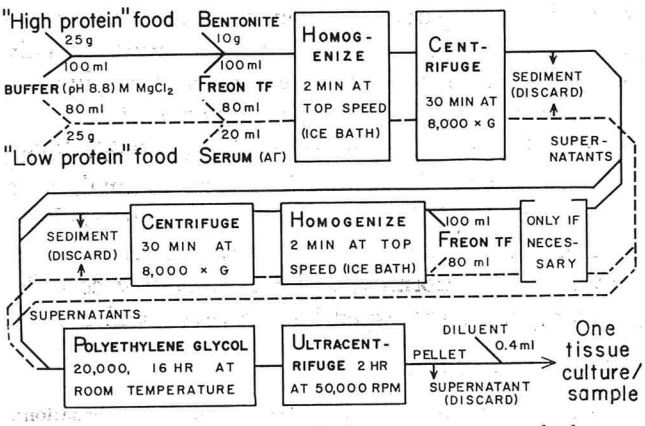

Figure 1. Detection methods for enteroviruses which contaminate foods in depth (schematic summary).

procedures will have to be adapted further to permit their use in detecting agents other than the enteroviruses. Larger or more labile viruses tend to be inactivated or lost during the processing.

Surface contaminated foods

The situation is somewhat different with surface-contaminated foods. It seemed that it should be possible to dislodge the virus from the contaminated surface into a fluid suspension without including any of the substance of the food. This, in turn, should simplify preparation of the sample for concentration and might even permit use of simpler, more rapid methods of concentration than those applied to clarified food suspensions. Model foods used in the initial studies on surface sampling included tomatoes, peaches, and (in the winter) well-worn tennis balls. These were contaminated with standard quantities of feces from a child who was undergoing immunization with trivalent oral poliomyelitis vaccine. The fecal specimens had titers in excess of 10^6 plaque-forming units (PFU) of poliovirus per gram as determined in tissue culture by methods described previously *(8)*.

The exploratory phases of the study included tests of surface-active agents, an ultrasonic probe, and brushing as means of dislodging the virus from the food. It was found that essentially all of the virus could be dislodged by rubbing a glass stirring rod over the inoculated area, and that none of the more elegant techniques listed was quite as good. The disadvantage of the glass rod was that it was not well suited to sampling the entire surface of a food when the point of contamination was unknown. An apparatus devised for this purpose is shown in Fig. 2. The sample (a tomato) is placed in a 2-qt., wide-mouth fruit jar of square cross section. The lid of the fruit jar has been punctured off-center and fitted with a steel shaft which is driven through a flexible rubber connection by an electric motor and continuously variable transmission. The shaft on the jar lid is passed through an improvised bearing, and the butt end of the jar is supported by floating it in a pan or sink of cold water (not shown). Also inside the jar are approximately 20 common glass marbles and 100 ml of 0.03 *M* phosphate buffer (pH 7.2). The jar is turned at approximately 100 rev/min for 5 min, and the fluid washings are collected.

It was found that essentially all of the contaminating virus was recovered from the surfaces of the test foods by this method. Limited tests were also done with pears, strawberries, and lettuce. Periods of storage between contamination and testing were found to have little influence upon the tenacity with which the virus adhered to the food surface. Food solids were not present in the washings unless portions

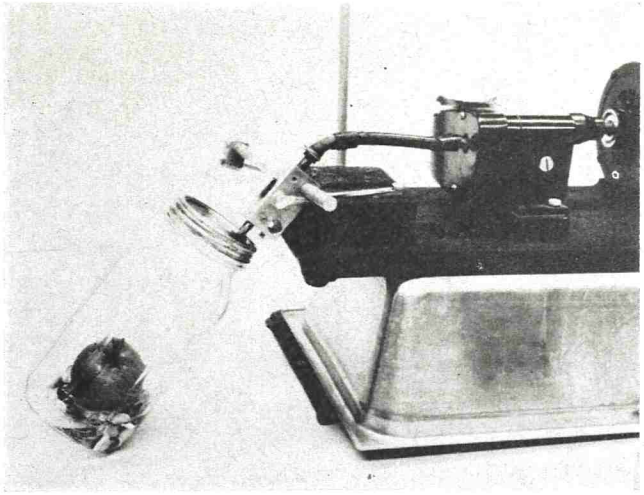

Figure 2. Apparatus for dislodging viruses from contaminated food surfaces.

of the surface were badly decayed. Surface dirt, fecal solids from the model contaminant, and a variety of bacteria appeared in the washings, but these were of no concern at this stage of the work.

The next task was to concentrate the washings to permit detection of lower levels of contamination. Since there were no food solids in the suspension, it seemed possible that the virus could be collected by adsorption to membrane filters and eluted in a small volume of fluid *(6)*. Unfortunately, dirt from the food surface made the suspension very difficult to filter; and even when enough of the dirt had been eliminated to permit filtration, the virus did not adsorb efficiently to the membrane filters. The aqueous polymer two phase system, which has been used to concentrate viruses from water and sewage *(19, 24)*, was also tested. The dextran sulfate in the virus concentrate was found to inhibit strongly some of

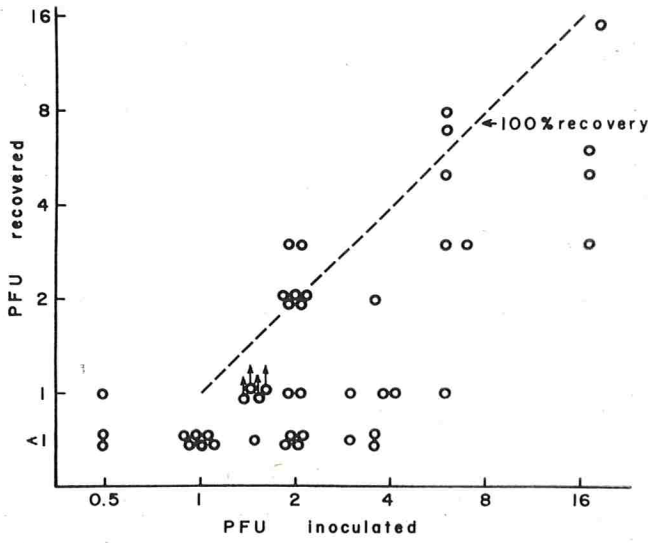

Figure 3. Recovery of CB-3 virus from experimentally contaminated tomatoes.

the enteroviruses used in evaluation experiments *(14)*. It was decided that for the time being the concentration procedure outlined in Fig. 1 would be used with surface washings. The first model agent in these studies was Coxsackie virus type B-3 (CB-3), strain Nancy.

Two problems were encountered at times with the concentrated samples. First, the quantity of inoculated feces used to contaminate the tomato surface was sometimes sufficient to interfere with concentration. Second, surface microflora were sometimes encountered which concentrated with the virus and could not be suppressed by any of a number of antibiotics. Serial membrane filtrations solved neither of these problems very efficiently. Centrifugation at 7,500 rev/min for 20 min was found to eliminate fecal solids when necessary. Microbial contaminants were suppressed by adding 1 ml of chloroform to the wash water at the time that dislodgement of virus from the sample surface was begun. This complicated the process somewhat, because the chloroform is incompatible with the polyallomer ultracentrifuge tubes used in concentration and is highly toxic to tissue cultures. Cellulose nitrate tubes were used during the polyethylene glycol concentration step with samples containing chloroform. Surplus chloroform was removed at the end of this step by centrifugation at 3,000 rev/min for 20 min, and the supernatant fluid was collected by aspiration. Air, sterilized by membrane filtration, was sparged through each sample for 10 min to entrain any residual, dissolved chloroform. The final ultracentrifuge concentration shown in Fig. 1 was then carried out as usual.

Over a period of time, a total of 32 tomatoes contaminated with low levels of CB-3 virus have been tested in this manner. Chloroform was used in the majority of these tests. The results are shown in Fig. 3. The 50% end-point for this series of determinations is somewhat less than 2 PFU per tomato, and the ratio of virus recovered to virus inoculated is of the order of 49%. This suggests that the method is somewhat more sensitive than that developed for internally contaminated solid foods, as judged by either parameter; however, recoveries in limited trials with another enterovirus, ECHO virus type 6 (EC-6), strain D'Amori, have not been as good. EC-6 had proved to be an unusually difficult enterovirus in past studies involving other properties *(8, 14, 16)*. Tests are continuing.

DISCUSSION

Methods are now available for the surveillance of many foods for enterovirus contamination. Neither the procedure for internally contaminated foods, reported previously, nor the method for surface contaminated foods, described here for the first time, entirely fulfills the hopes expressed in Table 1. Direct costs have been estimated at $4 to $8 per sample for the methods summarized in Fig. 1, and those for the surface sampling procedure should be comparable. Though the sensitivities of the two systems still leave something to be desired, they appear to be significantly better than any of those described by others. The size of an internally contaminated sample in a field testing situation was arbitrarily standardized at 25g, while tomatoes for the surface sampling procedure have been as large as 100 to 140g. These can be tested at the rate of 12 to 24 per operator-day. Larger samples, such as might be acquired when investigating an outbreak, would have to be divided. This would result in a proportionate loss in the number of samples which could be tested per day.

There are several growing points for future development of detection methods for food-borne viruses. One of the most significant is speed. Any serological, physical, or chemical method which would detect virus would be faster than the infectivity process, though probably with a sacrifice of 100-fold or more in sensitivity. These alternative methods might well require more expensive equipment, whereas the authors' research program is dedicated to devising procedures which allow use of less expensive facilities, but the trade-off might be justified in some circumstances.

More immediate goals are to adapt the test methods to a broader range of foods and to permit detection of a broader spectrum of viruses. Many of the direct test methods will permit detection of a broader range of viruses at some loss of sensitivity to any one type. The manipulations involved in preparing samples and concentrating them would inactivate some viruses outside the enterovirus group. The laboratory hosts in which infectivity is demonstrated also impose a significant limitation upon spectrum: there is no one of them which is susceptible to all of the viruses which infect man.

It seems highly unlikely that the perfect test method, able to detect all viruses in all foods at a low cost per sample, will ever materialize. Still, the means at hand could be expected to provide some very worthwhile information if they were applied to foods in the field on a routine basis.

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CURRENT DEVELOPMENTS IN DETECTION OF MICROORGANISMS IN FOODS-*CLOSTRIDIUM PERFRINGENS*[1]

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ABSTRACT

Recent work on *Clostridium perfringens* foodborne disease has established that any type, whether classical or "food-poisoning," may cause illness if present in sufficiently large numbers. Cultural methods for detection and enumeration of the organism have been suggested, and some of these methods have proven satisfactory in field trials. New approaches, such as the cultural or serological examination of patients' feces or the direct examination of foods for toxin content, have been suggested. The problem has been shown to be of sufficient importance to warrant continued and increased study.

HISTORY

The type of foodborne illness that results from the microorganism, *Clostridium perfringens*, is not a new disease, although most of the work on its detection has been carried out in the last 15 to 20 years. As early as 1895, Klein *(21)*, in England, described outbreaks of foodborne illness caused by an organism that he called *Bacillus enteritidis sporogens*. His description of the isolates, however, indicate that he was probably dealing with *C. perfringens*. Over the years, many similar outbreaks have been reported from England by Andrews *(1)* in 1899, Knox and MacDonald *(22)* in 1943, Duncan *(6)* in 1944, and by Hobbs and associates *(19)* in 1953. In 1933 Nelson *(26)* reported an outbreak in Fargo, North Dakota, involving infants who had became ill from milk contaminated with dust containing spores of *C. perfringens*. The first well authenticated outbreaks in the United States, however, were described by McClung *(24)* in 1945. These outbreaks, which resulted from contaminated chicken and gravy, occurred in 1943, 1944, and 1945. Since McClung's report, many outbreaks have been studied in Great Britian, Europe, Japan, and the United States.

THE ILLNESS

Clostridium perfringens food poisoning is a relatively mild disease. After an incubation period of 8 to 22 hr, patients develop acute abdominal pain and diarrhea. Nausea and vomiting are rare, and pyrexia, shivering, headache, and other signs of infection are seldom present. The illness is of short duration, usually one day or less, and is followed by complete and uneventful recovery.

THE CAUSATIVE ORGANISM

This type of foodborne illness is caused by toxicological type A strains of *C. perfringens*. The classical strains of this type that cause gas gangrene in man are characterized by significant production of the alpha and theta toxins and, therefore, produce both complete and partial hemolysis on blood agar. They also produce spores that are sensitive to heating at 100 C for a few minutes, and, serologically, they are almost strain specific. In the years immediately following World War II, Dr. Betty Hobbs and her associates *(19)* made comprehensive studies of the outbreaks resulting from this organism in Great Britian. They discovered that they were isolating strains of *C. perfringens* with characteristics that differed from the classical strains. These food isolates have been referred to as the "food poisoning type." They produced low levels of alpha toxin, and little or no theta toxin. Therefore, they were nonhemolytic on blood agar or produced only narrow zones of partial hemolysis. They produced heat resistant spores, many of which would withstand boiling for as long as 1 to 5 hr, and they could be typed serologically using 13 antisera *(19)*.

In the early 1960's researchers in the United States Public Health Service in Cincinnati began studying outbreaks of *C. perfringens* foodborne illness and isolates from foods and other sources in the United States. From these studies it was concluded that, in this country at least, there was no specific group of *C. perfringens* strains that could be referred to as "the food poisoning type." Rather, it appeared that foodborne disease was caused by classical strains, the English type, and strains with intermediate charac-

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teristics *(12)*. These observations have been substantiated by excellent work in Canada by Dr. Hauschild and his associates *(15, 16, 17, 18)*. These investigators showed that experimental food poisoning could be produced in human volunteers by a food poisoning isolate that produced nonheat resistant spores *(17)*. Furthermore, using lambs as test animals these workers were able to demonstrate experimental enteritis with both classical and food poisoning strains of *C. perfringens (15)*. Using both types of organisms *(18)*, they also have produced typical gas gangrene in guinea pigs. Using the "intestinal loop technique," they have demonstrated that *C. perfringens* cells, but not culture medium, produce reactions and fluid accumulation and that the reactions were not prevented by alpha-antitoxin *(16)*. Similar results also have been obtained by Duncan et al. *(8)*, using the "rabbit ileal loop technique."

The friendly disagreement between English workers and ourselves concerning the characteristics of the causative organisms of *C. perfringens* foodborne disease has now been resolved. In 1967, Sutton and Hobbs *(30)* published a description of five outbreaks caused by heat-sensitive strains of *C. perfringens* in England. It thus becomes manditory that all of us look for both heat-resistant and heat-sensitive strains when studying such outbreaks.

The question that next comes to mind is quite obvious. What is it about this organism that leads to foodborne disease? The work of Dack *(3)*, Hobbs *(19)*, and Dische and Elek *(4)*, and most recently Hauschild and Thatcher *(17, 18)*, prove that it is not one or another of the preformed toxins. The clinical syndrome of the illness makes it unlikely that it is a true infection. All work to date indicates that a very high level of viable vegetative cells, several millions per gram, are needed to cause symptoms. Dr. Hauschild in a personal communication states, "Our conclusion is that, although the disease is produced by *C. perfringens* cells, the food poisoning factor is produced *in situ* and is probably not a cellular constituent."

It has been postulated that the large number of cells required to produce illness result from mishandling of meats, meat dishes, or gravies. Poor sanitation practices may allow contamination after cooking, and growth of the organism or residual spores may germinate after cooking and grow to substantial levels of contamination. Two recent publications emphasize this latter possibility. Strong and Ripp *(29)* showed that spores of heat-resistant strains of *C. perfringens* survived after cooking of hams, turkey rolls, and ground beef casserole. Futhermore, the number of viable spores was only slightly reduced by refrigeration for 48 hr. Mishandling, such as slow cooling or warming after refrigeration, could allow the outgrowth of the organisms. Pivnick et al. *(27)* showed that the spores of *C. perfringens* survived in barbecued chicken and that outgrowth to significant levels would occur in 12 to 16 hr at 45 C.

CULTURAL DETERMINATION OF OUTBREAKS

It is not the purpose of this paper to discuss all of the recent publications on various aspects of work on *C. perfringens*. A few of these, however, seem quite pertinent to our interests.

The sporulation of *C. perfringens* has been a knotty problem for those interested in studies on the survival of the organism in foods and in time-temperature relationships. Two publications on the problem of spore production have recently appeared. Kim, Cheney, and Woodburn *(20)* proposed a new sporulation medium that combined the relatively large spore crops obtained in Ellner's *(9)* medium with the production of normally heat-resistant spores obtained in SEC broth *(2)*. Their medium contained peptone, trypticase, starch, NaCl, $MgSO_4$, and thiamine hydrochloride. Incubation at 37 C for 24 hr yielded excellent spore crops. Similiarly, Duncan and Strong *(7)* reported on a medium containing yeast extract, proteose peptone, starch, sodium thioglycollate, and Na_2HPO_4 that gave superior results. The addition of activated carbon resulted in additional increases in spore numbers and in heat resistance with some strains. It seems possible that the use of these new media may allow investigators to obtain spore crops for much needed studies on spore survival.

Enumeration of *C. perfringens* in outbreak foods is a necessity in determing the etiology of the disease. Methods for this purpose have appeared in the literature in recent years. Marshall, Steenbergen, and McClung *(23)* published a report on a rapid technique in which a medium containing tryptone, sulfite, yeast extract, polymyxin, and neomycin was used. These authors utilized an incubation temperature of 46 C and reported the medium to be practically specific for *C. perfringens*. To my knowledge, the use of this medium in the study of outbreak foods has not been reported. Green and Litsky *(10)* described a new medium and a Most Probable Number (MPN) technique for enumerating *C. perfringens*. They reported a significantly higher recovery rate for this method as compared to a plating procedure and suggested that it could be of value in quality control work where the number of *C. perfringens* is usually quite low. Hall *(11)* reported the results of a collaborative study of a quantitative method for *C. perfringens* in foods. This method is essentially the same as that described by Angelotti et al. *(2)* in 1962. It was adopted as official, first

TABLE 1. SEROLOGICAL TYPING OF *C. PERFRINGENS* ISOLATES FROM OUTBREAKS

Type of patient	Number of patients tested.	Type of C. perfringens in food.	Type of C. perfringens in patients.	Frequency of occurrence	
				Number	Per cent
Normal	9	None	PS35, PS49, PS14	2	22.2
Outbreak 1	14	(PS72, PS76, PS81A)	(PS72, PS76, PS81A)	12	85.7
Outbreak 2	38	PS89	PS89	24	63.1
Outbreak 3	6	None	(PS66, PS88)	6	100
Outbreak 4	10	None	Hobbs 12	7	70
Outbreak 5	11	PS55, H9, H13	PS24	6	54.5
Outbreak 6	22	H 12 (PS66, PS88)	H 12 (PS66, PS88)	16 4	72.7 18.1
Outbreak 7	15	PS90 PS75	PS40, H13, H3 PS20, PS78, PS89, H4, PS52	2	13.3

action, by the Association of Official Analytical Chemists. The method uses the Sulfite-Polymyxin-Sulfadizene (SPS) agar developed by Angelotti et al. *(2)*. Suitable dilutions of a 1:10 blend of the food are plated in duplicate in SPS agar and incubated anaerobically for 24 hr at 35 C. The black colonies appearing on the SPS agar plates are counted and recorded as the total clostridial count. A suitable number of colonies picked to a nitrate-motility medium and fluid thioglycollate in which they are incubated for 24 hr at 35 C. The nitrate-motility medium is observed for type of growth and tested for nitrate reduction. Sporulation broth is inoculated and tested for spore production. The presence of nonmotile, anaerobic, spore forming organisms that produce H_2S and reduce nitrate is considered to provisionally identify the organisms in the food as *C. perfringens*. This method has been field tested by a number of workers and found to be satisfactory for the examination of outbreak foods.

