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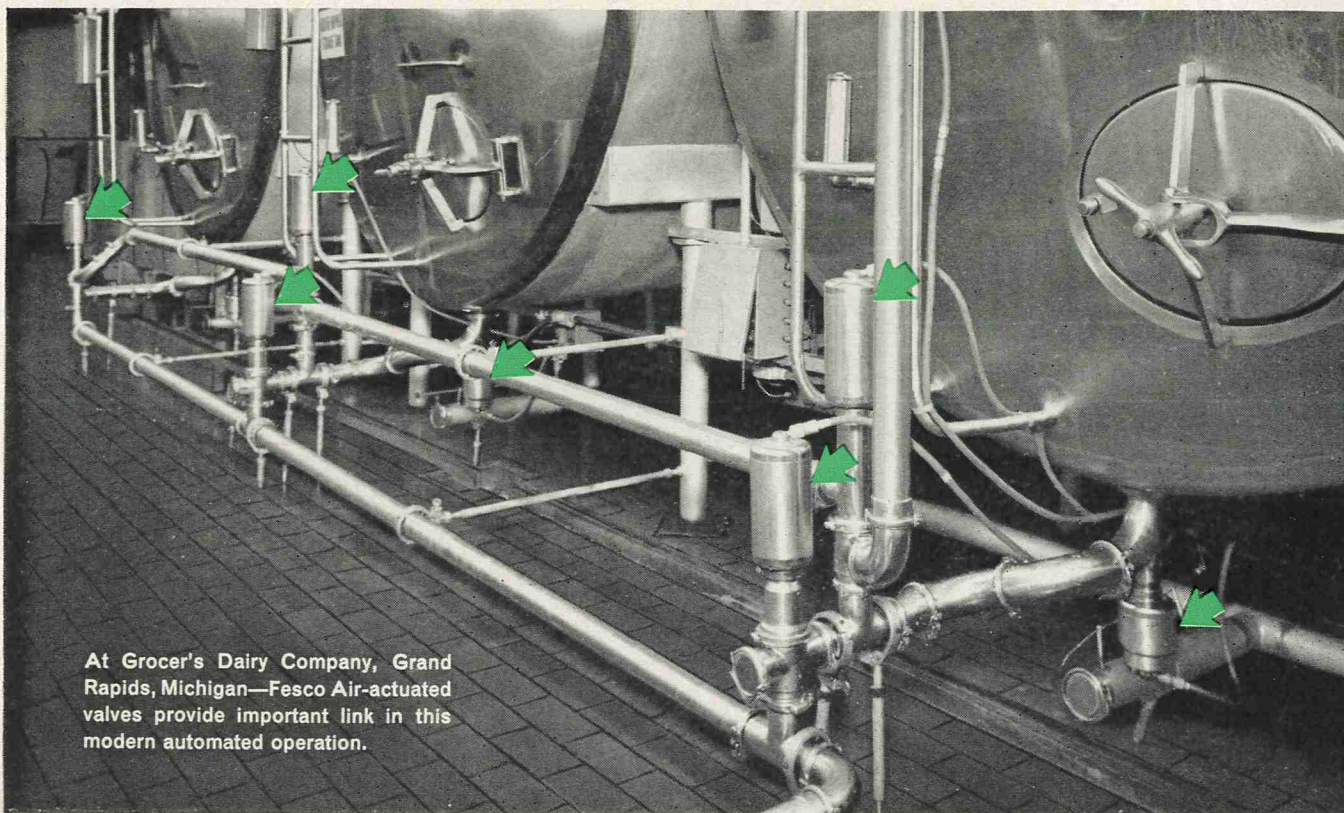
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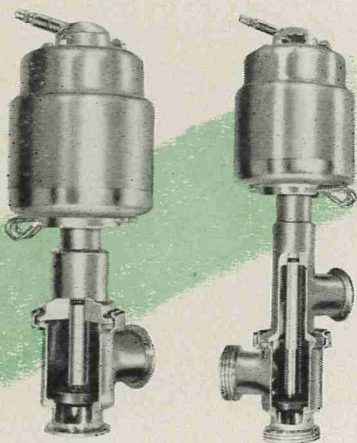
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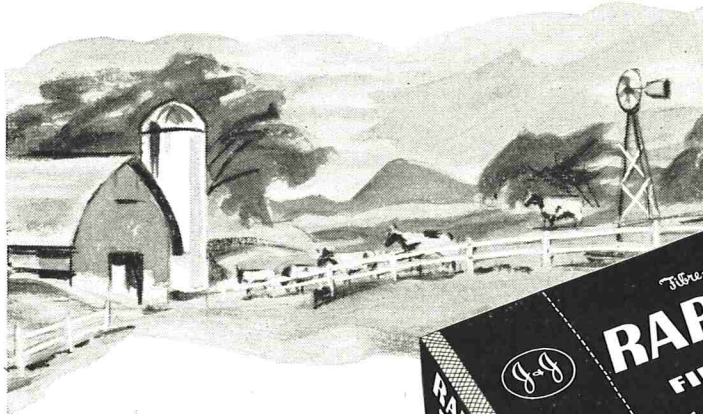


