SYSTEMATIC TESTING OF INHIBITORY SUBSTANCES IN MILK*

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A portion of a 30 ml sample of milk is tested for general inhibition by a starter activity test and if positive results are obtained the remainder of the sample is subjected to a disc assay test for antibiotics and specific chemical test for quaternary ammonium compounds and sulfonamides. All of these tests are readily adaptable to a systematic laboratory procedure. Their significance and limitations are discussed.

DURING the past few years there has been a great deal of discussion concerning current practices in the dairy field whereby milk might become contaminated with starter inhibitory substances. These practices include the treatment of dairy cattle for mastitis with antibiotics, such as penicillin, and with sulfa drugs and also the sanitization of dairy equipment with quaternary ammonium compounds. It has been pointed out by a number of investigators that these compounds may carry over into the milk and if the concentrations are large enough these milks cannot be properly made into such fermented dairy products as cheese, buttermilk, and sour cream. Inhibition of lactic starter organisms by milk containing these compounds has been conclusively shown in the laboratory. [7,8,9]

There is at the present time no evidence to indicate the importance of this problem in the field. Over the past year a survey has been undertaken to define the extent of the problem in the New York State area by this laboratory. During this study a definite need was shown to exist for a schematic procedure whereby it would be relatively simple to establish the presence, nature, and concentration of inhibitory substances on a large number of milks.

This paper is concerned with such a suggested procedure, and lists a tested flowsheet of methods for total inhibitory substances, antibiotics, sulfa drugs, and quaternary ammonium compounds along with observations on the characteristics and effectiveness of the individual tests. A number of the methods outlined are well established either in the dairy or medical field and needed only slight changes to fit into the systematic pattern of testing. The procedures which were used in the following order for the survey are presented in this report.

Preparation of Milk Samples

The total quantity of milk required for all the tests concerned with the systematic analysis for inhibitory substances is 13 ml. It is preferable, however, to have at least a 30 ml milk sample for duplicates. In analyzing total inhibitory substances and antibiotics, the milk, raw or pasteurized, is heated to 180°F for 5 minutes, cooled to 92°F if it is to be tested immediately or otherwise cooled to 40°F. For sulfa drugs and quaternary ammonium compounds the test is applied directly to raw or pasteurized milk.

Total Inhibitory Substances in Milk

Principle: This method measures the acid increase by starter organisms in an unknown milk compared to the acid increase of a known control milk or milk powder under comparable incubation and testing conditions. It is commonly referred to in the fermented milk products field as the 'starter activity test' and in this study it was similar in principle to the methods suggested by Horral and Elliker[5] and by Krienke[10].

Procedure: Ten ml of milk are heated to 180°F for 5 minutes in sterile test tubes and cooled to 92° F. Then 1 ml of active commercial starter which was first diluted 1:1 with cold highly heated milk for easier pipetting (180°F 30 min) is added. The tube of inoculated milk is shaken well, and 5 ml is withdrawn for the initial alkali titration. The tube with the remaining 6 ml is placed in a water bath at 92°F for 4 hours after which all of its contents are titrated directly in the tube, using N/20 NaOH with 4 drops of phenolphthalein as the indicator. In testing a large number of tubes, time intervals must be so set between each tube that the incubation period is always 4 hours. The first titration of the 5 ml portion is recorded as *Initial value*. Four hours later the titration of the 6 ml or the second titration is recorded as *Final value*.

Mr. G. J. Silverman is a graduate assistant at Cornell University. He was born in New York City and after serving in the United States Armed Forces, was accepted at Cornell University, receiving a B.S. and M.S. in Dairy Industry and hopes to eventually obtain a Ph.D. from this institution.

*The authors wish to express their appreciation to A. H. Robertson, Department of Agriculture & Markets, New York, for his interest and advice in regard to this problem and to C. W. Christensen, Difco Co., Detroit, Mich., for his technical assistance relative to the disc assay method.