The most recent suggested medium and method is the SFP agar of Shahidi and Ferguson *(28)*. This medium contains antibiotics and has been used by the New York City Department of Health for the examination of outbreak and other foods.

TABLE 2. CASES OF FOODBORNE ILLNESS OF BACTERIAL ORIGIN REPORTED IN 1968 *(25)*

Bacterial	Cases	
	Number	Per cent
C. perfringens	5,966	40.8
Staphylococci	4,419	30.2
Salmonella	1,287	8.8
Streptococcus	1,282	8.8
E. coli	1,234	8.4
Shigella	407	2.8
Brucella	12	<1.0
C. botulinum	10	<1.0
TOTAL	14,617	

OTHER APPROACHES TO DETERMINATION OF OUTBREAKS

As all those involved in the study of foodborne disease outbreaks know, there are many occasions when no suitable food is available for examination. Either the food has been discarded, or it has been refrigerated, frozen, or mishandled. Because of this, other approaches to the determination of a *C. perfringens* etiology have been suggested. It has been shown, by using enrichment techniques and resampling, that practically 100% of human fecal specimens contain *C. perfringens*. In normal individuals not exposed to outbreak conditions, the level of *C. perfringens* in the feces may be quite low, but, following an outbreak, these levels rise significantly. Dr. Sutton and Dr. Hobbs in England have suggested a simple method of evaluating this situation. A 1:10 suspension of feces is streaked on blood agar containing neomycin in such a manner that three successive streaks are made from one original small drop. The plates are incubated anaerobically at 46 C. If the patient has been recently exposed to a *C. perfringens* outbreak, there will be over 10 colonies in the third streak, indicating a level of about 10^6 organisms per g. Normal individuals, on the other hand, will show growth of *C. perfringens* only in the first streak. The very simplicity of this method indicates that it deserves some trial to determine its usefulness.

As was mentioned earlier, foods for examination may be refrigerated or frozen before receipt by the laboratory. This can reduce the numbers of viable *C. perfringens* by as much as 2 or more logs, thus yielding low results that might be interpreted as

being below the level necessary to cause disease. Harmon *(14)* has suggested a method of testing such foods for the presence of lecithinase. Using either the hemolysin indicator plate or the lecithovitellin test, he was able to detect the alpha toxin (lecithinase) when the viable count of *C. perfringens* had reached levels of 1.8 to 2.3 million cells per gram of food. Such levels and higher are usually found in outbreak foods. It would seem, therefore, that the method has merit for the examination of foods and would allow the determination of a *C. perfringens* etiology when the viable count has been reduced by freezing or subsequent reheating.

Another approach to the determination of an outbreak of *C. perfringens* foodborne disease is the serological typing of isolates obtained from the food and the feces of patients. Furthermore, in those instances in which no food is available for examination, such studies on cultures from the feces of the patients appear to yield equally valid information. If one makes 3 to 4 isolates from the feces of each of a group of normal nonexposed individuals, one can expect that about 10 to 20% of the individuals will be carrying the same serotype. If, on the other hand, a group of individuals have had a common source of exposure to large numbers of a single strain of *C. perfringens*, as occurs in outbreak conditions, one or more of the isolates from most of the individuals will be of the same serotype.

At the present time, a cooperative study is being conducted by the Anaerobic Laboratory of the National Communicable Disease Center in Atlanta and the Milk and Food Sanitation Research of the Division of Microbiology in Cincinnati *(13)*. The NCDC laboratory is studying the serologic properties of strains from outbreaks and our laboratory is examining the cultural characteristics of the same strains. A comprehensive study of the serotypes is being made with the use of over 90 antisera prepared at NCDC *(5)*. Some representative results are shown in Table 1. It can be noted that the first line shows the results with 9 normal nonexposed individuals. Three serotypes—PS35, PS49, and PS14—occurred more than once. In each instance, the serotypes were detected in the feces of two individuals, which indicated that 22.2% were carrying the same serotypes. In the first outbreak, a strain that reacted to three sera—PS72, PS76, and PS81A—was found in the food. This same strain occurred in the feces of 12 of the 14 individuals, or 85.7%. This indicates a common source of fecal contamination. Outbreak two was a very large one, involving over a thousand people. Data on isolates from 38 of those who became ill are shown. The food contained serological type PS89, as did 24, or 63.1%, of the fecal specimens of the ill patients. This percentage appears rather low, but some of the specimens were obtained nearly 2 weeks after the outbreak. Six patients were studied from outbreak 3, but no food was available. All six of the fecal specimens contained a strain reacting with PS66 and PS88 antisera, again indicating a common source. Outbreak 4 is similar. No food was available, but 7 of the 10 patients, 70%, were carrying Hobbs type 12 *C. perfringens*.

Outbreak 5 is interesting in that the food examined contained three serotypes PS55, Hobbs 9, and Hobbs 13. Six of the patients, on the other hand, were carrying serotypes PS24. It is suspected that in this outbreak a different food served as the source of the organism. Outbreak 6 is the first one that we have encountered in which the food contained two serotypes that were also found in the feces of the patients. Hobbs strain 12 occurred in 16 of 22 patients, or 72.7%, and the strain reacting to PS66 and PS88 occurred in 4 or 18.1% of the patients. Outbreak number 7 gave inconclusive results. The food, roast beef, contained two serotypes, PS90 and PS75. The fecal specimens from 15 patients gave reactions to at least 18 serotypes. Eight of these occurred more than once, but none occurred in more than two individuals. The result was a frequency of occurrence of only 13.3%. It seems doubtful that this group received their *C. perfringens* strains from a single source.

A total of 20 suspected outbreaks have been studied to date, and in 13, or 65.0%, of them it has been possible to determine that a common source of *C. perfringens* was present.

Regardless of the methods that may be used to determine that outbreaks of *C. perfringens* foodborne disease are occurring, it is obvious that such determinations must be made if a complete picture of the incidence of such disease in a country is to be seen. Table 2 shows the number and percentage of cases of foodborne illness of bacterial origin reported in the United States in 1968 *(25)*. *Clostridium perfringens* tops the list with 5,966 cases or 40.8% of the total of 14,617 cases. The staphylococci caused 4,919 or 30.2% of the total and were second on the list. *Salmonella* (1287), streptococci (1282) and *E. coli* (1234) were very similiar in their levels and accounted for 8.8, 8.8, and 8.4%, respectively. *Shigella* accounted for 407 cases or 2.8% of the total while the brucellae and *C. botulinum* with 12 and 10 cases accounted for less than 1%.

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THE NATIONAL CONFERENCE ON INTERSTATE MILK SHIPMENTS–PAST, PRESENT, AND FUTURE[1]

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The program of the National Conference on Interstate Milk Shipments (NCIMS) is young in years but old in accomplishments and, I hope, wise for the future. A good analogy would be a farmer hauling his milk to market in cans, in an insulated truck, over mediocre blacktop or gravel roads, to a local market as compared to the farmer of today with the gleaming, stainless steel, bulk tank for milk storage and milk picked up by a stainless steel tanker, hauled over roads many of which are four lane superhighways, to markets, counties, or even states away.

In 1950 the problems confronting the dairy industry in its production, processing, and marketing operations and regulatory agencies, local, state, and federal, in devising livable regulatory programs seemed almost insurmountable. Thank goodness the representatives from the 22 states attending this first NCIMS meeting in St. Louis in 1950 did not think they were insurmountable and got to work on the problems at hand.

To name a few, these very important problems included: *(a)* absence of recognized uniform standards of quality; *(b)* differences in laws and regulations relating to production, processing, labeling, and marketing of dairy products; *(c)* even more important were the differences in interpretation of laws and regulations even though their component parts were often in harmony; *(d)* lack of faith between authorities in shipping areas; and *(e)* multiplicity of product sampling and inspections of farms, processors, etc., which brought on, in many instances, inspections reprisals, general confusion, and hard feelings with a high cost of dairy production without a concurrent improvement in quality.

These 22 representatives had very *simple* but important goals to accomplished, among which were: *(a)* to promote the best possible milk supply for all people, which became and still is the NCIMS slogan; *(b)* to simplify standards for dairy products, inspection requirements, sampling procedures, labeling requirements, and interpretation of findings; *(c)* to develop principals, methods, and practices for industry receiving states, shipping states, and the Public Health Service to follow that would promote acceptability and usuage for all; in simple terms, a *voluntary interstate milk shippers program;* and *(d)* even though not stated as such, the one thing all were searching for was and still are today is spelled out in two words: *re - cip - ro - cate,* which defined in the dictionary means: A. to give and take in exchange; B. to give something in return; C. to interchange—make an exchange with one another; and *rec - i - proc - i - ty,* meaning: A. condition in which there is mutual action giving and taking, etc., between two parties.

The progress of the NCIMS program, beginning in 1950 through today and any progress expected in the future was, is, and will be in direct proportion to the degree that shipping and receiving states are willing to reciprocate one with the other in milk regulatory control programs. I will be referring to reciprocity in this paper and in the future while I am chairman of the NCIMS.

I want you to remember this statement: *Reciprocity* is the foundation upon which the NCIMS program is built. It is not practiced fully by all members of the NCIMS other than by lip service, I am sorry to say. We have trouble ahead unless there is a reassessment by those members in a positive nature and reciprocity is practiced more fully between all members. Further progress of the NCIMS program, as a voluntary organization, will be slow indeed unless this is accomplished.

Industry is moving rapidly today. It, in my opinion, will not be content to wait another 19 years for the States to develop reciprocity. If we fail, our voluntary type of regulatory control in the dairy field will be replaced by Federal legislation.

I am not going to deal with a long history of the NCIMS program developed during 1950 to 1969 other than to point out that meetings held during those years developed:

I. A good set of NCIMS by-laws and conference agreements which were accepted by most member states either in whole or in part.
II. The task force method of problem solving was developed and includes task forces on:
    A. Standards
    B. Supervision
    C. Rating and certification
    D. Uniform bill of lading
    E. Responsibilities of participating states
    F. Responsibilities of United States Pub-

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lic Health Service

G. Procedures for handling complaints and challenging the validity of ratings

H. Application of conference agreements and special problems; plus,

III. Special standing committees that operate on special problems that are of such nature that they cannot be solved at the biannual meeting of the Conference.

For the next 2 years, we have 11 such special committees that are charged with specific problems of great interest; they are as follows:

1. A committee with Dudley Conner, Kentucky State Health Department, as chairman to study the wide scope of survey ratings; this problem faces State rating and certification officers today because of the tremendous areas covered by a milk supply. Just how big can an area rating be; a local area, a state area, or a 16-state area?
2. A committee to study in depth the lack of reciprocity; a most important committee, in my opinion, with Chairman, K. G. Weckel, University of Wisconsin, and Co-chairmen, John Speer of the Milk Industry Foundation, and Richard M. Hoyt, National Milk Producers Foundation.
3. Single Service Containers—this committee has done an outstanding job during the past 2 years and it is being continued under the able leadership of Richard Parry, Connecticut Department of Agriculture, for the next 2 years.
4. Laboratory—a committee with many possibilities but not too effective in the past. It is hoped the new chairman, W. W. Ullman, will be able to get the committee in operation so that it can fill a much needed service to the Conference.
5. Credentials—S. O. Noles, Florida Department of Health—one would think this to be an easy task; however, last May in Denver we had a number of questionable state representatives and one state had to have a letter from its attorney general before the official delegate could be determined.
6. A committee on broadening the scope of the Conference to include milk and milk products other than Grade A. This is a very controversial issue with strong feelings on many levels of operation including local health departments, state health departments, state departments of agriculture, Food and Drug Administration, United States Public Health Service, and industry. I feel we have selected wisely in our chairman, C. K. Luchterhand, Wisconsin State Department of Health, and Co-chairmen, Roy Perkins, Memphis, Shelby County Health Department, and M. W. Jefferson, Virginia Department of Agriculture. I feel if these problems can be ironed out, these men will do so. My best wishes to them.
7. Over the Road Tankers—we feel we have a problem here; this committee is going to study the use of over the road tankers to find out if there is a problem. Our committee chairman for this one is Enos Huffer, Illinois State Department of Health.
8. Resolutions—our committee chairman for this committee was born writing resolutions. No one could be this good in just a short period of time. He is Professor Evert Wallenfeldt, Department of Food Science, University of Wisconsin.
9. Program Committee—chairman, Earl Wright, Department of Food Technology, University of Iowa, did such a good job last year he was drafted to serve again.
10. Local Arrangements—Chairman, John Schilling, St. Louis City Health Department, also another draftee based on past performance.
11. Nominating—Not appointed as yet.
12. Abnormal Milk—John Flake of Evaporated Milk Association.

If any reader is interested in participating on any committee, please notify me or the committee chairman directly as the committee membership has not yet been selected.

From nothing the NCIMS program has grown to 1,450 plants in 47 states rated for milk and cream and 60 plants in 24 states rated for nonfat dry milk processing, all since 1950.

The dairy industry has grown in stature but has declined in number of cows, farms, and plants. According to a paper presented at the NCIMS meeting in Denver this year by Dr. K. G. Weckel, 25 years ago there were 2,200,000 dairy farms in the United States and now we have about one-half million. Some have estimated that if this trend continues, the number will be reduced to one-quarter million farms within the next 10 years. We have about the same picture in dairy processing plant numbers as we have painted in relation to the dairy farms.

There has been a complete revolution of marketing procedures accompaning this production and processing phenonmenon. Milk producer organizations are organizing so they will have more to say about marketing operations; this is being accomplished by combining into larger milk producer organizations which control larger volumes of milk.

In Kentucky, for instance, we have one producer organization today, Dairymen, Inc.; and it has producer cooperative members in Virginia, Mississippi, Tennessee, Louisiana; and, according to the *Courier Journal*, John Mouser, President, was quoted as saying, "the talking stage is now going on of making one marketing cooperative for 16 states immediately east of the Mississippi River extending all the way from Michigan to the Gulf of Mexico." What is the answer? We only have two answers, in my opinion: *(a)* We really must understand this word, reciprocity, and practice it as if it were our Bible and, therefore, make the NCIMS a truly workable organization through voluntary compliance; or, *(b)* We face Federal laws and regulations for regulating milk production, processing, labeling, and sales from the Federal level and lose one of our most important state and local control programs.

We must keep pace with the industry. The industry has modernized its milk production, processing, packaging, labeling, and marketing techniques. We in the regulatory field on the state and local level

also must modernize our system of regulatory control.

Milk produced today in Kentucky may well be packaged and sold in Florida within the next 48 hr; this may be true for any milk produced east of the Mississippi River and it is asinine, in my opinion, for any regulatory body to think it can inspect each farm from which the milk orginated. There are a number of reasons why this cannot be done: *(a)* actually, the identification of the farm from which milk came is often difficult; *(b)* the cost of manpower, to say nothing of the total cost of making the inspections, is prohibitory for us in Kentucky; and *(c)* with time spent away from our own milk control programs inspecting someone else's there is a good possibility that our own would deteriorate.

There is only one answer, it is reciprocity. With the increase in marketing area, there will be duplication of inspection, agency overlap, and increased cost of inspection. We must stop these undesirables. We can best do this, in my opinion, in a voluntary program such as that of the NCIMS.

There are those who say that milk will be produced from a red cow if it sold in my state. I would advise you to look around and to ask yourself if the meat being sold in your state comes from a red cow and is labeled as coming from a red cow or if in reality, the Truth in Packaging Law and the Wholesome Meat Act have taken away your right to say these things? I believe when you truly answer this question for yourself you will find you have actually little to say because these two acts have taken this right from you. Then ask yourself if it is too much to believe that a national milk sanitation act would take additional program authority from the state and local level? Where and when will it stop?

There was great concern regarding future operation of NCIMS with consolidation of the Food and Drug Administration and Public Health Service into one Consumer Protection Program. In reply to this, Administrator, C. C. Johnson, had this to say in Denver this year regarding future operation; and I quote:

"When any reogranization is carried out within the Government, there is always interest, and even concern, among those who are obliged to deal with the agency or agencies involved. Certainly, the creation of CPEHS has been no exception. What does it mean? How will it affect me? These are natural questions to ask—and they have been asked, I assure you.

Some of you have made known to me your own interest in this subject, particularly with regard to the consolidation within the Food and Drug Administration of food protection programs which have been located elsewhere in the Public Health Service. One of these is, of course, the milk program with which this Conference is so intimately involved.

Let me say first, that I am much more interested in program functions than I am in the layout of organizational charts. Food protection programs are being brought together within FDA, not to 'neaten up' these management charts, but to provide unified planning and support for these functions. Broadening the base of scientific support for these programs is particularly important in the light of the complex technology which is part of today's environment.

Having said that, let me add that the milk program is not going to change in purpose or direction. Some members of the dairy industry have expressed concern about being brought within the jurisdiction of a regulatory agency. I can only tell them that they have been within the jurisdiction of the FDA—and for many years.

The FDA has the same statutory responsibility to insure the wholesomeness of milk in interstate commerce as it does for other food products. I suspect that milk producers have been less aware of this than some other elements of the food industry simply because State, local, and voluntary programs have been so effective in giving the consumer high-quality milk.

The National Conference on Interstate Milk Shipments has contributed greatly to this achievement since its first meeting in St. Louis 19 years ago. This is an outstanding example of what can be attained through Federal, State, and industry cooperation.

Over these two decades, you have improved the sanitary quality of milk shipped in interstate commerce. Greater uniformity has been achieved in applying sanitary standards. Laboratory methodology has been advanced. There has been progress in eliminating wasteful, multiple inspections. All of the participants in this Conference can be proud of the positive results produced by this cooperative effort. And I use the word 'effort' deliberately because I know, as you do, that it has taken far more than good intentions and pretty phrases to solve some of these difficult problems.

There are some problems, of course, that still await solutions. The assignments you have given your Task Forces reflect your awareness of the job still to be done. We in CPEHS are ready to work with you in every way that we can to ensure a sound milk supply. And we will continue to encourage broader participation in this voluntary program."

One of these problems referred to by Administrator Johnson is the lack of reciprocity. I know I am asking a superhuman effort to promote reciprocity in these days when: *(a)* the Russians are fighting the Chinese;*(b)* Americans are fighting Vietnamese; *(c)* Castro is fighting the good guys; *(d)* Catholics are fighting Protestants; *(e)* Democrats are fighting Republicans; *(f)* poor are fighting the rich; *(g)* teenagers are fighting the cops; *(h)* college kids are fighting the establishment; and *(i)* one-half of the male readers are afraid to tell their wives truly where they were last night.

Nevertheless, I am asking you, both industry personnel and regulatory officials, to join with me in developing this spirit of reciprocity. Reciprocity of faith in our counterparts, may it be industry or regulatory, will permit all of us to develop the NCIMS program into a truly productive program that will be strong enough to promote the best possible milk supply for all the people. Then we will be abie to say to Washington that additional laws are not needed and our control will remain on the state and local basis.

DEVELOPMENT AND EVALUATION OF THE DIRECT MICROSCOPIC SOMATIC CELL COUNT (DMSCC) IN MILK[1]

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Abstract

The Direct Microscopic Somatic Cell Count (DMSCC) was evaluated in two counting trials. The within film variance was found to be 130% of the mean and the film component of variance was 15% of the mean. These statistics were used to estimate the expected precision of the method. These estimates should be used as guidelines by users until sufficient data is collected to allow reliable estimates for a particular laboratory. Constant reevaluation of the precision achieved in a laboratory can indicate areas of laxity in execution of laboratory procedures.

The value of quantitative determinations depends, to a large degree, on the reliability of procedures employed. In research and regulatory programs requiring the assessment of somatic cell concentration in milk, it is essential to know something of the expected accuracy and precision of the counting procedure employed.

