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Journal of

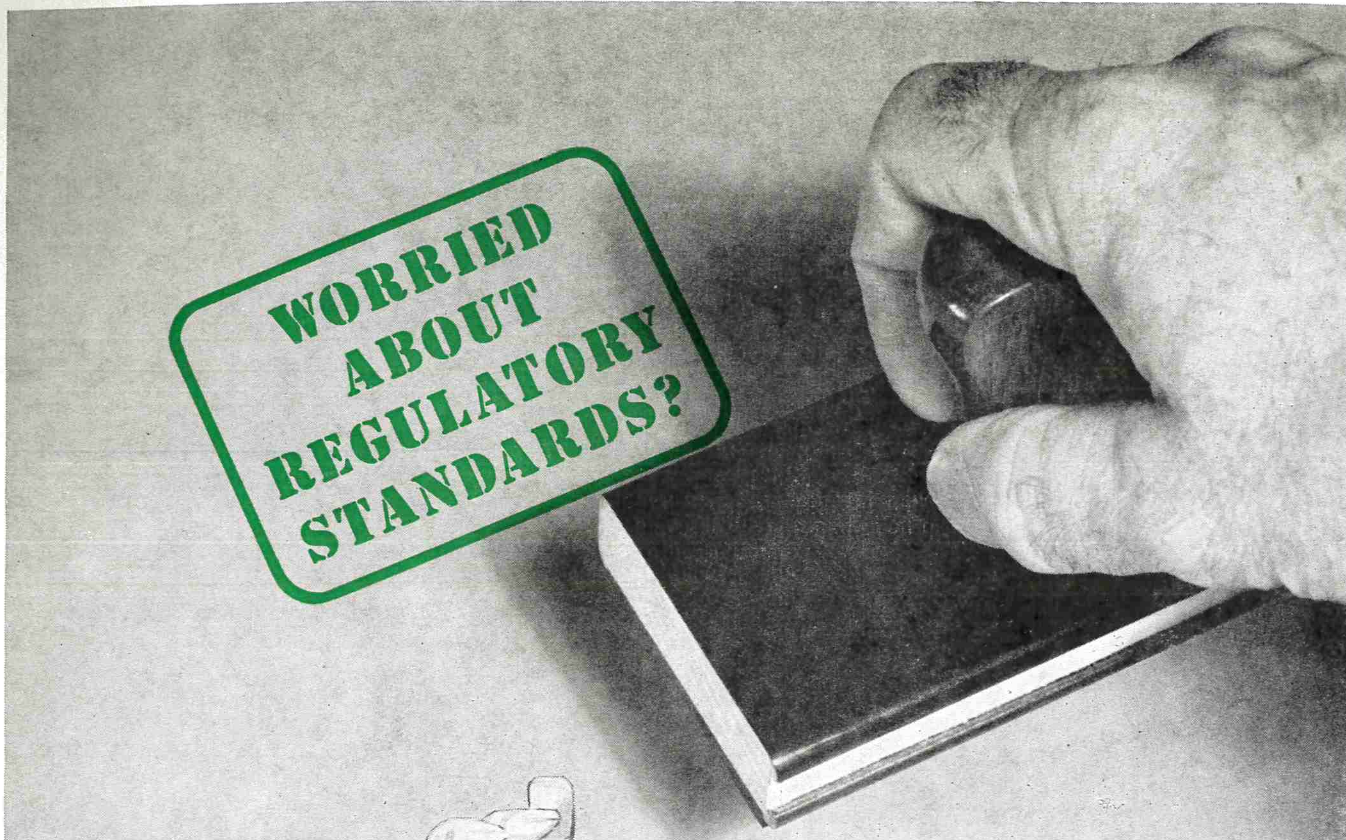
MILK and FOOD TECHNOLOGY

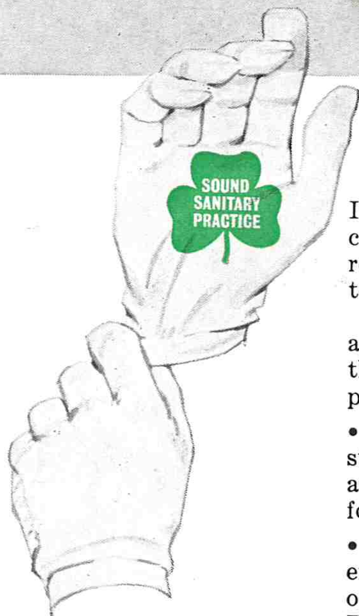
57TH ANNUAL MEETING
August 17, 18, 19, 20, 1970
Roosevelt Motor Hotel
Cedar Rapids, Iowa

NOTICE
Page I and II
National Mastitis Council Meeting

Official Publication

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





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Everyone interested in prevention and control of bovine mastitis is cordially invited to attend the ninth annual meeting of the National Mastitis Council.

The program this year will place major emphasis on preventing and controlling the disease.

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Featured speakers on research topics will include Dr. F. H. S. Newbould, Ontario Veterinary College; Dr. W. D. Schultze, Dairy Cattle Research Branch, USDA; Dr. R. P. Natzke, Department of Animal Science, Cornell University.

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2. Mastitis Therapy.

3. Milk Quality Control Programs.

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Sherman House—Chicago, Illinois

February 24-25, 1970

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INCLUDING MILK AND FOOD SANITATION

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

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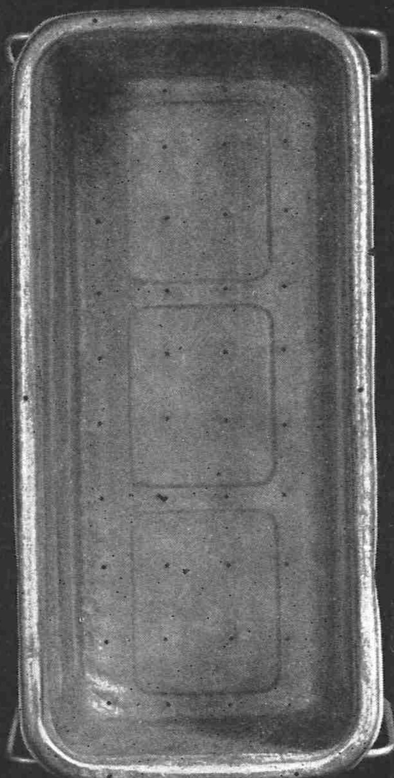

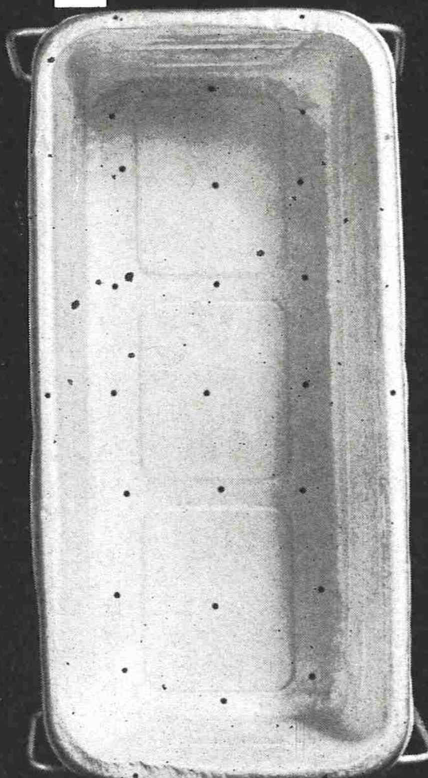

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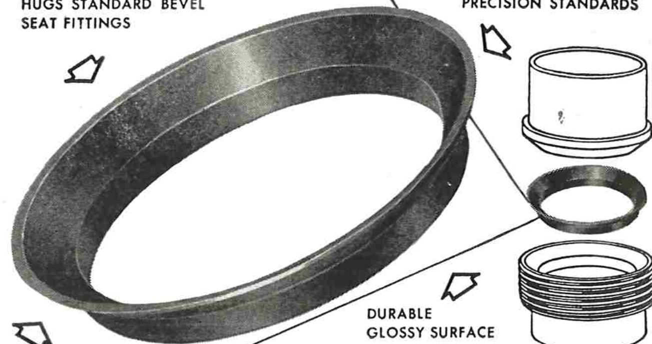

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SALMONELLAE AND THE FLUORESCENT-ANTIBODY TECHNIQUE: A CURRENT EVALUATION[1]

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and

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Battle Creek, Michigan

(Received for publication September 3, 1969)

Abstract

The increased microbiological surveillance of foods by government regulatory agencies and industry has shown that salmonellae are an important cause of food-borne disease. The significance of salmonellae in food-borne disease has made it mandatory for regulatory agencies as well as industry to develop a rapid, reliable, and reproducible method for the recovery of salmonellae in feeds and foods.

Standard cultural procedures have become increasingly sensitive but not without a proportional increase in the time necessary for salmonellae detection and confirmation. This test may require from 5 to 7 days elapsed time with all of the implied disadvantages.

One of the most promising areas of activity is the rapid microbiological-serological technique employing an improved fluorescent-antibody procedure. While much has been done by the medical technologist, the application of this technique is relatively new in food microbiology. The published data show the method to be highly promising and possibly even more sensitive than the standard cultural procedure.

This technique is not without its inherent problems. Like all methods under investigation, there are differences in procedure, validity, and interpretation of the data. These are cited, with a description and critique of the work done, in order to give the reader an opportunity to evaluate the state of the art.

The purpose of this paper is to summarize data regarding use of the fluorescent antibody (FA) technique for salmonella detection with special emphasis on its application to food and feed analysis. Many of the references and much of the information presented have their origin in the work of clinical microbiologists. Since its inception in 1941 *(9)*, the FA technique has become a powerful diagnostic tool in the clinical laboratory. Without the untiring efforts of people in this field, the principles and procedures would not have been suitably developed to permit its extension to food analyses. It should be pointed out that the problems associated with the analyses of food for the presence of salmonellae can be and often are markedly different from those associated with clinical analyses. Because of this, the approach, the methods, and the interpretation must be altered to fit the particular food-associated situation.

There has been a significant increase in the surveillance of human foods and animal feeds by the food industry and governmental regulatory agencies. This emphasis has resulted in a profound increase in the number of salmonella analyses that are performed yearly throughout the industry. Consequently, salmonella methodology has come under intensified study to shorten and simplify the present cultural procedures. Many modifications and procedures have resulted from these studies *(4, 17, 18, 26, 28, 29)*. The immunofluorescence procedure is one. The fluorescent antibody method has received considerable attention and a number of publications and data have been made available since the fine review of the subject by Ayres *(3)*.

It is hoped that this publication will stimulate investigators outside this field and those actively engaged in FA research to mold this procedure into one that can be readily applied throughout industry. The authors hope to accomplish this by bringing together references and defining the current problems, advantages, disadvantages, and approaches under consideration.

To achieve this end, a literature review is presented, followed by a discussion of the important facets of the methods being studied. It is not our intention to review the principles and details of fluorescence microscopy since this has been the subject of several excellent texts *(15, 23)*. It will be necessary to describe in detail only those steps inherent in the direct and indirect techniques that are necessary for adequate discussion of the problem.

Very briefly, the direct method involves staining the salmonellae in a single step with fluorescein-conjugated salmonella serum. In contrast, the indirect method is a two step procedure. In the first step the smear is exposed to untagged salmonella serum (prepared in rabbits). Next a fluorescein-conjugated antiglobulin (prepared in sheep or goats)

[1]Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

is applied. Both methods are described in more detail in the Discussion.

LITERATURE REVIEW

Two reports by Coons et al. *(9, 10)* introduced the fluorescent antibody (FA) technique as a successful diagnostic procedure. Coons et al. demonstrated that a fluorochrome could be attached to antibody molecules without affecting the specificity of the antibody. Antibody tagged in this fashion could then be used to detect specific antigen in clinical specimens. In this way, Coons et al. were able to demonstate the presence of pneumococcal capsular antigen in diseased tissue by use of fluorescent anti-pneumococcal type III serum.

Application of the FA technique to the study of salmonellae was first reported by Thomason et al. *(32)* in 1957. These investigators were able to stain the H, O, and Vi antigens of broth-grown strains of *Salmonella typhosa*. A subsequent report by Thomason et al. *(31)* indicated that the FA procedure was rapid and sensitive for examining fecal material but that it lacked the desired degree of specificity. Using broth-grown pure cultures of selected strains of *Escherichia coli, Escherichia freundii, Aerobacter* sp. and certain paracolons, fluorescing cells were observed when a conjugated polyvalent salmonella serum was applied to the smears. Similar results were obtained when feces were examined by these techniques. These findings led the authors to suggest that the application of the fluorescent antibody procedure to salmonella detection in clinical material might be limited.

Truant *(34)* then reported use of a direct conjugate of salmonella O groups A, B, and D. His work suggested that there was little problem in the examination of urine and blood samples, but that testing of fecal material was less reliable. Truant also reported that the globulin fraction of the serum stained the cells brighter and that, in his opinion, the direct technique was preferable to the indirect method.

In 1962, Arkhangelskii and Kartashova *(2)* reported successful application of the fluorescent antibody method for detection of salmonellae in artificially contaminated milk. Several years later the use of FA to screen food materials for the presence of salmonellae was described in the work of Georgala and Boothroyd in England, and Haglund et al. in the United States.

In their initial paper on use of the FA technique for screening meats, Georgala and Boothroyd *(13)* introduced several important procedures that are discussed in detail in a later section. Working with the indirect technique and somatic antisera, the authors examined 158 meat samples in which the enrichment broth was not centrifuged prior to making the smear and 128 samples in which the enrichments were centrifuged. The centrifugation step increased the percentage of false-positives[1] (13%) but did not lower the false-negatives[2] (6-7%). A subsequent paper *(14)* described use of the direct method in examining enrichments of 706 fresh meat samples. The criterion for a positive smear was: an average of one or more fluorescing salmonella-like cells in each microscopic field. The results showed a single false-negative and the false-positive rate was lessened to 7-8%. The test was completed within 24 hr after the sample was collected.

Haglund et al. *(16)* employed the indirect method to examine 20 strains of *Salmonella* and 82 isolates from dried egg material using both O and H serum. As had been earlier indicated by Thomason and associates *(31)* numerous cross reactions were obtained when somatic sera were used. Four of 5 *E. coli,* 8 of 23 *Escherichia* intermediates, 1 of 3 *Aerobacter* sp. and 2 of 15 gram-positive rods fluoresced when stained by the 'multivalent' O sera. No cross reactions were obtained when H antibodies were used. The indirect procedure was then used to screen 8 egg yolk and 12 egg albumen samples. There were no false-negatives and only a single false-positive. Most Probable Number analyses of the positive materials yielded a range of 3-240 salmonellae per 100 g of sample. The time elapsed from sampling until microscopic examination was 24 hr.

Shortly thereafter, Thomason and co-workers *(33)* suggested that typhoid carriers could be detected rapidly by the use of conjugated anti-Vi sera. In that study the FA technique detected 68.5% of a group of known carriers as compared to 69.2% by the longer standard cultural procedures.

Stulberg and associates *(30)* described the use of the direct procedure to follow a nosocomial outbreak of salmonellosis among infants. Broth cultures were formalinized and reacted with conjugated O-H antiserum in a small tube prior to applying the smear to a slide. More success was obtained with fecal samples from children than from adults, presumably because of differences in the intestinal flora.

More recently, Bissett et al. *(6)* used the conjugated Vi serum to study an outbreak of typhoid fever. Good correlation with the cultural technique was obtained when smears were prepared from the selenite-F broth enrichment. Direct examination of fecal smears was not as efficient as the enrichment method.

---

[1]A false-positive is defined as positive by the FA technique but negative by cultural methods.

[2]A false-negative is defined as negative by the FA technique but postive by cultural methods.

Interestingly, a problem was encountered in choosing an appropriate 'working dilution' of the serum. A four-fold variance in the dilution at which the serum could be used was reported when several strains of *S. typhi* were tested. This was attributed to a difference in quantity of Vi antigen in the culture at different periods of the growth cycle.

Silliker et al. *(27)* used the indirect test and commercially available reagents to examine 420 samples of dried foods (mostly egg products) and 45 liquid egg samples. The dried foods were preenriched for 24 hr, incubated in enrichment media for 8 hr and subsequently in Brain Heart Infusion broth for 16 hr. Twenty-six positive samples were detected by the cultural and FA methods with a 100% correlation between methods. A similar degree of success was not obtained with the liquid egg that was inoculated directly into enrichment media. Thirteen false-positive and two false-negative results occurred. Subsequent analyses of the egg samples by the MPN procedure yielded salmonella counts 10-100-fold greater by the FA technique than by the cultural procedure. This led the authors to postulate that the contaminating flora (coliforms) were overgrowing the salmonellae on the plating media leading to deceptively low or negative results by the cultural method. Silliker et al. did not encounter difficulty with fluorescing yolk material as reported by Haglund and co-workers *(16)*. This may have been due to transfer dilutions that were a part of Silliker's procedure. Preliminary results reported by these authors suggested that the indirect procedure did not produce the high level of cross reactions when examining feces as had been reported by Thomason et al. *(31)* and Truant *(34)*.

Caldwell et al. *(7)* conjugated both O and H antibody preparations with fluorescein. They compared the brightness of fluorescence with agglutination titer of the unconjugated sera for homologous, partially related as well as heterologous salmonella antibody-antigen systems. Good agreement could be obtained with homologous and unrelated systems but this was not consistent when partially related systems were tested. A conjugated 'multivalent' serum was used to examine broth cultures of mouse pellets for salmonellae. The authors considered a sample positive if they saw a fluorescing cell with attached fluorescing flagella. Of 714 samples examined after 16 hr of incubation, 51 positives by both FA and cultural means, 13 FA false-positives and 2 FA false-negatives were obtained. In 367 of the 646 culturally negative samples somatically fluorescing cells (mostly coccobacilli) were seen. Moreover, 20 samples had organisms that showed flagellar but not somatic fluorescence. This paper also reports conjugation of pools 1-4 of the Spicer-Edwards 'kit' and use of these sera to effect a presumptive identification of salmonellae in clinical material.