Along with the samples of milk of unknown history, samples of skimmilk (10 percent T.S.) from reconstituted spray powder known to be free of inhibitory substances, are heated to 180°F for 5 min. and tested in identical fashion. These controls always were incubated as the first and last samples of the group being tested. A composite milk sample from a mixed herd known not to have been treated with antibiotics or other drugs may be used as a supplementary control.

Calculations and Interpretation:

$$\text{Final value} - \text{Initial value} = \text{Acidity increase}$$

$$\frac{\text{Acidity increase of test milk}}{\text{Acidity increase of control milk}} \times 100 = \text{Total activity value expressed as percent}$$

Total activity values of 100 percent or higher are not inhibitory. If value is below 80 percent, then definite inhibitory characteristics are predicted and the milk was submitted to more specific tests for inhibitory substances listed in the following pages.

Disc Assay Method for Penicillin and other Antibiotics in Milk

Principle: The disc assay method for penicillin determination is an evolution of the cup assay methods as developed by Vincent and Vincent[14], Foster and Woodruff[2,3], Schmidt and Moyer[13] and by Loo *et al.*[11] This type of method is much simpler in operation and in some respects is considered more accurate than cup assay methods. Application of the penicillin disc assay to milk on a practical basis has been under development at Difco Laboratories, and much of the detail of their method is presented in this paper.

The method is based upon the observation that if a paper disc containing penicillin is placed on a hardened agar layer previously seeded with bacteria sensitive to pencillin or other antibiotics, the antibiotics will diffuse from the disc radially and where inhibition occurs a circular clear zone is formed, indicating no bacterial growth, whereas the rest of the agar layer in the petri dish is of turbid nature. The size of the diameter of the clear zone is directly related to the concentration of antibiotics up to a certain level.

Procedure: Six drops of standardized *B. subtilis*** spore suspension are added to 12 ml of sterile melted whey agar at 50°C. After shaking, the seeded agar is poured into a standard sterile petri dish and allowed to solidify as a level layer.

A small quantity of milk, 0.017 ml, is placed on ¼" diameter filter paper discs** by means of a micro pipette, and the disc, held by tweezers, is saturated with milk but without any excess. Good results have also been obtained in this laboratory by saturating ½" diameter paper discs simply by dipping an edge in the milk until the the entire disc is impregnated by capillary action thus eliminating the need of pipetting. The smaller ¼" disc also may be used with this latter technique but more care is needed to prevent surplus milk from being retained on the disc. After the disc absorbs all of the liquid it is quickly and firmly placed on the surface of the solid agar, and as more discs are placed on the plate they are arranged in a circle on the petri dish. About 8 to 12 discs depending upon their size may be placed on a standard petri dish. The petri dish is inverted and incubated at 37°C for 4 to 6 hours after which observations are made on the plate. All results in this paper pertaining to disc assay methods were obtained using ¼" discs.

A standard disc containing known concentration of penicillin should always be placed on each plate to serve as a check on the sensitivity of the method. Larger sterile dishes can be substituted for the standard petri dish if large numbers of milk samples are to be analyzed.

Calculations: As shown in figure 1, clear circular zones around the discs indicate inhibition of the test organism and the presence of antibiotics in milk. If a small ruler and a set of calipers are used to measure the diameter of zones obtained by standard discs of varying concentration this can be translated into a standard penicillin curve. Prepared standard penicillin discs purchased from commercial sources or disc impregnated with milk containing known amounts of penicillin are satisfactory for producing such a curve.

Specificity of Disc Assay Method for Penicillin.

Penicillin can be specifically identified in this method by using penicillinase disc** where positive results are obtained. When a zone occurs, the milk which produced it is placed (0.017 ml) on a disc impregnated with penicillinase. This disc is placed on seeded whey agar and the analyses repeated as for penicillin. If a zone is not reproduced penicillin was present in the milk, and it is assumed that the penicillinase inactivated the penicillin. If a zone still persists it indicates that other antibiotics or inhibitory substances are present.

Free Sulfa Drugs in Milk.