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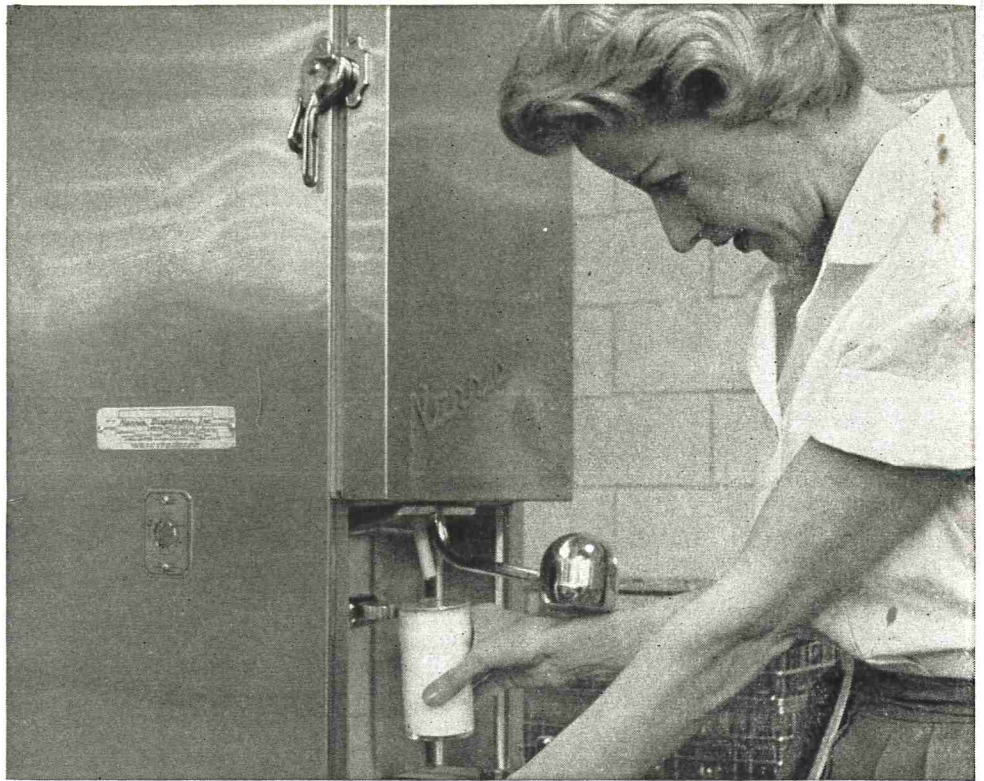
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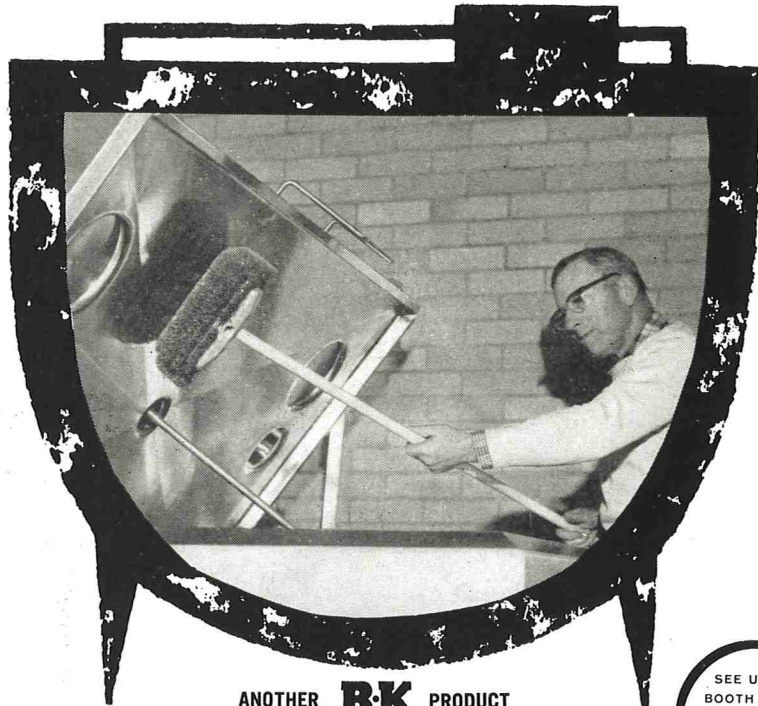
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"Standard Methods" – Time for a Change?

Soon the 11th edition of *Standard Methods for the Examination of Dairy Products* will be available (see the preview of certain changes published in the August issue of the *Journal*). This will culminate a tremendous effort contributed by many people and spearheaded by Dr. L. A. Black. A task of such magnitude is not accomplished without difficulty and personal sacrifice. Undoubtedly, many will be pleased and others disappointed. This is to be expected. As is the case with most books, certain portions become out-of-date or obsolete even before publication. Technological developments and research findings, let alone other factors, make this inevitable. If past editions may be used as an indication of the quality of the forthcoming edition, and considering the mechanisms in effect for assembling the material and getting it into final form for publication, only praise is in store for Dr. Black and his associates. No one knows better than they the limitations which may be evident.

It is not too early to look ahead. There is good reason to think seriously relative to a change in the mechanism by which successive editions of "Standard Methods" are prepared. Does it not seem incongruous that a task of such importance and magnitude as that of preparing successive editions of "Standard Methods" be coordinated and supervised by an individual who takes on this task almost as an extracurricular activity? Continuity of effort to the extent desirable is impossible by such a procedure – and continuity of effort is essential for best results in the selection, evaluation, and preparation for publication of the numerous methods, directions and interpretations which make up the contents of "Standard Methods." This is too much to ask, even of one as dedicated and as competent as Dr. Black.

Consider for a moment the mechanism of preparation of the PHS *Milk Ordinance and Code*. This is a specifically assigned and integral function of the Public Health Service. The writer maintains that "Standard Methods" is as much a part of the Code as anything included therein. The Code is the interpretation of the Ordinance. The Ordinance spells out the requirements; the Code specifies what constitutes conformance or compliance with the provisions of the Ordinance. "Standard Methods" is merely an extension, and a most essential one, of the Code. Here we find the directions to be followed in making the numerous bacteriological, chemical and physical examinations so as to determine whether or not a milk supply is in conformance with standards specified in the Ordinance. Does it not seem logical that the preparation of "Standard Methods" deserves more than a periodic concentrated effort confined largely to a relatively short time prior to each revision? It would seem so. Methods which are in a sense official should be the best available; they need to be proven before their establishment as "official." This has not always occurred to the extent desirable. There is great need for a continuing effort which could be systematically spread over the intervening years between editions of "Standard Methods." If such were effected, research and development work could be more effectively planned and results evaluated. That which is found applicable could be assembled and prepared for publication (a variety of publication media is available) as recommendations to supplement the current edition, and in effect and in due course be included in a subsequently revised edition.

It is suggested that one effective means of facilitating improvements in the preparation of "Standard Methods" would be for the Public Health Service to assume a greater role in this regard. This would be no more than an extension of work already being done by the PHS in connection with the continual up-dating of the Ordinance and Code. "Standard Methods" deserves the same degree of attention. The use of "Standard Methods" transcends any local or circumscribed application. It serves the public, industry and governmental agencies alike. Expenditure of public funds for its preparation, at least in manuscript form, would seem fully justified. With someone assigned this task as a specific and major responsibility within the Milk and Food Program of the Public Health Service, and with the help of a broadly based advisory committee, continuity of effort could be expected. Coupled with this, efforts should be made to assure the Milk and Food Program of the Public Health Service sufficient funds for both an internal and external research program designed to stimulate research and development in the area of methodology applicable to milk and milk products.

The writer is cognizant of the interests of the American Public Health Association and other Associations in "Standard Methods." These interests need be no deterrent to the proposal outlined. The problem does not lie in *publication* of "Standard Methods"; the problem rests in the mechanism of its preparation. Let us settle only for the best. To attain the best, sometimes a change is necessary.

FURTHER OBSERVATIONS ON TESTING MILK FOR PENICILLIN¹

C. K. JOHNS

Dairy Technology Research Institute

Canada Department of Agriculture, Ottawa

(Received for publication March 31, 1960)

Positive results can be obtained most rapidly by using penassay seed agar or equivalent in not over 6 ml. amounts per plate, preincubating for one hour, using 0.5" discs and incubating at 37°C. after "spotting." Lower incubation temperatures prolong the time required for zone formation. Heat-shocking of the spore suspension also generally hastens growth. Refrigeration of poured plates before use is not necessary, but with penassay seed agar has little delaying effect until after 5 days.

Some workers have felt that the standard disc assay method (9), which calls for incubation of the disc-planted plates at 35°C. for 4-6 hours, is too time-consuming. Arret and Kirshbaum (1) have described a "simplified and rapid method" which they claim will detect concentrations of penicillin as low as 0.05 I. U./ml. in 2½ hours at 37°C. Johns (4) has criticized their original method as being less simple, less reliable and less sensitive than the standard procedure. Arret and Kirshbaum (2) have since recommended that poured plates be *refrigerated*, instead of being held at 15°C., for 3 to 5 days before being used. This has removed the writer's chief objection to the method. There still remain, however, other objections such as their requirement of a 37°C. incubator, the thicker layer of agar (10 ml. vs. 6 ml. in the official method), and the failure to indicate that the plating medium, spore suspension and penicillinase discs are all available commercially. They also failed to recommend heating milks to avoid false positive zones due to naturally occurring inhibitory substances (8).