Insalata, Schulte, and Berman *(19)* reported that a commercially available conjugated salmonella serum was efficient for the rapid detection of salmonellae by the direct method in seven artificially contaminated food prototypes. Although the level in most of these foods was rather high (10/g) the method was able to detect *Salmonella senftenberg* at a level of <1 organism per 25 g of egg yolk solids. The somatic sera that were used covered groups A, B, C_1, C_2, D, E_1 E_2, E_4, and H and were absorbed with several strains of *E. coli* and *Citrobacter*. In addition, a conjugated flagellar serum comprised of the 1 complex was used and considerable weight was given to organisms that were stained with this serum. Perfect correlation between the FA method and the standard cultural procedure was obtained with all 58 samples. Schulte et al. *(25)* continued this method and improved the antigen coverage by including group G in a modified serum. Examination of 1173 samples using the improved serum yielded 34 false-positives and only a single false-negative. The primary cause of the false-positives was determined to be *Proteus* sp. Insalata and Sunga *(20)* described a more efficient technique wherein agitation during the preenrichment and enrichment phases permitted the analysis to be completed within 24-32 hr.

Laramore and Moritz *(21)* applied the method of Insalata et al. *(19)* to a study of animal feeds and feed ingredients. A false-positive rate of 5.7% and a false-negative rate of 2.2% occurred during the examination of 1013 samples. The percentage of false-positives could be decreased by subculture of the FA-positive culturally-negative samples. This finding strongly indicates that the FA procedure is more sensitive than the standard cultural method. Since the serotypes isolated from the FA false-negative samples were not specified, these may have been isolates from somatic groups not covered or covered at a low titer by the conjugated sera. The total elapsed time for these analyses was 20-23 hr.

Several other examples of the success of the direct method have been reported recently. Fantasia *(11)* examined 592 samples representing 34 different food and feed materials and obtained no false-negatives and only 10 false-positives. Again, repeated subculture of the enrichment broths that yielded the false-positives resulted in the subsequent isolation of salmonellae. This is in agreement with the work of Laramore and Moritz *(22)* and further documents the sensitivity of the FA method.

Reamer et al. *(24)* were able to increase the sensitivity of milk analyses by the FA procedure by incorporating trypsin in the preenrichment broth. The

enzyme prevented formation of a curd during the incubation period, thereby eliminating entrapment of salmonellae in coagulated casein.

Ellis and Harrington (*personal communication*) described a method that permits the microscopic examination of feed materials within 9-12 hr after beginning an analysis. The method involves use of gradient centrifugation, pentane extraction, and application of a flazo orange counterstain to mask undesired fluorescence of debris and organisms other than salmonellae. A conjugated polyvalent H serum was used as the diagnostic reagent. Although the qualitative criteria for a positive smear were not defined, the authors state that both *Salmonella pullorum* and *Salmonella gallinarum* were stained. Any smear that showed a single fluorescing cell was considered positive. A study of 200 feed samples yielded 9% false positive and 1% false negative.

The FA technique was coupled to a selective motility enrichment system by Abrahamsson et al. *(1)*. The indirect procedure employing a polyvalent H preparation and sheep anti-rabbit globulin was used to screen 332 samples that were mostly meat meal. Complete agreement between FA and the standard method was obtained. The total time involved ranged from 24-50 hr. The design of the culture vessel minimized interference by food material.

Goepfert and Hicks (*in press*) investigated the nature of the cell surface staining that occurs when unabsorbed flagellar sera are used in the FA procedure. By absorption and other procedures, it was established that the site of this staining is the cell wall or somatic antigen. The significance of these data as they affect the choice of sera and establishment of criteria for a positive interpretation will be discussed.

DISCUSSION

Basically, the goal of the FA procedure as discussed here is the rapid, positive identification of *Salmonella* in foods. The simplicity of the statement belies the difficulty of achievement. Immediately several questions arise. How rapid is rapid? How positive is positive? Certainly, any new method must be able to supply an answer earlier than the classical procedures.[3] The positivity of the procedure depends on (and determines how) the method is to be used. To date, virtually all investigators have chosen to use the FA method as a screening tool.

The efficiency of the method is dependent on a number of factors. One must consider the equipment, the available sera, the personnel, the criteria of definition and the culture and fixation techniques. These factors are discussed below.

Equipment

The equipment that is available today is relatively equivalent in efficiency of performance. Investigators have specific preferences and the names Zeiss, Leitz, and Reichert, will be heard more often than any others. The performance of the equipment does not vary greatly and thus is not a major consideration. If one is skilled in general microscopic procedures it is unlikely that results will be misjudged because of the optical equipment. Conversely, an expensive research microscope with numerous attachments does not ensure successful use of the procedure. Implicit in these statements is the assumption that the equipment will be kept in proper repair and the light source changed frequently enough to ensure its optimum performance. The optical equipment that is used does not merit an elaborate discussion in this paper.

Sera

One of the most critical aspects of the FA technique is the sera used in the analyses. To meaningfully discuss the sera it is necessary to briefly describe the mechanisms of the two methods in use today. The direct method employs a salmonella serum much the same as is used for routine slide and tube agglutination reactions but to which a fluorescent dye (fluorescein) has been chemically linked. A smear of the material to be examined is exposed to the fluorescent-labeled salmonella serum. The serum is removed by washing and the smear is examined while exposed to ultraviolet-blue light. If salmonellae are present, they will adsorb the specific serum which, because of its attached fluorochrome, will cause the cell or the flagella to fluoresce yellow-green against a dark background.

The indirect method is a two step staining procedure. The smear is first exposed to specific salmonella sera (unconjugated) which are then removed by washing. If salmonellae are present they fix the specific antibody. Next a drop of fluorescein-tagged antiglobulin that is specific for the carrier of the salmonella antibody (i.e. rabbit globulin) is applied to the smear. The salmonellae will then fluoresce in a fashion similar to those stained by the direct method.

Three major considerations concerning the sera are the specificity, availability, and sensitivity.

Specificity. The specificity of salmonella sera depends on two factors. First, the type of antibody preparation that is chosen, i.e. H or O antibodies.

[3]For purposes of discussion, classical procedures include pre-enrichment (when necessary) enrichment, selective plating, biochemical tests, and serological confirmation.

Somatic antigens of the salmonellae are shared to some extent with other *Enterobacteriaceae*. In addition, although some O antigens in other enterics may not be identical with those in *Salmonella*, they may be closely related and cause some degree of nonspecific reaction with salmonella serum. In contrast the flagellar antigens are not commonly shared with other organisms. One exception is the genus *Arizona* which has flagellar antigens in common with salmonellae. This is an advantage inasmuch as these organisms are pathogenic and have no place in foods. It is on this specificity of flagellar antigens that most definitive identifications of isolates are based. Many rapid method approaches are based on flagellar agglutination in both pure and mixed culture *(5, 12, 26, 28, 35)*.

The second factor of importance regarding the specificity of the sera is the dilution level at which they can be used. It is advantageous to use sera at as high a dilution as possible to minimize undesirable reactions. This is particularly important in the fluorescent antibody procedure where two types of unwanted reactions can occur if the serum cannot be properly diluted. First, use of low-titer conjugated serum can result in staining the entire background thereby reducing contrast between fluorescing cells and the remainder of the smear to a minimum and preventing interpretation of the slide. Also, the number of cross reactions with non-salmonellae can be minimized by use of higher dilutions of the sera. Those investigators using the direct method have found it necessary to absorb the somatic conjugate with strains of *E. coli* and/or *Citrobacter* sp. It would be impossible to absorb the serum with all strains of these genera and indeed this would be undesirable. There are reports that document the presence of identical antigens in certain somatic groups of *E. coli* and *Salmonella*. Absorption of the serum with one of these *E. coli* strains would remove antibody specific for *Salmonella* thereby reducing the effectiveness of the serum. Alternatives to absorption should be developed to reduce nonspecific cross staining.

Availability. For a method to enjoy widespread application, the reagents must be reliable and commercially available. At present, there is only one commercial source of conjugated salmonella sera for use in the direct procedure. These sera include a polyvalent somatic serum that covers O groups A through H and flagellar sera against a, b, c, d, i, and the 1 complex. These sera are of low titer and should not be used at greater than a 1:4 dilution. Although unconjugated polyvalent salmonella sera for use in the indirect method are available from several firms, there is only a single source of the serum that provides the desired coverage of antigens. This serum, prepared against flagellar antigens, also contains somatic antibodies representing groups A-H (Goepfert and Hicks. *In press*). This eliminates the need to use a polyvalent O preparation in the indirect test. This serum can be used at 1:500 dilution. The second reagent, i.e. the conjugated anti-rabbit globulin prepared in either sheep or goat is available from numerous supply houses. Although some preparations are excellent and can be used at 1:35 dilution, other sera are weak and cannot be used at greater than 1:2. These latter products usually impart a bright background fluorescence and are unacceptable for use. For this reason a source of suitable reagents should be chosen and new individual lots of the material titered before use. All of the sera are stable when stored as suggested by the manufacturer.

Sensitivity. The sensitivity of the sera is directly related to their antigen coverage. At present, conjugated *Salmonella* somatic sera purport to cover groups A-H. The flagellar antisera include antibodies to only a few flagellar antigens. Contrastingly, the unconjugated serum used in the indirect method has excellent flagellar and somatic coverage.

Therefore, at this time, the sera for the indirect method offer a specificity and sensitivity greater than that of the direct method. Efforts are being made in several laboratories, governmental and industrial, to produce a high quality all encompassing O-H serum for use in the direct FA method.

Personnel

The phase of the procedure most difficult to assess is the personnel applying the technique. Problems are associated with subjective interpretation of results and may limit the usefulness of the method. The initial training in use and handling of the microscope can be readily absorbed. The potential problem lies in application of the criteria to a given specimen; in other words, the subjectivity of the technician. Judgment of pure cultures grown in laboratory media is relatively straight forward but is far removed from scanning a slide containing autofluorescent or nonspecifically stained material. In the authors' experience, each food material has its own manner of behavior in FA analysis. Although there are certain problems that are common to all foods, there are also differences which make it difficult to be expert with a certain food material solely on the basis of prior experience with other materials. Confidence with the technique as it is applied to a given substance will come only after repeated examination of that material. As the technique is studied more intensively and the procedures are improved and simplified, it may well be that the problem of subjectivity can be reduced to a controllable minimum. Knowledge of the limitations of the procedures and reagents

is necessary to avoid the technical difficulties incurred by earlier investigators. This information should reside in at least one person in the laboratory where FA analyses are being conducted.

Criteria for positivity

In establishing the criteria for positivity, we must determine how we are to define a positive reaction. This definition depends, in part, on several things. Ideally, each field should show numerous short, fluorescing, rods bearing attached fluorescent flagella. Unfortunately, this is not always possible because of: serum used, cultural methods employed prior to examination, and fixation procedures. Consequently, we must say that any cell that shows the correct morphology and is fluorescing is a presumptive positive whether or not flagella are present. This is the most rigid criterion that may be applied. But what about other situations? Do fluorescent 'strands of material' that may or may not be attached to the cell, have significance in the absence of cell wall staining? How many fluorescing cells must be present in order to be called positive? These are important questions and require further discussion.

The minimum number of fluorescing cells necessary to define a positive depends on the procedure used in culturing the organisms and preparing the specimen for examination. One important consideration is the possibility of interference by dead salmonellae. To dispel these fears let us consider the following calculations. For the observation or scanning of smears a 40-54X objective is most commonly used. The microscopic factor[4] of these objectives is approximately 200,000 (assuming 0.01 ml is spread over an area of 1 cm^2 on the slide). This means that in order to see 1 organism per field there must be 200,000 organisms per ml in the broth from which the smear is made. Most food materials are tested at the 10% level (i.e. a 1:10 dilution into preenrichment broth) and further diluted 1:10 upon transfer of an aliquot to the enrichment broth. This would necessitate an initial content of 20 x 10^6 dead salmonellae per gram of food in order to see one dead cell per field. Even if the enrichment broth was concentrated up to 20-fold prior to making the smear, 1 x 10^5/g would be necessary and then only if no cells were lost during the fixation procedure (an unlikely event). These numbers are reduced if preenrichment is not used but it remains extremely difficult to believe that at any stage in food processing a product contains 1 x 10^5 – 20 x 10^6 salmonellae per gram that are subsequently killed in processing. For these reasons, the authors are of the opinion that dead cells do not interfere in an analysis.

If we accept that the interference by dead cells is of little or no consequence, we are justified in establishing criteria for positivity as rigid as necessary to: (*a*) increase the sensitivity of the method, and (*b*) to make sure that if error is introduced it will result in a false-positive rather than a false-negative. Thus, most investigators agree that a single fluorescent short rod in the smear is sufficient to consider the sample as positive. Cultural confirmation of such interpretations has been obtained in many laboratories. The morphology of the fluorescing cell and the specificity of the serum used in staining will affect the validity of such criteria. For example, if the single observed organism bears attached fluorescing flagella, this is strong presumptive evidence that it is a *Salmonella*. Such an observation would only be possible in a smear stained with a serum containing both O and H antibodies, such as Spicer-Edwards or other unabsorbed poly H sera. Moreover, one would feel more confident that a cell was *Salmonella* if it were stained somatically with a serum diluted 1:500 or more than if the dilution were only 1:2 to 1:4.

The successes reported for the direct method using commercially available reagents make it difficult to suggest that alternate means of analyses be considered. However, it would be advantageous to include the specificity of flagellar antigens in an FA method. Assuming that flagella were present on the cells at the time of analysis, their observation would lend weight to any judgment on the salmonella content of the sample. It is possible that the use of sera containing H antibody will reduce the percentage of false-positives associated in the past with the direct technique.

Culture and fixation techniques

The last part of this paper deals with the preparatory work leading to examination of the slide, i.e. the culture and fixation techniques. These phases influence the efficacy of the FA procedure.

There are two requisites to be met in order that the FA technique can be performed. First, if salmonellae are present in a given sample, the number of salmonellae must be increased by cultural procedures to a minimum level to be detectable microscopically. To observe one cell per field there must be approximately 200,000 salmonella cells per ml in the broth from which the smear is made. But if the criterion is established that a single cell in 100 fields is sufficient to be considered positive, then only 2000 cells per ml of broth are necessary. These figures are based on the assumption that no cells will be lost during the preparative manipulations of the slide. This is

[4]Microscopic factor is the reciprocal of the quantity of broth being examined in a single field.

an assumption that has been made in order to calculate approximate values for minimum salmonellae concentration. Therefore, we can state that the cultural procedures preliminary to making the smear must result in an increase in salmonellae from as few as one cell per sample to 2000 or more cells per ml of suspending fluid.

The second requisite is that the salmonellae on the slide must be antigenically developed. Any method that employs an antigen-antibody reaction must have adequate antigen development in order to succeed. To a certain extent, antigenic development and cell number are interrelated.

At present, the more pressing of the two requisites is adequate cell populations. There have been several approaches to ensure that this minimum is met. All investigators have employed the basic enrichment method for increasing the salmonella population. Some have omitted either preenrichment or incubation in selenite and tetrathionate because of the nature of the product being tested or for other reasons. But in all instances, the sample was incubated in a manner that allowed proliferation of salmonellae.

One technique to increase the cell number prior to making the smear has been concentration of the organisms by centrifugation *(11, 13, 20)*. Most often 10-15 ml of the broth medium is centrifuged and the resultant pellet is resuspended in a small volume of buffer or in the liquid that remains after decanting the supernatant fluid. By this process, a 20 to 30-fold concentration of cells (and debris) can be accomplished. This technique necessitates additional equipment and may result in the loss of attached flagella. To date the evidence for the necessity of centrifugation is inconclusive.

More recently, an effort to increase cell numbers and shorten the time invested in the preenrichment and the enrichment steps has been described *(20)*. This was accomplished by agitation of the broth cultures on a mechanical shaker to introduce oxygen into the media. The time for the analyses was thus shortened to 24-32 hr. Additional equipment is necessary, and this process would almost certainly shear flagella from cells thus negating specificity gained from the use of flagellar serum.

Several other cultural modifications employed are worthy of mention. Use of an elevated temperature for incubation of the enrichment medium has been suggested *(8)*. Others have noted that this may be deleterious when dealing with low numbers of salmonellae. The effect of the combination of selective media and elevated temperature on somatic and flagellar antigen production merits close examination.

The principle of selective motility as a separatory method has received recent attention. Several reports have appeared that describe the apparatus, media, and protocol for this technique *(1, 4, 29)*. At least one paper describes the use of FA identification of the isolates procured by this technique *(1)*. One attractive feature of selective motility is that the cells recovered are flagellated and, if proper fixation techniques are employed, the examiner should detect cells with attached fluorescing flagella; a finding that lends considerable weight to decisions regarding the positivity of the sample. It should also be realized that non-motile salmonellae will not be detected using this system. Further evaluation of the method is warranted.

One of the critical phases of the method involves the fixation or attachment of the cells to the slide. Equally important is the retention of the cells during the staining and washing steps. Several fixation and washing schedules based on the individual author's experience and preference have been described. One recent study (Goepfert and Hicks. *In press*) compared five fixation methods and their efficiency of retaining broth-grown cells on the slide during staining and washing. This study indicated that, under the circumstances tested, the most efficient method was that of Kirkpatrick *(22)*. This involved immersion of the smears in a ethanol-chloroform-formalin (60:30:10) mixture for 3 min. The food material examined may play a considerable role in influencing the ease with which a smear is fixed, and it is difficult to extend results obtained for broth cultures to a food-broth situation.

Care in preparing and handling smears cannot be over-emphasized. It is imperative that maximum retention of cells as well as the structural integrity of the cells be effected to achieve optimum results for the detection of salmonellae by the FA technique.

FUTURE RESEARCH

It should be evident from the discussion presented that additional work is needed. There are several facets of the procedure that merit further development before the method can be applied on a widespread basis.

There is need for further evaluation in comparison to establish cultural methods. In establishing the sensitivity of what may be an improved fluorescent-antibody technique, comparisons are necessarily made between established cultural methods for salmonellae recovery and this newer procedure. However, it should be recognized that while the scientific community may accept the cultural method at this time, it has not been established as the most sensitive procedure and consequently may not be the basis for a valid comparison with the fluorescent-antibody tech-

nique. To elaborate: recovery by fluorescent-antibody, and lack of cultural confirmation, may not be false-positive for fluorescent-antibody but rather an inability of the cultural method to detect levels as low as one fluorescing salmonella cell per 100 microscopic fields. There are data to indicate that so-called false-positives in FA may actually be "additional-positives," over and above those not confirmed by initial cultural methods.