Principle: The presence and concentration of free sulfa drugs in milk were obtained by modifying the chemical method of Bratton and Marshall[1] which is an accepted method for blood and urine. According to these investigators the following principles govern this test. Trichloracetic acid precipitates the protein in blood. Nitrous acid produced as a result of the addition of sodium nitrite diazotizes the free sulfonamide or any other free sulfa drugs which are derivatives of the parent compound, sulfanilamide. Excess nitrous acid is destroyed by ammonium sulfamate and the diazotized sulfonamide combined with added N-(1-Naphthyl-ethylenediamine) to form a stable vivid red color.

Procedure: To 1 ml of raw or pasteurized milk is added 10 ml of fresh 1.5 percent trichloracetic acid. The resulting precipitate is filtered off with Whatman No. 42 paper. One ml of the clear filtrate is added to a clean test tube followed by the addition of 10 ml of 0.01 percent fresh sodium nitrite solution.

**Whey agar, *B. subtilis* spores, plain and standard ¼" discs can be obtained from Difco Co., Detroit, Mich. Preparation of *B. subtilis* spores in the laboratory is also discussed by Loo *et al.*[11] Plain ½" disc No. 740-E may be obtained from Schleichter and Schuell Co., Keene, N. H.

The solution is allowed to remain at room temperature for 3 minutes. Then 1 ml of 0.1 percent ammonium sulfamate solution is added and the solution is allowed to stand for 2 minutes. Finally 1 ml of fresh 0.5 percent N-(1-Napthyl)-ethylenediamine is added. A clear red color results almost immediately if sulfa drugs are present in the milk.

Calculations: A standard color curve indicating concentrations of sulfa drugs can be developed from the red color using a photocolorimeter. Though there is some difference in standard curves for the individual free sulfa drugs the differences are not extremely great.

Quaternary Ammonium Compounds.

A method for these compounds in milk was presented recently by Miller and Elliker[12]. This method involved the use of eosin yellowish dye and citric acid buffer. As the method was used exactly as suggested by these authors reference to the details of the principle and procedure should be made to the original article[12]. One ml of milk is used for this test.

RESULTS

Detection of Penicillin and Other Antibiotics in Milk.

Methods outlined in this study were applied to milk from mastitis-free cows of the college herd. Varying concentrations of penicillin G, aureomycin, and dihydrostreptomycin were added to separate lots of raw milk. These milks were then heated to 180°F for 5 minutes, cooled to 40°F and held for 24 hours before analyses were made. This treatment was considered to approach the treatment of milks for some commercial fermented dairy products.

In table 1 are shown data obtained by the total inhibitory substances method and by the disc assay method on milks which contained these three antibiotics. Although three commercial lactic acid starters from different sources were run, table 1 lists results from only one of these starters. Milks containing penicillin in concentrations of 0.1 unit of penicillin per ml exhibited a slight inhibitory effect upon this multi strain starter of about 10 percent. Between 0.1 and 0.3 unit of penicillin per ml, however, the effect was very pronounced. With this starter, slight activity persisted even at the highest concentration of penicillin. Most of the other commercial starters studied were affected to about the same degree as starter L1 but one starter (data not shown) was somewhat more sensitive being inhibited 20 percent at 0.1 unit per ml penicillin concentration and showing zero activity at 5 units per ml and higher.

Aureomycin and dihydrostreptomycin are not nearly as effective inhibitors of lactic acid starter bacteria, table 1, as is penicillin at levels below 3.0 units per ml. Above this concentration differences in potency between the various antibiotics were not too pronounced.

Results in the laboratory on the disc assay method, table 1, indicate it is capable of a great deal of sensitivity when specifically applied to penicillin in milk. Under optimum test conditions, concentrations of penicillin in milk as low as 0.05 unit per ml were detected. For the average laboratory analyses sensitivities of 0.1 unit per ml could be expected, fig. 1. A good indirect relationship was shown to exist between milk activity and zone diameter when the total inhibitory method and the disc assay method were compared on milk containing only penicillin as the inhibitory agent, table 1. Experience in the field on a large number of raw and pasteurized milk samples have not indicated as good a correlation. In particular blended pasteurized samples from commercial dairies did not show such correlation. This may have been due to the fact that most positive cases contained low concentrations of penicillin and that such factors as natural inhibitors for the test organism may have entered into the picture.