Milk plant laboratories are now doing the bulk of the testing for antibiotics in milk. They would like the results as soon as possible. However, when milk supplies continue to arrive until late in the day, it is not convenient to complete all tests the same day. For the late arrivals at least two alternatives suggest themselves. Poured plates may be "spotted" with the milk-soaked discs and either (a) incubated overnight, or (b) refrigerated overnight, then placed in the incubator the first thing next morning. The former method suffers from a slight disadvantage in that the zones of inhibition are sometimes partially obscured by a secondary growth of colonies of the test organism, and the zones are slightly smaller than

when first visible. It has, however, been employed successfully by H. P. Hood & Sons of Boston since July 1959, using whey agar and incubation at 32°C. for 14 to 24 hours (10). Results are available first thing in the morning and positive findings are reported to fieldmen when they telephone in for other laboratory results.

With the latter method the sensitivity is increased, as the antibiotic has a greater opportunity to diffuse into the agar layer before the test organisms begin to grow.

The present paper reports recent studies on modifications of the standard method (9) aimed at getting results in less than 4 hours. These will be discussed individually. With few exceptions, tests were run on milk shown to be free from antibiotics, to which penicillin G was added to give a concentration of 0.05 I. U./ml. Incubation was ordinarily at 35°C., using 6 ml. of Bacto penassay seed agar per plate and 0.5" No. 740-E, S & S discs. Bacto subtilis spore suspension B453 was used in all tests.

Plating Medium

The standard method (9) gives a choice of whey agar, Bacto B34, or penassay seed agar, Bacto B263, or penicillin assay seed agar, BBL 176, for poured plates. Growth on whey agar has been very much slower than on penassay seed agar (Table 1). Only rarely have zones been detectable at 35°C. in under 5 hr. while many have only shown up after 7 hr., particularly where plates were previously refrigerated. Concurrent tests on penassay seed agar showed measurable zones in 3 to 3½ hrs. Because growth on whey agar is so much slower, zones are generally larger than on penassay seed agar. However, with 0.5" discs on the latter we have been able to detect 0.025 I. U. penicillin/ml. quite readily.

Temperature of Incubation

Most milk plant laboratories have incubator space at 35° and/or 32°C., but few also have space at 37°C. Comparative tests, some of which are shown in Table 1, indicated that with penassay seed agar zones were usually detectable with 0.5" discs in 2½ to 3 hr. at 37°C., in 3 to 3½ hr. at 35°C., and in 3½ to 4 hr. at 32°C. Similar differences were observed with whey agar; thus, there is no serious objection to the use of temperatures lower than 37°C.

¹Contribution No. 36 from the Dairy Technology Research Institute.

TABLE 1 — RATES OF ZONE DEVELOPMENT ON PENASSAY SEED AGAR AND ON WHEY AGAR. MILK CONTAINED 0.05 I. U. PENICILLIN/ML.; 0.5" DISCS; PLATES INCUBATED AT 32°, 35° AND 37°C.

	Days refrigerated at 4°C.	Hours required to detect zone of inhibition at —		
		32°C	35°C	37°C
Penassay Seed Agar	0	3.5 (3.0-4.0)	3.2 (3.0-3.5)	2.8 (2.5-3.0)
	1	3.5	2.9 (2.5-3.5)	2.5
	2	3.7 (3.5-4.0)	3.0 (2.5-3.5)	2.5
	3	3.9 (3.5-4.5)	3.1 (3.0-3.5)	2.8 (2.0-3.0)
Whey Agar	0	—	5.0 (4.5-5.5)	—
	1	—	5.8 (5.5-6.0)	—
	2	>7	6.8 (6.5-7.0)	5.5
	3	>7	7	6.5

Note: Figures in parentheses represent ranges of values encountered.

Overnight incubation at room temperature has been successfully employed (5a) using penassay seed agar.

Refrigeration of Poured Plates Before Use

Arret and Kirshbaum now (2) recommend refrigerating poured plates not less than 3 or more than 5 days before using. In our earlier studies (5) we found a definite slowing down with refrigerated plates when compared with freshly poured ones. In later, more extensive, studies (Table 2) this effect was scarcely noticeable with penassay seed agar until after 5 days refrigeration at 4°C. Whey agar plates, on the other hand, have required 7 hours after 2 and 3 days refrigeration compared with 5 hours when freshly poured (Table 1).

There was no apparent advantage in using *only* refrigerated plates as prescribed by Arret and Kirshbaum (2) for penassay seed agar, while with whey agar there was a definite disadvantage. For most laboratories it would be convenient to pour a batch of penassay seed agar plates at one time, use some the same day and refrigerate the remainder for subsequent use within 5 days.

Preliminary Incubation of Poured Plates Before "Spotting"

When plates were incubated at 35°C. for 1 hour

before the milk-soaked discs were "spotted" thereon, zone formation was detectable from 30 to 90 minutes earlier. In this laboratory this procedure has not resulted in a loss of sensitivity, as reported by Arret and Kirshbaum (1).

Heat Shocking of Spores

The standard procedure (9) specifies melting the plating medium, cooling to 50-55°C., then introducing the spore suspension and pouring the plates. If the spore suspension is heat-shocked by adding it to the melted medium at 70°C. and maintaining the medium at this temperature for 15 minutes, zones are frequently detectable 30 to 60 minutes earlier than with the official procedure. This effect has been observed even in plates refrigerated for 8 days before use. However, considerable variability has been noted. In one test heat-shocking saved over 90 minutes, while in another 30 minutes longer were required. There are evidently some unrecognized variables here which have not been controlled; nevertheless, this procedure does seem to be of value in hastening growth of the test organism.

Sensitivity

The increased sensitivity obtained by using (a) thinner layers of seeded agar and (b) 0.5" paper discs has been reported by various workers (3, 5, 6, 7). The standard procedure (9) calls for the use of 6 ml. per plate. Recent comparisons between plates poured with 6 and with 10 ml. of seeded medium showed a sharp drop in sensitivity with 10 ml., especially when 0.25" discs, as recommended by Arret and Kirshbaum (1), are used. In fact, in several tests we were unable to detect 0.05 I. U./ml. penicillin with the small-

TABLE 2 — EFFECT OF PREVIOUS REFRIGERATION OF Poured PLATES ON RATE OF ZONE FORMATION. PENASSAY SEED AGAR; 0.5" DISCS; MILKS CONTAINING 0.05 I. U. PENICILLIN/ML.; INCUBATION AT 35°C.

Days refrigeration	No. of tests	Hours incubation to detect zone of inhibition	
		Average	Range
0	6	3.33	3.0-3.5
1	6	3.5	3.0-4.0
2	6	3.7	3.0-5.0
3	5	3.5	3.0-4.5
4	5	3.6	2.5-5.5
5	4	3.63	3.0-4.0
6	4	4.0	3.5-4.5
7	3	4.5	4.0-5.0
8	2	5.25	5.0-5.5

ler discs on 10 ml. plates, and on one occasion on a 6 ml. plate.