Counting techniques have two general types of error: *(a)* systematic errors that produce results which are generally too high or too low (i.e. bias the data) and, *(b)* random errors, which introduce dispersion into the results. Systematic error controls accuracy, and random error controls precision. The evaluation of both types of error is extremely important. This paper is primarily concerned with the characteristics of random errors encountered in counting somatic cells in dried milk films. However, factors affecting the accuracy of the count will be discussed where appropriate.

Initially, these studies were undertaken to bring together the developed theory applicable to the problem and to develop a general counting procedure with measurable and predictable precision for all levels of cell count. The organization of a National Mastitis Council Research Subcommittee on Screening Tests and the adoption of the Abnormal Milk Control Program by the National Conference on Interstate Milk Shipments emphasized the need for reliable counting procedures. The studies have continued as a contribution to the Subcommittee's effort.

Errors in the Breed method

The errors involved in estimating the concentration of particles in a liquid (cells per ml of milk, etc.) may be grouped into two categories. First, there are errors in sampling, subsampling, and sample preparation. Secondly, there are errors in estimating the number of particles in the subsample. The specific errors associated with an estimate of the number of body cells in milk by the Breed method *(8)* include the following:

Variation in sampling, subsampling, and sample preparation. The sample collected from the bulk may not be representative. This source of error must be kept in mind, but the random variation should be extremely small when a sample is drawn from a well-mixed batch. In theory, the sampling error will follow the Poisson distribution and if the sample is large enough to include a great number of cells, the percentage error will be negligible.

The subsample (0.01 ml droplet) that is taken from the sample may not be representative of the sample. Again, the expected sampling errors follow the Poisson distribution and, as will be shown later, the coefficient of variation (C.V.) is expected to exceed 1% only as cell concentration decreases below 1,000,000 cells per ml.

The droplet of milk delivered to the slide is not likely to be precisely 0.01 ml. Errors in measurement may be attributed to variation between and within items of equipment used for measuring and operators. Measurement errors may be determined and expressed in quantitative terms, but differences in

[1]A contribution of the Subcommittee on Screening Tests, National Mastitis Council Research Committee:

cell count resulting from such errors must be considered as a percentage error. For example, if a film is prepared with 0.0105 ml instead of 0.0100 ml milk, the resulting counts are expected to be 5% too high regardless of the cell concentration in the sample.

Milk drying on a surface tends to form a film of nonuniform thickness. This disparity may be compounded by failure to spread milk evenly over the entire area or drying the films on an unlevel surface. The control of this error requires care in film preparation and an adequate systematic procedure in the selection of areas to be counted.

Errors in estimating the concentration of cells in the film. Theoretically, if the cells are dispersed at random, the counting errors will follow the Poisson distribution unless crowding or clumping becomes a factor. The applicability and the consequences of this distribution in the analysis of counting errors is the major subject of this paper and will be discussed in detail later.

The number of cells counted is multiplied by a factor derived from the area of the film examined to determine the concentration of cells in the sample. Errors in the factor will bias all samples counted with a given microscope but will not affect the precision of the count.

Theoretical considerations

Student *(13)* gave the theoretical basis for evaluating the precision of counting cell suspensions with a microscope. Subsequently, numerous papers have been published on various aspects of the counting problem. Plum *(7)* and Lancaster *(2)* have reviewed the history of development and evaluation of microscopic counting procedures. In the light of the published literature, it is almost a statistical truism that the number of cells on a given area of a properly prepared slide is distributed in a Poisson series.

The Poisson distribution has been the subject of considerable research and many statistical text books devote space to its derivation and application. Probabilities for a Poisson distribution are given by:

$$P\ (y = k)\ =\ \frac{e^{-u}u^{k}}{k!}$$

This is read as "the probability that the random variable y takes the value k is equal to . . . ". The value of k may be 0, 1, 2 —; there is no stopping point. The mean of the distribution is u; the variance s^2 is also equal to u.

It is customary to make a number of observations. The mean of these, $\bar{y}$, provides an estimate of both u and s^2. Since the mean and variance, u and s^2, are both estimated by $\bar{y}$ the coefficient of variation, (C.V.), for an estimated count is a function of the total number of events observed, (SY), and independent of the number of observations, N, as will be shown in the following equation.

$$\text{C.V.} = \sqrt{\frac{s^2}{N}} \div \bar{y} = \sqrt{\frac{SY}{N^2}} \div \frac{SY}{N} = \frac{1}{\sqrt{SY}}$$

Where: C.V. is the coefficient of variation for the estimate of the mean number of particles per area examined. N is the number of areas examined. SY is the total number of particles counted. The relationship between the total number of particles counted and the expected coefficient of variation is shown in Fig. 1.

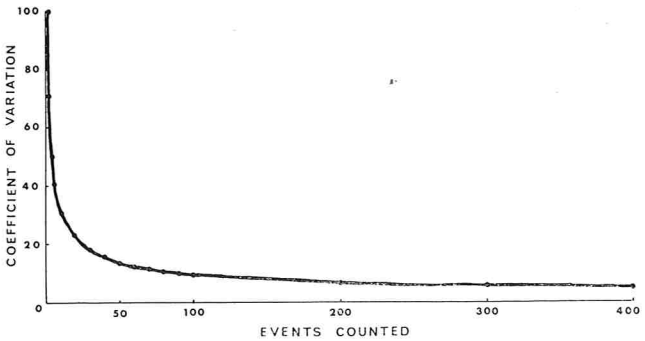

Figure 1. Theoretical coefficient of variation for Poisson distribution.

The studies reported here were undertaken to determine the relationship between the theoretical counting error and the observed error in making routine counts, to develop a reliable procedure for counting cells, to develop procedures for evaluating count precision, and to establish tentative confidence limits for cell count estimates based on experience.

RESULTS OF INITIAL STUDIES

In order to check the validity of the assumption that cells in a dried milk film are distributed at random, we recorded the number of cells in each microscope field counted for a number of milk films. The milk films were prepared and stained by procedures described by Prescott and Breed *(8)* and Levowitz and Weber *(4)*. Commercially available 2 x 3 inch slides with 15 circles of 1 cm^2 area surrounded by a frosted surface were used. Milk was placed on the slide with a 0.01 ml pipet and spread with the point of a bent needle. Chi-square goodness of fit test, as outlined by Steel and Torrie *(12)* was applied to individual field counts from 15 films that averaged less than two cells per microscopic field, and 14 films containing two or more cells per field. Consistent with our routine procedure at the time, 20 microscope fields, chosen at random across the central portion of the film, were examined in each of the low count films, whereas only 10 fields were examined on those with an average of two cells or more

TABLE 1. COMPARISON BETWEEN COUNTS OBTAINED BY TWO COUNTING PROCEDURES[a]

Group	Grand Mean	Sample Range		Routine Count			Radial Count		
		Low	High	Mean Count	Coefficient of Variation[b] Observed	Theoretical	Count Mean	Coefficient of Variation[b] Observed	Theoretical
1	.010	.005	.016	.017	96	128	.007	190	200
2	.020	.016	.025	.027	91	100	.016	127	133
3	.030	.025	.035	.039	72	83	.024	100	108
4	.049	.037	.063	.061	61	67	.043	80	83
5	.083	.063	.107	.078	54	59	.086	53	59
6	.145	.108	.197	.150	41	43	.142	41	45
7	.577	.198	1.107	.621	25	21	.555	26	22
8	4.427	1.117	16.912	4.572	12	8	4.354	15	8

[a]Cell count per ml x 10^{-6}
[b]Compilations are based on 20 field counts

in the first 10 fields examined. Relying on the fact that $\sqrt{2y^2} - \sqrt{2n-1}$ is approximately normally distributed with zero mean and unit variance [Fisher *(1)*] the data were pooled for all samples in each group. The mean deviations of 0.17 and 0.27 for the low and high count groups were not significantly (P.05) different from zero. Therefore, we assumed that cells in a milk film, at least in the range of interest, tended to be randomly distributed and we set up a rigorous counting procedure to evaluate the various sources of error influencing cell counts.

This evaluation was conducted using cell counts from milk samples collected in conjunction with other studies. The bulk of the data has been described in a previous publication by Schultze and Smith *(11)*. Duplicate films were made from each sample on separate slides using the procedures described above.

Initially, two separate types of counts were performed on each film. First, each film was evaluated twice by our old routine procedure of moving well into the film and counting 20 fields chosen from a random path across the central portion of the film; however, if there were more than 200 cells in the first 10 fields, the count was terminated. Secondly, we began a more rigorous procedure consisting of a 20 field count along each of the horizontal and vertical radii beginning five fields from the periphery. Using all of the information from the two random counts and the four radial counts from each of the two films, we estimated the cell content for each of 383 milk samples. The samples were ranked according to cell content and divided into eight groups. The first seven groups contained 48 samples whereas group eight contained only 47 samples. These data are summarized in Table 1. The grand mean and the sample range for each group were derived from all the information available on each milk sample. Counts by the routine procedure were consistently higher than the radial count beginning five fields from the periphery indicating that there was a tendency for the cells to be concentrated near the center of the film. The between count coefficient of variation for the two procedures was in excellent agreement with expectation, assuming random dispersion of cells between areas sampled. It should be pointed out that there may have been an overlap of areas considered in the two counts since the slide was simply counted on two different occasions using the same procedure, but the radial counts were made from different quadrants on the circle. The tendency for cells to be more concentrated near the center of the circular area was further investigated by counting cells in a continuous series of fields across the horizontal and vertical diameter of prepared films. The pattern of cell distribution varied from film to film; however, there was a definite tendency for cells to concentrate in the center of the film. Slight increases in concentration were also noted around the periphery. These observations were in general agreement with the findings of Schneider and Jasper *(9)*. Therefore, if the Poisson distribution is to be completely applicable, each observation (count) made on a film must include a uniform sample of the film area.

Realizing that the 20 field counts along each of the horizontal and vertical radii did not include the areas of greatest concentration, we considered the total number of cells counted along each radius as an observation (count); thus, we had counts on four uniform areas per film and two films per sample. We conducted a number of trial analyses to determine the advisability of using counts obtained along the radii or across the diameter of the circular film for routine counting. In our laboratory, where the films were prepared on a level surface, there were no differences among the four radial counts considered. A comparison between the theoretical coefficient of variation, considering a Poisson distribution, and the observed coefficient of variation indicated that these counts were slightly more vari-

able than expected. The methods of analysis used here consisted of comparing the total observed variation with the expected variation for the number of cells observed; therefore, we were unable to partition the variation for between and within films. While we were considering other methods of examining films and of analyzing cell count data, the National Mastitis Council's Research Committee appointed a subcommittee to study cell counting and screening tests, and the work was continued as a part of the committee's effort.

COMMITTEE CONSIDERATIONS

The Subcommittee on Screening Tests was appointed at the 1967 Meeting of the National Mastitis Council and began work immediately. Before meeting in Guelph, Ontario, in April of 1967, several members prepared slides using their routine procedures. These slides were distributed to the Committee for examination. This preliminary trial served to focus our attention on a number of problems that would have to be resolved. The Committee has given detailed consideration to all aspects, but emphasis has been placed on problems relating to counting of cells in milk for regulatory purposes. Before attempting to develop a counting procedure, the Committee agreed on a definition of cells to be counted and the purpose of the proposed procedure. The term "somatic cells" was chosen to indicate that all nucleated cells of body origin were to be counted without attempting to distinguish between leucocytes and other cell types. In recognition of this, the procedure was named "Direct Microscopic Somatic Cell Count" (DMSCC) *(5)*. This new procedure was to be as accurate as practical, have a definable precision, and should become the reference standard to which all other cell counting procedures are compared.

In the development of the DMSCC procedure, the Committee considered a number of alternatives for each step and based the final choices on the published literature, research experience, results of the Committee's research, and consideration of the user. Some of the major considerations in film preparation included the following. Slides with circular areas of one cm^2 were chosen because the drying pattern and cell distribution are more uniform than on square areas. In addition, it is easier to locate accurately horizontal and vertical strips that pass through the center on circular films. If films are prepared and dried on a level surface, a narrow strip of uniform width passing through the diameter of the circle is expected to be representative of all such diameter strips which could be observed on a particular film.

The Levowitz-Weber *(4)* staining procedure was selected because it gives a deeply differentiated nucleus, is reliable, requires a minimum of skill on the part of the technician, and is in common use in dairy laboratories.

One of the most important considerations facing the Committee was the development of a method of examining the film to produce representative counts. To some extent, this was taken into consideration in the selection of the circular format of the slide. Work referred to earlier indicated that counts should be made along a radius or across the full diameter of the circle. If we select individual fields for counting, a considerable amount of time is spent in aligning the microscope, making the procedure very slow and necessitating enumeration of both cells and fields. Two members of the Committee, Levowitz *(3)* and Newbould [Newbould and Phipps *(6)*], had previous experience in counting continuous strips, the latter using a metal reticle in the eyepiece of the microscope. An experimental glass reticle was developed for the Committee's use in evaluating the DMSCC procedure, and Schultze *(10)* gave a detailed discussion relating to the specifications and use of such reticles with various microscopes. The strip method was tested in the laboratories of Subcommittee members and received enthusiastic approval of the tech-

TABLE 2. HIERARCHAL ANALYSIS OF VARIANCE FOR EQUAL NUMBERS OF DETERMINATIONS ON EACH FILM

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Square	Expected Mean Square
Total	$n - 1$	$Sy^2_{ij} - (Y..)^2/N = SS_1$	$SS_1/n - 1$	----------
Films	$f - 1$	$\frac{Syi.^2}{c} - \frac{Y..^2}{N} = SS_2$	$SS_2/f - 1$	$s^2_e + c(s^2_f)$
Counts in film	$f(c - 1)$	$SS_1 - SS_2 = SS_3$	$SS_3/f(c - 1)$	s^2_e

Where n = Total number of counts
f = Number of films
c = Number of counts per film
Y_{ij} = The jth count on the film

TABLE 3. EXAMPLE CELL COUNTS OBTAINED FROM ONE SAMPLE OF MILK

Film No.	Count j = 1 Horizontal	Count j = 2 Vertical	Total
i = 1	87	90	177
i = 2	89	111	200
Total (Y..)			377
Mean ($\bar{y}$)			94.25

TABLE 4. ANALYSIS OF VARIANCE FOR EXAMPLE CELL COUNTS

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	3	378.75	
Film	1	132.25	132.25
Count in film	2	246.50	123.25

TABLE 5. EXPECTED VARIATION IN CELL COUNT

$$s^2_{\bar{y}} = \frac{\bar{y}(1.3)}{4} + \frac{\bar{y}(.15)}{2} = .4\bar{y}$$

Mean Strip Count $\bar{y}$	Variance of Mean $s^2_{\bar{y}}$	Standard Error $s_{\bar{y}}$	Sample Coefficient of Variation C.V.	Sample 95% Limit in %
1	.4	.63	63.00	123.48
10	4	2.00	20.00	39.20
20	8	2.83	14.15	27.73
30	12	3.46	11.53	22.60
40	16	4.00	10.00	19.60
50	20	4.47	8.98	17.60
60	24	4.90	8.16	15.99
70	28	5.29	7.56	14.82
80	32	5.66	7.07	13.86
90	36	6.00	6.67	13.07
100	40	6.32	6.32	12.38
110	44	6.63	6.03	11.82
120	48	6.93	5.78	11.33
130	52	7.21	5.55	10.88
140	56	7.48	5.34	10.47
150	60	7.75	5.17	10.13

nicians on the grounds of increased speed and greatly reduced eye strain for counts attaining comparable levels of precision.

STATISTICAL EVALUATION OF THE DMSCC

The basic statistical considerations in the evaluation of the precision of cell counts have been discussed previously. Procedures set forth in this section were developed and used initially to evaluate the precision of counts made in the laboratories of the Committee members during the development and testing of the DMSCC.

The factors affecting the precision of the estimate of the cell concentration of a particular sample of milk may be divided into two categories as discussed previously. These include errors in film preparation which give rise to the film component of variance (s^2_f) and errors in estimating the number of cells in a particular film that gives rise to the counting error variance (s^2_e). In order to partition the total variance into these two components, multiple films must be prepared from each milk sample and multiple determinations must be made on each film. The method as prescribed for regulatory purposes specifies two films per sample and two determinations per film. In these terms, a determination is defined as the total number of cells observed in a defined strip across the diameter of the film. One determination each is made across the horizontal and vertical diameter of each film.

The determinations made on a sample of milk can be compared using an analysis of variance as shown in Table 2. Cells observed in one milk sample are shown in Table 3. The computations for the analysis of variance are as follows: Total sum of squares $= SY_{ij}^2 = (87)^2 + (90)^2 + (89)^2 + (111)^2 = 35{,}911$. Correction factor $= Y_{..}^2/N = (377)^2/4 = 35{,}532.25$. Total corrected sum of squares $= 35{,}911.00 - 35{,}532.25 = 378.75$. Sum of squares for films $=$

$$\frac{S(Y_{i.})^2}{n_{i.}} = \frac{(177)^2}{2} + \frac{(200)^2}{2} = 35{,}664.50.$$

Corrected sum of squares for films $= 35{,}664.50 - 35{,}532.25 = 132.25$. The count within film (error) sum of squares is the difference between the total corrected sum of squares and the corrected sum of squares for films which is $378.75 - 132.25 = 246.50$. Dividing this latter by $2 = 123.25$, estimates the error mean square (s^2_e). The film mean square estimates $s^2_e + 2s^2_f$, therefore, $s^2_f = \frac{132.25 - 123.25}{2} = \frac{9}{2} =$ 4.5. If the counting errors were normally distributed and independent of the mean, we could pool the estimates for a large number of samples and obtain reliable estimates from which confidence limits could be calculated in the usual manner. However, since the mean and both components of variance are closely related, we cannot pool directly the variance estimates for samples with different concentrations of cells. We can, however, take advantage of the strong tendency toward a definite relationship between the mean and components of variance and express the components as a ratio of the mean. Using the data from the sample,

$$\frac{s^2_e}{\bar{y}} = 123.25/94.25 = 1.3077 \text{ or } 130.77\%, \text{ and } \frac{s^2_f}{\bar{y}} = .0477 \text{ or } 4.77\%$$

TABLE 6. EXPECTED VARIATION AND 95% CONFIDENCE LIMITS FOR COUNTING CELLS WITH A STRIP MICROSCOPIC FACTOR OF 15,000 AND $s^2_{\bar{y}} = .4\bar{y}$

Total No. Cells Counted	Mean Count Per Strip	Standard Error $s_{\bar{y}}$	Co-efficient of Variation	95% Limits in Percent	Factored Cell Count (10^{-6})	95% Confidence Limit No. cells (10^{-6})		
						$\bar{y} \pm$	Lower Limit	Upper Limit
360	90	6.00	6.67	13.07	1.350	.176	1.18	1.520
364	91	6.03	6.63	12.99	1.365	.177	1.188	1.542
368	92	6.07	6.60	12.94	1.380	.178	1.202	1.558
372	93	6.10	6.56	12.86	1.395	.179	1.216	1.574
376	94	6.13	6.52	12.78	1.410	.180	1.230	1.590
380	95	6.16	6.48	12.70	1.425	.181	1.244	1.606
384	96	6.20	6.46	12.66	1.440	.182	1.258	1.622
388	97	6.23	6.42	12.58	1.455	.183	1.272	1.638
392	98	6.26	6.39	12.52	1.470	.184	1.286	1.654
396	99	6.29	6.35	12.45	1.485	.185	1.300	1.670
400	100	6.32	6.32	12.39	1.500	.186	1.314	1.686
404	101	6.36	6.30	12.35	1.515	.187	1.328	1.702
408	102	6.39	6.26	12.27	1.530	.188	1.342	1.718
412	103	6.42	6.23	12.21	1.545	.189	1.356	1.734
416	104	6.45	6.20	12.15	1.560	.190	1.370	1.750
420	105	6.48	6.17	12.09	1.575	.190	1.385	1.765
424	106	6.51	6.14	12.03	1.590	.191	1.399	1.781
428	107	6.54	6.11	11.98	1.605	.192	1.413	1.797
432	108	6.57	6.08	11.92	1.620	.193	1.427	1.813
436	109	6.60	6.06	11.88	1.635	.194	1.441	1.829
440	110	6.63	6.03	11.82	1.650	.195	1.455	1.845
444	111	6.66	6.00	11.76	1.665	.196	1.469	1.861
448	112	6.69	5.97	11.70	1.680	.197	1.483	1.877
452	113	6.72	5.94	11.64	1.695	.197	1.498	1.892
456	114	6.75	5.92	11.60	1.710	.198	1.512	1.908
460	115	6.78	5.89	11.54	1.725	.199	1.526	1.924
464	116	6.81	5.87	11.50	1.740	.200	1.540	1.940
468	117	6.84	5.85	11.47	1.755	.201	1.554	1.956
472	118	6.87	5.82	11.41	1.770	.202	1.568	1.972
476	119	6.90	5.80	11.37	1.785	.203	1.582	1.988
480	120	6.93	5.78	11.33	1.800	.204	1.596	2.004

Factor for total count = 3,750.