Aureomycin and dihydrostreptomycin, on the other hand, both exhibited lower sensitivities to the disc assay method when *B. subtilis* was used as the test organism. This in effect increases the specificity of the disc assay method for penicillin when this organism is used with this agar.

Detection of Sulfa drugs in Milk.

Five sulfa drugs were added to different lots of the same milk in the various concentrations listed in table 2. Acid activity, zone diameters, chemical readings and taste reactions were noted. Though sulfapyridine had the greatest effect upon the activity of the starter organisms used it is apparent that as a group the sulfa drugs are not highly effective against normal starter organisms in the concentrations that might be expected in a milk which has been obtained from a cow treated with this drug.

Except for sulfathiazole, very little sensitivity to *B. subtilis* was exhibited in the disc assay method by sulfa drugs. Even in the case of sulfathiazole zoning was apparent only at the highest concentration

TABLE 1. The Detection of Antibiotics in Whole Milk as Shown by the Starter Activity Test and the Disc Assay Method Using a Starter Culture of High Activity. (L1)

Type & Concentration of inhibitory Substances	Activity*	Zone diameter of inhibition** by disc assay
units	percent	CM (includes disc diameter)
Penicillin		
0.00	100	0.0
0.10	92	1.1
0.30	56	1.5
0.70	27	1.8
1.00	20	2.0
3.00	17	2.4
5.00	16	2.5
10.00	15	2.6
Aureomycin		
0.00	100	0.0
0.05	92	0.0
0.10	95	0.0
0.30	79	0.0
0.70	53	0.0
1.00	41	0.0
3.00	23	1.15
5.00	20	1.45
10.00	18	1.60
Dihydrostreptomycin		
0.00	100	0.0
0.05	103	0.0
0.10	96	0.0
0.30	82	0.0
0.70	62	0.0
1.00	45	0.0
3.00	21	0.0
5.00	17	1.4
10.00	15	1.8

* Increase in acidity of control milk in 4 hours at 35°C using 5% (L1) starter was 0.21% to 0.73% = 0.52% increase. Contols in this table were of same lot of milk containing no antibiotic.

** *B. subtilis* test organism used on whey agar.

TABLE 2. The Detection of Sulfa Drugs in While Milk as Shown by the Starter Activity Test, Disc Assay Method, Chemical Test, and Taste.

Type and concentration of sulfa drug in milk	Activity*	Diameter zone of inhibition** by disc assay	Chemical Test	Taste
percent	percent	CM		
0.000	100	0.0	no color	O. K. (sl. cooked)
Sulfanilamide				
0.001	104	0.0	no color	sl. puckery
0.005	98	0.0	pink	sl. puckery
0.010	94	0.0	sl. red	puckery
0.025	88	0.0	red	sl. bitter
0.050	82	0.0	red	bitter
Sulfamerizine				
0.001	101	0.0	no color	O.K.
0.005	101	0.0	pink	sl. puckery
0.010	98	0.0	red	sl. puckery
0.025	91	0.8	red	bitter
0.050	85	0.8	red	strong bitter
Sulfathiazole				
0.001	97	0.0	no color	sl. puckery
0.005	97	0.0	pink	sl. puckery
0.010	95	0.0	red	sl. bitter
0.025	82	1.8	red	sl. bitter
0.050	80	2.2	red	sl. bitter
Sulfamethezine				
0.001	94	0.0	no color	sl. puckery
0.005	89	0.0	pink	sl. puckery
0.010	83	0.0	red	sl. puckery
0.025	87	0.0	red	bitter
0.050	96	0.0	red	strong bitter
Sulfapyridine				
0.001	98	0.0	no color	sl. puckery
0.005	92	0.0	pink	sl. puckery
0.010	90	0.0	red	puckery
0.025	81	0.0	red	sl. bitter
0.050	70	0.8	red	sl. bitter

* Controls in this table were milk of same lot containing no sulfa drugs or antibiotics.