In our recent studies the difference in sensitivity between the two sizes of discs has been less than we found in tests with aureomycin (5), or that reported by others (3, 7) with penicillin. However, with the 0.5" disc we have invariably detected a concentration of penicillin of 0.025 I. U./ml; with the 0.25" disc this concentration sometimes gave a negative or doubtful reaction, even when incubation was continued for several hours longer. Up to 2 hours longer incubation was frequently required to detect zone formation with the smaller disc.

While it is recognized that more of the smaller discs can be accommodated on the surface of a poured plate, this must be balanced against the greater ease of handling the 0.5" disc, and the larger quantity (some six times as great) and greater uniformity of the amount of milk absorbed.

ACKNOWLEDGEMENT

I am indebted to D. J. Swan for help in running the tests in the present series of studies.

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PROPOSED MODEL ACT FOR THE REGISTRATION OF SANITARIANS

Editor's Note: One of the projects of the Sanitarian's Joint Council has been the development of a model act for the registration of Sanitarians. At its meeting on June 18, 1960, the Council approved this Act printed below. Representatives of APHA, NAS and IAMFS constitute the Joint Council.

AN ACT RELATING TO THE PRESERVATION AND PROTECTION OF THE PUBLIC HEALTH AND PROVIDING FOR THE REGISTRATION OF SANITARIANS AND SANITARIANS-IN-TRAINING; PROVIDING FOR THE ESTABLISHMENT OF A BOARD OF REGISTRATION AND PRESCRIBING ITS POWERS, DUTIES AND FUNCTIONS; DEALING WITH QUALIFICATIONS, APPOINTMENT, REMOVAL, COMPENSATION, AND EXPENSES OF MEMBERS THEREOF; PROVIDING FOR QUALIFICATIONS, EXAMINATIONS, AND REGISTRATION OF SANITARIANS AND SANITARIANS-IN-TRAINING; AND FOR ISSUANCE, RENEWAL, AND REINSTATEMENT OF CERTIFICATES OF REGISTRATION; AND FIXING FEES THEREFORE; AUTHORIZING REVOCATION OF CERTIFICATES; PROVIDING FOR EXPENDITURES OF FUNDS COLLECTED UNDER PROVISIONS OF THIS ACT; FIXING PURPOSES FOR WHICH SUCH FUNDS MAY BE USED; AND PROVIDING A PENALTY.

BE IT ENACTED BY THE LEGISLATURE OF THE STATE OF -----:

Section 1. State Board of Registration: There is hereby created a Board of Registration to register qualified sanitarians whose duties in public health and environmental sanitation require a knowledge of physical, biological, and sanitary sciences and whose professional pursuits and duties are necessary to the promotion of life, health, and prosperity of the State's citizens.

Section 2. Definitions: The words and phrases defined below shall, when used in this Act, have the following meaning unless the context clearly indicates otherwise:

- (a) "Board"—means the Board of Registration for Sanitarians, hereby created.
- (b) "Sanitarian"—is a person who by education and experience in the physical, biological, and sanitary sciences, is qualified to carry out educational, investigational and technical duties in the field of sanitation.
- (c) "Registered Sanitarian"—is a sanitarian registered in accordance with the provisions of this Act.
- (d) "Sanitarian-in-Training"—is a person registered as a sanitarian-in-training under the provisions of this Act.
- (e) "Certificate of Registration"—is a document issued as evidence of registration and qualification to practice

as a sanitarian or a sanitarian-in-training under this Act and bearing the designation "Registered Sanitarian" or "Sanitarian-in-Training," and showing the name of the person, date of issue, serial number, seal, and signatures of the members of the Board hereby authorized to grant such certificates.

Section 3. Qualifications for Registration as a Sanitarian: Any person desiring to be registered as a sanitarian may make application to the Board on a form prescribed by the Board. The Board shall accept such application when submitted if accompanied by the required fees. Persons meeting the following qualifications shall be eligible for registration under this Act:

- (a) Graduate with a baccalaureate, or higher, degree from an accredited college or university who has satisfactorily completed at least 45 quarter hours, or 30 semester hours, of academic work in the basic natural sciences; employed full time as a sanitarian for a period not less than two years; and having passed an examination given and conducted by the Board under the provisions of this Act; provided, that persons holding a degree higher than a baccalaureate degree and who has satisfactorily completed at least 45 quarter hours, or 30 semester hours, of academic work in the basic natural sciences may qualify when employed as a sanitarian for a period of not less than one year.
- (b) Any person who on or before _____ 19____ has passed an official civil service examination as certified by an official agency qualifying him as a sanitarian, given by the State or political subdivision thereof, provided that such person makes application for registration within eighteen (18) months of the effective date of this Act, or
- (c) Any person who on or before _____ 19____ has been employed as a practicing sanitarian for a period not less than three (3) years, may at the discretion of the Board be considered for registration, provided such person applies for registration within eighteen (18) months of the effective date of this Act.

Section 4. Qualifications for Registration as a Sanitarian-in-Training: Any person meeting the educational qualifications of Section 3 (a) but who does not meet the experience requirements of said section may make application to the Board on a form prescribed by the Board for registration as a sanitarian-in-training. The Board shall accept such application when submitted, if accompanied by the required fees.

Section 5. Examination for Registration as a Sanitarian:

- (a) Only persons who meet the educational and experience requirements in Section 3 (a) shall be eligible for admission to examination for registration as a sanitarian.
- (b) Examinations for the registration of sanitarians-in-training may be required by the discretion of the Board.
- (c) Examinations for registration as a sanitarian under this Act shall be administered not less than once each calendar year, in the State at such times and places as may be specified from time to time by the Board. Such examinations may be written, oral, or both, and shall include applicable subjects in the field of sanitary science and such other subjects pertinent to the qualifications of sanitarians as the Board may prescribe. The examination shall be objective and practical in character. The examination papers shall not disclose the name of any applicant but shall be identified by a number assigned by the secretary of the Board. The

preparation of the examination shall be the responsibility of the Board, provided that the Board may use material prepared by recognized examination agencies.

- (d) A person shall not be registered if he fails to meet the minimum grade requirements for examination specified by the Board. If an applicant fails to meet such minimum grade requirements in his first examination, he may be re-examined at any time and place specified by the Board for the administration of such examination and upon resubmitting his application accompanied by the prescribed fees.
- (e) The examination papers and records pertaining thereto shall be filed with the secretary of the Board and retained for at least one year.

Section 6. Board Membership: A Board for the registration of sanitarians and sanitarians-in-training is hereby created. It shall consist of six members appointed by the Governor, one of whom shall be the State Health Officer or his designated representative; one shall be a public-spirited citizen; and four shall be sanitarians who qualify by education and experience for registration as a sanitarian under this Act; provided that on and after _____ 19____ each sanitarian member appointed by the Governor shall be a registered sanitarian under the provisions of this Act.