Meaningful data regarding the efficiency of the FA procedure can only be obtained by the examination of naturally contaminated foods and feeds. Samples of this material are extremely scarce. Few industrial laboratories will admit to having these and governmental laboratories are seldom in a position to share their samples. Availability of contaminated material to researchers will enhance the development of the rapid procedures.

It is desirable to accelerate the cultural phase of the FA method to shorten the recovery time and bring about greater sensitivity. The media and procedures should be examined with the intent of stimulating cell yield and antigenic development. Approaches that have been suggested include use of higher temperatures of incubation, development of media that are nutritionally adequate and in which the pH is closely controlled, and aeration of inoculated media. This facet of the procedure has only recently been accorded its degree of importance. There is a need for more intensified research in this area.

Improvements can be made in the immunofluorescent phase of the procedure. Although many fixation techniques have been described, there seem to be reservations attached to their use. Modifications should be developed that are simple to perform and which effect a maximum retention of cells on the slide and little or no alteration in the integrity of important structural features of the cell, e.g. flagella.

There is a need for improved conjugated antisera of high specificity and titer. There is strong evidence to indicate the advisability of using "O" and "H" antisera separately or in combination to take full advantage of the somatic and flagella antigens of salmonellae. Unpublished lists of the antigens necessary to afford maximum serological coverage in an antiserum have included 38 somatic antigens and 41 flagellar antigens representing 31 significant serotypes. This list is comprised of those serotypes which have been recovered from human salmonellosis in the past five years. As intensive screening continues, this list will inevitably increase and become more complex.

The scope of this paper has been to describe, in some detail, the technical problems that exist in the method at present and to suggest alternative approaches. However, this recognition of the problems does not promise that the scientific community will readily overcome them. The state of the art at this time does not permit resolution of all of these problems with certainty.

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LETTER TO THE EDITOR

More on the Wisconsin Mastitis Test

DEAR SIR:

This is in response to the invitation for suggestions which appeared on p. 74 of the February, 1969 *Journal* regarding the next edition of *Standard Methods for the Examination of Dairy Products*. I should like to comment on the section dealing with tests for abnormal milk.

Apparently split-samples will be used by various groups in collaborative studies designed to evaluate these tests. Our experience indicates the samples to be used for comparison of the direct microscopic somatic cell count (DMSCC) with the Wisconsin Mastitis Test (WMT) should be AUTHENTIC BULK TANK SAMPLES. They should be collected from the farm bulk tank on the day of split-sample shipment and maintained continuously at 32-40 F (pilot bottle used for temperature control) during preparation for shipment to participating laboratories. The receiving laboratory should conduct the WMT within 20 min after removal from the refrigerated shipper.

Also, it seems logical that when the WMT is used, the results should be reported to the producers in the more easily understood terms of somatic cells or leucocytes rather than WMT values. Consistent checks within means of ±50,000 cells have been experienced in our laboratory when the DMSCC is compared with the direct reading scale calibrated according to Table D-1, p. 291, of the current *Standard Methods*. No detectable difference has been noted in the results obtained with the newer LAS detergent as compared to the original ABS reagent. The former has been used in the standardized WMT reagent since May, 1967.

An additional advantage of reporting cells is that it eliminates confusion with per cent oxygen results in states that also use the catalase test. Then, too, there may be a problem in explaining to producers why a reading of 20 mm indicates a little over 500,000 somatic cells, whereas a reading of 27 mm indicates a million cells per milliter.

The direct reading scale permits an easy way to estimate counts in excess of 1,500,000. This is done, for example, by adding 1 ml of cold pasteurized milk to the WMT tube plus 1 ml of sample and multiplying the test result by 2. The pasteurized milk acts as a diluent.

A further observation has been the tendency for the mean DMSCC values obtained by untrained personnel to be considerably higher than those obtained by trained microscopists. This should be considered in evaluating results from split-sample programs.

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VENDING SANITATION AND SAFETY—A CURRENT REPORT[1]

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Abstract

While the automatic vending of food and beverages—and non-food merchandise—has been a part of The American scene since the late 1880's, problems of public health and safety did not enter the picture until the end of World War II when the first hot beverage vending machine went on location.

In 1947, the vending industry and health officials first came together to discuss the development of uniform national standards for vending machines and vending operators. In 1957, after necessary research and preparation, The Public Health Service published *The Vending of Food and Beverages—A Recommended Ordinance and Code.* In the same year, the industry launched a full-time public health and safety program, guided by a nationally-constituted Health-Industry Council.

The progress in sanitation and safety made as a result of cooperative industry and agency efforts over the past 12 years is discussed in terms of vending legislation, internal and external training, machine standardization and testing, and research.

Concluding, a review of current problems in such matters as microwave safety, copper poisoning safeguards, temperature controls, icemaker design, and commissary layout is made, together with a "crystal ball" look at the future.

Twelve years ago the author discussed the then new Public Health Service *Vending Code* and the vending industry's plans to initiate a full-time program of machine standardization, testing, education, legislation, and research based on Code requirements.

It seems most appropriate now to present a progress report on each of these activities together with a review of current problems and a "crystal ball" look at vending's future.

Progress Report

Public health service vending code

At the time of its adoption in July, 1957, the Vending Code was totally new to machine manufacturers, machine operators, and agency officials. While it was offered as the best thinking of over 800 industry and public health participants in its creation, no one thought that it could prove to be perfect. But in retrospect, the 1957 Code was very nearly that.

By 1965, there were sufficient changes in the industry and experiences in applying the Code to warrant a revision in something over 50 Code requirements, most of them very minor in impact.

Judging from its continuing—even increasing—acceptance by industry and health agencies, the 1965 Code must be considered still adequate as a machine design guideline and for purposes of controlling vending field operations.

NAMA's health-industry council

Founded in 1957, the Automatic Merchandising Health-Industry Council (AMHIC) has met annually or more often to advise the National Automatic Merchandising Association (NAMA) on its programs of machine standardization, legislative cooperation, training, safety, and research.

Representing the major public health associations, observers from the U.S. Public Health Service (PHS) and the military departments, NAMA's evaluation agencies (universities), and an industry group, the AMHIC organization in 1969 has a number of active committees to recommend to NAMA standards and actions relating to such matters as icemaker design, temperature controls testing, carbonation backflow prevention, field sanitation procedures, and standards for renovated machines.

Vending standards

Acknowledging that the Vending Code is not a complete "standard" for use in actual testing and machine approval, AMHIC developed in 1959 a *Vending Machine Evaluation Manual* for the guidance of manufacturers and machine evaluators.

The *Manual* is now at 50 pages and has a cross-referenced, 25-page Checklist used by the NAMA machine evaluation agencies during machine testing.

In 1968, an industry task committee worked with the National Sanitation Foundation to amend its 1958 Vending Criteria C-1 and develop the new NSF Standard No. 25. The new Standard and the NAMA Manual are now equivalent in their coverage and content.

Legislation

A mark of the need for the Vending Code and its acceptance is the fact that, since 1957, 26 states and the District of Columbia have adopted it uniformly —by law, regulation, or administrative action. Over 120 cities and counties have also adopted the Code, principally in states which, at the time, had not made a state-level enactment.

The Code also is a medical regulation of the Army, Air Force, and Navy and is in the process of

[1]Presented at the Fifty-sixth Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Inc., Louisville, Kentucky, August 17-21, 1969.

enactment in 3 additional states during the 1969-70 legislative period. A gratifying aspect is that state vending groups have actively participated in practically all state enactments and many local ones.

Education and training

To familiarize health officials with various aspects of vending machines and vending operations, over 300 seminars and program appearances—from an hour to 2 days—have been made available by NAMA to agencies and sanitation groups since 1957. In the same period, NAMA has presented over 250 clinics, workshops, and lectures on sanitation for its operator members and their employees—meetings ranging from popular routeman workshops to owner clinics on commissary safeguards.

The vending industry generally recognizes sanitation training of employees as its own responsibility. To this end, NAMA training booklets on such subjects as machine sanitation procedures, commissary sanitation, and microwave oven safety have been provided to operators on a free basis for many years. New booklets on labeling and commissary layout are now under way. (A listing of free health and safety publications is available on request from NAMA, 7 South Dearborn Street, Chicago, Ill. 60603.)

Research

With the rapid growth of the industry in the perishable food field and increased equipment sophistication, many research studies have been carried out in recent years to ensure that new techniques in machine design are consistent with public health needs. At Michigan State, Clemson, and Ohio University, research projects have dealt with potentially-hazardous food machine performance—one project sponsored by PHS and two by the industry.

Other studies have involved carbonation backflow in copper tubing, copper formation in brass pumps, and cooling rates of sandwiches under different ambient temperatures performed at the Indiana University Department of Public Health. A current 7-month project has studied vending machine icemaker design, construction, and new methods of in-place cleaning.

RECENT DEVELOPMENTS

If we were to line up several typical 1957 and 1968 machine models—particularly those for cup soft drinks and hot beverages, the changes and improvements would be readily visible (See Fig. 1 and 2). For example:

(*a*) Machines designed to brew one cup of coffee at a time (14 sec or less) have been developed —offering optional chocolate, tea, and soup and buttons for extra cream and sugar. These newer models have far less product-contact area and are all designed with flush buttons for rinsing each product system with 200 F water.

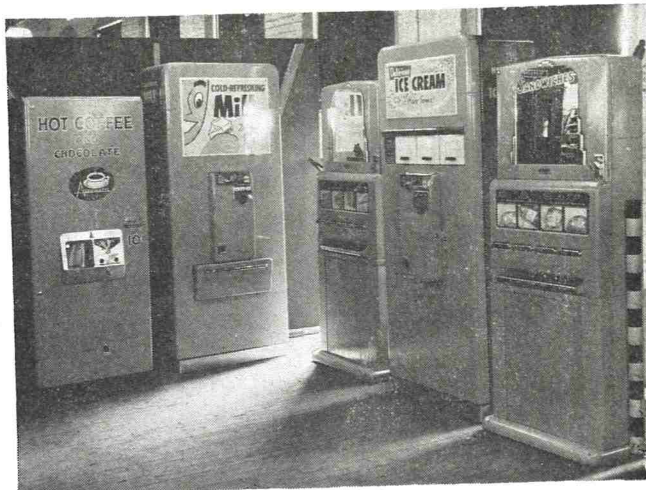

Figure 1. A 1957 industrial vending location with a typical product offering of that period.

Figure 2. A modern "full-line" bank of machines featuring the decor and auxiliary equipment widely used in industrial, office, and institutional locations.

(*b*) In about 1962, icemakers made their appearence in cup soft drink machines. At the same time, water connections between the machine system and the external water supply were equipped with air gaps to prevent back-siphonage into the supply system.

(*c*) A vast majority of hot beverage vending machines made since 1963 have been equipped with non-pressurized water heaters and incoming water supply air gaps, thus providing an added measure of safety both against explosions and back siphonage.

(*d*) Since 1957, every American-made post-mix cup soft drink machine has been equipped with

double checkvalves or venting devices to preclude copper poisoning. In 1969, the industry has voluntarily completed the phase-out of machines which rely exclusively on checkvalves to prevent CO_2 backflow into copper pre-cooler tubing. Some manufacturers have gone to all-stainless-steel water systems throughout the machine while others have elected to design in positive venting valves.

(*e*) Bulk milk vending machines have been totally phased out, as also have coffee venders which use refrigerated liquid cream. Both decisions were based largely on sanitation considerations.

(*f*) Heated machines to vend prepared sandwiches and casseroles are being used less frequently than in the past, because of the short shelflife obtained in a 155-165 F environment. These products are now held, largely, at under 45 F and heated by microwave ovens at the time of sale. Because of the increase in microwave oven usage, NAMA has published a Microwave Oven Safety Bulletin and is working with Radiological Health officials to develop effective monitoring programs.

Many other improvements could be reported, ranging from first-in first-out confection venders to the new NAMA Evaluation Program for "Remanufactured Vending Machines". It is sufficient here to acknowledge that the industry is still growing and diversifying—and that the NAMA Public Health Activity is designed to keep vending machine safety and sanitation features abreast of the other operational advances.

REMAINING PROBLEMS

It would not be accurate to imply that all vending sanitation problems have been solved, either with equipment or with operating personnel. Here are some continuing projects which the industry and its health advisors now have under way:

Carbonation backflow prevention

An educational program has already been initiated to promote double checkvalve conversion or more frequent examination and servicing of checkvalves on older machine models which are not vented. The program includes publication of a new *Manual for Post-Mix Machine Sanitation Practices* plus operator notices and program presentations.

Health code cut-off controls

Long a problem, a committee of AMHIC is working with machine and control manufacturers to improve accessibility of controls for field testing and development of a rapid field test for each machine model to determine operability of the control and its ability to interrupt vending when temperatures are not within the safe zone.

Icemakers

In many parts of the country, vending operators experience extreme problems of bacterial growth in icemakers. A 7-month research project underwritten by NAMA (just completed) covers many aspects of icemaker design and servicing. The results of the study—and further studies that are indicated—should provide many answers to the present cleaning problem in the "bad water" areas of the country.

Commissary standards.

The Federal "Wholesome Meat Act" and "Wholesome Poultry Products Act" place many vending-type commissaries under the Federal Meat Inspection Program or under equivalent state programs by early 1970. It is already apparent that the plant layout and installation views of meat inspection officials are not entirely consistent with the commissary layout and operational guidelines which have long been administered by health-type agencies. At present, it appears that vending and restaurant commissaries which prepare meat food products or poultry products for sale or service *away from the commissary* will come under both the health and the agriculture authority in many states. The vending industry has worked, and will continue to work, with these agencies toward the end that requirements and interpretations will not conflict from state to state or agency to agency.

Labeling

The vending industry's rapid growth in the area of pastry, sandwich, salad, dessert, and entree preparation leads directly to problems of labeling which formerly rested with the purveyor who supplied these packaged products to the vending operator. At the present time, NAMA is assembling a "Labeling Guide for the Vending Operator" which will be submitted both to FDA and USDA for concurrence.

THE FUTURE

There is no doubt that the vending industry will continue to show sales volume increases in the next few years well above the gross national product average. Further, much of this continuing growth will be in the food service area—vended, combination, and manual.

The industry has conducted its own internal public health and safety programs for 22 years with the goal of creating the least possible burden to health and safety officials. Current long-range plans envisage a continuation of this approach which, thus far, has paid tangible dividends to both the vending industry and the consumer public.

A PROCEDURE TO MINIMIZE DIFFERENCES IN EXPECTED COEFFICIENT OF VARIATION IN THE DIRECT MICROSCOPIC SOMATIC CELL COUNT

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(Received for publication July 28, 1969)

ABSTRACT

The Direct Microscopic Somatic Cell Count (DMSCC), developed to meet the need for a closely standardized reference method in an abnormal milk control program, requires the use of a specified single-band eyepiece reticle chosen to yield optimum precision for cell counts in the region of the control limit. We have adapted the techniques of the DMSCC and the statistical model for its evaluation to mastitis research, in which quarter milk samples may require precise counting over an extreme range of cell concentrations.

A special reticle provides a choice of 2 band widths, and 1, 2, or 4 strips are counted. The area of milk film to be examined differs for each of 6 concentration ranges, identified by a preliminary estimation of cell density and by the count on the first strip. Except in the lowest range, in which the number of cells counted may be as low as 0, total cells counted vary only between 400 and 800 and the expected coefficient of variation between 5.0 and 3.5%. Above the maximum concentration of 18 x 10^6/ml a field-strip method extends the usable range to 70 x 10^6/ml at a constant coefficient of variation of 5.0%.

Particle counting theory specifies that the coefficient of variation of a microscopic cell count will vary inversely with the number of cells actually enumerated *(3)*. This presents the difficulty that if the samples encountered in a given investigation span a very wide range of cellular concentrations, the counts will differ tremendously in precision. Such is frequently the case in mastitis research, especially if quarter samples are employed. Usually, sample variance is extremely great at low cell concentrations. An adjustment in counting procedure to reduce this (and also reduce the magnitude of the range of coefficient of variation, or CV) increases the number of cells to be counted at higher concentrations to an unacceptable extent.

The solution, of course, lies in some form of regenerative control, in which the area of the milk film to be examined (and thus the Working Factor) is made variable and dependent upon the density of cells observed during the count or during a preliminary estimation. A choice is available to users of the Direct Microscopic Somatic Cell Count (DMSCC) *(1)* procedure for research purposes as to how the film area is to be modified. The option taken for abnormal milk control may be accepted, namely, the selection of that one of a series of single-band eyepiece reticles for which the region of optimum count precision falls closest to the estimated cell concentration of the milk sample *(2)*. This, however, may require an impractical amount of reticle switching between milk samples.

We have elected to use the two-band reticle[1] developed originally by the Subcommittee on Screening Tests, National Mastitis Council and described by Schultze *(2)*. The choice of a wide or narrow strip width is combined with variation from one to four in the number of strips to be examined, and finally with a narrow strip-field count at extremely high cellular concentrations. Six concentration ranges are identified and counted to a predetermined area. Figure 1 shows the numbers of cells counted by each of the film area modifications, the portion of the cellular concentration range covered by each modification, and the expected CV. Except in the lowest range, in which the number of cells counted may be as low as zero, the total number of cells counted varies only between 400 and 800, approximately. In the highest concentration range, the total of 400 cells in the

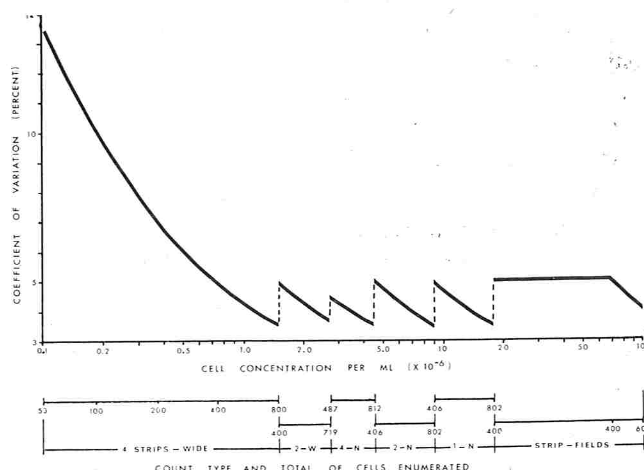

Figure 1. The Numbers of Cells Counted and the Expected Coefficient of Variation over the Applicable Range of Cell Concentration.