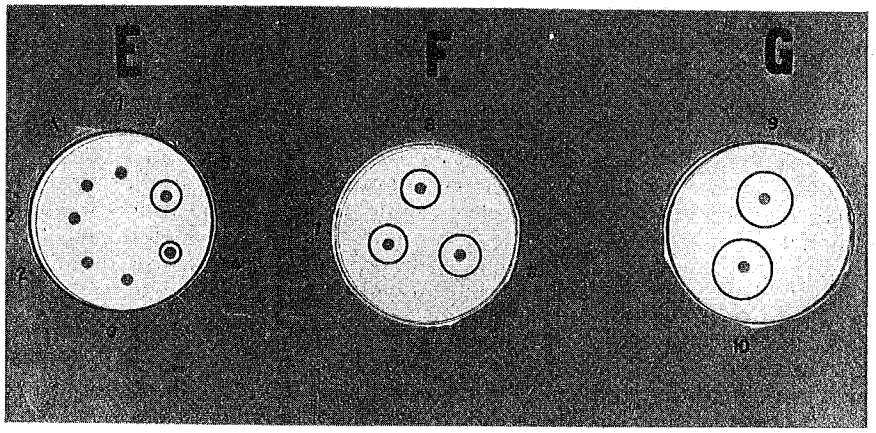

FIGURE 1. Results of Disc Assay Method on Milks Containing Known Quantities of Penicillin and Expressed as Units/ml Milk. 1. 0.00, 2. 0.01, 3. 0.05, 4. 0.10, 5. 0.30, 6. 0.70, 7. 1.0, 8. 3.0, 9. 5.0, 10. 10.0.

TABLE 3. The Detection of a Quaternary Ammonium Compound (Roccal) in Whole Milk as Shown by the Starter Activity Test, Disc Assay Method, and Chemical Test.

Quaternary ammonium compound	Activity*	Zone of inhibition by disc assay
	percent	CM
0.0	100	0.0
3.0	95	0.0
5.0	89	0.0
10.0	70	0.0
50.0	15	0.0

*Controls in this table were milk of same lot containing no quaternary ammonium compounds.

used. When discs dipped in solutions containing p-amino-benzoic acid were dried and then treated with milk containing sulfathiazole, no zone appeared. This application of p-amino-benzoic acid might serve as a specificity test for sulfa drugs if the latter were suspected on a disc assay check plate.

The chemical test of Bratton and Marshall for adaptation to milk provides a high degree of sensitivity. Milks containing as low as 0.005 percent sulfa drugs could be detected by a pink coloration. Very small amounts of sulfa drugs also were indicated by the off-flavor in milk ranging from slight puckery to extremely bitter.

Detection of Quaternary Ammonium Compounds in Milk

Relatively small concentrations of these quaternaries affected the activity of the starter organism, table 3. However, amounts greater than 10 ppm were required before any serious problem with commercial starter inhibition was encountered. The disc assay method using *B. subtilis* was without sensitivity of quaternary ammonium compounds employed herein.

DISCUSSION

This study was undertaken to evaluate and coordinate methods for the systematic analysis of inhibiting substances in milk. It does not touch on methods for bacteriophage because at the present time no practical method exists which can test for bacteriophage in large numbers of milks obtained over wide areas. Therefore, the impor-

tance of this problem to the dairy industry cannot be fully evaluated until such time when the incidence of such cases can be measured.

All the methods finally adopted in this systematic approach showed some promise for their particular function. The starter activity test showed good sensitivity. If this test is carried out with an active starter in a thermostatically controlled water bath it should provide in five hours an answer as to whether the milk contains inhibitory substances. To achieve reproducibility the choice of a proper control is imperative, and the manner of adding starter and titrating the milks should be standardized. Extreme heating of the milks for the starter activity test is necessary as in its raw or even pasteurized state such factors as contaminants or natural inhibitors might come into the picture.