Section 7. Term of Office: Board members who are sanitarians shall be appointed for a term of office as follows: one shall be designated for a term expiring December 31, 196____; one shall be designated for a term expiring December 31, 196____; one shall be designated for a term expiring December 31, 196____; one shall be designated for a term expiring December 31, 196____. Thereafter the term of office of each sanitarian board member appointed by the Governor shall be four years. Vacancies shall be filled by appointment by the Governor for unexpired terms. The Governor may remove an appointee member for misconduct in office, incompetency, neglect of duty or other sufficient cause after due notice and a hearing.

Section 8. Board Organization, Duties of the Board, Officers, Compensation, Seal and Meetings:

- (a) The members of the Board shall, as soon as appointed, organize and annually thereafter in the month of _____, elect from their number a Chairman, Vice Chairman and a Secretary. The Secretary shall continue in office at the pleasure of the Board.
- (b) The Board shall make such rules as are necessary to carry out the provisions of this Act.
- (c) The Board shall hold at least one (1) meeting each year to review and evaluate applications for registration as sanitarians and sanitarians-in-training, conduct examinations, review and approve all bills, prepare and approve reports, and transact all other business as may be necessary to carry out the provisions of this Act.
- (d) The Board shall issue certificates of registration to applicants who have been found qualified as sanitarians or sanitarians-in-training, to which the official seal of the Board has been affixed.
- (e) Four (4) members of the Board shall constitute a quorum and special meetings of the Board shall be called by the Secretary upon written request of any two (2) members of the Board, or upon a written request signed by ten (10) registered sanitarians.
- (f) All Board meetings shall be open to any registered

sanitarian with the exception of executive Board sessions.

- (g) The Secretary of the Board shall receive compensation to be fixed by the Board, also traveling and other expenses necessarily incurred in the discharge of official duties. The other members of the Board shall receive a per diem allowance at the established State rate for each day actually engaged in official Board meetings plus transportation expenses; provided that no funds shall be dispersed for such purpose without the approval of the Board.
- (h) The Secretary of the Board shall receive and account for all money received from the operation of this Act and shall pay it to the State Treasurer who shall keep such money in a separate fund to be known as "Board of Registration for Sanitarians Fund."
- (i) Funds collected under the provisions of this Act shall be used to pay compensation and expenses of the Board and to administer the provisions of this Act. Any surplus at the end of the fiscal year or biennium shall be retained by the Board for future expenditures.
- (j) An annual audit shall be made of the Board's finances and incorporated in an annual report to the Governor. Copies of the annual report shall be mailed to all registered sanitarians.

Section 9. Record of Proceedings—Register of Applications—Register of Registered Sanitarians and Sanitarians-in-Training—

- (a) The Board shall keep a record of its proceedings.
- (b) The Board shall maintain a register of all applications for registration, which shall show:
 - (1) the place of residence, name and age of each applicant; (2) the name and address of employer or business connection of each applicant; (3) the date of application; (4) complete information on education and experience qualifications; (5) the action taken by the Board; (6) the serial number of the certificate of registration issued to the applicant; (7) the date on which the Board reviewed and acted upon the application; (8) such other pertinent information as may be deemed necessary by the Board.
- (c) The Board shall maintain a current registry of all sanitarians and sanitarians-in-training in the State of _____ that have been registered in accordance with the provisions of this Act.

Section 10. Applications—Fees—Renewals—Etc.: The Board shall prescribe and provide an application form for the use of all applicants. Applicants for registration as sanitarians shall deposit a fee of _____ dollars, and applicants for registration as sanitarians-in-training shall deposit a fee of _____ dollars at the time of making application for registration. The Board may also assess an additional fee for the cost of examination when deemed necessary. A sanitarian registered under the provisions of this Act may renew his certificate by paying the Board an annual renewal

fee of _____ dollars. Said fee shall be due and payable on or before the date to be fixed by the Board for which a renewal certificate for the current year shall be issued. All certificates shall expire on the renewal date unless renewed prior to such date. Registrations expired for failure to pay renewal fees may be reinstated under rules and regulations adopted by the Board.

Sanitarians-in-training shall be exempt from payment of a renewal fee.

Section 11. Suspension or Revocation of Registration: The Board shall have the power to suspend or revoke, after due notice and proper hearing, a certificate of registration when the holder is found guilty of unprofessional conduct, the practice of fraud or deceit in obtaining a certificate of registration, dereliction of duty, incompetence in the practice of sanitation, or for other good and sufficient cause. Notice of hearing in writing shall be given not less than ten (10) days prior to the date of the hearing, designating the time and place of hearing and providing the certificate holder with a copy of the charges against him. The person charged shall be entitled to be represented at the hearing and present evidence in his defense. Every order of the Board causing the suspension or revocation of a certificate of registration shall be predicated on findings based upon the record of the hearing; the determination of the Board may be reviewed by a court only to determine whether the Board abused its discretion or exceeded its jurisdiction.

Section 12. Reciprocity: Agreements for reciprocity with those States having an Act for the registration of sanitarians, whose provisions are equivalent, may be entered into by the Board under such appropriate rules and regulations as may be prescribed by the Board.

Section 13. Use of Title: Only a person who has qualified as a registered sanitarian and who holds a valid current registration certificate for use in this State shall have the right and privilege of using the title, "Registered Sanitarian," and to use the abbreviation "R. S." after his name.

Section 14. Violation—Penalty: It shall be unlawful for any person to represent himself as a registered sanitarian without being duly registered and the holder of a currently valid certificate of registration issued by the Board.

A person who violates the provisions of this Act shall, upon conviction, be guilty of a misdemeanor and may be fined not to exceed _____ dollars, or imprisoned for not more than _____ days, or both.

Section 15. Constitutionality Clause: If any provision of this Act, or the application thereof to any person or circumstances, is held invalid, such invalidity shall not affect other provisions or applications of the Act which can be given effect without the invalid provision or application, and to this end the provisions of this Act are declared to be severable.

Section 16. All laws and parts of laws in conflict with this Act are hereby repealed.

LABORATORY ASPECTS OF STAPHYLOCOCCAL FOOD POISONING FROM COLBY CHEESE

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Outbreaks of food poisoning in Wisconsin in 1958 were traced to Colby-type cheese, as were the outbreaks of greater magnitude in Indiana and Michigan. Laboratory examinations were made on a total of eighty seven samples from cheese, milk from producer farms and from swabs from factory equipment. Seventy-eight cultures of staphylococci were isolated. Seventy-two of these were coagulase positive, and forty-five of the coagulase positive cultures had phage patterns.

During the summer and fall of 1958 there were outbreaks of food poisoning in three families in Wisconsin, and outbreaks of greater magnitude in Michigan and Indiana. Epidemiological investigation revealed that cheese was the food involved in these outbreaks, and all of it was produced in one cheese factory in Wisconsin between May 15 and Nov. 6 of that year (3). A single type of cheese called Colby cheese was involved. This is a washed curd product of low-acid cure, and in this particular factory it was made from raw milk. The laboratory investigation confirmed the nature and source of the outbreak; *Staphylococcus aureus* was isolated from cheese, from milk from the producer farms and from swabs taken in different areas in the factory. This report relates the laboratory aspects of the investigation.