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[1]Manufactured by American Optical Company, Instrument Division, Buffalo, N. Y. 14215. Catalog No. K-2058.

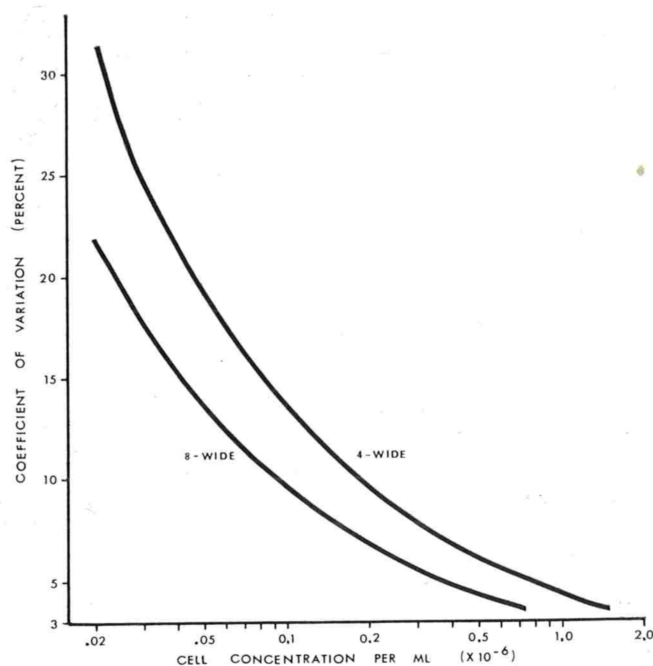

Figure 2. The Difference in Expected Coefficient of Variation Between 4-WIDE and 8-WIDE Counts at Low Cell Concentrations.

narrow strip-field count is exceeded only at a concentration of 70,000,000 per ml.

In the lowest concentration range, the CV drops with increasing count from 100% when one cell is counted to 30.7% at 20,000 per ml (11 cells counted) to 3.5% at 1,500,000 per ml. In the succeeding categories the CV is permitted to range only between 5.0 and 3.5%. It should be borne in mind that, whereas the relationship between particles counted and count variance is independent of magnification, the conversion of particle count to cellular concentration is not. In this paper, a factor of 7,510 for conversion of counts on a single wide strip has been used for illustrative purposes. Microscopes in common use will yield a factor close to this value.

In practice, the relevant film area modification is selected through a preliminary estimation of cellular concentration and further refined according to the magnitude of the count on the first strip. The following counting protocol is in use in our laboratory.

Using the WIDE reticle band, quickly scan the horizontal diameter of film #1 and estimate the average number of cells within the strip area of a microscope field. (The strip-field extends completely across the visual field.)

Estimated 0-4 cells per WIDE strip-field: use WIDE band and count the horizontal strip of film No. 1.

Count totals <200 cells: count also the vertical strip on film No. 1 and the horizontal and vertical strips on film No. 2 (4-W Count).

Count totals 200 cells or more: count also the horizontal strip on film No. 2 (2-W Count).

Estimated 5-27 cells per WIDE strip-field: use NARROW band and count the horizontal strip of film No. 1.

Count totals <200 cells: count also the vertical strip on film No. 1 and the horizontal and vertical strips on film No. 2 (4-N Count).

Count totals 200-399 cells: count also the horizontal strip on film No. 2 (2-N Count).

Count totals 400 cells or more: do not count any additional strips (1-N Count).

Estimated 28 or more cells per WIDE strip-field: use individual NARROW strip-field count.

Make a count within such fields across the horizontal diameter of film No. 1, skipping every other field, and accumulating the total of cells and of fields counted. Terminate the count after that strip-field in which the total of cells reaches 400. In any event, count a minimum of ten strip-fields. Do not count film No. 2.

For manual conversion of cell counts to concentration per ml, we supply the microscopist with three Strip Factors: for single WIDE strip; single NARROW strip, and for single NARROW strip-field. The DMSCC per ml is estimated by the product of total cells counted and the appropriate Factor, this product divided by the number of strips or strip-fields examined.

Although extension of this system to extremely high cellular concentrations is mathematically valid, there are practical objections. The crowding of cells on the film decreases the reliability of identification and enumeration. When the estimated cell concentration indicates the need for individual NARROW strip-field counting, it may be preferable to prepare a ten-fold dilution of the milk sample for counting.

We have devised a further modification of the DMSCC to meet situations in which increased precision is critical in the range of cell concentration below 750,000 per ml. Studies of variation within uninfected quarters or of the protective role of the leukocyte against microbial challenge illustrate the need for such a technique. One could simply double the 4-W Count by moving to an adjacent strip at the completion of each of the four strip counts, reversing direction of scanning, and repeating the count. The slight decrease in strip length is not detectable. The gain in precision is also not very great. Figure 2 shows the expected CV's for 4-W and 8-W counts.

We consider it preferable for studies in this cell concentration range to make four films and count the horizontal and vertical strips on each. The decrease in count variance is the same by this method, of course, but in addition the film component of variance is halved, as shown by Smith *(3)*. The smaller the count variance becomes, the greater is the proportion of the total variance contributed by the film component. In using this method, four films are prepared and stained. The previously described count-

ing procedure is followed. However, when the total of cells in the 4-W Count is less than 400, the count is continued through the third and fourth films. The Working Factor for such a count would be in the neighborhood of 950.

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2. Schultze, W. D. 1968. Design of eyepiece reticles for use in the direct microscopic somatic cell count method. J. Milk Food Technol. 31:344-349.

3. Smith, J. W. Development and evaluation of the direct microscopic somatic cell count (DMSCC) in milk. J. Milk Food Technol. *In Press.*

REPORT OF THE 3-A SYMBOL COUNCIL, 1968-1969

The Board of Trustees of the 3-A Symbol Council held two meetings since the 1968 Report was presented to this Association at the St. Louis Annual Meeting. The first of these meetings was held in Chicago, on October 16, 1968, during the Dairy and Food Industries Exposition; the second was held here in Louisville on the evening of Monday, August 18, 1969.

All the Trustees attended the Chicago meeting, which was marked by the initial attendance of Trustee Dr. Warren S. Clark, of the American Dry Milk Institute, who succeeded Mr. R. P. Zelm as a representative of the Sanitary Standards Committee of the Dairy Industry Committee. The principal business of the Chicago meeting was the consideration of two amendments to the By-Laws of the 3-A Symbol Council. Both were adopted.

The first of these amendments provides that, after May 1, 1969, a Trustee may not concurrently serve as a member of any of the 3-A Sanitary Standards Committees. The object of this amendment is to make the Board of Trustees of the 3-A Symbol Council an entity completely separate from all other aspects of the 3-A program. Because of the adoption of this amendment, Trustees D. C. Cleveland and James A. Meany have submitted their resignations from the Committee on Sanitary Procedure.

The other amendment to the By-Laws authorizes the Board of Trustees to designate an individual, who need not be a Trustee, to serve in the capacity of Assistant Secretary-Treasurer, or individuals for each of these offices.

A committee was appointed to name a panel of individuals capable of serving, and in position to serve, in this capacity, from which the Board would make its selection. This selection is reported among the actions of the Board during its meeting of August 18, 1969.

Seven of the Trustees attended the August 18, 1969 meeting of the Board. Fred Uetz, Past-President of this Association, who was named a Trustee to succeed H. S. Christiansen as a representative of the Sanitary Standards Subcommittee of the Dairy Industry Committee, was installed as a Trustee, and designated Assistant Secretary-Treasurer.

Several applications for authorizations were reviewed by the Board and action decided. An amendment of the By-Laws of the Council, involving procedure in a review of negative action on applications, was discussed; but action was deferred until the next meeting of the Board.

TABLE 1. NUMBERS OF AUTHORIZATIONS IN EFFECT

Equipment	Serial No.	7/31/68	7/31/69
Storage tanks	0102	17	16
Pumps	0204	12	13
Homogenizers	0402	3	3
Transportation tanks	0506	19	19
Piping fittings	0808	15	17
Thermometer fittings	0902	1	1
Filters	1002	1	1
Plate-type heat exch.	1102	7	7
Tubular heat exch.	1202	3	3
Farm bulk tanks	1303	19	17
Leak-detector valves	1400	4	4
Evaporators	1603	7	6
Fillers and sealers	1702	5	5
Freezers	1901	2	2
Silo-type storage tanks	2201	8	8
Packaging equipment	2300	3	3
Batch pasteurizers	2400	6	7
Batch processors	2500	5	6
Dry milk sifters	2600	5	5
Dry milk packaging equipment	2700	—	0
		142	143

Between August 1, 1968 and July 31, 1969, seven initial authorizations were issued, and six were relinquished (not renewed), resulting in an increase of one in the number of authorizations in effect—143. (Another has been added since August 1.)

A tabulation of authorizations in effect on July 31, 1968 and on July 31, 1969 will accompany this report when published.

A roster of Holders of Authorizations, as of February 20, 1969, was published in the March number of the *Journal*, and the roster, as of August 20, 1969, is ready for submission to the Managing Editor.

D. C. CLEVELAND, *Trustee*
JAMES A. MEANY, *Trustee*
K. G. WECKEL, *Chairman*
C. A. ABELE, *Secretary*

FACTORS AFFECTING SURVIVAL OF *SALMONELLA* IN CHEDDAR AND COLBY CHEESE

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(Received for publication August 7, 1969)

Abstract

Factors affecting the growth and survival of salmonellae in the experimental manufacture of 7 lots of Colby and 65 lots of Cheddar cheese were studied. Milk was artificially contaminated just prior to making of cheese. Manufacturing variables tested included effects of milk pasteurization, size of starter inoculum, titratable acidity and cheese pH, type of lactic culture, chemical additives, salt and moisture content, supplemental cheese microorganisms, and curing temperatures. The rate and amount of acid produced during making, the pH of the cheese, and the type and size of starter inoculum had significant effect in suppressing the growth and survival of salmonellae. Salt, moisture, chemical additives, and pasteurization of the milk, had little or no effect on the salmonellae. Addition of large numbers of *Propionibacterium* and *Leuconostoc* seemed to favor survival of salmonellae. Lactobacilli and enterococci tested had no effect.

Salmonellosis is a continuing health problem and its apparent increase can be attributed to an increasing awareness and more extensive surveillance. Salmonellae contamination in manufactured dairy products has become of increasing concern since 1966 when 11 serotypes were isolated from nonfat dry milk (NDM) from nine states *(8)*. Consequently, the Food and Drug Administration has indicated that it plans to screen all dairy products. In anticipation, these laboratories began a survey of a variety of dairy products to determine which manufacturing variables contribute to growth and dissemination of salmonellae and how procedures could be modified to insure safety of the product. Reports for Cottage cheese *(5)* and NDM *(6)* have been published.

A review of cheese-borne infections up to 1947 is given by Fabian *(1)*. More recently Price et al. *(7)* reported an outbreak from infected Cup cheese. In all instances for which background information is available, the cheeses were made from raw milk or from milk which was recontaminated after pasteurization. Pasteurization of cheese milk is not required providing the cheese is aged for at least 60 days prior to selling. Tucker et al. *(9)* reported survival of *Salmonella typhimurium* in Colby cheese for 302 days at 43 to 48 F. Goepfert et al. *(2)* reported survival of the same species in stirred curd Cheddar cheese for at least 12 weeks at 7.5 to 13 C.

Information is generally lacking concerning the effects of the many variables on salmonellae survival during manufacture and curing of Cheddar and Colby cheese. This paper reports the results of a study to determine such effects.

Materials and Methods

Cultures

Four test species of *Salmonella* were selected on the basis of their heat resistance and frequency of occurrence in dairy products. They were *Salmonella senftenberg* 775W, *Salmonella typhimurium* TMl, *Salmonella new brunswick* 1608, and *Salmonella newport*. In most trials, 18-hr cultures of each, grown in trypticase soy broth, were added to the cheese milk 1 hr prior to manufacture. The inoculum ranged from 300 to 5000 per ml of milk.

Commercial mixed-strain lactic cultures were used in most instances as the starter. Additional cultures, used singly or as supplementals, included one or more species of *Leuconostoc, Streptococcus, Lactobacillus, Micrococcus, Pseudomonas,* and *Propionibacterium*. All lactic cultures were subcultured in sterile 10% NDM prior to use. The *Propionibacterium* was grown in lactose broth and the *Micrococcus* and *Pseudomonas* in nutrient broth.

Salmonellae determinations

Analyses for salmonellae were made on the following: *(a)* cheese milk at time of addition of salmonellae and lactic starter, *(b)* milk at setting (addition of coagulating enzyme), *(c)* curd and whey at cutting, *(d)* whey at cooking, *(e)* whey at draining, *(f)* curd at draining, *(g)* curd at salting, *(h)* curd at hooping (packing curd in molds), and *(i)* the 21-hr cheese. The cheeses were subsequently tested in two weeks and at monthly intervals thereafter.

Two procedures were employed to detect and enumerate salmonellae. When counts exceeded 10/g, direct plating on a selective, modified, lysine iron agar (Difco) was used. The antibiotic novobiocin[2] (5-10 μg/ml) was added aseptically to the cooled lysine agar just prior to plating to suppress the growth of many gram-positive microorgansims and select strains of *Proteus* and *Escherichia coli*. A very thin underlayment and overlayer were used in conjunction with conventional pour techniques. Plates were incubated at 37 C for 24 to 48 hr. All salmonellae used in this study grew readily in the agar, producing hydrogen sulfide and developing into large black lens-shaped colonies. Samples were prepared for plating by blending 11 g of cheese with 99 ml w/v of a sterile 0.2% sodium citrate solution. When counts were lower than 10/g, quantities of the blended samples were preenriched in a selective lysine-iron broth

[1]Agricultural Research Service, U. S. Department of Agriculture.

[2]Novobiocin, sodium (Albamycin UpJohn Co.)

(3) and identified by standard cultural procedure.

Make procedure

Seven lots of Colby cheese and 65 lots of Cheddar cheese were made in small steam-jacketed vats, each having a capacity of 50 lb of milk. Manufacturing variables studied included the effects of: milk pasteurization, size of starter inoculum, titratable acidity (TA) and cheese pH, type of lactic culture, chemical additives, salt and moisture content, supplemental cheese microorganisms, and curing temperatures. The manufacturing methods were essentially those prescribed by Lochry et al. (4) for Cheddar cheese and Wilster (10) for Colby cheese. Variations in the "make" procedure were necessary to compensate for some of the test variables.

Fresh whole milk, standardized to 3.3% fat, was usually pasteurized at 163 F for 15 sec and cooled to 90 F before inoculation with the *Salmonella* test organisms. Two vats were made with raw milk as the variable.

Both mixed and single strain cultures of *Streptococcus lactis* and *Streptococcus cremoris* were compared. Inoculum size was varied from 0.1 to 3%. In an attempt to maintain comparable pH values and TA in the cheese during manufacture and curing, the make time-schedule and other variables had to be adjusted. For example, the ripening time for the milk with 3% starter was reduced from 1 hr to 5 min, the time between cooking and draining was shortened to 15 min, cheddaring time was reduced to 30 min, the pressing temperature was lowered to 40 F rather than 70 F and the curing temperature during the first month was 32 F rather than 40 F.

In an attempt to pinpoint the role of pH, various methods for controlling acid were utilized. These included variations in schedule to mill at TA from 0.25 to 0.55%, buffering the cheese milk with phosphates, the use of age-attenuated lactic starters and direct acidification of cold cheese milk with edible acids.

The role of salt in the finished cheeses was studied by varying the salt content from 1.4 to 2.7%. The curd was usually dry salted, but in two experiments salt was added directly to the cheese milk at the rate of 1 and 1.5% w/v.

Make procedures were altered to control moisture levels within a range of 35 to 48%. Other variables included the use of inhibiting agents such as diacetyl, potassium sorbate, nisin, and sodium lactate.

Goepfert et al. (2) reported evidence that the production of volatile fatty acids during curing, especially acetic, may be a major factor in elimination of salmonellae from cheese. To explore this theory, cheeses were made by direct acidification using lactic and acetic acids. Sufficient acid was added to the cold cheese milk (40 F) to lower the pH to 5.35. Rennet and 0.1% starter were also added while cold. The temperature was then slowly raised to 80 F without stirring by circulating 100 F water through the jacket of the vats. After coagulation, in about 0.5 hr, the coagulum was cut and cooked to 100 F. The curd was then drained, cheddared for 1 hr, and salted.

Most cheeses were hooped and pressed at 75 F for 21 hr and were sealed by dipping in melted cheese wax. Most were stored at 40 F, but some were divided so that portions could be stored at 32, 40, and 50 F.

The pH of the cheese was determined after 21 hr in the press, after two weeks curing, and at monthly intervals thereafter.

RESULTS AND DISCUSSION

A typical pattern of salmonellae growth during manufacture is shown in Table 1. There was very little growth up to the time of cutting. Then a thirtyfold increase occurred during the 2-hr interval between cutting and draining. Since one would expect an approximate tenfold increase from cell concentration in the curd, the difference indicates a moderate growth rate of about two generations during the 2-hr period. Multiplication continued, resulting in a sevenfold increase (approximately three generations) during the 3-hr interval between draining and hooping. Following hooping, active growth stopped sometime during the pressing operation as evidenced by an 80% decline between 6-1/2 and 21 hr. These data are in good agreement with those of Goepfert et al. (2) who reported approximately 4.5 generations between cutting and hooping, followed by a 75% decline during pressing.

An assumption that salt initiated the rapid initial decline between hooping and 21 hr was generally disapproved by the fact that subsequent vats of cheese having salt contents from 1.4 to 2.7% showed no difference in decline during the same period. Additional vats of cheese were made in which 1.5% salt was added to the cheese milk. Resulting inhibition of the lactic starter necessitated increasing the inoculum to 3% or lengthening the cheddaring time by about 15% before milling. No inhibition of salmonellae was observed. Additional salt was then added to the curd to raise the final level to normal values. Figure 1 compares salmonellae survival during curing as affected by differences in salt content. Here again, no differences were observed. It can be concluded that salt plays little or no part in hastening the death of salmonellae during cheese manufacturing and curing.

Survival of salmonellae during curing varied from 2 to 9 months with an average of 6.5 months for all vats. The variables significantly affecting survival were pH and type and amount of starter. These data are presented in the accompanying figures.