These percentage figures are expected to be rather stable over a wide range of cell counts. Therefore, these percentage values or ratios can be pooled and the results used to compute confidence limits over the entire range of cell counts encountered. Once an average or pooled estimate of the percentage relationship between the mean and the two components of variance is obtained, the relative value of the variance of a sample mean can be determined as follows:

$$\frac{s^2}{\bar{y}} = \frac{s^2_e}{\bar{y}}\left(\frac{1}{c}\right) + \frac{s^2_f}{\bar{y}}\left(\frac{1}{f}\right)$$

Where c is the number of observations per sample and f is the number of films per sample. It may be convenient to express the variation of the sample in terms of the coefficient of variation as follows:

$$CV = \sqrt{s^2_{\bar{y}}} \div \bar{y}$$

RESULTS AND DISCUSSION OF CELL COUNTING TRIALS CONDUCTED BY THE COMMITTEE

Committee members made over 1,300 cell counts in two trials, evaluating the procedure over a wide range of cell counts. These trials contained data derived from two sampling procedures. A majority of the counts were from bulk samples collected specifically for the evaluation of the DMSCC Procedure. Each laboratory selected three or four batches of milk representing a wide range of cell concentrations (i.e. approximately 500,000; 1,000,000; 1,500,000; and 2,000,000). A large sample of milk was collected from each batch and then subdivided into 12 samples for processing and counting. The remainder of the counts were from samples collected for routine evaluation in existing research or control programs. The initial analysis of these data included 1,270 counts which

averaged 122.96% and 14.8% for $s_e^2/\bar{y}$ and $s_f^2/\bar{y}$, respectively. There were wide variations in precision between and within laboratories which will be discussed later. First, we will consider the consequences of this variation on the expected precision of cell counts. In order to add a small margin of safety, the figures were rounded to 130% and 15% for $s_e^2/\bar{y}$ (100) and $s_f^2/\bar{y}$ (100), respectively. As shown previously, the variance of a sample mean is given by the formula:

$$s_{\bar{y}}^2 = \frac{s_e^2}{c} + \frac{s_f^2}{f}$$

where c is the total number of observations and f is the number of films counted. Therefore, since we have the two variances expressed as ratios of the mean, the equation becomes:

$$s_{\bar{y}}^2 = \frac{1.30\,(\bar{y})}{4} + \frac{.15\,(\bar{y})}{2} = \frac{1.60\,(\bar{y})}{4} = 0.4\,\bar{y}$$

Using 0.4 $\bar{y}$ as an estimate of the variance of the mean cell count obtained on each sample of milk the sample coefficient of variation is $\frac{\sqrt{.4_{\bar{y}}}}{\bar{y}}$

The expected 95% confidence interval expressed in terms of cells is $\bar{y} \pm \sqrt{0.4\bar{y}}\ (1.96)$, where 1.96 is the appropriate value selected from statistical tables of t. This means that if a sample is observed to have a mean determination of 100 cells per strip for each of two strips on each of two films, 95% of the time the mean for that sample is expected to be $100 \pm \sqrt{0.4(100)}\ (1.96) = 100 \pm 6.32\ (1.96) = 100 \pm 12.39$, or between 87.61 and 112.39. Now, if the microscope factor for a strip count is 15,000, the 100 cells per strip represents 1,500,000. Ninety-five percent of the time the cell concentration in the sample will be between 1,314,000 and 1,686,000.

In order to translate from the observed mean count and the factored count, it is simpler to again express the variation in terms of the mean using the coefficient of variation. Considering a similar example, the computation is as follows: The 95% confidence limits in percentage $= \bar{y} \pm (1.96)\ (\sqrt{s_{\bar{y}}^2/\bar{y}})$; where $s_{\bar{y}}/\bar{y}$ = coefficient of variation. A sample of milk counted as above was observed to have 120 cells per strip, so the 95% confidence limits are:

$$120 \pm \frac{\sqrt{0.4(120)}}{120}\ (1.96)$$

$120 \pm 5.78\%\ (1.96) = 120 \pm 11.33\%$.

Now, the expected value for the cell count is within $\pm$ 11.33% of the observed count expressed either as an observed or factored count, that is 120 ± 13.59 or $1{,}800{,}000 \pm 204{,}000$. A tabulation of the variance of the mean, $s_{\bar{y}}^2$, standard deviation of the mean, $s_{\bar{y}}$, the coefficient of variation, C.V. and the 95% confidence limits in terms of percentage are shown in Table 5.

Table 6 gives a detailed tabulation for counts around the 1.5 million level for a microscope with a strip factor of 15,000. A milk sample with 100 cells per strip is expected to contain 1.5 million cells per ml. However, we must observe 114 cells per strip estimating 1.71 million cells per ml before we have a 95% probability of the cell concentration in the sample exceeding 1.5 million. Each laboratory using the method will need for each microscope a table similar to Table 6 including the range of cell counts of interest. This can be accomplished by substituting the appropriate factored count in column 6 and using the values in computation of the remainder of the table.

The individual groups of data included in this analysis exhibited a wide range of variation between and within laboratories. Although a detailed statistical evaluation of the distribution properties of the values of $s_e^2/\bar{y}$ and $s^2/\bar{y}$ has not been completed, some general observations may be useful. The fact that the error variance averaged almost 130% of the mean caused some concern initially, since we expected the mean and variance to be equal. If the counting errors followed the Poisson, $2\ s_e^2/\bar{y}$ would be distributed as Chi^2 with 2 degrees of freedom, and 95% of the values would fall between .056 and 7.38. Therefore, 95% of the values for $s^2/\bar{y}\ (100)$ are expected to fall between 23 and 369. Approximately 10% of samples included in the data exceeded the higher value. One group of data consisting of 114 samples had one sample with $s^2/\bar{y}\ (100) = 747.42$; it was observed to have had a number of clumps of cells. Another sample had a $s^2/\bar{y}\ (100) = 1236.70$ which resulted from a detectable recording error. These two samples were deleted prior to the analysis but other equally suspicious values in this and other groups of data were included in the analysis since the cause of the excessive variation was not determined. Therefore, it is reasonable to assume that the counting errors observed in this evaluation are inflated by operator counting mistakes, recording errors, and abnormal distribution of cells in the milk film that may be attributed to clumping of cells. To some degree, these types of errors will be present in all large groups of data; however, extreme caution must be exercised to minimize their occurrence. The film component represents the portion of the variation in cell counts that may be attributed to differences between milk films. The estimates derived from the individual samples vary widely and include large negative values. In general, the mean of pooled values for the film component of variance will indicate the precision with which the 0.01 ml of milk is transferred to the slide. If the proper care is exercised, $s_f^2/\bar{y}$ should be very small when large groups of data are pooled. Finally, it is conceivable that the relation between the mean and the components of variance would change

as cell concentration increases. However, there was no evidence of such a change in the counts considered in this paper. Should this prove to be true in other sets of data, especially where a wider range is considered, corrections could be developed from an appropriate regression equation.

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LETTER TO THE EDITOR

DEAR SIR:

As Harold Barnum so ably pointed out in his letter in the June 1969 issue of the *Journal*, it is high time we re-appraised the value of the Standard Plate Count (SPC) in sanitary milk control.

Bacteriological tests of raw milk are intended to give some assurance that it has been produced under sanitary conditions. With bulk tank cooling restricting bacterial growth, and a huge dilution effect, a low SPC is no assurance of this. As an alternative, some have advocated testing for specific groups of bacteria—coliforms, enterococci, thermodurics, etc. As psychrotrophs are by all odds the most important organisms in both raw and pasteurized milk these days, it has been suggested that there should be a standard for these. While the psychrotroph count was the only bacterial test that showed a significant correlation with the farm score in the Iowa studies (*JMFT* 31:388, 1968), the 10-day incubation required is a serious handicap to its routine use.

A new approach has recently been suggested by Blankenagel and Okelle-Uma (*Canad. Inst. Food Technol.* 2:69, 1969). Since the udder flora is predominantly Gram-positive, it may be concluded that Gram-negative bacteria present in milk are external contaminants. Utilizing the finding of Freeman et al. (*JMFT* 27:304, 1964) that sodium desoxycholate (SDC) at 0.5% concentration was most effective in inhibiting Gram-positive organisms without affecting Gram-negative ones, they proved that samples from individual quarters were either free from Gram-negative bacteria or counts were very low. They then applied this procedure to 163 bulk tank samples and found that it provided more valuable information about dairy farm sanitation than either coliform tests or SPCs. As might be suspected, many samples with low SPC contained over 1,000 Gram-negative bacteria per ml. Many of these "blew up" when subjected to Preliminary Incubation (PI) at 12.8 C for 18 hr.

This new approach offers several advantages over PI (*a*) there is no need for an incubator or water-bath at 12.8 C; (*b*) results are obtained as quickly as with the SPC; (*c*) samples are tested "as received;" and (*d*) a uniform count limit can be more easily agreed upon. There is abundant evidence that milk produced with equipment in good physical condition, properly cleaned and sanitized, has counts of Gram-negative bacteria approaching zero. Such a test deserves extensive trials in many areas to confirm these workers' findings.

However, Gram-negative bacteria are not the only type of contaminant. Most thermoduric bacteria are Gram-positive. Consequently, Blankenagel and Okello-Uma recommend that their numbers also be determined. The combination of these two tests should furnish a much more reliable indication of sanitation shortcomings.

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AN AUREOMYCIN-ROSE BENGAL AGAR FOR ENUMERATION OF YEAST AND MOLD IN COTTAGE CHEESE

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Abstract

A peptone dextrose agar containing 20 ppm of aureomycin and 20 ppm of rose bengal was compared to acidified potato dextrose agar for the enumeration of yeast and mold. Mold and yeast isolates, as well as yeast and mold from Cottage cheese, were compared on the two agars. The results of the study showed no statistically significant difference between the mean counts on the agars and tests for bacterial growth of five species were all negative. The aureomycin-rose bengal agar had several advantages over the potato dextrose (PD) agar; spreading mold colonies were eliminated, there was no precipitation of casein to interfere with counting colonies, the possibility of some yeast and mold species not growing because of the low pH of the acidified PD medium was eliminated, and the red background aided in detecting colonies of yeast and mold.

The use of an acidified medium for the separation of yeast and mold from bacteria has been practiced for many years. Thom and Church *(14)* used an acidified malt agar in their studies of the aspergilli as did Fulmer and Grimes *(5)* studying yeast growth on synthetic agar media. The Committee for the Microbiological Analysis of Butter of the American Dairy Science Association in their reports of 1930 *(1)* and 1933 *(2)* recommended acidified malt or whey agar. Parfitt *(12)* also recommended acidified malt, whey, or wort agar for obtaining comparative yeast and mold counts in butter. However, acidified potato dextrose agar has replaced other acidified agars and is presently the standard medium *(3)* for determination of yeast and mold in dairy products, including Cottage cheese. Several investigators *(7, 8, 10)* have reported that acidified media cause fungi to sporulate poorly and generally depress the numbers and kinds of soil fungi. Tyner *(15)* reports that an acid medium has little effect on restricting the colony radial size of fungi and Butler and Hine *(4)* found the acid medium did not eliminate all bacteria.

Papavizas and Davey *(11)* found peptone dextrose agar modified by the addition of rose bengal and streptomycin to be satisfactory for isolating fungi from soil. Johnson and Manka *(6)* found peptone dextrose agar with rose bengal, as suggested by Martin *(7)* and modified by substituting 2 μg aureomycin per ml for streptomycin, to be superior for detection of soil fungi. Olson and Bonner *(9)* recommended use of 100 ppm aureomycin in potato dextrose agar for enumeration of yeast and mold in Cottage cheese and reported higher counts with aureomycin agar than with acidified potato dextrose agar.

Much of the dairy literature on the enumeration of yeast and mold has dealt with their detection in butter and the problems encountered in their enumeration in Cottage cheese have received little attention. Problems inherent with acidified potato dextrose agar are: excessive spreading of mold colonies; distinguishing yeast colonies from curd particles; failure of some yeast and mold to grow at the low pH; and a re-precipitation of the casein which makes counting difficult at the low dilutions necessary for examination of Cottage cheese. The objective of this study was to determine if a medium containing aureomycin and rose bengal and without the above objections could be developed and used for Cottage cheese.

Procedure

Preparation of media

The basal peptone dextrose medium contained the following ingredients.

Agar	20.0 g	Peptone	5.0 g
KH_2PO_4	1.0 g	Dextrose	10.0 g
$MgSO_4$	0.5 g	Distilled H_2O	1000 ml

The constituents were solubilized by heating in a steam cabinet and 100 ml of the medium was dispensed in screw-cap bottles and sterilized by autoclaving at 121 C for 15 min.

A 1% rose bengal solution in distilled water was sterilized at 121 C for 15 min and served as the stock solution.

An aureomycin capsule containing 100 mg was split open in 100 ml sterile distilled water and dissolved by shaking. Both the rose bengal and aureomycin solutions were stored at 4 C. Fresh aureomycin solutions were prepared weekly.

The potato dextrose (PD) agar was obtained from a commercial source (Difco Laboratories, Detroit, Michigan) in dehydrated form. It was resuspended according to the manufacturer's directions and sterilized. A sterile 10% solution of tartaric acid was used to adjust the pH to 3.5 just prior to pouring of plates.

Preparation of yeast and mold isolates

Yeast isolates were prepared from PD agar plates of Cottage cheese by picking colonies into tryptose phosphate broth and incubating at 21C for 5 days. Microscopic examination showed the typical budding of yeast.

Mold isolates were likewise prepared from PD agar plates of Cottage cheese by picking colonies and streaking on nu-

TABLE 1. YEAST COUNTS ON PD AGAR AND ON SIX FORMULATIONS OF AR AGAR

Replication	ARA[1]	ARA[2]	ARA[3]	ARA[4]	ARA[5]	ARA[6]	PDA
	x10[5]						
1	20	90	90	34	23	14	27
2	60	50	100	26	19	21	25
3	70	40	60	37	24	17	17
4	20	60	80	28	26	13	24
5	30	80	80	25	27	11	22
Average	40	60	80	30	24	15	23

ARA[1] = 10 ppm aureomycin + 10 ppm rose bengal in basal medium.
ARA[2] = 20 ppm aureomycin + 10 ppm rose bengal in basal medium.
ARA[3] = 30 ppm aureomycin + 10 ppm rose bengal in basal medium.
ARA[4] = 10 ppm aureomycin + 20 ppm rose bengal in basal medium.
ARA[5] = 20 ppm aureomycin + 20 ppm rose bengal in basal medium.
ARA[6] = 30 ppm aureomycin + 20 ppm rose bengal in basal medium.

TABLE 2. MOLD COUNTS ON PD AGAR AND ON SIX FORMULATIONS OF AR AGAR

Replication	ARA[1]	ARA[2]	ARA[3]	ARA[4]	ARA[5]	ARA[6]	PDA
	x10[5]						
1	32	29	28	34	20	22	23
2	36	33	34	25	16	20	19
3	24	24	18	16	19	13	16
4	38	22	34	38	25	17	26
5	27	25	39	27	21	15	25
Average	31	26	31	28	20	17	22

ARA[1] = 10 ppm aureomycin + 10 ppm rose bengal in basal medium.
ARA[2] = 20 ppm aureomycin + 10 ppm rose bengal in basal medium.
ARA[3] = 30 ppm aureomycin + 10 ppm rose bengal in basal medium.
ARA[4] = 10 ppm aureomycin + 20 ppm rose bengal in basal medium.
ARA[5] = 20 ppm aureomycin + 20 ppm rose bengal in basal medium.
ARA[6] = 30 ppm aureomycin + 20 ppm rose bengal in basal medium.

trient agar slants. After incubation at 21C for 5 days a mold suspension was prepared by flushing the agar surface with nutrient broth and using a sterile glass rod to scrape the mold growth into the broth.

Sampling procedure

Sampling of the yeast suspension and the nutrient broth mold suspension was accomplished by making serial dilutions using 99 ml water blanks *(3)*. Duplicate platings were made using aureomycin-rose bengal (AR) and PD agars. Plates were incubated at 21C for 5 days and counted with the aid of a colony counter.

Five species of bacteria *(Escherichia coli, Streptococcus lactis, Aerobacter aerogenes, Lactobacillus casei,* and *Pseudomonas fragi)* were grown in pure culture in litmus milk. These cultures were plated undiluted on AR agar. Plates were incubated for 48 hr at the optimum temperature of each organism. Comparison counts of these cultures were made on Plate Count agar *(3)*.

Cottage cheese was sampled by weighing 10 g into a sterile Virtis Homogenizer flask with 10 ml of sterile 2% sodium citrate solution. The samples were homogenized at low speed for 1 min and plated in duplicate using AR and PD agars. Plates were incubated at 21C for 5 days and counted using a colony counter.

Statistical analyses of the data were based on the methods outlined by Steel and Torrie *(13)*.

RESULTS AND DISCUSSION

The work of Johnson and Manka *(6)* indicated that peptone dextrose agar with 33 ppm rose bengal and 20 ppm aureomycin gave good results with soil fungi; therefore, these concentrations were used in preliminary trials on Cottage cheese. In nine trials the PD agar counts were considerably higher than the AR agar counts. Also, the yeast and mold colonies were much smaller in radial growth on the AR agar than on PD agar.

In order to establish the possibility of using other concentrations of aureomycin and rose bengal in a medium for enumerating yeast and mold six different

TABLE 3. YEAST COUNTS WITH AR AGAR AND PD AGAR

Replication	ARA[1]	PDA
	x10[4]	
1	32	28
2	28	29
3	34	33
4	31	30
5	29	25
6	25	38
7	29	34
8	37	30
9	34	35
10	40	36
11	31	27
12	38	32
13	44	30
14	28	28
15	32	33
16	26	39
17	30	24
18	34	31
19	46	34
20	29	30
Total	656	626
$\bar{x}^2$	32.4	31.2

[1]ARA = 20 ppm aureomycin + 20 ppm rose bengal in basal medium.
[2]$\bar{x}$ = Mean.

TABLE 4. MOLD COUNTS WITH AR AGAR AND PD AGAR

Replication	ARA[1]	PDA
	x10[5]	
1	48	35
2	45	39
3	42	45
4	41	42
5	43	46
6	35	41
7	39	48
8	47	45
9	41	43
10	36	66
11	55	46
12	47	43
13	51	42
14	57	52
15	54	49
16	84	48
17	62	55
18	49	65
19	57	53
Total	983	968
$\overline{x}$[2]	49.15	48.40

[1]ARA = 20 ppm aureomycin + 20 ppm rose bengal in basal medium.
[2]$\overline{x}$ = Mean.