MATERIALS AND METHODS

Cheese

Small portions were cut from the center of each specimen, emulsified with saline and a drop plated on Litmus Lactose Agar (LL), SS Agar (Difco), blood agar made with ten percent defibrinated sheep blood in nutrient agar containing 0.85% salt, and on Staphylococcal Medium No. 110 (Difco). Plates were incubated 48 hours at 37°C. When there were crowded colonies on the LL Agar and blood agar suggestive of being staphylococci, colonies were isolated from the No. 110 Agar plates. In each case a number of colonies were picked which showed some pigment. These were pooled and replated on blood agar for purification purposes. Again, several colonies were picked, pooled and inoculated on nutrient agar (containing salt) slants.

Milk

Each milk sample represented a pooled sample from each producer farm. These samples were collected at the cheese factory as each producer brought in this milk. They were brought to the laboratory

under refrigeration the same day. Each specimen, undiluted and diluted 1:10, was streaked on the above media and treated in the same fashion. Each culture spoken of in the body of this paper is a pool of several isolated colonies.

Swabs

Swabs were moistened with veal infusion broth and rolled across the above media.

Cultural characteristics

Pigmentation. No attempt was made to tabulate the degree of pigment. As stated above colonies with some color were picked from LL Agar, blood agar or No. 110 medium. The pooled cultures after twenty-four hours at 37°C. showed some degree of pigmentation, and the color was enhanced in some cultures after sitting at room temperature for awhile.

Hemolysis. Blood Agar Base (Difco) containing five per-cent blood (sheep, human, rabbit) was used for streak plates to demonstrate alpha, beta and delta lysins. Hemolysis was read at eighteen hours.

Mannitol fermentation. Mannitol broth (Difco) containing 0.004% bromocresol purple was used. Production of acid was recorded after twenty-four hours at 37°C.

Coagulase. Two drops of a twenty-four hour trypticase soy broth culture were mixed with 0.4 ml. of rehydrated plasma (Difco) and incubated three hours at 37°C. Clotting was checked at one hour as well as three hours, and any degree of clotting was considered a positive test. Known positive and negative controls were included.

Phage typing. The twenty-four phages recommended by the National Reference center on phage typing at the Communicable Disease Center, Georgia, were used. Four to six hour trypticase soy broth cultures were used to inoculate trypticase soy agar plates, and drops of the appropriate dilutions for each phage were dropped on these plates within the hour. After overnight incubation at 30°C., lysis was read. The routine test dilution for each phage was checked on its own propagating strain whenever typing was done. One-plus reactions were recorded, but only two-plus and three-plus reactions were reported.

Antibiotic sensitivity. Pour plates were made with nutrient agar (containing 0.85% salt) and 0.1 ml. of a

TABLE 1—CHARACTERISTICS OF CULTURES OF STAPHYLOCOCCI ISOLATED FROM VARIOUS SOURCES

	No. of specimens		No. of cultures		Coagulase	
	+	-	+	-	+	-
Milk	31		24		24	
Cheese samples A	5		5		5	
Cheese samples B	11		11		11	
Cheese samples C	32		6		1	5
Swabs	8		32		31	1
Totals	87		78		72	6

	Coagulase		Mannitol		Hemolysis		Phage:	
	+	-	+	-	+	-	Typable	Non-Typable
Cheese samples A	5						3	2
Cheese samples B	11		9		9		8	3
Cheese samples C	31	1	32		32		23	9
Swabs	1	5	1	5	2	4	1	5
Milk	24		21	3	18	6	10	14

Legend:

Staphylococci isolated from 89.6% of the samples
 Coagulase positive cultures 92%
 Cultures with phage patterns 62.5%

Samples A cheese - Misc. cheese samples for presumptive food poisoning.

Samples B cheese - Received from northern hospital. Later found to be from same source as Samples A.

Samples C cheese - Collected from factory which produced Samples A and B.

suspension of each culture. Bacto-Unidiscs (Difco) were gently secured to the surface of the agar. Zones were recorded after twenty-four hours incubation. The high concentration discs were used: Penicillin, 10 units, erythromycin, 15 mcg.; aureomycin, terramycin, chloromycetin, tetracycline, polymixin and dihydrostreptomycin were all 30 mcg.

RESULTS

The overall results are summarized in Table 1. Gram stains of the cheese emulsions showed a predominance of small Gram positive cocci, and the majority of plates showed a heavy seeding of cocci with some streptococci, a few Gram negative bacilli and some spreaders. No colonies picked from the

SS agar belonged to the *Salmonella* group. Direct smears were not made from the milk samples.

Five specimens of cheese received at different times during the fall showed *Staphylococcus aureus* (designated as Samples A). These cultures were hemolytic and coagulase positive. Mannitol fermentation was not done. Three of these cultures showed phage patterns (see Table 2).

There were eleven samples of cheese sent in by a northern hospital for examination, though no one was reported to have eaten any of this cheese (designated as Samples B.) Coagulase positive staphylococci were isolated from each of the eleven cheese samples, and eight of these cultures had phage patterns. Nine of the eleven cultures were hemolytic and fermented mannitol, two cultures were not checked.

Thirty-two samples of cheese were collected at the cheese factory (designated as Samples C). Staphylococci were isolated from each sample. Of thirty-two cultures, (a) all were hemolytic, (b) all fermented mannitol, (c) one failed to coagulate plasma, (d) all were sensitive to antibiotics, and (e) twenty-three showed phage patterns.

Eight swabs were submitted from areas in the factory. Two, from the gate valve on the weighing can and the raw milk pump and pipe, showed no growth. Four cultures from inside the bottom of a cheese vat, conductor trough, sides of deep tank and filter, and the interior of electric cooler showed no hemolysis, were coagulase negative, fermented mannitol and were non-typable. A culture from the gate valve on the cheese vat was hemolytic, coagulase negative, failed to ferment mannitol, and it was not typable. The eighth culture, from the rennet container, was hemolytic, coagulase positive and had a phage pattern.

Thirty-one samples of milk were examined from the producer farms of this cheese factory. Coagulase positive staphylococci were isolated from twenty-four of the milk samples. Of twenty-four cultures tested, eighteen were hemolytic and twenty-one fermented mannitol. One culture which showed evidence of hemolysis did not ferment mannitol. The ten cultures of this group which showed phage patterns were hemolytic, coagulase positive and fermented mannitol. All twenty-four cultures were sensitive to antibiotics with one exception, one culture was resistant to terramycin.