Figure 2 shows the effect of cheese pH on survival of salmonellae throughout curing. Zero time represents the salmonellae count of the cheese after 21 hr in the press. The pH range given indicates the pH of the cheese from 21 hr to final sampling. Cheeses with an abnormally high pH, caused by starter failure (dead vats) obviously have little or no inhibitory effect on the salmonellae. The most rapid decline was associated with pH 5.0. Such cheese made from pasteurized milk would probably be criticized for acidity and bitterness. However, raw milk cheeses with pH values from 5.0 to 5.1 are common and are usually acceptable in flavor and acidity.

Figure 3 shows dramatic differences in effects of amount of starter on survival during storage. Zero time in the figure represents the salmonellae count

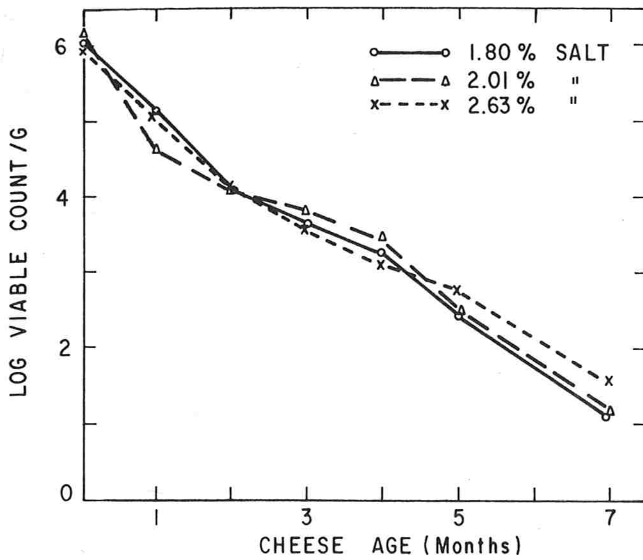

Figure 1. Effect of salt on *Salmonella* survival in Colby cheese.

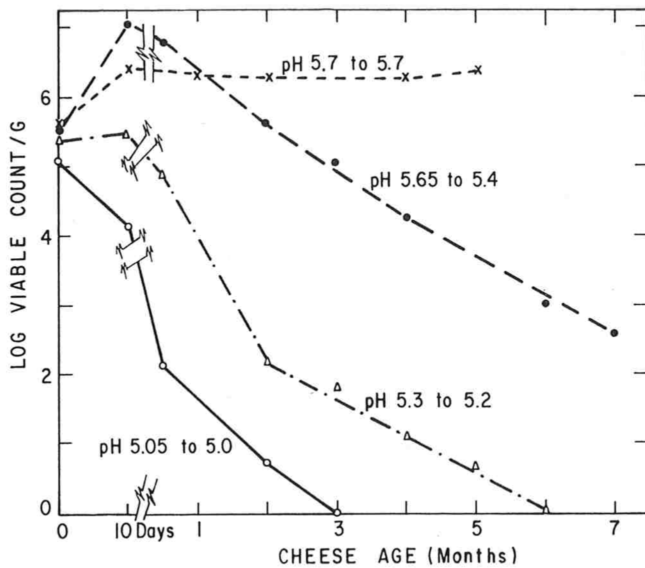

Figure 2. Effect of pH on *Salmonella* survival in Cheddar cheese.

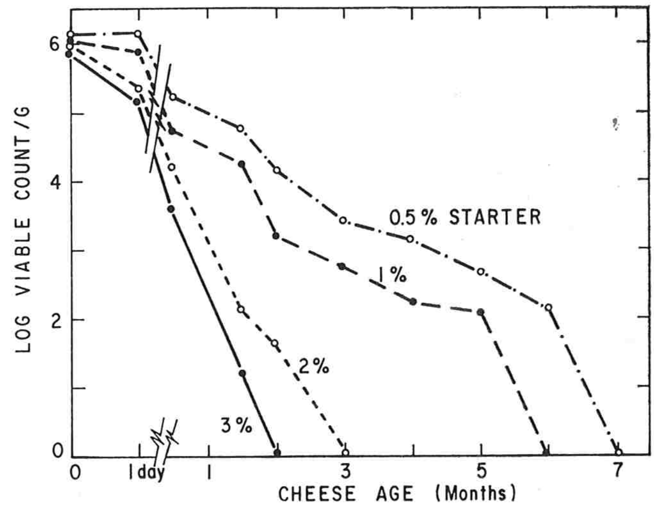

Figure 3. Effect of lactic starter inoculum on *Salmonella* survival in Cheddar cheese.

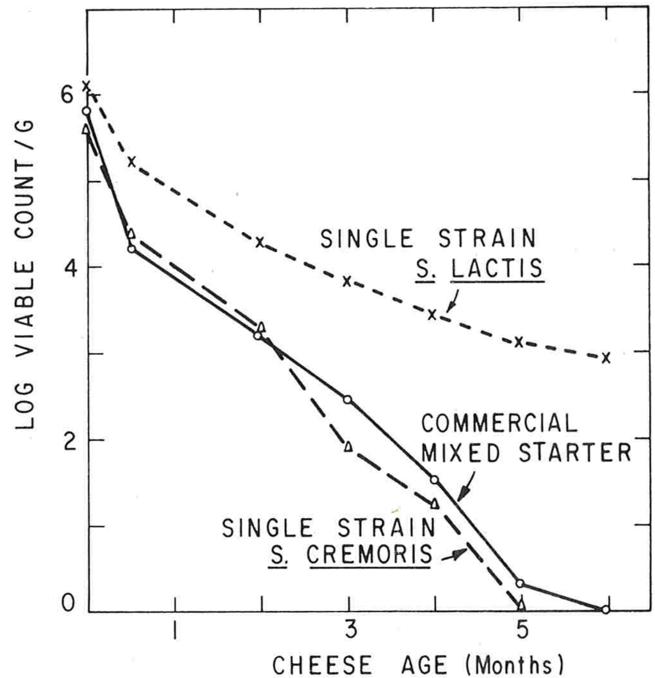

Figure 4. Comparison of cheese starter on *Salmonella* survival in Cheddar cheese.

of the cheese curd just prior to pressing. The count of the cheese made with 0.5% starter showed no decline during the first day of storage. In contrast, an initial reduction of about 80% occurred in the cheese made with 3% starter. All of this decline was not caused by the acid. The various control techniques mentioned earlier succeeded in maintaining essentially identical pH development through pressing. At milling, the TA of all four lots was 0.53%, and the 21-hr pH values were 5.55, 5.55, 5.45, and 5.45, respectively, for 0.5, 1, 2, and 3% starter. The differences at two months were greater, varying from pH 5.31 (0.5% starter) to 5.00 (3% starter). Thus the control of acid development until the cheeses with less starter could "catch up" to that with 3% starter indicates that the lactic starters were somewhat inhibitory.

In order to study possible differences in natural inhibitory effects of different species of lactic starter, several species of *S. cremoris* and of *S. lactis* were compared to commercial multiple-strain cultures. Efforts were made to develop comparable acidities and to produce cheese of similar pH and composition throughout manufacture and curing, thus eliminating variables other than starter. The pH of these cheeses was within the range of 5.18 to 5.29 after one day and 5.19 to 5.25 after two months of curing. Figure 4 is representative of the results obtained. The species of lactic starter used in cheesemaking appears

TABLE 1. SURVIVAL OF SALMONELLAE IN CHEDDAR CHEESE MANUFACTURE[1]

Manufacturing operation	Time hr	Salmonellae No/ml - g
Starters added	0	1,400
Milk at setting	1	1,500
Curd + whey at cutting	1 1/2	1,560
Whey at cooking	2	230
Whey at draining	3 1/2	410
Curd at draining	3 1/2	47,100
Curd at salting	5 3/4	310,000
Curd at hooping	6 1/2	318,000
Cheese after pressing	21	60,000

[1]0.75% lactic starter; milling TA 0.40%; 21 hr pH 5.4.

TABLE 2. EFFECT OF TEMPERATURE ON SURVIVAL OF SALMONELLAE DURING STORAGE

Cheese age months	32 F		40 F		50 F	
	No./g	pH	No./g	pH	No./g	pH
0	61×10^4	5.52	61×10^4	5.52	61×10^4	5.52
1	18×10^3	5.38	11×10^3	5.38	16×10^3	5.39
2	39×10^2	5.31	32×10^2	5.23	20×10^2	5.16
3	65×10^1	5.30	60×10^1	5.22	6×10^1	5.15
4	60	5.25	18	5.18	0	5.10
5	30	5.21	2	5.15	0	5.10
6	10	5.22	0	5.15	0	---

to have a marked effect on survival, probably because of the production of an unknown inhibitory factor. Species of S. *cremoris* were much more inhibitory than S. *lactic* species. These results were confirmed by comparing lactic streptococci grown on agar plates seeded with salmonellae. S. *cremoris* strains produced greater zones of inhibition than either S. *lactis* or S. *thermophilus.* The antibiotic "nisin" and nisin-producing strains of S. *lactis* had no effect on salmonellae. Supplemental starter microorganisms, *Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus casei, Pseudomonas fragi,* and *Micrococcus caseolyticus* had no apparent effect on salmonellae when added to the cheese milk. In contrast, large numbers of *Leuconostoc* bacteria and *Propionibacterium shermanii* added as starters resulted in the survival of salmonellae at higher levels and for longer periods of time than in controls. The pH of these cheeses was slightly higher which may account for this effect.

None of the additional variables tested were of value as a means of salmonellae control. No apparent differences were detected when raw milk cheese was compared with pasteurized milk cheese. Moistures had no direct influence. Potassium sorbate and sodium lactate had no inhibitory effects while high levels of diacetyl slowed the growth of both salmonellae and lactic starter.

Results of storage at 32, 40, and 50 F, shown in Table 2 revealed slight differences with a more rapid decline at higher temperatures. These variations were not considered significant and appeared to be related more to greater acid development at the higher temperatures.

The results obtained from the cheeses acidified with lactic and acetic acids confirm the roles of both pH and starter. Both cheeses were inoculated with only 0.1% starter, much less than would normally be used. The starter organisms grew slowly in the lactic acid cheese to a maximum of 2,000,000/g. This cheese became gassy in three months with a coliform count of 180,000/g. The salmonellae declined slowly during storage but the rate of decline was about one-half that of cheeses with a comparable pH developed by normal amounts of starter. The cheeses acidified with acetic acid gave the same pattern for salmonellae; the decline was about half as fast as expected. This cheese, however, indicated inhibition of lactic starter and coliforms. The starter grew to a maximum of 16,000/g while no coliforms were evident. It appears that a pH of 5.2 to 5.3 was sufficient to cause death of salmonellae during storage, but the lower rate of decline in the absence of normal amounts and development of starter offers further evidence of the natural inhibitory nature of starter organisms. While acetic acid appears to be inhibitory to some organisms, it had no apparent effect on the salmonellae.

The results of this study revealed no variables that could be used to insure freedom of Cheddar or Colby cheese as a source of salmonellosis should these organisms enter cheese milk as post pasteurization contaminants. Only pH and starter significantly influenced salmonellae survival. All other variables had indirect effects; that is, their role was obvious only to the degree that they influenced the development of acid. Thus, factors which contributed to subnormal acid production enhanced growth and survival of salmonellae and those that contributed to normal or excessive acid production were detrimental. It should be pointed out that the degree of salmonellae contamination used in these studies far exceeded that which would likely occur from post-pasteurization contamination. However, it does reflect the need for quality control.

Although the incidence of salmonellosis is apparently increasing, the dairy industry has maintained a good record. United States Public Health officials have found no salmonellae in their survey of Cheddar cheese. In addition, no outbreak of salmonellosis has been traced to Cheddar type cheese

since 1945. This paper, by pointing out the possibility of salmonellae survival for long periods of time, reemphasizes the need to maintain strict adherence to proper and sanitary procedures.

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REPORT OF IAMFES REPRESENTATIVE TO THE NATIONAL MASTITIS COUNCIL, 1968-1969

As the official representative of the International Association of Milk, Food, and Environmental Sanitarians, Inc., on the Board of Directors of the National Mastitis Council, the following is a brief summary of events to date:

At the annual meeting of the National Mastitis Council held in Chicago in February, 1968, I was appointed to the Board of Directors. I attended only the last day of the Board meetings, mainly to become acquainted with the members.

At the International Association's annual meeting held in St. Louis in August, 1968, I submitted a request to the officers of the Association asking that consultants be appointed to advise me regarding requests and recommendations which I should submit to the National Mastitis Council. This request was granted. Following are the consultants which were appointed from the Farm Methods Committee: M. W. Jefferson, Ben Luce, Leon Townsend, David Monk, and Glenn Cavin.

Attached is a copy of the subjects discussed and submitted by this Committee. These subjects were presented to the proper committees at the National Mastitis Council's annual meeting held in Chicago in January, 1969:

(*a*) No action. I was advised that this should be presented to the Interstate Milk Shippers Conference. It is the opinion of many that the National Mastitis Council is only interested in research regarding the cause and treatment of mastitis as well as the laboratory procedures and analysis of the number of somatic cells in the milk supply. There are a great many variations in the opinions of the members of the National Mastitis Council on these two subjects.

(*b*) This request was presented to the Committee on Screening Tests, A. R. Brazis, Chairman. It was accepted and placed in the minutes of the meeting.

(*c*) I presented this recommendation to the veterinarian's section and asked for more cooperation between the veterinarians and the regulatory sanitarians. There is some question as to the acceptance of this recommendation. The veterinarians feel that they are the only people who are directly involved in a mastitis program and that the regulatory agencies should be interested in the quality of milk only. They could be correct regarding this matter.

At this time, may I request that the Board of Directors of the International Association submit guidelines and advice in regard to the direction we should take with the National Mastitis Council. I hope we can continue to show constructive results for the organization through our combined efforts.

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EFFECTS OF TIME AND TEMPERATURE ON SALMONELLAE IN INOCULATED BUTTER[1, 2]

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Abstract

Three batches of butter made in small experimental lots from commercial cream were contaminated with *Salmonella typhimurium* var. *copenhagen* by inoculating the cream and wash water. Contaminated butter was held 10 weeks at 77, 40, 32, 0, and −10 F. *Salmonella* increased at 77 F and decreased at ≤ 40 F. Most significant decline in viable *Salmonella* was at 0 or −10 F, in unsalted butter followed in order by lightly salted butter and moderately salted butter.

Salmonellosis is considered one of the most important zoonotic diseases; it affects as many as 2 million persons annually in the United States *(7)*.

The most important reservoirs of human salmonellosis are livestock and poultry. It therefore follows that the most important vehicle of *Salmonella* is human or animal food, the most suspect foods being those lightly cooked and subjected to much handling.

While butter is not commonly considered a source of salmonellosis, little concern for its potential suggested that it be studied. Experimentally, sweet cream butter has been reported to support survival of *Salmonella* at room temperature and at 0 C *(9)*. Zagaevski reports *Salmonella* viability of up to 9 months in butter *(10)*.

Variations in salt content as well as commercial utilization of lower storage temperatures (0 to −10 F) that have been studied, made it appear feasible to attempt to determine effects of salt content and time and temperature in storage in butter inoculated with *Salmonella*.

Materials and Methods

Bacterial cultures

A *Salmonella* species which is commonly incriminated in food-borne salmonellosis, was used. The culture (*Salmonella typhimurium* var. *copenhagen*) was procured from the Division of Biology, Kansas State University, and maintained on trypticase soy agar slants.

The inoculum was prepared by washing the cells from 24-hr slants with sterile 0.1% tryptone solution *(8)*. The suspension was centrifuged at 500 x gravity for 30 min and the supernatant fluid discarded. Cells were resuspended in sterile 0.1% tryptone solution and adjusted to an optical density of 0.4 at 520 mμ on a spectrophotometer (Bausch & Lomb Spectronic 20). This resulted in a cell concentration of 6.5 x 10^8/ml (variance of 34.8) as predetermined by the least squares regression technique of Fryer *(1)*. The inoculum for batches 1 and 2 consisted of 5.0 ml of the suspension. Batch 3 was inoculated using 500 ml water-tryptone cell suspension (O.D.–0.4) as wash water. The concentration was cinfirmed by plating on Brilliant Green sulfadiazine (BGS) agar (Difco) using the spreader technique.

Manufacture and sampling of butter

Butter was manufactured in a sterile glass electric churn. Pasteurized grade A cream, 4.5 pints per churning, was used. Fat content, standard plate count, and coliform count were determined on the cream as described in *Standard Methods (6)*. All cream was confirmed by BGS agar plating to contain no *Salmonella* prior to use.

Batch 1 (unsalted butter, cream inoculated). To 4.5 pints of 36.5% fat cream (45 F) was added 5.0 ml tryptone cell suspension of *S. typhimurium* var. *copenhagen* (O.D.–0.4). The inoculated cream was churned approximately 45 min and the butter placed in a sterilized pyrex bowl after discarding the buttermilk. The butter was washed with 300 ml sterile deionized distilled water (40 F) and worked with a sterile wooden paddle to distribute the moisture evenly. The butter was placed in sterile screw-top jars of 130 ml capacity, each jar receiving 10 g of butter. Five lots were formed (13 jars/lot) and one lot placed at each of these temperatures: 77, 40, 32, 0, and −10 F (25, 4.44, 0, −17.77, and −23.33 C). Samples were withdrawn and placed in jars for initial *Salmonella* count and chemical analyses. Chemical analysis was performed by the Kohman method *(3)*.

Batch 2 (salted butter, cream inoculated). To 4.5 pints of 37.5% fat cream (45 F) was added 5.0 ml of tryptone cell suspension of *S typhimurium* var. *copenhagen* (O.D.–0.4). Working, churning, and washing were accomplished as in batch 1, except that 20 g of sterile NaCl was added to the butter. The butter was divided, stored, and sampled as batch 1.

Batch 3 (salted, wash wated inoculated). Churning was accomplished in 45 min using 4.5 pints of 30.0% fat cream. Buttermilk was discarded and the inoculated wash water (500 ml) prepared by adding 75 ml tryptone cell suspension to 425 ml sterile deionized distilled water (40 F). The resulting cell suspension (O.D.–0.4) was used to wash the butter and to act as another possible contamination route. Nineteen grams of sterile NaCl was worked into the butter mass after it was washed. The butter was divided, stored, and sampled as with batches 1 and 2.

Table 1 summarizes composition of the cream, the resulting butter, and inoculum used.