TABLE 5. YEAST AND MOLD COUNTS OF COTTAGE CHEESE WITH AR AGAR AND PD AGAR

Trial	ARA[1]	PDA
	per gram	
1	50	50
2	60	50
3	60	70
4	50	40
5	80	70
6	70	70
7	90	90
8	80	60
9	40	30
10	50	50
11	30	40
12	40	30
13	110	90
14	100	70
15	80	90
Total	990	900
$\overline{x}$[2]	66.0	60.0

[1]ARA = 20 ppm aureomycin + 20 ppm rose bengal in basal medium.
[2]$\overline{x}$ = Mean.

formulations of AR agar were compared with PD agar. The results are presented in Tables 1 and 2. With both yeast and mold suspensions the formulation containing 20 ppm aureomycin and 20 ppm of rose bengal consistently produced results most nearly comparable to PD agar. The radial growth of both yeast and mold colonies on the AR agar, although not measured, was observed to be smaller than the growth on PD agar.

In Table 3 are given the yeast counts on AR agar (20 ppm of aureomycin and 20 ppm rose bengal) and on PD agar. The mean count (of 20 replications) was slightly higher on the AR agar. However, this difference was not significant. A similar comparison was made with a mold suspension and the results are shown in Table 4. Here again the mean count was slightly higher on the AR agar but this difference was not statistically significant.

Since these results with yeast and mold suspensions indicated the AR agar (with 20 ppm aureomycin and 20 ppm rose bengal) gave results comparable to PD agar, a trial (15 replications) using Cottage cheese was made. The results appear in Table 5. Here again there was no significant difference between AR and PD agars but the AR agar had a slightly higher count and the radial growth was smaller.

An additional comparison of the two agars was made on 17 samples of Cottage cheese made by different manufacturers and which had been held at refrigeration temperature (4 ± 1 C) for more than 2 weeks. These results are shown in Table 6. No attempt was made to analyze these data but one can see the similarity of the counts on the two agars.

In order to test for bacterial growth on AR agar, 20 replications were made with each of the 5 species. No visible growth appeared on any of the plates after

TABLE 6. YEAST AND MOLD COUNTS WITH AR AGAR AND PD AGAR ON COTTAGE CHEESE

Samples	ARA[1]	PDA
	per gram	
1	870	830
2	7	5
3	2,500	2,800
4	4,700	3,800
5	3,800	3,300
6	2	4
7	5	3
8	TNC[2]	TNC
9	400	400
10	2	3
11	12	10
12	TNC	TNC
13	13,000	13,000
14	2,600	2,600
15	TNC	TNC
16	2	4
17	TNC	TNC

[1]ARA = 20 ppm aureomycin + 20 ppm rose bengal in basal medium.
[2]TNC = Too numerous to count.

incubating 48 hr at each optimum temperature for the species. From these results one may conclude that AR agar inhibits bacterial growth to the extent that yeast and mold counts can confidently be reported without interference of bacterial colonies.

Aureomycin-rose bengal agar has several definite advantages over PD agar. First, the problem of spreading mold colonies is virtually eliminated by the use of the rose bengal in the medium. Secondly, there is no precipitation of casein with AR agar to interfere with distinguishing colonies from curd particles. Thirdly, the possibility of some yeast and mold species not growing because of the low pH agar is eliminated. Finally, the red background imparted by the rose bengal aids in detecting yeast and mold colonies.

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INTERNATIONAL EXECUTIVE SERVICE CORPS SERVES FOOD AND FOOD RELATED INDUSTRIES AROUND THE WORLD

Veterans of U. S. food and food-related industries—serving abroad on short-term volunteer assignments for the International Executive Service Corps—are helping to strengthen the food production-distribution chain in developing countries around the world. By introducing advanced production, technical and distribution concepts as well as sound management practices, they are contributing to the achievement of food self-sufficiency and general economic progress within those countries.

An article in the current issue of IESC's International Executive News describes their work in countries throughout Latin America, the Middle East, Africa, South and East Asia.

Some of the volunteers serve on temporary leave from their U. S. companies. Others have recently retired, and several have been "activist" septuagenarians. Many have had management experience with well known U. S. food firms, ranging alphabetically from A&P to Wilson and Company. And several have had U. S. government or university research backgrounds.

They have advised grain millers, warehousers and processors from Iran to Singapore; bakeries in a dozen countries; supermarkets in Latin America and East Asia; dairies; refrigeration and farm equipment firms; chemical fertilizer producers in countries including those which have traditionally relied on night soil; poultry growers; slaughterhouse and meat packing firms; fisheries and seafood packers; fruit and vegetable growers and packers; edible oil producers; container manufacturers; lithographers who produce package and can labels . . .

In Panama, a baking concern was so pleased with the assistance and advice of one volunteer that it named a new premium line of products for him—Miller's Special, for Ross D. Miller, retired president of Freihofer Baking, Philadelphia.

In five years of operations, IESC volunteers have completed more than 1,200 projects in 45 of the developing countries. Projects cover practically the entire spectrum of business and industrial activity. About 15 per cent of them have been in the agricultural and food processing field, with important additional percentages in other food-related fields.

A Research Note

MICROBIOLOGY OF IMITATION AND FILLED DAIRY PRODUCTS AND THEIR COMPONENTS[1]

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ABSTRACT

Eighty-four imitation and filled milk products and 31 components used to fabricate such products were examined for total plate counts, coliform count, and the incidence of staphylococci and salmonellae.

All purchased commercial products were free of staphylococci and salmonellae, but 4 of 84 (4.7%) contained coliforms. Products purchased in retail outlets as a powder had high total plate counts (average 107) more often than products purchased frozen, refrigerated or in aerosol cans (average 78).

Fat or oil components had total plate counts of <10 per gram, whereas plate counts of powdered components ranged from <10 to 14,500 per gram. No components examined contained salmonellae, staphylococci, or coliforms.

Dairy and bakery products often have been implicated in food poisoning *(1, 5, 7, 8, 10, 11)*. In recent years imitation and filled products also have been implicated *(3, 12)*.

Imitation and filled milk products were examined for microbiological quality. Thirty-one components used to fabricate such products also were examined as possible sources of contamination.

EXPERIMENTAL PROCEDURE

Eighty-four imitation and filled milk products purchased in local markets in Manhattan, Kansas, and 31 components obtained from manufacturers were analyzed. Commercial samples purchased consisted of frozen and powdered whipped topping, liquid and powdered coffee whitener, cereal topping, imitation whipping cream, aerosol whipped topping, puddings, and frosting. Samples of frozen and liquid products were examined within 24 hr after purchase.

Frozen products were allowed to thaw in the refrigerator at 4 C before sampling. Other products were sampled exactly as purchased. Eleven grams (or 11 ml) of sample were aseptically removed and transferred to a 99 ml sterile water blank. The 1:10 dilution was shaken vigorously for thorough mixing and a further dilution of 1:1000 was prepared.

Plate count agar (Difco)[5] was used to determine total plate counts. *Standard Methods (2)* was followed except for an incubation temperature of 37 C instead of 32 C. This incubation temperature was chosen so that results could be compared with those obtained from other studies on the microbiology of malted milk shakes, the adult human mouth, cottage cheese, margarine, precooked frozen dessert type foods, ice, and drinking water.

Violet red bile agar (Difco) was used for the coliform count. *Standard Methods (2)* was followed except for an incubation temperature of 37 C. Isolates were streaked on eosin methylene blue agar and confirmed by lactose fermentation and IMViC reactions.

Mannitol salt agar (Difco) was used to isolate staphylococci. One and 0.1 ml of 1:10 dilution were transferred to plates and Snyder's surface spreading technique *(13)* was used to spread samples over the plates. Plates were incubated at 37 C for 24 hr, then yellow colonies surrounded by a yellow zone were examined by Gram stain.

Determinations for salmonellae were carried out as suggested by Galton, et al. *(4)*.

TABLE 1. BACTERIAL COUNT OF COFFEE WHITENERS, IMITATION HALF AND HALF, AND CEREAL TOPPINGS

Products	No. Samples	T.P.C./gram Range	T.P.C./gram Average	Coliforms/ gram (ml)
Frozen liquid coffee whiteners	8	<10-260	45	1/ml[1]
Powdered coffee whiteners	13	<10-200	50	10/ml[1]
Cereal toppings	3	<10-1500	500	<10
Imitation half & half	1	<10	<10	<10
Non-frozen liquid coffee whiteners	12	20-160	66	2/ml[1]

[1]In one sample only

RESULTS AND DISCUSSION

Of 84 samples from commercial retail sources, none contained salmonellae or staphylococci, and only 4 (4.7%) contained coliforms. Table 1 shows ranges and average total plate counts and coliform counts per gram (ml) of coffee whiteners, imitation half and half, and cereal toppings. The coliform organisms found in the liquid coffee whitener and non-frozen liquid coffee whitener were *Aerobacter* species.

Table 2 shows ranges and average total plate

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TABLE 2. BACTERIAL COUNT OF NON-DAIRY TOPPINGS

Product	No. Samples	T.P.C./gram Range	T.P.C./gram Average
Pre-whipped	6	<10-70	17
Aerosol	8	<10-150	19
Powders	6	20-700	220
Liquids to be whipped	12	<10-800	66

TABLE 3. BACTERIAL COUNT OF MISCELLANEOUS FOODS

Product	No. Samples	T.P.C./gram Range	T.P.C./gram Average	Coliforms/ gram
Desserts	3	<10-380	180	<10
Pudding	4	<10-190	78	<10
Frosting	2	20-170	95	<10
Pie filling	1	<10	10	<10
Pastry filling	5	<10-1800	550	130[1]

[1]In one sample only

TABLE 4. PLATE COUNT FOR NON-FAT COMPONENTS

Sample	T.P.C./gram
Imitation half and half	<10
Imitation milk	<10
Sour cream products	150[1]
Filled milk	<10
Coffee whitener	40
Vegetable component	<10
Vegetable component	40
Vegetable component	4700
Vegetable component	<10
Sodium caseinate	<10
Beverage base	14,500[1]
Sodium caseinate	<10
Sodium caseinate	<10
Enhancer	5,000
Imitation milk base	300
Sodium caseinate	400
Cream base	100
Vegetable component	<10
Sodium caseinate	<10

[1]Mold Growth

counts of non-dairy toppings. Coliform counts were <10 per gram and are not shown in Table 2. In several instances total plate counts were quite high, but no samples contained coliforms, staphylococci, or salmonellae.

Ranges and average total plate counts per gram of miscellaneous foods such as desserts, pudding, pie filling, and pastry fillings are shown in Table 3. Coliforms found in one pastry filling were *Aerobacter aerogenes.*

Table 4 shows total plate counts per gram of non-fat components. Coliform counts were <10 per gram and are not shown in the table. Several non-fat components contained bacteria and several showed mold growth, although none contained coliforms, salmonellae, or staphylococci. Fats do not readily support the growth of most bacteria *(6).* Total plate counts of fat and oil components were all <10 per gram and are not shown in a table.

Although not subject to public health regulations as rigid as those governing Grade A fluid milk production and processing, the imitation and fluid products studied in this report were generally of good bacteriological quality.

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FOOD AND DRUG ADMINISTRATION ATTITUDES ON IMITATIONS[1]

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As the industry is concerned about the impact of substitutes, regulatory and public health officials are concerned with protecting consumers against substitute dairy products which are unsafe, or nutritionally inferior, or which do not bear informative, non-misleading labeling. In carrying out our obligation we must, of course, act in accordance with applicable laws and regulations presently in effect or which may be enacted to deal with new problems. It is the administration of these laws that is discussed in this paper.

TWO CATEGORIES OF SUBSTITUTE DAIRY PRODUCTS

FDA consideration of substitute dairy products can basically be broken down into two categories: *(a)* products defined by specific Congressional acts, such as the Filled Milk Act or the Filled Cheese Act and *(b)* other substitute products—not covered by these specific acts—but which are subject to labeling requirements for imitation foods under provisions of the Federal Food, Drug, and Cosmetic (FDC) Act. This last category covers a wide range of products—and the marketing of these products in interstate commerce is somewhat confused because of the different labeling requirements under the FDC Act plus many different state laws.

FILLED MILK ACT

There is one subject that should be clarified now—before proceeding with our discussion. The Filled Milk Act is a Congressional Act—not a Standard of Identity promulgated under the FDC Act. A Congressional Act can only be changed by Congress. Regarding the Filled Milk Act specifically—we in FDA cannot change this Act—we can only enforce the Filled Milk Act as written and passed by Congress.

The Food and Drug Administration has taken the position that the Filled Milk Act is quite specific in defining the compositional framework in which a food is violative of the Act. In evaluating whether a product is violative, we consider three phases. First, does the product consist of " . . . any milk, cream or skimmed milk whether or not condensed, evaporated, concentrated, powdered, dried, or desiccated . . . ?" Second, " . . . to which has been added or which has been blended or compounded with any fat or oil other than milk fat . . . " Third, " . . . so that the resulting product is imitation or semblance of milk, cream or skimmed milk whether or not condensed . . . "

With all the words of the English language available, Congress in its wisdom chose to use the words, "milk, cream or skimmed milk," in describing the base to which a fat or oil other than milkfat is added to make a product in imitation or semblance of milk, cream or skimmed milk. This wording provides the Administration's basis for determining the status of these products. We consider the quantitative formula and usually examine the product before issuing an opinion on a product.

In our opinion, products on the market fabricated basically from water, vegetable oil, and non-fat dry milk which imitate or simulate milk or cream are "filled milk" products, and as such, cannot be shipped in interstate commerce under any form of labeling unless meeting the specific exemption in the Filled Milk Act.

Products which purport to be or resemble milk but which do not violate the Filled Milk Act since they contain no milk, cream or any dairy ingredient specified in the Filled Milk Act should—in our opinion—be clearly labeled as "imitation milk" and must comply with the general labeling requirements of the FDC Act.

Many of the milk and cream substitutes referred to consist of sodium caseinate in combination with vegetable oil or fat and certain other non-dairy ingredients. We believe that it is important that these combinations of sodium caseinate, vegetable oils and other ingredients should be marketed for what they are—imitation milk or imitation cream.

SODIUM CASEINATE

This brings us to the sodium caseinate question and its relation to the Filled Milk Act. We are aware that within the dairy industry, there is some

[1]Presented at the Fifty-sixth Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Inc., Louisville, Kentucky, August 18-21, 1969.

misunderstanding of the Food and Drug Administration's position on sodium caseinate. The situation is this: We have not considered sodium caseinate to be a "dairy product" in the same sense that you would consider market milk, ice cream, butter, or cheese to be a "dairy product."

You may have heard that the Administration considers sodium caseinate to be a chemical product. We do consider sodium caseinate to be a chemical product—a chemical product derived from milk, or saying it another way, a milk derivative.

We are of the opinion—and have so stated—that when sodium caseinate is used as a food ingredient in the fabrication of another food—the ingredient statement of the final fabricated food would be more informative if, in addition to the usual or common name required by the FDC Act, in this instance sodium caseinate, the phrase "milk derivative," or similar wording was used—thus, "sodium caseinate, a milk derivative." Incidentally, this position would apply to any of the various salts of casein.

In relation to the Filled Milk Act, however, the chemical and physical properties of sodium caseinate are different from the three products specifically named in the Filled Milk Act and from non-fat dry milk as defined by Congress. Accordingly, sodium caseinate is not one of the three products included in the Filled Milk Act. This, I believe, is the area where the dairy industry has misunderstood the Food and Drug position on sodium caseinate. We do consider sodium caseinate to be a milk derivative even though not a "dairy product." But, regardless of the "flag" that you attach to sodium caseinate, that is, "milk derivative" or "dairy product," etc., the product, sodium caseinate, is still not one of the three basic products specifically mentioned by Congress in the Filled Milk Act.

Therefore, products are not "filled milks" that are made using sodium caseinate as the base to which a fat or oil other than milkfat is added to make products that are imitations of milk. Such articles may, therefore, be shipped interstate provided they are not adulterated or misbranded in violation of the Federal Food, Drug, and Cosmetic Act.

THE FOOD, DRUG, AND COSMETIC ACT

This brings us to consideration of how that Act—the FDC Act—applies to these imitation products. The first provision to which I would like to direct your attention is Section 403(c) under which a food is deemed to be misbranded if it is an imitation of another food, unless it's label bears, in type of uniform size and prominence, the word "Imitation" and immediately thereafter, the name of the food imitated.

Many state laws include a similar provision, and some of these in which the sale of filled milk is not illegal require that filled milk products be labeled as "imitation milk," "imitation cream," "imitation half-and-half," etc. We exercise no jurisdiction over such articles, which are marketed solely in intrastate commerce.

DISTINCTION BETWEEN FILLED MILKS AND "NON-DAIRY" PRODUCTS

There are some who believe that there should be a distinction between the designation of these filled milks and the so-called "non-dairy" products. If the "non-dairy" substitutes are, in fact, imitations we have no choice under the Act—we are bound by the Act and the decision of the Court in the case of U. S. vs. 651 Cases, More or Less, Each Containing 24 Boxes of "Chocolate Chil-Zert." The food involved here was a frozen dessert labeled in part "Rich's Chocolate Chil-Zert, not an ice cream, and contains no milk or milk fat." The claimant contended that this labeling was truthful and more effectively informed consumers than would the words "imitation ice cream." The Court held that it was not for the claimant to choose the means or method of advising the public that his product was not ice cream; Congress had specified the method of advising the public that a product is not in fact the one which is imitated.

In applying Section 403(c) the first step is to determine whether the article is, in fact, an imitation of another food. The best guidelines we have received from the Courts in making such determination were included in the Chil-Zert decision. The Court declared that resemblance alone is not enough to establish that a food imitates another, and stated, "As indicated above, Chil-Zert is identical with ice cream in its method of manufacture, packaging, and sale. It is similar in taste, appearance, color, texture, body, and melting qualities. It has identical uses; its composition differs only from ice cream in the substitution of a cheaper ingredient, namely, vegetable oil in place of milk products. It is, therefore, something less than the genuine article, chocolate ice cream. It is inescapable that the ordinary understanding of English speech would denominate it as an imitation of ice cream." The Court also held that the food imitated need not be one for which a standard of identity has been established.

STANDARDS OF IDENTITY

As I mentioned earlier, we are concerned with protecting consumers against substitutes which are nutritionally inferior or which do not bear informative, non-misleading labeling. We believe these problems could best be solved by establishing, in accordance with Section 401 of the Act, standards of identity and of quality for imitation milks and creams. Most of you are probably acquainted

with the proposed standards which were published in the Federal Register of May 18, 1968.

We believe that standards of identity will promote honesty and fair dealing in the interest of consumers by requiring that ingredients be safe and suitable; by specifying the substances derived from milk which may be included; by requiring that the food be pasteurized, sterilized or sealed in a container and so processed by heat as to prevent spoilage; by specifying that the name must include the word "imitation" followed by the common name of the food imitated; and by requiring the listing of ingredients except that artificial flavor or color may be declared as "artificial flavor added," or "artificial color added."

The prescribed names include the word "imitation" followed by the name of the dairy product imitated. The names of the dairy products follow the definitions in the 1965 revision of the Grade "A" Pasteurized Milk Ordinance. We believe the required labeling is informative and not misleading.

The proposed standards of identity specify levels of fats the same as those prescribed for their dairy counterparts by the Pasteurized Milk Ordinance.

The purpose of the proposed standards of quality is to assure consumers the imitations will either be nutritionally equivalent to milk (in terms of specific nutrients for which milk is a significant food source) or will warn purchasers about nutritional deficiencies by a prominent statement "Below Standard in Quality" followed by an explanation of the deficiency such as "Low in Protein." The proposed standards specify not only the amount of protein but require that this be of a biological value equivalent to casein. The published proposal also prescribes levels of calcium, phosphorus, vitamin A, and riboflavin. Addition of vitamin D is optional, but if added there must be 100 U.S.P. units per 8-fluid oz serving.