Throughout this study 87 samples (cheese, milk and swabs) were examined, and 78 showed staphylococci. Seventy-two of the 78 cultures examined were coagulase positive (47 from cheese, 24 from milk, and one from a swab), and 62 of the 78 cultures showed evidence of alpha, beta or delta lysins or combinations of them. The combination of the three lysins appear-

TABLE 2—PHAGE PATTERNS FROM 45 CULTURES

Source	No. of Culture	Phage Groups				
		I	II	III	IV	Misc.
Cheese A	1			6/7/42-E/47/73		81
	4			6/42-E		81/83
	5			7		44-A/81
Cheese B	6			6/7/42-E/73		81
	7					44-A
	8					44-A
	9					44-A
	10				42-D	44-A
	12					44-A
	13			7/42-E		81
	16					44-A
Cheese C	17			47/53/54/73/75/77	42-D	83
	18				42-D	
	19			54/73	42-D	81
	21			47/53/54/73/75/77	42-D	83
	22				42-D	81/44-A
	23	29		42-E/47/53/54/73/75/77	42-D	83
	24			53/77	42-D	
	26					83/44-A
	28				42-D	
	29			42-E/54/73/	42-D	81
	32			6/42-E/47/54/73	42-D	81
	33				42-D	
	34	29		6/7/42-E/47/54	42-D	81
	36	29		6/7/42-E/47/54/73	42-D	81
	37				42-D	81
	38					83/44-A
	39			53/54/77	42-D	81
	40	79	3A/3C		42-D	81
	41					44-A
	44			42-E		81
45					44-A	
46					44-A	
Swab	53		3A			
Milk	56		3A/3B/3C	6/7/42-E/47/54/73/75		81
	58			54		
	61					44-A
	62		3C	6/7/42-E/47/54/73		81
	65		3A/3B/3C	6/7/42-E/47/54/73/77	42-D	44-A/81
	66		3A/3C	6/7/42-E/47/54/73/75		81
	68				42-D	44-A
	69				42-D	81
	70				42-D	44-A
	75					44-A

ed most frequently; however, alpha-delta lysins were seen in combination more than the alpha-beta lysin combination.

Phage patterns were demonstrated in 45 (62.5%) of the coagulase positive cultures; 42-D appeared twenty-one times and 44-A appeared eighteen times among these forty-five cultures. Representatives of the phage lytic Group III appeared twenty-one times, but it must be remembered that this is the group with the greatest number of phages. Lytic Groups I and II appeared less frequently.

DISCUSSION

In the early fall of 1958 five specimens of cheese were received. These have been spoken of as Sample A cheese. There was no particular significance attached to them, and after they were examined, the cultures isolated were discarded.

Sample B Cheese came from a hospital laboratory early in November, and they were examined in the same manner. It was later learned that this group of cheese samples came from the same factory as those

in Sample A. That same week we learned of the outbreak of food poisoning in Indiana and Michigan from Wisconsin Colby-type cheese which had been produced in the same factory that supplied the cheese in Samples A and B.

Retrospective inquiry revealed three family outbreaks of food poisoning (3). Three families had purchased cheese from the same country store which in turn had purchased it from the cheese factory in question: Case histories were as follows:

(a) Mr. R ate cheese at noon one day in July and by 4:30 p.m. he became ill characterized by vomiting and diarrhea. He was hospitalized 24 hours. Two days later he again ate some of the cheese in a sandwich and became ill again with milder symptoms.

(b) In August the L family purchased six pounds of cheese. Mr. and Mrs. L, their five children and the handyman, ate some of the cheese for dinner, and all eight became ill with vomiting and diarrhea occurring. Six days later Mrs. L, her daughter and the handyman again ate a small portion of the cheese, and all three became ill again.

(c) In September Mrs. S and her two children ate some of the cheese and within four hours developed the same symptoms.

By this time an investigation of this particular cheese factory was undertaken as well as the cheese produced there. Our district health officer submitted thirty-two samples of cheese which were selected at random from cheese made in this particular factory between May 15 and Nov. 6, 1958. These specimens have been referred to as Sample C cheese. At this time milk samples from the producer farms of the cheese factory were collected with the co-operation of a representative from the Department of Agriculture.

Coagulase positive staphylococci were found among 72 of the 78 cultures isolated, and phage patterns were demonstrated in 45 of the 72 cultures. These phage reactions indicated cultures of bovine origin. Williams-Smith (9) in England found 42-D the most common type from milk, and Thatcher and Simon in Canada (6) found it the predominate type in butter and cheese. However, in Australia, McLean (2) showed the predominate type to be 44-A, and that was the experience of Seto and Wilson (5) in Wisconsin. These phage patterns among the milk and cheese cultures were not identical but there was a similarity. However, they did not point out the enterotoxigenic strains. Blair (1) has stated that the staphylococcus cultures from food poisoning usually fit into the lytic Group III, and this was the experi-

ence of Williams and Rippon in England (8) as well as Saint-Martin in Canada (4). However, Thatcher and Simon in their work in 1957 (7) found enterotoxigenicity in phage Groups II, III, IV and Misc., as well as among some of the non-typable cultures.

In retrospect it is likely that enterotoxigenic strains occurred among the cultures isolated from milk, and since this Colby-type cheese was made from raw milk, these cultures undoubtedly continued to produce enterotoxin. Colby-type cheese is a washed curd cheese resulting in a low-acid cure, and it has an open texture; therefore it might be a good vehicle for the maintenance of staphylococci. Furthermore, it was said that this factory had trouble with cheese starters which failed to produce their normal acidity.

Cultures were requested by the Robert A. Taft Sanitary Engineering Center, and six from Sample C were sent. Two of these six cultures, No. 17 and No. 24, were positive for enterotoxin assay by the cat procedure. Thus it was shown that there were enterotoxin producing staphylococci among the cheese cultures.

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AN EVALUATION OF SIMPLIFIED METHODS FOR DETERMINING VIABLE COUNTS OF RAW MILK

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When statistically analyzed, the results of comparative examinations of raw milk samples revealed that bacterial counts by oval tube and microplate methods were essentially equivalent to standard plate counts. The oval tube provides an economical and accurate method for determining the count of producers' milk and may be used in the laboratory or on the platform. The microplate provides an equally accurate but more rapid method than either the oval tube or standard plate since counts can be obtained in 20 hours.

Producers' milk is generally graded by standard plate count, direct microscopic count, or by reduction tests. The standard plate count provides a reproducible estimate of bacterial numbers but is relatively expensive and time consuming. Experiences in a number of States have shown that direct microscopic counts and reduction tests are not sufficiently discriminating for grading or for differentiating between lots of low-count raw milks. These studies of simplified methods were initiated to provide economical but fairly accurate bacterial counts of producers' milk. They describe a modification and evaluation of oval tube and microplate methods.

Myers and Pence (7) reported that an oval tube method produced reliable counts of laboratory pasteurized milk. Tubes containing melted agar were each inoculated with a loopful (0.01 or 0.001 ml) of milk and incubated at 35°C for 48 hours before they were counted. Heinemann and Rohr (6) substituted a 1-oz medicine bottle for the oval tube and obtained counts of raw milk which compared favorably with standard plate counts.

Frost (4, 5) described a microscopic colony count for milk in which he used a mixture of 0.05 ml of milk and 0.05 ml of agar, spread over a 4-sq cm area on a flame-sterilized slide. After incubating 12-16 hours, these microplates gave counts equivalent to standard plate counts. Bryan, *et al.*, (3) using a modification of the Frost method, reported that microplates of pasteurized milk and ice cream incubated for 4 and 8 hours, respectively, gave counts equivalent to the standard plate count.