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[5]Professor, Division of Biology, Kansas State University, Manhattan, 66502. (Deceased, September 15, 1969).

TABLE 1. COMPOSITION OF BATCHES OF EXPERIMENTAL BUTTER

Constituent	Batch 1	Batch 2	Batch 3
Cream			
% Fat	36.5	37.5	30.0
Standard plate count/ml	6000	3300	3000
Salmonella cell suspension			
Concentration	8.5 x 10^8/ml	1.0 x 10^9/ml	6.4 x 10^8/ml
Volume added (ml)	5.0	5.0	500
Butter			
% Fat	85.8	82.0	84.1
% Moisture	13.3	15.3	14.1
% Salt	0.0	2.2	1.7
% Curd	0.9	0.5	0.1
Initial salmonellae	1.1 x 10^5/g	2.7 x 10^3/g	2.2 x 10^5/g

TABLE 2. ANALYSIS OF VARIANCE TABLE FOR BUTTER STORED AT 40 F.

Source of variance	Degrees of freedom	Mean square of variables
Time	12	0.52477**
Batch	2	1.21540**
Temp.	3	0.11278**
Time x batch	24	0.02654**
Time x temp.	36	0.01823*
Batch x temp.	6	0.10608**
Error	72	0.01104

*Mean square significant at .05 level
**Mean square significant at .01 level

Enumeration of Salmonella

Salmonella were enumerated by the BGS-spreader-plate technique. All lots were tested for *Salmonella* initially and at these intervals: 3, 6, 9, 14, 21, 28, 35, 42, 56, 63, and 70 days.

Samples were removed from storage at stated intervals. A 5-g sample was immediately aseptically removed by a heat-sterilized spatula and placed in a sterile 50 ml beaker. One milliliter of 10% tergitol No. 7 solution was added by sterile pipette to be emulsified (2). Tergitol No. 7 solution used per sample was increased to 3 ml in instances where undiluted butter was spread on BGS agar plates; otherwise even spreading necessary for uniform colony distribution was hampered. The beaker containing the butter-tergitol mixture was then placed in a 45 C (113 F) magnetically agitated water bath until the butter had completely melted (5). Concurrently warming in the same water bath were 9 and 99 ml sterile dilution blanks containing 0.1% tryptone solution. The blanks were warmed to prevent the melted butter from solidifying when dilution was in progress. The butter-tergitol mixture was then serially diluted and 0.1 ml from each dilution spread on BGS agar plates with sterile 3 mm bent glass rods. Duplicate plates were utilized. When undiluted butter was spread on BGS agar plates, the plates had been prewarmed to 39 C to enhance spreading.

Plates were incubated at 37 C for 24 hr; when colonies were small, the plates were allowed to incubate an additional

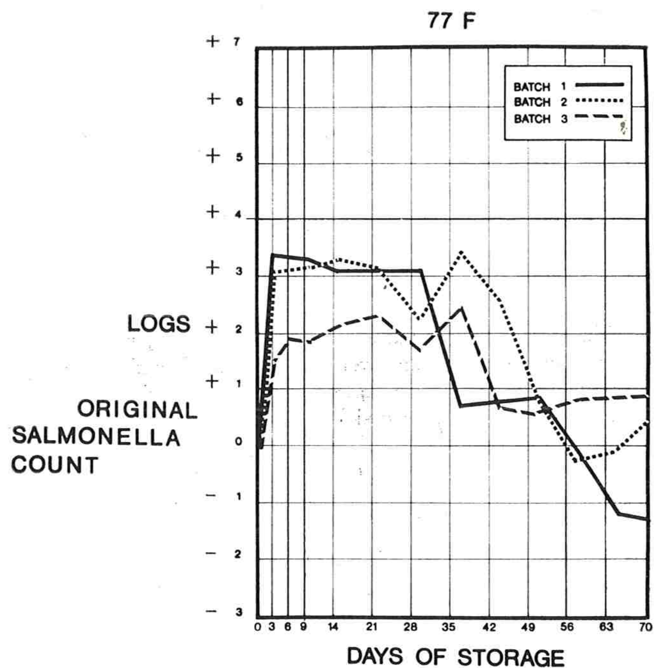

Figure 1. Population deviation in logs from original counts of butter batches 1, 2, and 3 stored at 77 F for 10 weeks.

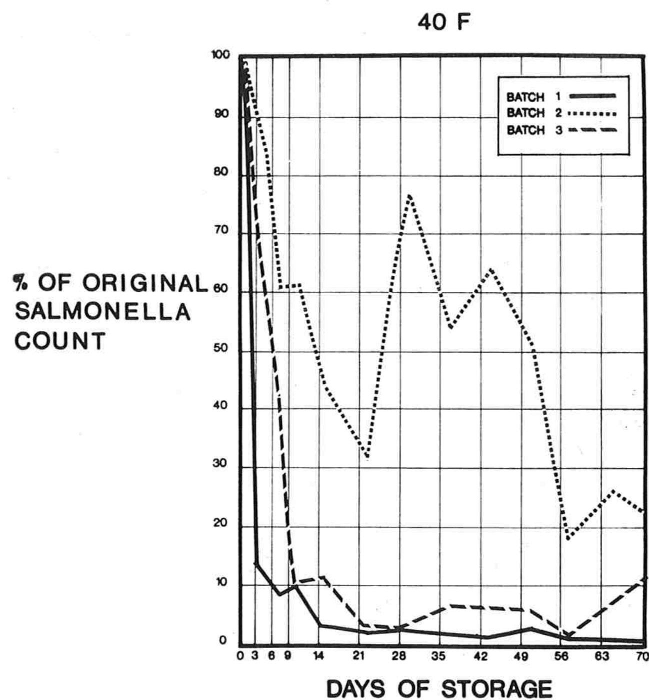

Figure 2. Percentages of *Salmonella* survival in butter batches 1, 2, and 3 stored at 40 F for 10 weeks.

24 hr before counting. A Quebec dark field colony counter was utilized counting only plates with 30-300 colonies of typical *Salmonella.*

Typical *Salmonella* colonies were streaked and stabbed on triple sugar iron and lysine iron agar slants and streaked on trypticase soy agar slants. The slants were incubated at 37 C for 24 hr and results recorded. Confirmation of isolates as *S. typhimurium* var. *copenhagen* was accomplished by slide

agglutination with group B antisera (Difco) and by flagellar agglutination with Salmonella H antiserum, Poly (Difco).

RESULTS AND DISCUSSION

Salmonella typhimurium var, *copenhagen* was recovered from all butter samples at all time intervals and at all temperatures. At no time did the recovery level at temperatures ≤40 F exceed the initial count of the came batch. All batches supported the growth of *Salmonella* when stored at 77 F as illustrated in Fig. 1.

Viable *Salmonella* of batch 1 (unsalted, cream inoculated) decreased to 5.26 to 0.71% of the original at temperatures ≤40 F by the end of 10 weeks storage. The same butter stored at 77 F became rancid and had nearly a 3.35 log increase in viable *Salmonella* within 3 days; then viable *Salmonella* gradually began to decline to 1.3 logs below the initial count at the end of 10 weeks storage.

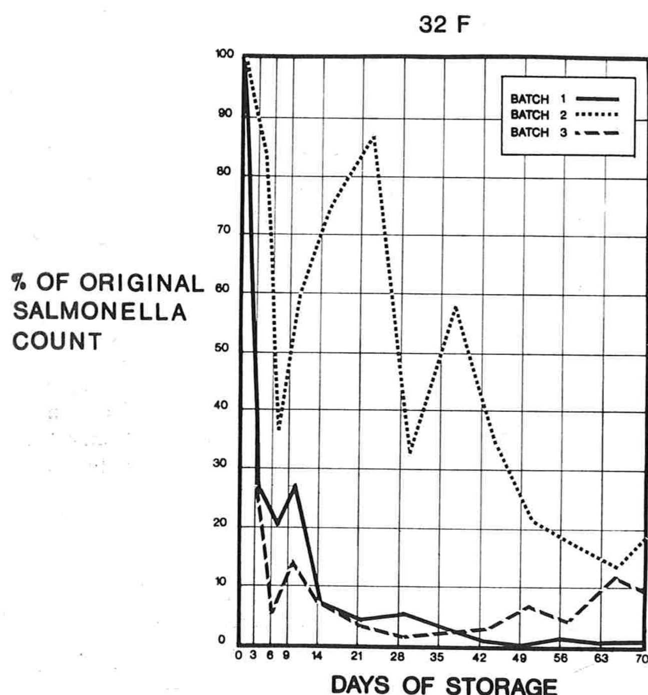

Figure 3. Percentages of *Salmonella* survival in butter batches 1, 2, and 3 stored at 32 F for 10 weeks.

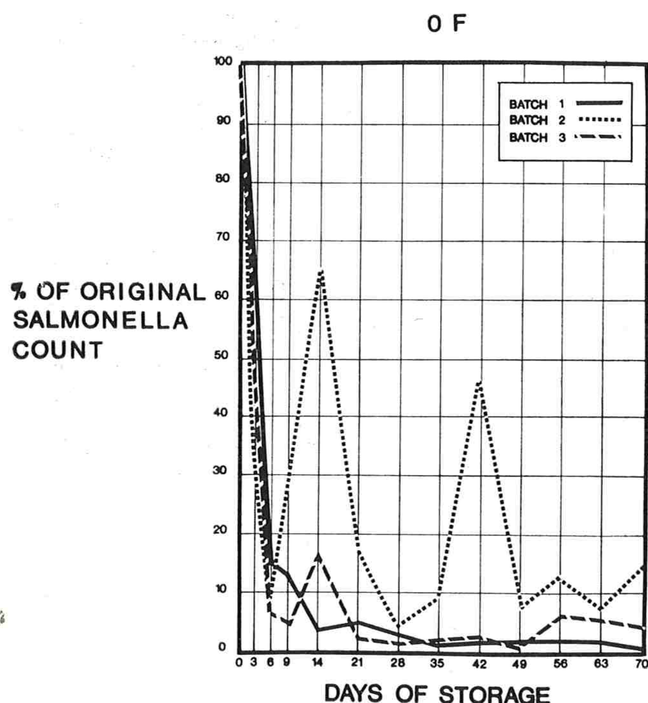

Figure 4. Percentages of *Salmonella* survival in butter batches 1, 2, and 3 stored at 0 F for 10 weeks.

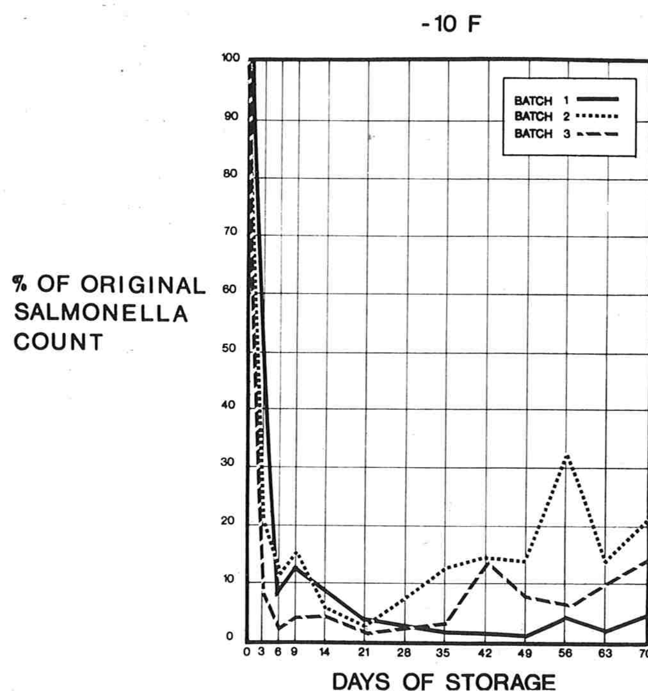

Figure 5. Percentages of *Salmonella* survival in butter batches 1, 2, and 3 stored at −10 F for 10 weeks.

In batch 2 (salted, cream inoculated) the *Salmonella* count declined to 22.52 to 13.04% of original at temperatures ≤40 F by the end of 10 weeks storage. A portion of the same butter stored at 77 F became rancid later than batch 1 and had a 3.1 log increase in viable *Salmonella* within 3 days, followed by a fluctuating recovery level, then declining to 0.2 log below the initial count at 56 days, followed by a slight increase of about 0.6 log by the end of 10 weeks storage.

Viable *Salmonella* in batch 3 (salted, wash water inoculated) decreased to 5.02 to 14.06% of original at temperatures ≤40 F by the end of 10 weeks storage. *Salmonella* in a portion of the same butter stored at 77 F increased by about 1.6 logs within 3 days and to 2.4 logs above original at 35 days. The recovery level then declined to about 0.8 log above the initial count at the end of 10 weeks storage.

The data were converted to percentages of original

count for all batches stored at ≤40 F. All counts from butter samples stored at 77 F were converted to log deviations from initial counts. Transformations were necessary to compare different original populations. Figures 1 through 5 graphically represent ranges of transformed populations for times, temperatures, and batches involved. We recognize some differences, because of manufacturing methods, among batches and between experimental butter and commercial butter. There were, theoretically, differences in moisture–droplet size and in dispersion as well as in salt distribution. Contamination levels—to enable observation of trends—were necessarily higher than probable under commercial conditions.

A three-way analysis of variance was computed on all counts derived from butter stored at ≤40 F. The data were converted to a squarefoot of the log of the percentage for programing. Table 2 presents the analysis of variance.

Computer analyses (Table 2) showed that all variables significantly affected *Salmonella* population means. In general, the longer a sample was held at temperatures ≤40 F, the fewer *Salmonella* survived. *Salmonella* in batch 2 declined less than in batch 3, where they declined significantly less than in batch 1.

Batch 2 had slightly more salt (2.2%) and lower initial count than batches 1 or 3 (Table 1). Theoretically, salt, being hydrophilic, can contribute to larger moisture droplets, which enhance *Salmonella* survival. Further, the salt (2.2% overall and approximately 11.5% in brine of batch 2) may have inhibited the natural flora and somewhat affected *Salmonella*. Batch 2 had more curd available for nutrients than batch 3 but less than batch 1. Batch 3 was significantly different from the other batches, having a mean survival rate between batches 2 and 1. Batch 3 was higher in salt (1.7%) than was batch 1; its original count differed only slightly. Apparently the low curd (0.1%) of batch 3 was not important, as batch 1 had 0.9% curd. Batch 1 was significantly less able to support *Salmonella* than were batches 2 or 3. Apparently the lack of salt may have allowed more natural flora to survive.

Temperature, as a variable, was significant; lower storage temperatures (0 and –10 F) significantly reduced survival means, compared with 40 and 32 F temperatures. Mean survival rate as it applies to temperature is listed here from high to low:

Temperature (F)	Means[1]
40	0.448
32	0.430
0	0.367
–10	0.333

[1]LSD = 0.04732 (Differences >0.047 are significant.)

Differences in survival means between 40 and 32 F and between 0 and –10 F were not significant. Combinations of variables were significant as time, temperature, and composition combined were significant.

Wide disagreement exists regarding what constitutes an infective dose of *Salmonella* organisms. Much depends on the host's general health. The U. S. Public Health Service recommends a 0 tolerance, but no experimental butter sampled during the 10 weeks storage met this standard.

Evidently, salt content, as usually employed by the butter industry (1-4%), is not significantly bactericidal to S. *typhimurium* var. *copenhagen*. Butter will readily support *Salmonella* growth at room temperature, whereas refrigeration or freezing for short periods offers no promise of eliminating *Salmonella* from butter.

ACKNOWLEDGMENTS

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INDIANA STATE UNIVERSITY'S ANSWER TO THE ENVIRONMENTAL HEALTH MANPOWER SHORTAGE[1]

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ABSTRACT

Indiana State University is attempting to meet some of the unmet needs for competent environmental health manpower by: (*a*) encouraging participation in and assisting the national and local sanitarian associations, (*b*) promoting a program of studies in Environmental Health that prepares a Generalist in the field of Environmental Health (instead of a General Scientist who then needs considerable training by future employers), (*c*) preparing students to understand the broader health needs and other needs of society to show where Environmental Health fits into the picture, (*d*) actively recruiting students into the field, (*e*) working closely with existing competent health departments in providing necessary work experiences as part of the undergraduate program of studies, and (*f*) informing the public that the Sanitarian does not refer to "privy sniffer," but rather to the Environmental Health practitioner who participates in community life and provides the services needed to prevent our environment from controlling us.

We urge other universities to heed Dr. Hilleboe's advice to reorganize their curricula to meet the diverse needs of our constantly changing society.

In Aristotle's ideal state "The Public Health," which was in fact Environmental Health, was the major concern in choosing the site of the city. Shattuck in 1850 explored our public health problems and anticipated our needs by saying that we should pass smoke ordinances, protect foods, establish schools for training sanitarian inspectors, etc. During the enlightened era of the 1880's to the 1920's we conquered yellow fever, typhoid fever, and dysentery. We then settled down to a well beaten path of complaint checking, milk inspection, and premises placarding. Unfortunately, although Aristotle and Shattuck, 23 centuries apart, pre-thought some of the environmental health needs of today and the future, we neglected for the most part to listen.

But change is inevitable and if we do not, at each point in the growth process of our society, mold the change to prevent disease and promote health, we will wallow in our pollutants and suffocate in our rat infested, poverty striken urban slums. Our children will sail their toy boats in streams of subdivision sewage in our suburban streets and parks. Our rural and semi-rural areas will continue to grow erratically without pre-thought direction, needing, but lacking, competent environmental health people to bring forth and nurture the basic environmental health programs that are our constitutional rights, rather than some hoped-for ultimate goal of our society. If we can circle the moon in 1968, should we not also apply the known basic environmental health principles to our lives and achieve the ultimate goal of good health?

INDIANA STATE UNIVERSITY PHILOSOPHY

In keeping with this desire to meet the continually changing needs and demands of our society and in an attempt to anticipate the future needs, Indiana State University (I.S.U.) has established a philosophy of service to the professional and non-professional communities that includes: (*a*) sponsoring of Sanitarian Association Conferences and other professional Environmental Health Conferences for the existing Sanitarians to promote a greater uniformity of recognition of the scope and nature of existing hazards and to use knowledge to alleviate them, (*b*) active participation in State and National Sanitarian and Public Health Associations, and (*c*) involvement in community health problems and informing thousands of I.S.U. students about potential careers in the various health and safety fields including Environmental Health.