During the comment period (ending October 15, 1968) about 85 comments were received. These comments were discussed with the NAS/NRC Food and Nutrition Board and additional suggestions were made. A revised draft of the proposed standard has been prepared and is currently being considered by the Commissioner of Food and Drugs. We anticipate a decision and publication in the near future.

New proposed standards of identity and quality for imitation milks were published in the *Federal Register* 34 (194:15657-15658 (October 9, 1969).

Because of the present interest in the substitutes for fluid milk and cream, I have devoted most of my discussion to these products. I would like, however, to make brief comments concerning certain other imitation or substitute dairy products.

MELLORINE

With regard to the frozen dessert sometimes called "Mellorine," we believe that this is definitely an imitation ice cream, meeting all the criteria of the Chil-Zert decision. When shipped in interstate commerce, it should be labeled as "Imitation Ice Cream" even when the shipment is between states which have established standards of identity for "Mellorine."

IMITATION MARGARINE

Sometimes, however, an imitation meeting the criteria of the Chocolate Chil-Zert decision receives recognition under another name. This has been the case with margarine which Congress, by the Statute for Colored Margaine of 1950, has defined as "(1) all substances, mixtures and compounds known as oleomargarine or margarine; (2) all substances, mixtures and compounds which have a consistency similar to that of butter and which contain any edible oils or fats other than milk fat if made in imitation or semblance of butter." But, the first definition of "margarine" by Congress was by Act of August 2, 1886.

The most recent ruling concerning use of the word, "imitation" resulted from a seizure involving an article labeled as "Imitation Margarine," which contained about half as much fat as required by the standard of identity for margarine. We argued that the article was made in imitation or semblance of butter, therefore, was "margarine" as defined by the Statute, and that it, therefore, should comply with the provision of the standard of identity for margarine. The article was intended for use by persons who wish to restrict their intake of fats or calories. The Court did not agree with our contention that there could not be an imitation of the food "margarine"—the ruling was that an article not complying with the standard but still "purports to be" could be labeled and sold as "Imitation Margarine."

LOW-FAT BUTTER

Some butter manufacturers have expressed an interest in marketing a low fat butter similar to the "imitation margarine." Congress, by the Act of March 4, 1923, defined butter as follows: "For the purposes of this chapter 'butter' shall be understood to mean the food product usually known as butter, and which is made exclusively from milk or cream, or both, with or without common salt, and with or without additional coloring matter, and containing not less than 80 percentum by weight of milk fat, all tolerance having been allowed for."

Any butter in interstate commerce found to contain less than 80% milk fat is subject to action. We have sometimes been asked whether a low fat butter might be marketed as "imitation butter." We

have commented that such an article would probably be subject to the taxes, licensing provisions, labeling, and other requirements of the Adulterated Butter Act of May 9, 1902, administered by the Internal Revenue Service. The definition of "adulterated butter" in the Act includes "any butter or butter fat with which there is mixed any substance foreign to butter as herein defined, with intent or effect of cheapening in cost the product, or any butter in the manufacture or manipulation of which any process or material is used with intent or effect of causing the absorption of abnormal quantities of water, milk, or cream." This however, would be for a decision by the Internal Revenue Service.

CULTURED DAIRY PRODUCTS

It might be well to briefly discuss the acid type or cultured dairy foods. For purposes of this discussion, let us consider that the various cultured dairy products fall in three categories. The first category consists of foods covered under the mandatory Federal Definitions and Standards of Identity. The second classification includes the foods coming under Advisory Federal Definitions of the *U. S. Public Health Service Milk Ordinance and Code*, while the third category or classification consists of foods not covered by any definition but for which Federal advisory opinions have been issued.

The primary concern regarding these cultured dairy products is, "What shall they be called?" From the FDA standpoint, "What are the labeling requirements for these various foods?" Mandatory standards of identity have been promulgated under the Federal Food, Drug, and Cosmetic Act for Cottage cheese, creamed Cottage cheese, Cream cheese, Neufchatel cheese, cream cheese with other foods, and Neufchatel cheese spreads with other foods. These standards are quite specific in their labeling requirements, composition, etc. For these products, as for any product covered by and in compliance with a standard of identity, it is not necessary to include an ingredient statement listing the mandatory ingredients used in manufacturing the product. In all instances when certain optional ingredients provided for in the respective standard of identity have been added, these optional ingredients must be listed conspicuously on the label preceding or following the name of the product, for example, "Vegetable gum added."

When these products, covered by a mandatory standard of identity, are mixed with other foods all other foods that have been added must be declared on the label. Using an example familiar to all—"Creamed Cottage Cheese with Chives." An ingredient declaration must include all components of the mixture by common or usual name in descending order of prominence.

The products having advisory definitions and the products for which advisory opinions have been issued all come under the general provisions of the FDC Act. The product known as "cultured cream" or "sour cream" or "cultured sour cream" as to its composition is based on the FDA Standard of Identity for cream class of food, Section 18.500. The product, cream, containing not less than 18% fat and containing no added non-fat dry milk, stabilizers, or flavors which simulate fermentation-developed flavors is soured by lactic acid-producing bacteria or similar cultures. The resulting product is a fluid or semi-fluid cream containing not less than 0.2% acidity expressed as lactic acid. Likewise, cultured, or sour half-and-half, or cultured buttermilk refer to products defined in the *U. S. Public Health Service Milk Ordinance and Code* and which have been soured by the action of lactic acid-producing bacteria or similar cultures.

The product made in the same manner as sour cream but from cream containing added non-fat dry milk, stabilizers, synthetic flavor, i.e., starter distillate, should have the term, "dressing" after the name —thus, "sour cream dressing." The ingredient statement for "sour cream dressing" should name all the ingredients. The reason for the term "dressing" is that the addition of non-fat dry milk, stabilizers, etc., has the net effect of substantially altering these products. Thus, they should have a different name. In other words, such additions to "sour cream" result in products with improvement in body, less syneresis or "wheying off," etc. These additions serve a useful purpose—but the consumer should be informed by appropriate labeling that the product is not simply cream which has been soured. The addition of the term "dressing" serves to alert the consumer that there is some difference.

PRODUCTS MADE BY DIRECT ACIDIFICATION

Acidified products made by the addition of food-grade acids and containing, of necessity, artificial flavors and possibly added non-fat dry milk, stabilizers, etc., should be qualified by a name such as "product." Thus, cream containing added non-fat dry milk, synthetic flavors, stabilizer, and acidified by the direct addition of acid would be properly labled by the name, "acidified sour cream product." The same type of labeling should be used for the other directly acidified products. For example, the buttermilk product when made by direct acidification is "acidified buttermilk product."

The term "product" should also be used even if on occasion the cream soured by direct acidification does not contain added ingredients other than the

acid. These products are not made by the normal culturing or souring process. Thus, the product is not exactly what the consumer has been purchasing under a given name over the years. For this reason, the product should have a distinguishing name and, of course, all of the ingredients must be listed.

Foods made in semblance of cultured cream, sour cream, or sour cream dressing but with the milk fat substituted whether in whole or in part by another fat are "imitation" products. This decision stems from Section 403(c) of the FDC Act. Although there are products on the market resembling sour cream or sour cream dressing which contain a vegetable fat and some form of milk, there has been no decision by the Federal courts as to whether such products come within the definition of "filled milk." We have indicated we will not seek a ruling from the courts so long as the articles are labeled as imitations and otherwise comply with the FDC Act.

IMITATION CHEESE PRODUCTS

I have said nothing so far about imitation cheese and cheese products. Many products which imitate standardized cheeses or cheese products are on the market. Most of these are properly labeled as imitations. As you know, the addition of any vegetable or other fat or oil to cheese brings it within the definitions of "filled cheese," making it subject to the Filled Cheese Act administered by the Internal Revenue Service. You may not be aware that this includes cheeses made with milk or skimmed milk admixed with butter. Filled cheese may be shipped interstate if it is manufactured and labeled in accordance with the Filled Cheese Act and complies with the Food, Drug, and Cosmetic Act and the Fair Packaging and Labeling Act.

Usually a filled cheese is made in imitation of one of the cheeses for which a Standard of Identity has been established. In addition to the labeling required under the Filled Cheese Act, such cheese should be labeled "Imitation————Cheese" (the blank being filled with the name of the cheese imitated). Since the article is not standardized, all ingredients should be listed.

SANITATION IN THE RETAIL FOOD INDUSTRY[1]

GALE PRINCE

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The subject of this paper involves a very important commodity, namely food. Throughout history food has been one of man's major concerns. Of the essentials of life, none is more vital. Yet over the years, mishandling of food has caused illness, and occasionally death. Thus, food distribution is an important part of our daily lives. Sanitation in the retail food distribution channels has been receiving more attention from both governmental agencies and industry. Before sanitation aspects in the retail food channels are described, some information on the magnitude of the retail food industry will be presented.

SIZE OF RETAIL FOOD INDUSTRY

The cans which you see on the shelves in your local supermarket do not appear there automatically as some people think. It takes people to put them there, and lots of them. In fact, of every seven people working in this country, one person works in some field of the food industry. Just the number of store employees alone is approximately 1.2 million or a population equivalent of about four of our states.

If all the eleven million shopping carts used in the nation's food stores were lined up, they would reach from Chicago to Paris. The 365,000 checkout counters would form a line 425 miles long *(1)*. Some 36 billion paper bags are used in the retail food stores in a year, and the register tape used for totaling customer transactions would reach to the moon and back twice, with a little to spare. Banking is an important aspect of food retailing. Food stores cash checks totaling about 150% of their gross sales; an amount second only to banks.

Food retailing is a big operation; for America has a big appetite. The average sized U.S. family of four persons consumes over 2.5 tons of food a year. *(1)*. Of this amount nearly 1.75 tons are of a perishable nature.

Profit is the life blood of business. It is through profit that a company obtains the capital needed to enlarge facilities and update equipment and products. Profits for the retail food industry averages only about 1.0% net profit compared with 11% for the tobacco industry and 4.3% for the mail order houses. The

[1]Presented at the annual meeting of the Illinois Association of Milk Sanitarians, Elgin, Ill., May 5, 1969.

retail food industry is very competitive. There are 28 food stores for every department store and 10 for every variety store.

Today the American consumer spends only about 19% of his take-home pay in a food store, compared to 26% in 1948. Only about 80% of this 19% spent in the food store is actually for food. The British spend about 29% while the Russians spend 50% of their take-home pay for food *(1)*.

With the abundance of food available in the United States, the consumer can choose freely from many types of products, different forms, and at different price levels. Thus we live in a consumer–oriented economy, and Mrs. Consumer is the boss. The typical supermarket shopper is a woman between 25 and 40 years old, with two children, who makes 150 shopping trips a year to the food store, and spends on an average of $26.69 a week. Her average purchase on a trip to the market is 12.4 items, but on the major shopping trip of the week she buys 30 items. Each stay in the store averages 22 min. On her tour of the market, she passes approximately 270 items a minute or 4.5 items every second *(1)*. This is the reason for increased emphasis on packaging methods. The manufacturer must attract the consumer's eye because only about one-third of the shoppers use a shopping list.

SELECTION OF A STORE BY CONSUMER

What determines why a shopper selects a given store? A recent survey indicated price is the main reason, followed by customer service, cleanliness of the store, and fresh high quality perishables.

These points are all taken into consideration when a new store is designed. It costs at least $250,000-$400,000 to build and equip and another $125,000 to stock a supermarket. Within this beautiful decor of today's supermarket, Mrs. Consumer does her weekend shopping with the confidence she is buying food which is free of foreign matter and microbiological contamination. She has developed this trust from the past record of the industry in providing high quality products. As the volume of food sold goes up because of the increase in population, so does the chance of a breakdown in the food distribution process. Eighteen months ago the Corporate Officers of the Jewel Companies foresaw this potential problem and the need for more emphasis to be placed on sanitation. With this decision came the author's assignment to develop a sanitation program for the Eisner Operation. Prior to this assignment, sanitation within the retail stores was handled by the store operations manager.

A SANITATION PROGRAM

Eisner Food Stores is one of the 11 divisions of the Jewel Companies with operations in Central Illinois and Western Indiana. The Eisner operation consists of 30 corporate retail stores, 40 franchise stores, a distribution center, 2 bakeries, a salad kitchen, an Institutional Foods Division, and a catering service. Each operation has its own sanitation problems and it is the author's responsibility to see that proper sanitation procedures are followed in all operations. In this paper we will be limited to a discussion of the sanitation aspects of the retail food stores.

The sanitation program for the retail food stores is quite involved. A store, depending on the size, will stock from 4,000 to 8,000 different items, all the way from soup to nuts. Nearly 50% of these items were not available 10 years ago. Each year our buyers are shown approximately 5,000 new items, and of this number they may select only 10%. The average life for a new product is about 3 years. Thus, the industry is continually faced with a rapid influx of new products. But for the store to be profitable and to maintain the product in a sanitary condition, it is necessary to have a rapid turnover of each item on the shelves. This means in the grocery department we need 13 turns of stock per year. In other departments the turnover rate expected is as follows: frozen food, 39 turnovers; dairy, 41; meat, 58; produce, 78; and bakery, 125. To control product it is necessary to have a coding system for every item. One that can be easily read. An example of this is our Hillfarm milk products. In the training programs various store employees find the F1F0 rotation method is continually stressed. The industry uses a large number of part timers and most of them are young people. In some instances 60% of the retail store employees are part timers. This creates an endless training job, but, we need this flexibility, because about 75% of the shopping is done on Thursday, Friday, and Saturday.

Our quality control program consists of sampling products with bacteriological and chemical analysis and taste testing to meet our specifications. Labeling and weight checks are also made. Perishable goods are constantly checked for adequate temperature at time of receipt and during distribution. Bacteriological swabs are taken from equipment in food processing areas to check on cleaning procedures and also as an educational tool. Visits also are made to supplier's or to prospective supplier's plants.

In conjunction with the quality control program previously mentioned, periodic in-store inspection tours are made before, during, or after working hours by supervisors. It is during these inspections that

checks are made to see if the recommended cleaning schedules are followed. Is the hourly, daily, weekly, and monthly dirt being kept up to date? Is any equipment in need of repair or replacing? What is the temperature of the various coolers and display cases? Is food being properly stored and handled? Are hazardous compounds stored away from food ingredients? Is product displayed within the load level limits of all display cases? Has damaged product been removed from the display shelves? Are there any peculiarities noted in the appearance of products or packaging? These are examples of the many things that need to be checked when inspecting a store operation. When problems are found they are discussed with the manager and area supervisor. The legal requirements and the reason why and what could happen are discussed along with preventive measures.

Meetings are held with managers where the sanitary science of food handling is discussed in detail. These meetings have been very helpful and have yielded many interesting comments. This is the first exposure most of these people have had to the growth of bacteria.

So far nothing has been mentioned about pest control. Just like humans, rodents and insects need food to survive; and where is there a better place than in a supermarket? Pest control is a science in itself, not and the job of our own store employees. Presently we are using an outside pest control service on a regular basis. However, this program must be constantly monitored to avoid complacency on the part of the service. Store employees have the responsibility to report any problems they may have with pests between visits to the sanitarian. In our distribution center we have a full time pest control employee.

We are constantly reviewing new cleaning compounds and cleaning processes which will do a better job at reduced labor costs. The present cost of clean up is far too high. New methods of cleaning for the retail store have to come about. With this comes new design for stores and equipment. Although great progres has been made by the National Sanitation Foundation and the Commercial Refrigeration Manufacturers Sanitation Code in equipment design for the retail operation, they still leave things to be desired. These standards are not the complete answer for better equipment design but do give us a basis with which to start.

Sanitation begins in the early planning stages on the drawing board. For sanitation to be effective and efficient it must be built in. In the past sanitation has not received the necessary emphasis in new building. Hence the author spends part of his time going over blueprints and making recommendations on new building and store remodelings.

Presently an educational sanitation program is being prepared which will be made available to all Eisner employees.

One is very fortunate if one has a good working relationship with the Public Health officials in the area; people with whom one can sit down and discuss solutions to sanitation problems. This is very important. Sanitation in the retail food industry is not a once a day job or a one man's job; it is everyone's job throughout every working day. The program described above is not necessarily the best, but it is giving results, and we will be able to accomplish even more in the future.

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LISTING OF SOURCES OF SHORT-TERM TRAINING FOR LABORATORY WORKERS

The Laboratory Consultation and Development Section of the National Communicable Disease Center is compiling a list of organizations which offer short-term training for persons working in clinical and public health laboratories. Academic training for full-time students will not be included. When completed, the list will be made available to all individuals interested in continuing education for medical laboratory personnel. The compilation will serve to alert training coordinators, supervisors and prospective students to the existence of many little-known sources of short-term training, some at no cost to the student.

Companies, professional associations, individuals, and schools who desire to furnish information for inclusion in the listing should write: National Communicable Disease Center, Attention: Mr. John H. Krickel, Education Specialist, Laboratory Division, Atlanta, Georgia 30333. No information will be included in the published list without prior clearance from the organization or individual offering the training.

MIXED SAMPLE TESTS FOR EXTRANEOUS MATTER IN MILK

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(Received for publication June 9, 1969)

Abstract

A relationship is sometimes assumed to exist between the amounts of extraneous matter in milk as measured by the mixed sample method and by the off-the-bottom method but the reliability of such a relationship may be so low as to seriously limit its usefulness. In a study of this relationship, extraneous matter measurements were performed by both methods on 149 cans of milk, and on samples taken from cans and dump tanks for a further 74 shippers. The results showed that no close relationship existed between the two methods. From a consideration of several factors it is concluded that the mixed sample method is more suitable and that it should be used in preference to the off-the-bottom method for estimates of extraneous matter in milk.

Dairy plant milk receipts, at one time entirely in cans, now arrive principally by tanker truck from farm bulk tanks. With the change, the off-the-bottom method of sampling and testing for extraneous matter has become much less useful. Only a mixed sample method can be employed satisfactorily on bulk milk *(3, 4)*.

While the Province of Alberta regulations respecting amounts of extraneous matter in milk provide for use of the off-the-bottom method for milk received in cans, they also permit tests for extraneous matter on milk accepted from farm bulk tanks to be conducted by an "acceptable and approved method that will yield results comparable to the off-the-bottom method for individual cans" *(1)*. Because of the expectation that a mixed sample method could yield results comparable to those obtained by off-the-bottom tests, it was considered that an investigation should be conducted into the relationship between results obtained by the two test methods under Alberta conditions.

A review of available literature suggested a low level of reliability would probably be associated with such relationships as might be observed. Watson *(7)*, after many trials on different tanks of milk and filtering all samples through an area of 1.125-inch diameter, reported (without giving more details) only that he found 1 gallon of agitated milk would give a disc which seemed to most nearly match the disc from 1 pint off-the-bottom of a settled 10 gallon can of the same milk (8 to 1 ratio). Liska and Calbert *(4)*, in experiments with various amounts of a standard sediment added to milk, and filtering all samples through an area of 1.125-inch diameter, found that the results obtained with 1 pint off-the-bottom samples from 10 gallon cans agreed with those obtained in 77% of the mixed sample tests conducted with 4-quart samples. When 3-quart mixed samples were taken, agreement was observed in 38% of the tests, and with 5-quart mixed samples, agreement was observed in 29% of the tests. Four-quart mixed samples thus showed the highest percentage of agreement with the 1-pint off-the-bottom samples (8 to 1 ratio). However, the agreement was far from perfect and if the composition of the sediment mixture had varied, as in real samples, the degree of agreement would undoubtedly have been lowered. Roman *(6)* also has observed that in many instances comparisons between off-the-bottom and mixed sample methods are far apart.