The disc assay method in the laboratory produced sensitive and reproducible results which supports to a great extent the findings of Gilcreas and Stewart[4] on a related type of method, the cup assay method, but any statements as to its ultimate success in the field must be qualified. Heating the raw or pasteurized milk to 180°F for 5 minutes and then cooling to 70° F or lower before use is a precautionary measure for this test. At certain times of the year natural inhibitory substances against *B. subtilis* appeared in the milk. The false positive zones which were created by these natural inhibitors were never over 1.0 cm in diameter and disappeared upon heating of the milk to a high temperature. If for the sake of convenience raw milks are tested directly, all values below 1.0 cm should be considered as possibly being caused by natural inhibitors.

When testing blended pasteurized fluid milk it is doubtful that the level of penicillin which one might expect to find is anywhere near the optimum working sensitivity scale of the disc assay method. Nevertheless, if blended pasteurized milk from commercial dairy plants contains penicillin between 0.1 and 1.0 unit/ml, this method should pick it out. For individual producer's raw milk the disc assay method may prove to be of greater usefulness as one might reasonably expect relatively higher concentrations of penicillin, if there is any present, than for the blended pasteurized type of milk sample.

Bacteria other than *B. subtilis* as a test organism may be employed. For example, *Staphylococcus aureus* which may be used instead does not seem as sensitive to natural inhibitors as is *Bacillus subtilis*. However, *B. subtilis* has the advantage in that it can be used as a spore suspension. This spore suspension can be held in a cold room for many months without need of further transfer, thus saving labor and at the same time introducing a test organism of uniform growth and viability characteristics. The latter organism also gives sharper edges to its zones.

Unless more sensitive test organisms for other antibiotics are found using whey agar it appears that this disc assay method is restricted largely to the detection of penicillin. A number of factors, however, are likely to change the sensitivity of the method for penicillin. The thinner the agar layer the greater the sensitivity. Irregularities in agar depth cause irregularities in results. Excess moisture in plates, uneven incubation temperatures, and varying amounts of inoculum also cause variation in final results.

Aseptic technic should be used throughout and includes such practices as flaming the tweezers for holding discs.

The sulfa drug test was sensitive enough to detect 0.005 precent sulfa drugs in milk whereas the test for quaternary ammonium compounds could readily detect 3 ppm in milk. In both tests successful analyses were made only when fresh reagents were used as instructed.

Work in our laboratory as well as elsewhere, has indicated that there is no satisfactory one-hour test for the detection of antibiotics, such as penicillin, in milk. It should be no deterrent, however, that several of the methods listed here require about 5 hours or more before an answer can be obtained. If a group of producers transport their milk to a plant and if after testing several of their milks are positive, it may be too late to prevent that milk from entering the supply. Yet on the following day those same producers with suspected milk can have their milk held up until completion of another test. The realization by the producer that the dairy management is aware of the presence of drugs or antibiotics in his milk then serves as an effective educational method to combat whatever problem exists in this field.

SUMMARY

A schematic procedure was compiled from a number of individual methods used for the detection of such inhibitory substances as antibiotics, sulfa drugs, and quaternary ammonium compounds in biological fluids. This systematic procedure was formulated for the large scale testing of fluid milks. Because of its simplicity and cohesiveness it should prove to be of importance in a large scale undertaking if all qualifications are considered.

A disc assay method for milk using *B. subtilis* as the test organism procedure showed promise in the laboratory for the detection of amounts of penicillin in milk as low as 0.1 unit/ml. Results from the field on mixed pasteurized milk samples did not indicate as good a correlation relative to antibiotics as results obtained in the laboratory on control milks. This was undoubtedly due to other variable factors inherent in some milks obtained at random. More work should be undertaken in the field to confirm the usefulness of the disc assay method as a standard test for milk. Penicillinase discs enabled this method to attain a specificity for penicillin. This disc assay method using *B. subtilis* was not very adaptable to other inhibitory substances likely to be found in milk due to the relative lack of sensitivity exhibited by the test organism toward these substances.