We hope to stimulate discussion among our Environmental Health and Public Health colleagues, also among the informed citizenry concerning desirable types of model programs which should be established for the urban, suburban, rural-suburban, and rural socities. We hope to encourage existing Environmental Health Personnel to upgrade their knowledge and abilities to function in the current times, and in relation to current problems. We recommend that reciprocity be established in *many* areas of Environmental Health inspection to eliminate duplication and to improve services.

I.S.U. HEALTH AND SAFETY PROGRAMS

But with all of the current recognition of the need

[1]Presented at 96th Annual Meeting of the American Public Health, Detroit, Michigan, November 10-15, 1968.

to improve our environment by government, industry, and interested citizens and the many millions of dollars being allocated for a variety of environmental health activities, including air pollution, water pollution, rodent control, hospital sanitation, etc., we are still woefully lacking the necessary manpower to implement these programs. Eighteen thousand new Environmental Health professionals are needed by 1970, when we have in the recent past produced only about 150 new Environmental Health graduates. Many additional thousands of professionals are needed in Safety and Health Education. Therefore, to assist in the remedy of this dire personnel shortage, we have established in our Department of Health and Safety as of 1966, new undergraduate non-teaching programs in Environmental Health, Safety Management, and Community Health Education. Within two years we have grown to 75 undergraduate majors including 35 in Environmental Health. We have 42 in our graduate Health Education Program and the program of studies leading to the Master's degree in environmental health is already being formulated.

ENVIRONMENTAL HEALTH PROGRAM OF STUDIES

Preparation for the field in the past has been heavily science-oriented. In fact, the demands of science were so great that the individual who graduated with a degree in Environmental Health was really, in many instances, the graduate of a general science, biology, or chemistry curriculum. The value of an extensive scientific background is debatable since, if the institution is to be accredited by the North Central Association of Colleges and Universities or the comparable accrediting body, depending on the geographic location of the school, to confer degrees in particular programs of studies, students must *(a)* usually take a minimum of 50 semester hours of general education; *(b)* be permitted to take a minor or desired electives; and *(c)* complete an extensive major. *Shall we,* as in the past, insist on extensive general scientific backgrounds at the expense of the humanities and the social and behavioral sciences? *Or,* should we reduce the concentration actually needed in Environmental Health Science until our students in fact receive only a token amount of credits in the most vitally needed phase of their professional preparation?

Our students are seeking the answer to how and why man reacts as he does and where the health professions fit into the value system established by our communities. To satisfy the needs of our students and to prepare a better qualified Environmental Health Specialist, we have established the following program of studies.

ENVIRONMENTAL AND COMMUNITY HEALTH CURRICULUM

Major Field Core, Including Professional Field Practice Education

Concentration of Basic Courses:

Course	Title	Hours
HLSF 221:	Community Health	2 Sem. Hrs.
HLSF 326:	Accident and Disaster Control	2 Sem. Hrs.
HLSF 312:	Intro. to Environmental Health	4 Sem. Hrs.
HLSF 324:	Probs. of Environmental Pollution	3 Sem. Hrs.
HLSF 329:	Envmntl Hlth Lab. Practice I	2 Sem. Hrs.
HLSF 412:	Appl. Prin. of Envmntl Hlth	4 Sem. Hrs.
HLSF 414:	Public Health and Epidemiology	3 Sem. Hrs.
HLSF 417:	Cmty Hlth and Sfty Resources	2 Sem. Hrs.
HLSF 429:	Envmtl Hlth Lab. Practice II	2 Sem. Hrs.
		24 Sem. Hrs.

Professional Courses:

Course	Title	Hours
HLSF 321:	Envmntl Hlth Fld Practice Observ. & Reptng	2 Sem. Hrs.
HLSF 392:	Educ. Meth. for Health and Safety	2 Sem. Hrs.
HLSF 426:	Health and Safety Supervision	2 Sem. Hrs.
HLSF 421:	Prof. Fld. Pract. Internship in Envmtl Hlth	6 Sem. Hrs.
		12 Sem. Hrs.

Cognate Foundations in Chemistry and Biological Sciences

Course	Title	Hours
CHEM 100:	Inorganic Chem. (Non-Chem Majors)	3 Sem. Hrs.
CHEM 150:	Organic Chem. (Non-Chem Majors)	3 Sem. Hrs.
LSCI 112S:	Intro. to the Life Sciences	3 Sem. Hrs.
LSCI 374:	Introductory Bacteriology	3 Sem. Hrs.
LSCI 474:	Applied Bacteriology	4 Sem. Hrs.
		16 Sem. Hrs.

Total Hours Required for the Major 52 Sem. Hrs.

The strong points of the program include directed study in basic biological and chemical sciences followed by specific courses which apply to Environmental Health and Laboratory Practice. Comprehensive studies of the total environment employ the systems concept. Mind stretching courses in Community Health, Public Health, and Health Education avoid a narrow approach to Environmental Health problems.

Professional field practice education during the course of studies enables integration of the art and science of the profession taught at the University with the practical existing problems in the community.

Recognition of the need for articulating professional study programs, provided in an academic atmosphere on campus, with supervised work experiences gained through the medium of related off-campus employment, is a concept that is emerging slowly but steadily in higher education. This practice has the

potential of becoming one of the more significant developments related to the preparation of individuals to serve in professional-vocational fields.

Actually, the principle is not new, since on-the-job experiences of an introductory nature have been provided by many employers for quite some time. However, in-service programs designed to enable adjustment to a particular vocation seldom prove to be sufficient unless an unusual amount of time is devoted to such matters. The latter was frequently felt to be unwarranted and employers, therefore, continue to be hopeful that a more acceptable means of solving the dilemma through pre-service preparation might be devised. In fact, many employers are highly interested in cooperative education programs as one way of identifying and recruiting effectively prepared professionals.

Student teaching programs and internships promoted by Schools and Departments of Education have contributed immeasurably to professional development of school personnel. Internships required of medical and allied practitioners have likewise proven to be of unquestionable value. Furthermore, numerous professional societies interested in matters of program accreditation either require or recommend the equivalent of professional field practice education as part of preparatory programs of study.

The Department of Health and Safety conceives that professional field practice education should serve the following functions regarding assistance to those students who elect one of the professional-vocational programs offered by the department: *(a)* provide practical introductory work experiences under appropriate supervision by experienced and competent personnel, *(b)* enable refinement of basic skills essential to practice of the professional field involved, *(c)* assure familiarity with the activities of practicing professionals in a particular vocational field, *(d)* allow for participation in the planning of various work activities, *(e)* fulfill certain individual needs that cannot be readily met in the usual classroom-laboratory environment, *(f)* provide essential contacts which are usually helpful to one entering a chosen profession, and *(g)* assist with the development of an applied professional philosophy.

Courses included in the Professional Field Practice area are:

HLSF 320, 321, or 322: Field Practice Observation and Reporting ________ 2 Sem. Hr.
HLSF 392: Education Methods for Health and Safety ____________________ 2 Sem. Hr.
HLSF 426: Health and Safety Supervision ____ 2 Sem. Hr.
HLSF 420, 421, or 422: Professional Field Practice Internship ______________ 6 Sem. Hr.

By staggering the practicum courses and field traineeships, all persons involved in the educational process: students, practitioners, and teachers, have an opportunity to grow professionally and keep up to date with the field.

The Health Departments of Oakland County, Michigan; Wayne County Michigan; Summit County, Ohio, Montgomery County, Ohio; Steuben County, Indiana; Vigo County, Indiana; Hendrix County, Indiana; Will County, Illinois; Lake County, Illinois; and the State of Illinois are to be congratulated for taking the lead in providing many positions for supervised, comprehensive work study experiences for I.S.U. Environmental Health majors on a continuing basis. Adequate wages are being paid to the students to provide enough money for room, board, and some left over for the following year's tuition and books. We urge other states to establish like cooperative agreements with universities.

THE PUBLIC HEALTH SERVICE'S REPORT TO THE NATIONAL CONFERENCE ON INTERSTATE MILK SHIPMENTS[1]

Harold E. Thompson, Jr.

Division of Food, Milk and Interstate Travel Sanitation
Bureau of Community Environmental Management
Environmental Control Administration
Consumer Protection and Environmental Health Service
Department of Health, Education and Welfare
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Progress Report—1967-68

Since the last Conference, we have continued to give attention to standardizing the rating procedures of the Public Health Service (PHS) regional personnel to insure a uniform approach. Headquarters personnel have worked with 10 regional milk consultants to assure a uniform approach to the Pasteurized Milk Ordinance and the correct application of its provisions.

In order to fulfill the responsibility of standardizing personnel. PHS regional milk consultants have certified or re-certified 84 state milk sanitation rating officers in 1967-68. This is over 50% of the 150 certified state milk sanitation rating officers currently listed in the quarterly publication, *Sanitation Compliance and Enforcement Ratings of Interstate Milk Shippers.* These 150 individuals, who are located in 43 states, bear the major responsibility for assuring that certified supplies are under adequate and full-time supervision and for conducting accurate and dependable milk sanitation ratings.

Since our last report, all designated state laboratory survey officers have improved their proficiency in laboratory approval programs through uniform use of PHS Publication No. 999-FP-3, entitled *Evaluation of Milk Laboratories,* which was published in 1965. Although some of the material in this publication has been updated during the past year through transmittal of a laboratory *Newsletter* to all survey officers, the administrative laboratory approval program requirements pertaining to sampling, laboratory surveillance, and split samples have not changed. We have been encouraged by the results of this laboratory certification program which stimulates local authorities as well as the dairy industry to improve facilities and laboratory procedures.

The 1969 edition of PHS publication 1925, which lists *Milk Laboratories Approved by Federal and State Agencies,* is now being distributed to participating state, municipal, industry, and educational personnel. This edition identifies 74 state designated milk laboratory survey officers in 50 states, the District of Columbia, and Puerto Rico, 55 of which have been certified by Public Health Service milk laboratory certification personnel. As of January 1969, the names of all certified state milk laboratory survey officers have been published in the *Sanitation Compliance and Enforcement Ratings of Interstate Milk Shippers;* this list is revised quarterly, predicated on evaluation of each milk laboratory survey officer's work by representatives of the PHS milk laboratory certification program. As of January 1, 1969, 1928 analysts in 885 laboratories were approved by state and federal laboratory survey officers for the examination of milk and milk products.

Most laboratory survey forms were revised and printed earlier this year to be in accordance with the requirements of the 12th edition of *Standard Methods for the Examination of Dairy Products,* the 10th edition of the *Methods of Analysis of the Association of Analytical Chemists,* and the *1965 PHS Pasteurized Milk Ordinance.* These survey forms have been distributed to state milk laboratory survey officers. The format of these forms has been revised to assist laboratory survey officers during surveillance of laboratory procedures.

During 1967 and 1968, 63 surveys were made of 42 state health and 19 state agriculture department central milk laboratories and the health department central milk laboratories in Puerto Rico and the Virgin Islands. PHS milk laboratory survey officers have made joint surveys of 30 local laboratories with 29 different state designated milk laboratory survey officers. All Interstate Milk Shipment states, except one, are currently in substantial compliance with PHS criteria for laboratory approval and split milk sample programs.

Although continued progress has occurred in the surveillance and standardization of sampling procedures, a number of states are no longer actively participating in this activity. Demonstrated progress has occurred in many states where milk rating officers have assumed full responsibility for completion of sampling surveys and standardization of raw milk sampling precedures through state training courses. As of January 1, 1969 sampling surveys apparently are not being conducted in 11% of the states, are in

[1]Presented at the Twelfth National Conference On Interstate Milk Shipments, Denver, Colorado, May 26, 1969.

arrears for the past two years in 32%, or only token participation exists in 18%. Cumulatively, 44% of 47 shipper states and D.C. do not appear to be substantially supporting the agreements of this conference pertaining to sampling.

PHS training sessions conducted in support of the Interstate Milk Shipments Program since the last conference included:

1. Fifteen sessions of "Milk Pasteurization Controls and Tests"—437 trainees;
2. Three sessions on the "Grade A Pasteurized Milk and Milk Products"—119 trainees;
3. Four sessions on "Laboratory Analysis of Milk and Milk Products"—71 trainees;
4. Two sessions on the "Direct Microscopic Somatic Cell Counts"—64 trainees;
5. Two sessions of the "State Milk Laboratory Survey Officers Work-Shop"—32 trainees;
6. One session on "Pesticide Residue Analysis"—13 trainees.
7. One Milk and Food Workshop—84 trainees.

The two-year totals to date are 28 presentations with an attendance of 820.

Of course, during this period there were 18 regional seminars held for certified state milk sanitation rating officers and milk laboratory survey officers.

Since our last report, regional milk consultants made 303 check ratings of listed shippers. As a result of these check ratings, it was necessary to request state certifying agencies to re-survey or reinspect 86 shippers because conditions indicated the compliance rating was significantly lower than the listed rating. This is not to be considered a reflection on the state certifying agency concerned, but it does point to the necessity of continued vigilance and points out the consequences of the neglect of any one phase of the program. It should be remembered that check ratings are not for standardization of procedures used by rating officers, but related to supervision. Check ratings are used to determine the validity of the sanitation compliance status of the shipper's supply between official ratings. In addition to the conduct of check ratings, regional milk consultants have evaluated the inspection and rating work of seven state milk sanitation programs.

At this point I would like to comment about our quarterly publication *Sanitation Compliance and Enforcement Ratings of Interstate Milk Shippers.* This publication has grown from a very nominal beginning to a circulation of over 4,000 and is used by many agencies in the procurement of milk.

It contains the names of approximately 1,600 milk shippers, 60 Grade A milk drying plants, and 113 plants manufacturing single-service containers. In addition, it contains the "rules" for listing of such plants, the national uniform coding system, and the names of certified state milk sanitation rating officers. We are quite proud of this publication and the important part it plays in the movement of milk and milk products from one state or community to another. The prompt and efficient submission of IMS reports by state milk sanitation rating officers enhances our efforts in publishing this important document.

For some time now, the Veteran's Administration has used this list to purchase milk and milk products for their hospitals. Only milk sources approved or certified under this program are served on interstate carriers. Public Health Service hospitals and Indian schools and hospitals purchasing milk under federal specifications, utilize this program and the list. Recently, at a conference with the Department of Defense, we were informed that this department had placed in their regulations a requirement that military establishments would use this list in procuring milk and milk products.

GRADE A DRY MILK ORDINANCE

The Conference (several meetings back) requested the PHS to revise and update its sanitary standards for manufacture of Grade A dry milk products and to promulgate standards for Grade A condensed milk. The Service agreed to this request. We have brought to the Conference the completed Supplement 1 to the 1965 Grade A Pasteurized Milk Ordinance, which covers Grade A condensed and dry milk products used in Grade A pasteurized milk products.

It is our hope that the Conference will see fit to adopt these standards at this meeting so that we will have comparable standards for use with the 1965 PMO that we had for the 1953 Milk Ordinance and Code. Several drafts of this Ordinance have been widely reviewed and the majority of your people received the final draft with a request for comments. Relatively few comments were received and as a result, except for a few minor technical changes and the necessary editorial changes which are always needed in this type of document, this Ordinance is very near the same as the final draft. As it is a revision of the 1958 Dry Milk Supplement, it applies only to dry milk products used in pasteurized milk products. It contains no reference to the consumer package.

The administrative and technical requirements of this Ordinance have been developed as a supplement to the Grade "A" Pasteurized Milk Ordinance, and, as such, the contents related specifically to the physical facilities, operation, and maintenance of a drying plant. The standards for the raw milk and milk products which are used in the manufacture of these dry milk products are regulated by the 1965 Grade "A" Pasteurized Milk Ordinance were followed close-

ly with deviations, for the most part, relating to practices which are specific to condensed and dry milk processing. Grades other than Grade A are not specified since these standards are intended to cover the production of dry milk products which can be accepted by state and local regulatory agencies for use in the preparation of Grade A pasteurized milk products.

CERTIFICATION OF PLANTS MANUFACTURING SINGLE-SERVICE CONTAINERS

In 1965 the National Conference on Interstate Milk Shipments (NCIMS) asked the PHS to prepare sanitation guidelines for the fabrication of single-service containers for milk and milk products and ultimately publish quarterly lists of acceptable single-service container manufacturing plants. Following a series of meetings with the NCIMS committee on Standards For Single-Service Containers and Closures, the PHS developed and published a brochure entitled *Fabrication of Single-Service Containers and Closures for Milk and Milk Products.* This will be used as a guide for sanitation standards to be used in the inspection of plants.

The Grade "A" Pasteurized Milk Ordinance provides, upon adoption, that the manufacture, packing, transportation, and handling of single-service containers and closures must comply with certain sanitation requirements and bacteriological standards.

Item llp of the PMO specifies that the Milk Control Authority or an agency designated by him shall inspect the manufacturers' facilities, perform bacteriological tests, and make other examinations to determine the sanitary quality of the single-service articles expected to be in contact with milk and milk products. In order to facilitate these procedures, a Guide for sanitation standards and an inspection form has been developed. The Guide calls attention to area, equipment, materials, and practices which may cause contamination of the single-service containers and closures during manufacture, packing, transportation, and handling. The Inspection Form has been designed to follow these guidelines.

The requirements of this Guide applies to all blank fabricators, closure manufacturers, plastic laminators, sheet formers, blow molders, vacuum formers, extruders, injection molders, and preformers, and include the installation and maintenance of equipment used in compounding materials for the fabrication products, handling, and storage of single-service containers and closures.

During this past biennium, administrative procedures for the certification of plants manufacturing single-service containers and closures for milk and milk products were developed by our headquarters staff in close liaison with the NCIMS committee on Single-Service Containers and Closures. These procedures provide the necessary guidelines for the inspection, certification, and listing of single-service manufacturing plants in the quarterly publication, *Sanitation Compliance and Enforcement Ratings of Interstate Milk Shippers.*

To provide for uniform inspection and to minimize the problems of multiple inspections by many jurisdictions, it is recommended that inspections for certification be carried out by: *(a)* state milk sanitation ratings officers, or *(b)* recognized competencies in the commercial field.

Provisions have been made for the state milk sanitation rating officers to accept industry inspection for certification in lieu of an actual survey by the state rating officers. Commercial enterprises have developed and maintained excellent routine sanitation programs within the single-service container industry and are able to supply, upon request, complete sanitation and laboratory data pertaining to each plant in their program.