Off-the-bottom tests are normally conducted with a device consisting of a cylinder and piston so arranged that on the upstroke of the piston a sample of 16 fluid oz is drawn past the filter pad into the cylinder and, on the down stroke of the piston, the sample is forced through an area of 1.125-inch diameter on the filter pad. This method was used in the tests reported in this study.

Mixed sample tests may be conducted in either of two ways. The first uses an off-the-bottom tester equipped with a special head having a filtering area of 0.40-inch diameter *(2)*. The second, which was used in this study, makes use of a filter funnel and a small vacuum *(5)*. Both methods can be applied to tests on milk in cans or in bulk tanks.

Methods and Results

Two series of tests were conducted. In the first, the mixed sample was taken from individual cans and, in the second, the mixed sample was taken from the dump tank so that it represented a composite of several cans. All filtration was performed in a "platform" situation with milk at temperatures as received at the plant.

Individual can samples

In the first series of tests, a normal off-the-bottom sediment estimation was made on each of 149 individual cans. The milk in the can was then thor-

oughly mixed with a dairy mixing rod. A 16-oz sample was removed and filtered through a 0.40 inch diameter area on a filter disc. Although the reverse order (taking the mixed sample first and sampling off-the-bottom last) would have been more appropriate, it was not practical in the milk receiving situation.

Discs obtained by the mixed sample method were scored according to the nearest matching disc on the "Guide for Sediment in Milk" prepared by the Alberta Dairymen's Association Research Unit *(5)*. Discs obtained by the off-the-bottom method were scored using the guide of the Department of National Health and Welfare. Interpolations were made between the values represented by the discs in this guide in order to record as precisely as possible the apparent amount of sediment on each disc. In Table 1 the results are presented showing the range in amounts of off-the-bottom sediment observed in milks receiving each of the mixed sample scores. Thus 30 samples, assayed by the mixed sample method, were assigned Score 1 (which represents an average of 0.05 mg of sediment in 16 fluid oz). In the off-the-bottom assay these 30 samples showed as little as 0.3 and as much as 2.5 mg in 16 fluid ounces.

Cans and dump-tank samples

In a second series of tests, estimates of amounts of extraneous matter were made by the off-the-bottom method on approximately one-half of the cans delivered by each of 74 shippers. As soon as all of each shipper's milk was in the dump-tank, a 16-oz mixed sample was taken for test. The amounts of extraneous matter were estimated as before. In Table 2 results are presented showing the range in amounts of off-the-bottom sediment observed in milks receiving each of the mixed sample scores.

TABLE 1. COMPARISON OF MIXED SAMPLE AND OFF-THE-BOTTOM METHODS FOR THE ESTIMATION OF EXTRANEOUS MATTER IN MILK: ESTIMATES FROM INDIVIDUAL CANS

Mixed sample method		Off-the-bottom method		
Score on Guide	Amount of extraneous matter represented by score (mg/16 fl oz)	Amount of extraneous matter found (mg/16 fl oz)		Number of samples
		Range of values	Mean values	
1	0.05	0.3-2.5	0.7	30
2	0.15	0.3-2.2	1.0	44
3	0.20	0.3-3.0	1.3	63
4	0.30	0.3-3.0	1.7	12
				149

TABLE 2. COMPARISON OF MIXED SAMPLE AND OFF-THE-BOTTOM METHODS FOR THE ESTIMATION OF EXTRANEOUS MATTER IN MILK: ESTIMATES FROM CANS AND DUMP TANKS

Mixed sample method		Off-the-bottom method		
Score on Guide	Amount of extraneous matter represented by score (mg/16 fl oz)	Amount of extraneous matter found (mg/16 fl oz)		Number of samples
		Range of values	Mean values	
1	0.05	0.3-1.1	0.5	30
2	0.15	0.3-1.5	0.7	26
3	0.20	0.4-2.0	1.0	15
4	0.30	0.6-2.3	1.2	3
				74

DISCUSSION AND CONCLUSION

It is apparent from these results that the mean value of the amounts of sediment found in off-the-bottom tests increased as the amount found by the mixed sample increased. However, no close relationship existed between the results obtained by the two methods. For any amount of extraneous matter found by the mixed sample method, there was a wide range of amounts found by the off-the-bottom method.

The nature of the extraneous matter on a number of discs examined under a microscope showed that the differences in amounts could be explained in part, at least, by differences in the kinds of extraneous matter detected by the two methods. Off-the-bottom sediments consisted largely of mineral particles while mixed sample filter discs showed both mineral and organic matter but tended to include a larger proportion of organic matter. Thus some samples which did not have excessive contaminations of high density particles received low sediment scores when examined by the off-the-bottom method. However, if they contained appreciable amounts of light suspended material they received relatively high sediment scores when examined by the mixed sample method.

To some shippers, mixed sample tests will appear more severe than off-the-bottom tests. Because farm filtering of milk may be expected to remove the larger (and usually the lighter) particles, this view may be less likely to arise when milk is filtered on the farm than when it is not.

Results obtained by the off-the-bottom method are subject to at least three sources of error: *(a)* suspended matter is not adequately measured, *(b)* the can may have been agitated shortly before testing, and *(c)* bottoms of cans may have variable surface con-

tours. The mixed sample method is not subject to these errors and is superior in two additional respects. First, it can be reproduced test after test and this is not possible with the off-the-bottom method. Second, results are more meaningful when converted to amounts of sediment present in each gallon, or in the total quantity of milk represented by the test.

It is considered, therefore, that the mixed sample method is not only necessary for bulk milk tests, but even for tests on milk delivered in cans it has several advantages over the off-the-bottom method. For these reasons it is recommended for adoption as the standard method for the estimation of extraneous matter in both bulk milk and milk in cans.

ACKNOWLEDGEMENTS

The authors thank the National Research Council of Canada and the Alberta Agricultural Research Trust for their financial support and Mr. Leonard Ewanyk for technical assistance in this investigation.

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ASSOCIATION AFFAIRS

EGG INDUSTRY ADOPTS SANITATION CRITERIA

Two additional E-3-A Accepted Practices and one E-3-A Sanitary Standard were adopted at the E-3-A Sanitary Standards Committees' second meeting held in Hartford, Connecticut on September 25. At its first meeting in Palm Springs last March E-3-A Standards for Pumps, Homogenizers, Sifters, and Thermometer Fittings for processing egg products were adopted. They were officially signed on June 23, 1969.

The two new Accepted Practices cover Air Under Pressure, and Permanent Pipelines. The new E-3-A Sanitary Standard is for Rubber and Rubber-like materials. These now will be subject to editorial review and prepared for signing and publication. They are believed to hold much interest to the egg processing industry where circulation cleaning, sources of purified air, and sealing materials are important processing needs. In 3-A parlance a *Practice* covers a complete system and thus relates to procedures as well as equipment. A *Standard* relates solely to the piece of equipment.

Up for review at the Hartford meeting were tentative drafts for E-3-A Sanitary Standards for Batch Pasteurizers, and for Leak Protector Valves, and a tentative E-3-A Accepted Practice for Spray Drying Systems. These next pass to the regulatory-sanitarian segment of the committees for consideration of industry proposals.

Participating in the E-3-A Committees common effort to develop sanitary criteria for cleanability of egg processing equipment are, representing industry, the Institute of American Poultry Industries, and Dairy & Food Industries Supply Association's technical committees. For the regulatory-sanitarian segment participants are USDA, U. S. Public Health Service, and International Association of Milk, Food and Environmental Sanitarians.

The E-3-A Committees for food processing equipment ("E" for egg) are an outgrowth of the 3-A Committees for food processing equipment which for some 25 years have been developing similar Standards for the dairy foods industry.

KANSAS ASSOCIATION OF ENVIRONMENTALISTS ANNUAL MEETING OCTOBER 29, 30, 31

The annual meeting of the Kansas Association of Environmentalists was held October 29, 30, and 31, 1969 at the Hilton Inn, Salina, Kansas with approximately one hundred in attendance. An outstanding program was presented, highlights of which were: "Animal Waste Control," Jim Current, Engineering Aide, Kansas State Health Department; Jim's talk dealt mainly with the many problems of the waste

disposal connected with the huge feed lots in Kansas; New Activities of the Environmental Health Division, Wichita-Sedgwick County Health Department, Jack E. Milburn and Don Dixon very ably presented a program for bird control and junk automobiles: Advancement in the control of Pollution of Air and Water, Howard F. Saiger, Director, Division of Air Quality Conservation; Labeling of Milk – Upgrading Manufactured Milk Products, Brace Rawley, Dairy Commissioner, Kansas State Board of Agriculture; Eating and Drinking Establishment Survey, O. L. Honomichl, Food and Drug Inspector, State Health Department; Housing for the Poor—How to Solve the Problem, Bill Atwater, Assistant Director, Local Housing Authority, Wichita and Reverend Jackson all were interesting features of the meeting and generated considerable discussion.

President Keith Nash presided at the Annual Awards Banquet at which time O. L. Honomichl received the outstanding sanitarian of the year award. Awards were also presented to Mr. Garland and Mr. Tichenor for many years of outstanding service.

WILLIAM A. DEAN, JR. RECEIVES 3-A HONOR PLAQUE

William A. Dean, Jr., associate technical director for the Dairy & Food Industries Supply Association, was presented with the bronze 3-A Honor Award plaque at the 3-A Committees regular fall meeting in Hartford, Connecticut, September 23-24. The award was made in recognition of his services to the 3-A Standards program over many years.

The award, instituted in 1963 and sponsored by the Dairy Industry Committee, is presented from time to time to outstanding participants among the various industry and government segments making up the 3-A Committees. Dean's award was based upon his 20-year participation on the Sanitary Standards Subcommittee for dairy processors while he was with Bowman Dairy Company, Chicago. Thus, his award was presented by Robert H. North, executive vice president, Milk Industry Foundation, at a special testimonial dinner held on September 23. The monumental contribution to the drafting of sanitary standards for dairy equipment has been continued by Dean since he retired from Bowman in 1966 and joined the DFISA staff. At the time of his retirement he was director of engineering for the company.

Five other 3-A participants have received the 3-A bronze plaque. They are E. H. Parfitt, DIC; Tom Burress, DFISA; C. A. Abele, International Association of Milk, Food and Environmental Sanitarians; Robert Everett, DFISA retired; and Fred Uets, DIC.

RECENT AFFILIATE ASSOCIATION MEETINGS

Twenty-Fifth Annual Conference, Wisconsin Association of Milk and Food Sanitarians in cooperation with Wisconsin Dairy Plant Fieldmen's Association, September 11-12, Conway Motor Inn, Appleton, Wis. Secretary-Treasurer, Wayne Brown, 4702 University Ave., Madison, Wis. 53702.

Annual meeting of Washington Milk Sanitarians Association, September 24. Yakima, Washington. Secretary-Treasurer, Ray Carson, 2505 S. McClellan St., Seattle Washington 98144.

Fifty-first Annual Meeting, California Association of Dairy and Milk Sanitarians held jointly with the California Fieldmen's Conference, October 13, 14, 15, at Pomona Valley Inn, Pomona, California, Secretary-Treasurer, Robert E. Abacherli, 870 S. Acacia, Rialto, Calif. 92376.

Nineteenth Annual Meeting, Indiana Association of Sanitarians, Inc., September 23, 24, 25, Ball State University, Muncie, Indiana. Secretary John D. Boruff, R. R. 1, Roachdale, Indiana 46172.

Program Committees of other affiliates wishing programs from other associations to aid them in making up their own program may obtain copies of the program from the affiliate Secretary-Treasurers.

BASIC MAINTENANCE COURSE FEBRUARY 9-27, 1970 AMERICAN INSTITUTE OF BAKING 400 EAST ONTARIO STREET CHICAGO, ILLINOIS 60611

WELCOME, ORIENTATION, REGISTRATION, BUILDING TOUR. (AIB Staff) 1½ hrs.

COMMUNICATIONS. (AIB Staff) 3 hrs.

PREVENTIVE MAINTENANCE VS. MAINTENANCE REPAIR. What is preventative maintenance? (AIB Staff) 1 hr.

EFFECTS OF POOR MAINTENANCE, MACHINE BREAKDOWN. And other delays on the finished product. (AIB Staff) 1½ hrs.

PHYSICS (BAKE SHOP MECHANICS). Density, relative humidity, boiling point, heat, specific heat, specific gravity and math. (AIB Staff) 21 hrs.

BULK HANDLING OF INGREDIENTS. Detailed explanation of different types of bulk systems, the use of various type blowers, controls and metering devices used in a bulk, dry or wet, ingredient handling system. (AIB Staff and Outside Lecturer) 4 hrs.

SCALES. Principals of a beam scale, maintenance requirements, how to check, liquid meters, volumetric scaling devices. Effects on cost and quality of finished product. (Outside Lecturer) 2 hrs.

CONTROLLING DOUGH TEMPERATURE. An explanation of why dough temperatures must be controlled and the maintenance personnel relationship to each. (AIB Staff) 1½ hrs.

BLUEPRINT READING. How to read a machine drawing. (AIB Staff) 2½ hrs.

VERTICAL MIXERS. Maintenance and operation, gear drive on variable speed. (Outside Lecturer) 2½ hrs.

HORIZONTAL MIXERS. Maintenance and operation of tilt bowl and stationary bowl mixers. Explanation of refrigeration timers, mixing timers and recording instruments. (Outside Lecturer) 2½ hrs.

AUTOMATIC ROLL MACHINE. Operation, maintenance and sanitation of an automatic soft roll machine (example Model "K"). (Outside Lecturer) 1½ hrs.

MAKEUP EQUIPMENT–CONVENTIONAL BREAD. Operation, maintenance and adjustment of dividers, vertical head and rotary head, rounders, intermediate proofers, panner moulders. (Outside Lecturer) 5 hrs.

DOUGH AND INGREDIENT PUMPS. Operation and maintenance of positive displacement pumps and degassing pumps, etc. (Outside Lecturer) 2 hrs.

ELECTRICITY. Basic electricity, principles of electric motors, starters, fuses, relay switches, how to read a wiring diagram and an electrical schematic. (AIB Staff and Outside Lecturer) 19 hrs.

REFRIGERATION. Principles of refrigeration, including explanation of dryers, liquid indicators, etc., water chillers, direct expansion, troubleshooting aids and hints. (Outside Lecturer) 6 hrs.

FERMENTATION ROOMS, PROOF BOXES. Operation and maintenance of fermentation rooms, proof boxes, automatic and manual. (Outside Lecturer) 3 hrs.

OVENS. Detailed explanation of various types of ovens in use today. Maintenance of each, direct and indirect. Types of fuel, loaders and unloaders. (Outside Lecturer) 5 hrs.

SAFETY. Why bakery safety? A discussion of the need for a safety program. (Outside Lecturer) 1 hr.

SANITATION OF BAKERY EQUIPMENT. A discussion of the problems of bakeries and bakery equipment sanitation. (AIB Staff) 2 hrs.

POWER TRANSMISSIONS. A discussion on the selection and maintenance of gear reducers, variable speed drives, oil seals, lubrication, etc. (Outside Lecturer) 4 hrs.

LUBRICATION. An explanation of the different types of lubricants and their uses (proper lubricant for each piece of equipment). (Outside Lecturer) 1 hr.

BAKING PANS AND COATINGS. Maintenance requirements of baking pans and pan coatings–how to get the most out of each. (How proper maintenance of equipment can reduce need for new pans and coatings.) (Outside Lecturer) 2 hrs.

CONVEYORS. Explanation of the various types of conveyors and maintenance requirements of each. (Outside Lecturer) 2 hrs.

BREAD COOLERS. Operation and maintenance of various types of bread coolers. (Outside Lecturer) 2 hrs.

SLICING, WRAPPING AND BAGGING. Importance of correct slicing, operation and maintenance of slicers, wrappers and baggers. (Outside Lecturer) 5 hrs.

BOILERS. Operation of burners and controls, maintenance of boilers and a discussion of feed water treatment. (Outside Lecturer) 2 hrs.

FIELD TRIP. A trip to one of the major bakeries to observe a plant in full operation. (AIB Staff) 4½ hrs.

CONTINUOUS CAKE MIXING EQUIPMENT. Operation and maintenance of the equipment for the continuous mixing of cake. (Outside Lecturer) 2 hrs.

CONTINUOUS BREAD MIXING. A discussion of the continuous bread mixing equipment now used in bakeries. (Outside Lecturer) 4 hrs.

SUMMARY. (AIB Staff) 1 hr.

HOWARD YOUNG

Howard Young, Assistant Extension Dairy Technologist, University of Florida Extension Service, Gainesville, Florida, passed away October 1, 1969 after a long illness.

Howard was a member of International and was active in the Florida Association of Milk, Food and Environmental Sanitarians for many years, holding several offices in the association.

VERNON D. FOLTZ

Vernon "Tiny" Foltz, 64, a professor of biology at Kansas State University, died on September 15, 1969. Foltz had leukemia for sometime although he continued to carry out his faculty duties.

Survivors include his wife, Pauline; two daughters, Mrs. Lewis Hitch of Wichita, and Mrs. William Hoffman of Westmoreland, Kansas; and a son, Paul, of Manhattan, Kansas.

Foltz not only served as a professor but also made unusual contributions outside his field. For more than 20 years he served as adviser to the University fraternity system. He was presented Acacia Fraternity's Award of Merit in recognition of outstanding professional accomplishments and service to the fraternity system. The Inter-Fraternity Council endowed the "Tiny" Foltz scholarship in his honor several years ago and memorial contributions to the scholarship fund have been suggested by the family.

Foltz was born in Belle Plaine, Kansas in 1905. He was associated with Kansas State University since 1923, when he entered as a freshman. After receiving B. S. and M. S. degrees, he was appointed as instructor at Kansas State University in 1929. In 1946 he became a full professor.

His research in food and dairy bacteriology received worldwide attention. He was a member of the Advisory Laboratory Commission of the Kansas State Board of Health since its inception and had been chairman of the commission for the past 17 years. He also was a member of the Sanitation Committee of the Association of Operative Millers.

Foltz was head of the Department of Bacteriology at Kansas State University from 1952 to 1956. He was selected by his colleagues in 1966 to be honored at the annual Faculty Lectureship Dinner. In 1968 he received the Gamma Sigma Delta distinguished service award for faculty. He was a past president of the Missouri Valley Branch of the American Society for Microbiology, a fellow of the American Public Health Association, member of International Association, Milk, Food and Environmental Sanitarians, Inc., and a member of numerous honorary and professional societies.

DR. JAMES J. JEZESKI RECEIVES CERTIFICATE OF ACHIEVEMENT AWARD AT MINNESOTA SANITARIANS ANNUAL BANQUET

Recipient of the Minnesota Sanitarians Certificate of Achievement Award for 1969 was James J. Jezeski, Professor, Department of Food Science and Industries, University of Minnesota.

In making the presentation, Leonard Waldock, Chairman of the Awards Committee noted that Dr. Jezeski was being honored for his outstanding contributions to milk and food sanitation programs, his accomplishments as an educator and his untiring efforts in behalf of the Minnesota Sanitarians Association.

ANNOUNCING--NEW MANUAL MICROBIOLOGICAL PROCEDURES FOR THE DIAGNOSIS OF BOVINE MASTITIS

Written by an expert committee for publication by the National Mastitis Council. First manual of its type outlining recommended procedures for the collection of milk samples and current methods for the detection and identification of the various types of mastitis pathogens including streptococci, staphylococci, coliforms, pseudomonads, corynebacteria, mycoplasma, and fungi. Contains color photographs of streptococci and staphylococci on blood agar plates to assist in identification or differentiation of these pathogens. This Manual should prove highly useful for laboratories involved in mastitis control programs.

Available in October or November 1969. Order from National Mastitis Council, Inc., 910 Seventeenth Street, N. W., Washington, D. C. 20006. Price of the Manual: Single copy $2,00 if payment is included with order; $3.00 if payment is not included with order; 10% discount on 10 or more copies.