A four-hour incubation starter activity test also showed promise for detecting total inhibitory substances in milk. This test was highly sensitive but accurate reliable results are largely dependent upon standardization of technic.

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NEW AFFILIATES

DAIRY SANITARIANS ASSOCIATION OF NORTH CENTRAL PENNSYLVANIA

President, James H. Eck, 1304 W. Southern Ave., South Williamsport, Pa.
Vice-President, Galen Furry, Martinsburg, Pa.
Treasurer, Earl F. Hack, Mexico, Pa.
Secretary, C. D. Herbster, 325 Orange St., Selinsgrove, Pa.
Executive Committee: Dr. S. M. Ross, V. M. D., 1828 E. Third St., Williamsport, Pa.
I. E. Parkin, 213 Dairy Bldg., State College, Pa.
G. C. Kern, Milton, Pa.
Harry T. Daddario, New Berlin, Pa.

DR. S. B. FISCHER

It was with a feeling of sincere regret that the friends and fellow-workers of Dr. S. B. Fischer heard of his death.

Dr. Fischer was graduated from Cornell University in 1931. His work as veterinarian continued until he was appointed Associate Sanitarian by the State Health Department. He served on several committees of the New York State. At the time of his death he held a position as Associate Director of Sanitation with the Suffolk County Health Department.

One of his greatest contributions to the County was his tireless effort toward obtaining pasteurization of the milk supply.

ADDITIONAL MEMBERS OF COMMITTEE ON COMMUNICABLE DISEASES

Dr. R. J. Helvig, Chairman, U.S.P.H.S., Washington 25, D. C.

Dr. E. R. Price, Public Health Veterinarian, State Health Department, Jefferson City, Missouri

Dr. Raymond Fagan, Kansas City Field Station, Epidemiology Branch, CDC, 3900 East St., Kansas City 3, Kansas

Dr. L. E. Burney, State Health-Commissioner, Indiana State Board of Health, Indianapolis, Indiana

John H. Fritz, Chief, Food Section, Kansas City Health Dept., 21st Floor, City Hall, Kansas City, Missouri

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CALENDAR

June 4-7—New York State Health Conference, Lake Placid.
June 7-8—Colorado Public Health Association, Estes Park, Colorado.
June 8-12—Institute of Food Technologists, Twelfth Annual Meeting, Grand Rapids, Mich.
June 10-12—National Conference on Interstate Milk Shipments, Statler Hotel, St. Louis, Mo.
June 16-19 — Canadian Public Health Association, Winnipeg.
June 18-19—Massachusetts Public Health Association, Amherst.
June 24-26—American Dairy Science Association, Davis, California.
June 23-27—Annual Meeting and Short Course of the South Dakota Association of Sanitarians, at Sylvan Lake, South Dakota.
Sept. 18-20—Annual Meeting, International Association of Milk and Food Sanitarians, Inc., Nicolett Hotel, Minneapolis, Minn.
Oct. 20-24—Annual Meeting of the American Public Health Association in Cleveland, Ohio.
Oct. 22-23—Annual Conference for Vermont Dairy Operators, University of Vermont, Dairy Department, Burlington, Vermont.

3A SANITARY STANDARDS COMMITTEE ADVANCES PROJECTS

A joint meeting of 3-A Sanitary Standards Committees was held at the Georgian Hotel, Evanston, Illinois, on April 29 and 30.

Final action was taken on an amendment to the 3-A Sanitary Standards for Pumps, on Sanitary Standards for Return Tubular Heat Exchangers, and an amendment to the Sanitary Standards for Piping and Fittings.

The formulation of sanitary standards for milking machines and for can washers, which have been under consideration for a number of years, was considerably advanced. Closer agreement was reached on the details of sanitary standards for the permanent installation of pipelines to be cleaned in-place. And the formulation of sanitary standards for farm storage and for cooling tanks was so far advanced that it may be anticipated that these standards will be ready for publication at an early date.