To encourage the promotion of this certification program, four special 3-day courses by a grant to the University of Syracuse from the PHS were held for regulatory personnel responsible for the inspection of single-service container fabrication plants. The courses covered the fabrication of all types of single-service containers for milk and milk products. We anticipate that additional courses will be held in the near future that will be devoted entirely to the inspection of single-service container manufacturing plants and the submission of data for publication.

One of the charges from the NCIMS to the PHS was that consideration should be given to the allocation of points to the items of sanitation as they appear on the inspection sheet. This charge has been taken under consideration, but it is the opinion of those on the committee that additional experience and study should be given to this particular problem before points are assigned to the items of sanitation. We are recommending that the NCIMS committee be held on "Recall Status" for the next two years pending further study in this area and to help solve any other problems that may arise.

In accordance with the recommendations of the NCIMS, the names of manufacturers of single-service containers for milk and milk products who have been certified by state milk sanitation rating authorities as being in satisfactory compliance with the requirements found in Fabrication of Single-Service Containers and Closures for Milk and Milk Products, PHS publication 1465, are now being listed in the quarterly publication, *Sanitation Compliance and Enforcement Ratings of Interstate Milk Shippers.*

This list, first published January 1, 1969, contained the names of 95 fabricating plants. This listing of certified single-service manufacturing plants includes the names of only those plants reported to the PHS as having been certified by state milk sanitation rating officers. At this point, the fact that a plant is not listed does not necessarily mean it does not comply with the requirements. We feel unlisted plants should not have their products barred from market until adequate time has been given to obtain certification. As this new certification program progresses, each succeeding list should contain the names of additional plants. States and communities desiring information regarding unlisted plants may obtain such information through the PHS regional offices.

As I indicated, we still need experience in this area of listing certified single-service manufacturing plants. One problem concerns a coding or marking system that will enable the sanitarian to determine a plant's location when more than one plant is listed under the same name. Steps are being taken now to supply this information to the states and eventually the coding or marking system used by single-service fabricating plants will appear on the listing.

We are, needless to say, well pleased with the cooperative spirit exhibited by the NCIMS committee and the help extended the PHS in developing this phase of the Interstate Milk Shippers certification.

NEWS AND EVENTS

OBJECTIVES AND PURPOSES OF UNIVERSITY OF GUELPH DEPARTMENT OF FOOD SCIENCE[1] BY A. N. MYHR, ASSOCIATE PROFESSOR

The Food Industry in Canada has undergone an almost unbelievable rate of economic growth in recent times. A survey has shown that the Canadian Food Processing Industry is currently marketing goods valued at over 7.3 billion dollars annually. To achieve this, the industry has developed highly sophisticated and complex processing systems. Industry executives recognize that for further rapid development and refinements in food processing and for the whole area of quality control and new product development, they are going to require graduates who are not only trained in such fields as Chemistry, Microbiology, Chemical Engineering and so on, but also in specialized food oriented courses such as microbiology and chemistry of foods, industrial microbiology, food engineering, food plant sanitation, food processing and quality control, and food evaluation.

With this in mind, the Department of Food Science at The University of Guelph has developed a program of studies designed to prepare students for the many exciting and challenging opportunities that exist in the food industry. The program has been made sufficiently flexible through the elective system to appeal to the varying interests of students who wish to prepare themselves for careers in the food industry, the many allied industries or government and other agencies involved in such things as regulations, marketing and public health aspects of food production and control. Thus, through an appropriate selection of elective courses, the student may specialize in the pure science aspects of the food industry or the technological and engineering phases or the business management side of the industry.

A large number of inquiries are being received from outside by the department and by this fall, at the start of the second semester, there will be an estimated enrollment of between 30 to 40 students in semesters 1 and 2. While the Department is well satisfied with this level of enrollment at the outset of the new program, it also recognizes that many more students will be required to meet the yearly demand by industry for food science graduates. Based on a cross Canada survey of 721 food industries made in 1967, it was predicted that industry would require 75 food science graduates per year. Many other graduates will find good employment opportunities with government services, allied industries and in teaching, and probably over 20% of the baccalaureates will continue on for post-graduate studies. The present enrolment figures therefore, would have to be greatly expanded to meet the needs for graduates of industry and other agencies.

The Food Science Department is working diligent-

ly at establishing a close working relationship with food companies to maintain close surveillance of important practical problems associated with the processing and preservation of foods; to assess research requirements; to gain greater industry financial support for graduate research projects and to maintain a close watch on the best job opportunities for placement of graduates.

The Food Science Department is engaging in extensive building renovations to provide a bright cheerful and functional environment for teaching and research in food science. Completed to date has been a food microbiology laboratory ,a sensory analysis laboratory for product evaluation, and a food chemistry research laboratory equipped with the most modern scientific instruments for rheological and chemical analyses. Pilot size food processing units are being installed and further renovations to teaching laboratories and classrooms are proceeding as rapidly as possible. We invite all interested students to tour the Department this fall to see the facilities and view the educational food science exhibits.

Faculty members in the Department will be most pleased to meet personally with students interested in obtaining detailed information about the Food Science program and to discuss the many career opportunities open to graduates.

[1]Reprinted from News and Views, Ontario Milk and Food Sanitarians Association

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BABSON BROS. LAUNCHES NEW MILK PROMOTION

"Milk—Good with Anything" stickers will be showing up on lots of mail in the weeks and months ahead. At least, that's the objective of a new promotion by Babson Bros. Co. of Oak Brook, Illinois.

Babson has prepared a brochure promoting milk as the nutritious food that is *downright delicious any time with anything.* The unique part of the promotion is the third page which features 24 individual stickers, each showing the versatility of milk.

It is hoped that everyone who gets the brochure will spread the word by putting these stickers on correspondence they send out. More than 100,000 brochures have been distributed to dairymen as an insert in Babson's new publication for dairymen, Dairy Illustrated. Extra copies of the brochure are also available from Surge dealers or can be ordered directly from Babson Bros. Co., 2100 S. York Road, Oak Brook, Illinois 60521.

MODERN BREAD IN INDIA

Six bakeries in India now producing "modern bread," a fortified "complete" food designed to improve the diet of developing nation populations, have spurred an important development in India's fight against hunger and malnutrition.

Dr. Aaron M. Altschul, Special Assistant for International Nutrition Improvement of the USDA's Foreign Agricultural Service said recently that with an ultimate goal of 100 million loaves annually, modern bread production has spurred other Indian bread producers to seek ways of fortifying their own products. Modern bread is fortified with vitamins, minerals, and lysine which is a biologically important amino acid containing protein.

IMPROVING QUALITY STANDARDS FOR MANUFACTURING MILK

BY HERBERT L. FOREST,

Director, Dairy Division, C & MS, USDA

Better quality butter, cheese, nonfat dry milk, and other dairy products, with better keeping ability, may be on the way.

A step in that direction is a revised set of recommended standards for manufacturing milk—the raw material for such dairy products—being proposed by the U. S. Department of Agriculture.

The better the raw material is the better the final product, assuming good manufacturing processes. Hence there is the proposal to help States, which have the primary responsibility, raise the quality of manufacturing milk throughout the Nation.

The revised standards, proposed for State adoption and enforcement, are the result of a cooperative effort between USDA's Consumer and Marketing Service and two agencies of the U. S. Department of Health, Education, and Welfare—the Public Health Service and the Food and Drug Administration.

These standards are modeled closely after those developed in a continuing USDA-State-industry project stretching over many years. The new proposal, however, is intended to unify the earlier standards issued in 1963, with proposals of other government agencies and thus help eliminate overlapping, duplicate, and conflicting requirements.

Major additions to, or changes in, the earlier standards included in the new proposal are:

Lowering the bacterial limit for "acceptable quality" manufacturing milk. The bacteria limit in the proposed standards would be retained for three years after adoption by a State. Then the limit would be lowered to one-third of that level. It is expected that processing plant fieldmen and State extension workers will help farmers improve sanitation to the extent necessary to meet this requirement.

Requiring more stringent regulations for farm water supplies, which must be enforced by the State.

Requiring the abnormal milk test program prescribed by the National Mastitis Council.

Retained in the newly proposed standards are provisions of the 1963 standards which provided for:

Farm inspection and certification, including careful checks on the health of the dairy herd, milking facilities and procedures, sanitation of utensils and equipment, and water supply.

Inspection of incoming raw milk at processing plants for odor, physical appearance, bacteria, and sediment.

Plant licensing, requiring adequate procedures and facilities for sanitation before issuance of a license by the regulating State agency.

USDA officials emphasize that the proposed standards are for voluntary adoption by States which have no such regulations in effect or which have less stringent regulations. Some States, they point out, already have equal or better regulatory laws in effect. And many dairy farmers and milk processing plants already meet or exceed the requirements of the recommended standards.

Booklets of date recipes printed in Japanese were also prepared and widely distributed.

An evaluation of the promotion activity showed that the program had created some interest in the use of dates, but additional effort was needed to develop large-volume sales. More date sales to Japan probably could be realized, the study indicated, by shifting to retail outlets where the Japanese could get acquainted with dates.

The olive industry was faced with changing an age-old process.

Juicy, black, ripe olives have always been stored in wooden barrels in salt brine to fully develop their distinctive flavor.

Recently this process was dealt a severe blow. A California water pollution control board decreed that the brine solution, which was dumped into streams when the storage cycle was completed, was polluting the water and had to be stopped.

The Olive Administrative Committee engaged a research team from the University of California to develop a new process for storing ripe olives without using salt solutions.

After two years of research, the team discovered that a benzoic acid solution could be used quite successfully and preliminary tests showed that the benzoic acid could later be removed from the water through the use of filters.

Now in its third year, the research is expanding to a commercial scale. Research also will continue to determine if there is microbial resistance to benzoic acids in storage solutions under air-tight conditions. Further research will be done to find other acceptable preservatives for olives in salt-free storage solutions.

State officials, as well as producers, processors, and anyone interested, now have opportunity to comment on the proposed standards. The extent to which they are adopted and enforced by the States will determine the effect they will have in assuring a continuously wholesome and high-quality supply of dairy products for American consumers. It could be a long step forward.

COURSE IN FOOD MICROBIOLOGY

The Training Institute of the Food and Drug Administration (formerly Environmental Sanitation Training, NCUIH) is offering a course in Food Microbiology in Cincinnati, Ohio on April 6-17, 1970.

The course presents advanced technical information of special interest to laboratory and supervisory personnel concerned with the bacteriological examination of food. It is designed to enable the trainee to organize two types of programs: surveillance of the sanitary quality of foods with emphasis on prevention of disease outbreaks, and examination of foods implicated in foodborne disease episodes. Instruction prepares the trainee to perform selected laboratory analyses and to interpret the results of such analyses.

In addition to a lecture on each specific group of organisms studied in the laboratory some of the other topics presented include: Sampling procedures and sample preparation; Staphyloccal enterotoxins; Time-temperature relationships in food processing; Morphological, cultural, and toxigenic relationships among *C. botulinum* types, and Naturally occuring poisons of marine animals and ways of distinguishing them from botulinal toxin. The laboratory work includes exercises on: Standard Plate Count, Coliform bacteria and enteropathogenic *E. coli,* Salmonellae, Shigellae, Staphylococci, *C.* perfringens, *C. botulinum* and animal testing for toxin, Fecal streptococci, and Examination of suspect food sample. Emphasis in all of the laboratory work is placed on the detection, isolation and enumeration of these organisms from foods.

All applicants for this course must present evidence of immunization against *Clostridium botulinum* Types A, B, and E. The immunization program requires 12 weeks, and the entire series must be completed prior to the course. There are no registration or course fees.

Inquiries should be addressed to: Robert B. Carson, Training Specialist, Food & Drug Training Institute, 1090 Tusculum Avenue, Cincinnati, Ohio 45226.

PSYCHOLOGIST TALKS TO FOOD PLANT MANAGEMENT CLASS AT TEXAS A&M

Dr. William Smith, head of the Psychology Department at Texas A&M was a recent speaker for the Food Plant Management Class. Dr. Smith's presentation was concerned with "Motivation of the Employee." His emphasis was placed upon the management of a busines to ensure that the employee receives all of the factors which contribute to motivation of the individual.

From left to right: Al Diorio, Robert Aragona, David Klavens, and Dr. William Smith.

DEPARTMENT OF DAIRY TECHNOLOGY THE OHIO STATE UNIVERSITY COLUMBUS, OHIO 43210

SCHEDULED EVENTS FOR 1970

February 13—Centennial Events Program: "Focus on the Future of the Dairy Foods Industry." A one-half day program with an analysis of the role of the Dairy Foods Industry from 1970—2000 by leading industrial executives and representatives of the Department of Dairy Technology.

February 17—Symposiums: "Food Proteins for a Hungry World". Co-sponsored by the Ohio State University Graduate School. The program will present an analysis and evaluation of two principal areas (1) Domestic and International Food Protein Programs, and (2) Protein Resources and Utilization by speakers from Agencies, Foundations, Industry, and educational institutions.

February 18 & 19—The 37th Annual Dairy Food Science and Management Conference. The program will deal with timely issues in the areas of Milk Supply, Food Development and Utilization, Engineering and Processing, Manufactured Products, Laboratory Control, and Management and Marketing.

March 19-23—The Annual Mid-West Workshop in Sanitary Science. In-depth information will be provided in five areas of Sanitary Science (1) Milk, (2) Other Foods, (3) Solid Waste Disposal, (4) Community Sanitation (private water supplies and sewage disposal), and (5) General Sanitation (camps and swimming pools). Sessions in the five areas will last from two to five days depending on the area. Preregistration is required.

OUTER SPACE FOODSERVICE MARKET OFFICIALLY RECOGNIZED

The foodservice industry has welcomed a new market into its fold-the Moon. The National Restaurant Association formally recognized Restaurant Intrepid via the following message sent by wire to the Apollo 12 crew shortly after the Intrepid set up operations in the Ocean of Storms.

COMMANDERS CHARLES CONRAD,
RICHARD GORDON, ALAN BEAN
"INTREPID" RESTAURANT
OCEAN OF STORMS
MOON
C/O NASA MANNED SPACE CENTER,

Dear Sirs:

In recognition of successful opening of another American eating establishment on the moon, we hereby extend a membership for your restaurant, the only food-serving operation in 240,000 miles, in the Lunar Division of the National Restaurant Association. On behalf of thousands of restaurants and member government and military food operations on earth, we welcome you and admire your foresight in site selection. Your National Restaurant Association seal and other membership materials will be forwarded promptly.

Sincerely,
ROBERT E. HEILMAN
President, National Restaurant Association
1530 North Lake Shore Drive
Chicago, Illinois

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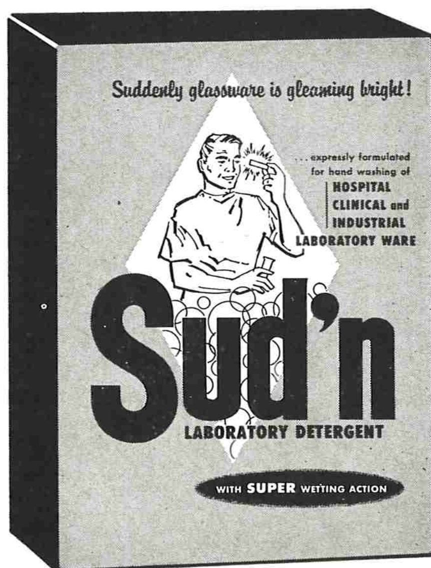

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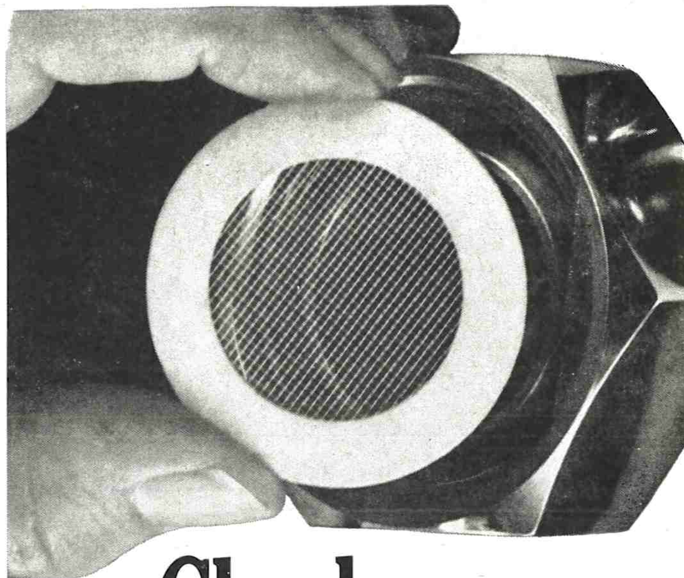

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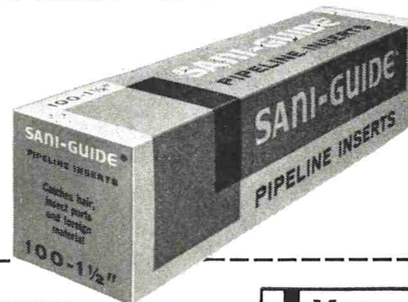

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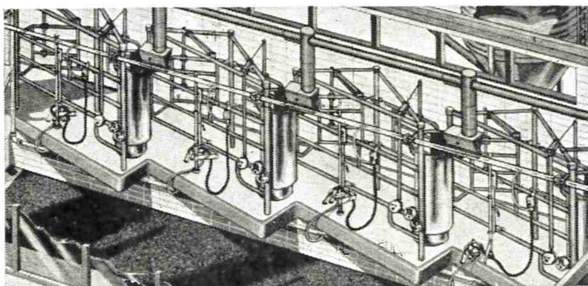

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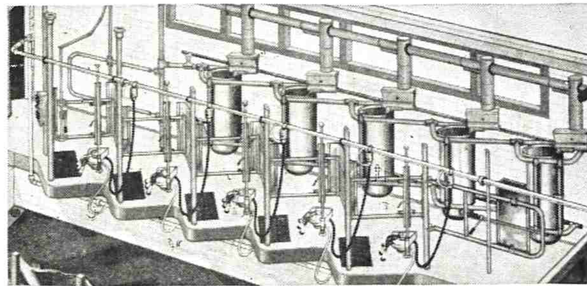

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