ANNUAL REPORT OF THE EXECUTIVE-SECRETARY AND MANAGING EDITOR, 1968-1969

In several respects this fiscal year has been the best year International has ever had. In fact, it is the best year we have had financially. Our income was over $56,000 and our expenses were $50,C00, making both gross income and the net income a record.

Our affiliates improved their collection and reporting of dues to an extent so that we sent out only about one-half the usual drop letters. Several affiliates showed a nice increase in membership. Florida doubled its membership, Iowa almost doubled its and Minnesota showed a good increase. Thus we had an increase in our affiliate membership. Our direct membership remained about the same, showing no gain for the first time in the last 4 or 5 years. Our turnover because of deaths, retirements, and drop outs, averages about 10%, so we have to enlist over 300 new members each year to show a gain. If all affiliates worked as hard at increasing their membership as Florida, Iowa, and Minnesota did this year, we could greatly increase our membership. It is well to remember that it is as important to keep old members as it is to get new ones.

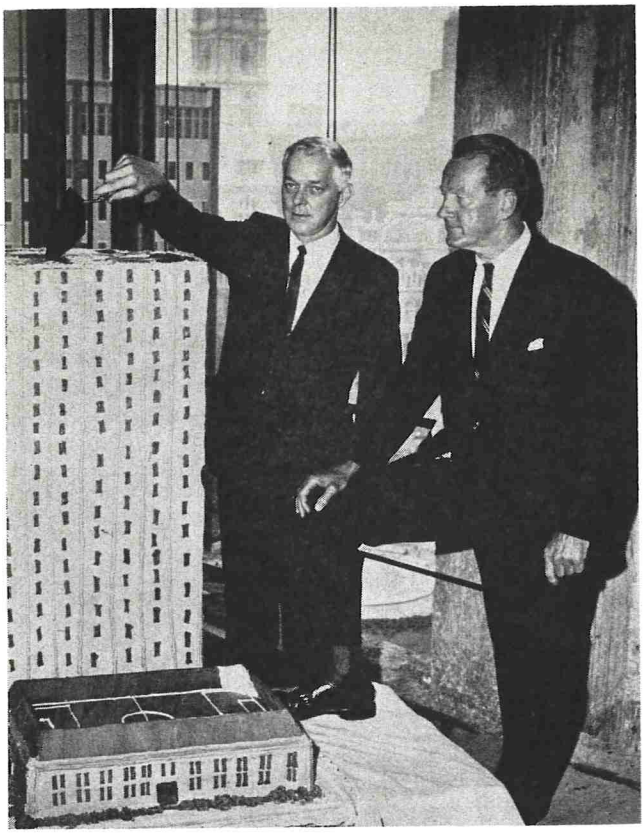
Our material for the *Journal* has been exceptionally varied and plentiful. We have had to run 8 to 16 pages additional in almost every issue to keep up. The institution of the page charge January 1, 1969 has been of great help and the full effects of this will be more evident in this coming fiscal year. The charge has had no apparent affect on receipt of research material and we will be able to publish this material more quickly, which is most important to do.

My attendance at meetings was even more extensive than usual this year. Important meetings were held with the local arrangements committees for this meeting, for Iowa 1970, and California 1971. The first meeting with the egg industry on 3-A sanitary standards and the finalizing of 4 standards at Palm Springs, Cal. was a good beginning of our expansion into the food standards field. I predict it is the beginning of one of our greatest contributions to sanitation. Our distribution, all over the world, of 3-A Standards, the Foodborne Disease booklet, and the *Journal,* increases each year. We are on the 12th 5000-copy printing of the Foodborne Disease booklet.

To me the future of International appears to be brighter than ever. The possibilities for important contributions to the members and the health of the public, is still unlimited. My hope is to still be able to help for some time to come. Again for the 18th time it has been a privilege to serve you.

Respectfully submitted,
H. L. "RED" THOMASSON

NEWS AND EVENTS



James E. Fike, Field Sales Manager, joins William P. Drake, Pennwalt Chairman, in taking first cut of a cake baked as a replica of the company's new 20-story corporate headquarters in Philadelphia, Pa. Pennwalt Corporation, formerly Pennsalt Chemicals, produces cleaners and sanitizers for the dairy industry. The company is also an international manufacturer of intermediate and specialty chemicals, specialized equipment, and health products.

APPLICATION FOR ENVIRONMENTAL HEALTH FELLOWSHIPS FOR 1970-1971

Applications for Environmental Health Fellowships are now being accepted for the 1970-1971 academic year at the Consolidated University of North Carolina (Chapel Hill and Raleigh campuses). Recipients participate in multidepartmental programs designed to prepare graduate students for careers in research, teaching, and practice in the various specialized fields in environmental health. This porgram is sponsored jointly by the Departments of Environmental Sciences and Engineering, Biostatistics, and Epidemiology of the School of Public Health; the Departments of Botany, City and Regional Planning, Geology, and Zoology of the School of Arts and Sciences; the School of Medicine and the Department of Food Science at North Carolina State University at Raleigh. Students generally enroll in the department of their basic specialty for training in depth in that area and then elect courses in other departments in order to obtain a broad understanding of the problems of the environment and the relation of their specialty to the solution of these problems.

Fellowships are available for U. S. citizens working for masters and doctoral degrees. They provide tuition, fees and a stipend, including a dependency allowance. Postdoctoral fellowships are also available.

Further information may be obtained by writing the Institute of Environmental Health Studies, Box 630, Chapel Hill, North Carolina 27514 or the head of any of the departments mentioned above.

PROGRAM TO PROMOTE COURSES IN FOOD PRODUCTION AND COMMISSARY MANAGEMENT

A program to promote and publicize courses in Food Production and Commissary Management at the University of Wisconsin is being planned by two of the foodservice industry's leading associations, the National Restaurant Association and the Institutional Foodservice Manufacturers Association.

It was pointed out by the two associations that there are many opportunities for employment in food production plants and commissaries, but there is difficulty in filling these positions with qualified people, educated for this purpose.

At a meeting held recently at the NRA headquarters in Chicago to discuss this problem, Alvin Cohn, Chairman of the Board of the Continental Coffee Company and a Director of IFMA said, "There is no question that there is a need for this type of curriculum. It is difficult to find qualified people, and what we want is someplace to go for future employees."

At the meeting, attended by representatives of industry, the NRA, IFMA and the University of Wisconsin, it was discussed that, although people trained in agriculture or restaurant/hotel management are being employed in food production and commissary management, these people are lacking in many facets of training that are needed for these positions. Some hospitality schools are deemphasizing preparation of food, and courses in quantity food preparation are not being offered.

Patrick O'Malley, President of Canteen Corporation and Chairman of NRA's Education Committee, stated, "It is important that we communicate with the high

school students and make them aware of the great service our industry renders. Some of the schools are not keeping pace with the fast changes being made by the food production and commissary segments of our industry, which means we have to completely retrain their graduates. Therefore, we should promote this curriculum at the University of Wisconsin."

Also discussed at the meeting was the feasibility of the University of Wisconsin establishing a central food production plant which would serve the entire school from a central freezing plant. This plant would also serve as a training ground for people studying Food Production and Commissary Management at the school.

Representing the National Restaurant Association, in addition to Mr. O'Malley, were Richard Brown, Executive Vice President, and George D. Hanby, of George D. Hanby Associates, an Honorary Director.

At the meeting for IFMA, in addition to Mr. Cohn, were Reuben Cordova, Executive Vice President; Ed Davis, Manager of Sales and Service, the Dover Corporation/Groen Division; Joseph Barclay, Seabrook Farms, Seabrook, New Jersey; and Dr. Jack Bloom, Research and Development, Continental Coffee Company.

Representing the University of Wisconsin were Professor D. C. Osterheld, Assistant Vice President of Business and Finance; Dr. Harold E. Calbert, Chairman of Food Sciences; and Professor Mary Mahaffey.

Further meetings will be held to discuss coordinated efforts involving NRA, IFMA and the University of Wisconsin to promote these programs to students and the industry.

1969 KENTUCKY DAIRY INDUSTRIES CONFERENCE

The 1969 Kentucky Dairy Industries Conference will be held at the Continental Inn in Lexington, Kentucky, on December 3.

Speakers include: Mr. Ed Sing, Managing Director, Moseley Laboratories, Indianapolis, Indiana; Mr. Fred Greiner, Executive Vice-President of the Evaporated Milk Association, Washington, D. C.; Mr. Jim Garrison, Manager of Ryan Milk Company, Murray, Kentucky; Mr. Henry Geisinger, Executive Vice-President of the Pennsylvania Association of Milk Dealers, Harrisburg, Pennsylvania, Dr. D. M. Graham, Professor of Food Science and Nutrition at the University of Missouri, Columbia, Missouri and Mr. Claude Harper, Director of Quality Control, Beatrice Foods Company, Chicago, Illinois.

For further information, please contact: Dr. C. Bronson Lane, 104 Dairy Products Building, University of Kentucky, Lexington, Kentucky 40506.

HEALTH OFFICERS NEWS DIGEST NOW "ENVIRONMENT"

The *Health Officers News Digest*, a pocket-size magazine familiar to many environmentalists, published by the Public Health Committee of the Plate, Cup and Container Institute, has given way to a new format and a new name, *ENVIRONMENT*.

The change to a larger pocket-size (4" x 8-1/2") and to the new name came as a result of a readership survey the editors conducted several months ago. The survey solicited the advice of *H.O.N.D.* readers about change. "They told us two things," says Executive Editor, William V. Hickey, "One, keep the magazine pocket-size; two, give us more material. We translated those wishes into the new long-look book which enables us to incorporate more material into each issue and at the same time to make effective use of photographs."

The survey also revealed that health officers were no longer the principal readers of the Digest. Sanitarians, health educators and even public health nurses outnumbered the H.O.s. Hence, the name change to *ENVIRONMENT*. "Environment is what this little magazine is all about," says editor Charles W. Felix. "We intend *ENVIRONMENT* to be a voice for the environmentalist, a news digest of what he is thinking, and saying, and—most of all—doing."

ENVIRONMENT, like its predecessor the *Health Officers News Digest* (which was published continuously since 1936), will be sent free of charge, six times a year, to public health professionals who request it. Editorial officers are at 250 Park Avenue, New York, New York 10017.

ANNUAL UNIVERSITY OF ILLINOIS-ISM ENVIRONMENTAL SANITATION & MAINTENANCE MANAGEMENT EDUCATION PROGRAMS ANNOUNCED FOR 1970

The 6th Annual Sanitation Management Short Course and the 2nd Advanced Sanitation Management Seminar will get under way on February 8-13, 1970 at the University of Illinois, Champaign. Held in cooperation with the Institute of Sanitation Management, this year marks the addition of a new short course for Hospital Environmental Sanitation Managers.

The Sanitation Management Short Course will include the following topics:

"Microbiology and the Environment," an illustrated discussion of the biological environment in which much of sanitation operations are conducted. Dr.

Lloyd G. Herman, Bacteriologist, National Institutes of Health, Bethesda, Maryland.

"Group and Individual Motivation," ideas which the manager can use in order to obtain desirable responses from his personnel. Paul M. Dauten, Professor of Management, University of Illinois.

"Written Communications for Sanitation Managers," how we can obtain greater effectiveness from our letters, directives and memoranda. Robert D. Gieselman, Assistant Professor of Business & Technical Writing, University of Illinois.

"Use of Time and Motion Concepts in Sanitation," suggestions for wider utilization of techniques which have proven of value in many applications. Raymond Wagner, Assistant Superintendent of Building Services, University of Iowa.

"Conference Leadership," increasingly plans are made and their implementation is discussed in group sessions. Your conduct of, or participation in, such meetings can be improved. Earl C. Wolfe, Professor of Labor and Industrial Relations, University of Illinois.

Prerequisite for registering for the Advanced Sanitation Management Seminar is completion of the Sanitation Management Short Course. Some of the topics at the Seminar include:

"New Ideas for Sanitation Management," this will not be a listing of new ideas but will present methods by which you can produce such new ideas. William H. Higginbotham, Higginbotham & McCoy, St. Louis, Missouri.

"The Disadvantaged in Sanitation," university and industry ideas as to how disadvantaged personnel might be trained for and utilized in sanitation work will be presented, followed by open discussion. For the University: Cecil H. Patterson, Professor of Educational Psychology and Michael Masucci, Assistant Professor of Educational Psychology. For the Industry: Max Cavanagh, Assistant Director of Physical Plant, Indiana University.

"Organizational Concepts," university and industry views of what "organization" can and should do for an organization, followed by open discussion. For the University: J. O. Weisenberg, Assistant Professor of Business Management. For the Industry: Richard F. Ehmann, The Port of New York Authority, New York.

"Work Simplification Techniques," a discussion of the application of methods which will improve your group productivity. Leo C. Pigage, Professor of Industrial Engineering, University of Illinois.

"Problems in Understanding," a review of the problems and discussion of solutions related to development of better rapport with your personnel. Ernest W. Anderson, Professor of Extension Education and Gertrude E. Kaiser, Associate Professor of Extension Education, University of Illinois.

The fee for the Short Course is $198. The fee for the Seminar is $167. Prices include housing, meals, applicable taxes, from buffet Sunday evening through lunch on Friday (Short Course) or Thursday (Seminar). Housing for the Short Course and the Seminar will be in the Illini Union. Courses will be held at the University' of Illinois, Champaign-Urbana, Illinois.

Complete descriptive brochures and additional information can be obtained by writing: Mr. Harold C. Rowe, Executive Director, The Institute of Sanitation Management, 1710 Drew Street, Clearwater, Florida 33515.

9,000 FOOD PROCESSORS EXPECTED IN WASHINGTON FOR 1970 NATIONAL EXPOSITION FOR FOOD PROCESSORS

The Nation's Capital, usually concerned with the affairs of state, will be the focal point for the affairs of taste during the 1970 National Exposition for Food Processors January 18-21.

An estimated 9,000 food processors from throughout the country and from many foreign nations are expected to gather in Washington for the four-day Exposition. According to the Food Processing Machinery and Supplies Association, which sponsors the annual Exposition, seven exhibit halls on two levels in the Sheraton-Park Hotel will be used to present an extensive array of supplies, equipment and services used in the production and processing of foods.

The theme for the 1970 National Exposition for Food Processors is "Serving the 70's" with emphasis on planning to meet the increasing demand for processed foods throughout the world. In addition to the Exposition displays and their inherent educational value, a series of technical conferences will be presented by the National Canners Association.

Hotel reservations for the Exposition and the Canners Convention are running higher than expected, although reservations are still being accepted by both associations.

Expected to be in Washington for the Exposition are representatives from every segment of the food processing industry. Canners, freezers, bottlers, brewers, dairy personnel, researchers and educators, specialty food processors and those in allied industries attend the NEFP each year to obtain a state of the arts review of the food processing industry. The broad-based Exposition covers every facet of processed food technology, from seed to shelf. Whether the product is liquid, granular or solid, the machinery or supplies needed to move it through the

production line and eventually onto the grocer's shelf will be represented at the NEFP.

A number of extra-curricular programs are also planned during the Convention-Exposition week, including the FPM&SA-sponsored Ladies Day Program and the Dinner Dance and Entertainment.

For complete information on the 1970 National Exposition for Food Processors write Food Processing Machinery and Supplies Association, 7758 Wisconsin Avenue, Washington, D. C. 20014.

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Executive Secretary
International Association of Milk, Food,
and Environmental Sanitarians, Inc.
Box 437
Shelbyville, Indiana 46176

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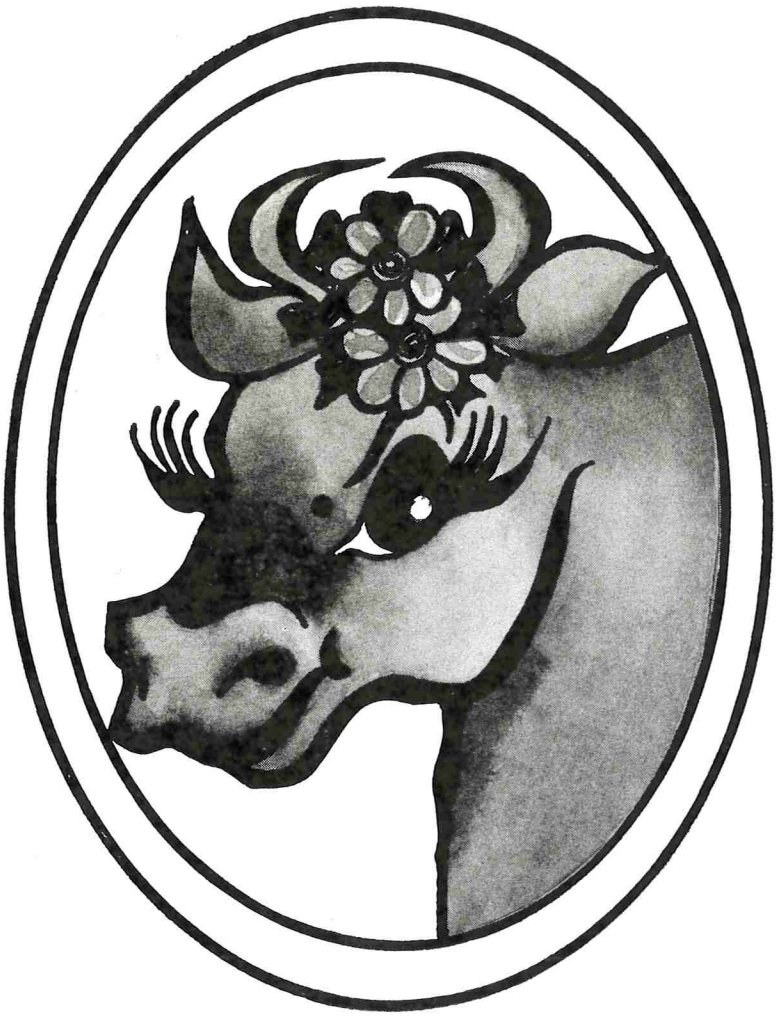

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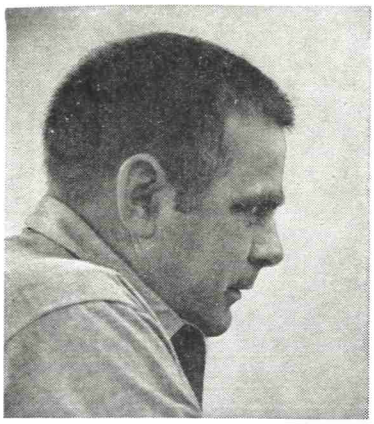

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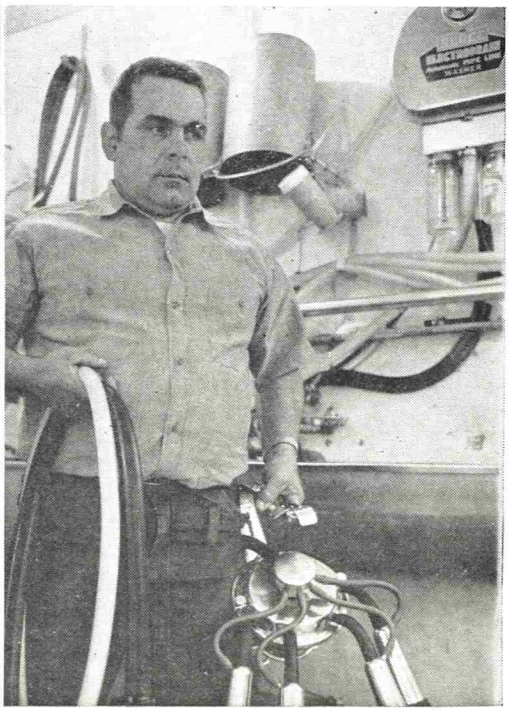

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