MATERIALS AND METHODS

In these studies, a microplate of 1 sq cm was used since slides with delineated 1-sq cm areas are com-

mercially available. Because of this smaller area, the quantity of milk examined was reduced to 0.01 ml. Slides 3" x 1" with delineated circular 1-sq cm areas were washed, sterilized in the autoclave, and two areas on each slide were circumscribed with a wax pencil to prevent spreading of the milk-agar mixture. The slides were flamed and placed on a warm plate controlled at approximately 45°C. One-ml portions of the milk were transferred to test tubes and tempered for 5 to 10 minutes in a 45°C water bath. Two ml of agar at 45°C were added to and thoroughly mixed with each 1-ml portion of milk; 0.03 ml of this mixture was transferred to the slide with a pipette and spread over a circumscribed 1-sq cm area as shown in Figure 1. The microplates were promptly incubated in a moist chamber at 35°C for 18-20 hours, dried on a warm plate at 70-90°C, and stained with thionine (8).

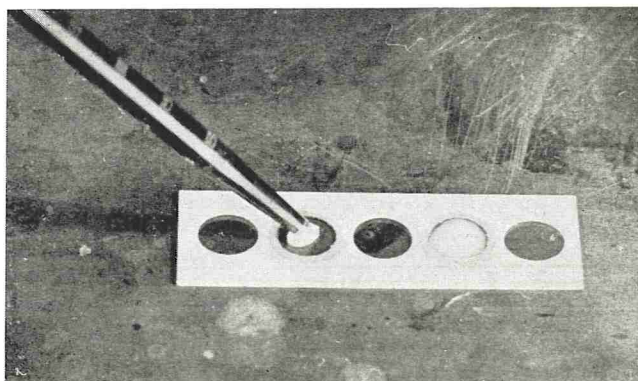


Figure 1. Preparation of microplates

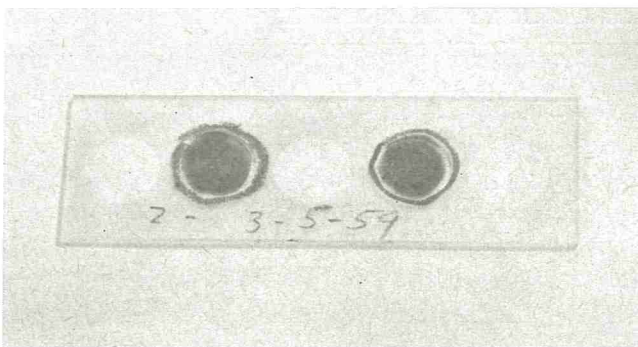


Figure 2. Stained microplates showing colonies

Figure 2 shows duplicate stained microplates. The colonies in three center fields were counted with a compound microscope using 100 X magnification, the average count per field was determined, and the count per ml of milk calculated as shown in the following example:

$$\text{Area of microplate} = 1 \text{ sq cm (100 sq mm)}$$

$$\text{Area of microscopic field} = 3.08 \text{ sq mm} \\ \text{(10 X objective and 5 X or 10 X WF ocular)}$$

$$\text{Volume of milk spread over 1-sq cm area} = 0.01 \text{ ml} \\ \text{(0.01 ml milk = 1:100 dilution)}$$

$$\text{Average number colonies per field} = 10$$

$$\frac{\text{Area: microplate}}{\text{Area: microscopic field}} \times \frac{100}{1} \times \text{Avg. No. of cols. per field} = \text{Count/ml}$$

$$\frac{100}{3.08} \times \frac{100}{1} \times 10 = 32.5 \times 100 \times 10 = 33,000/\text{ml}$$

The oval tube method is essentially the same as the original one (7) except that, in the present studies, tubes with shorter necks were used and metal closures were substituted for cotton plugs. The shorter necks facilitated inoculation and the metal closures prevented dehydration of the agar during incubation.

These modified oval tubes, each containing 4 ml of melted agar, were placed in a water bath and allowed to cool to 45°C. Using a standard loop, flamed and cooled between transfers, 0.001 ml of each milk sample was transferred to a tube. The milk and agar were mixed, and the tubes were laid flat on the bench top until the agar had solidified. They were then incubated in the horizontal position at 35°C for 48 hours. The resulting colonies (Figure 3) were counted with a Quebec colony counter.

RESULTS AND DISCUSSION

The microplate and the oval tube methods were compared with the standard plate count method (*Standard Methods for the Examination of Dairy Products*, 1953) in a series of 9 experiments in which a total of 114 raw milk samples were examined in duplicate by each of the three methods. All tests were made using Plate Count Agar (Difco), and were incubated at 35°C; the microplate for 18-20 hours and the oval tubes and agar plates for 48 hours. The tests were counted and the counts per ml computed for each method. The counts were compared statistically using logarithmic averages, since variation in the log count tends to be independent of the count level.

Table 1 shows the geometric means of the bacterial counts obtained by the three methods in each experiment. Experiments 1 through 5 were of a preliminary nature and represent efforts to standardize the two experimental techniques. Although the basic

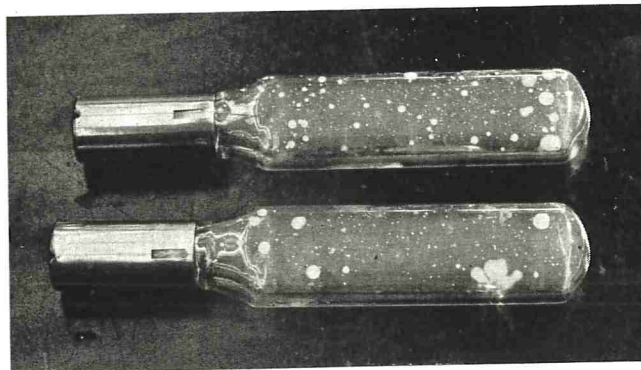


Figure 3. Oval tubes showing colonies

TABLE 1—GEOMETRIC MEANS OF BACTERIAL COUNTS PER ML OF MILK OBTAINED IN EXPERIMENTS COMPARING THE OVAL TUBE AND MICROPLATE METHODS WITH THE STANDARD PLATE COUNT

Experiments	No. of samples	Standard plate count	Oval tube	Microplate
Preliminary				
1	13	67,500	51,000	14,300
2	17	19,700	20,800	18,100
3	10	84,700	71,300	79,700
4	11	139,000	81,100	98,000
5	20	60,700	35,700	41,400
Field Trial				
6	10	57,900	57,000	55,900
7	11	28,500	39,900	23,000
8	10	58,600	48,200	40,600
9	10	27,100	26,800	23,300

procedures were the same for all experiments, improvements were made as the work progressed and greater dexterity of manipulation was acquired. For example, undue drying of the microplates before incubation, which may have caused the low counts in experiment 1, was avoided in subsequent experiments. Also greater care was exercised in removing cleaning solution from the small-bore pipettes and in warming them in a flame just before use. Relatively good agreement between methods was obtained in experiments 2 through 5, although 4 and 5 showed differences just barely significant statistically.

Experiments 6 through 9 were field trials (Chicago Board of Health laboratories) of the oval tube and microplate methods carried out by the senior author. The results (Table 1) show better agreement between methods in the last 4 experiments than in the preliminary five. Statistical analysis of the results revealed no significant differences between methods in the field trials. Also, statistical analysis of differences between duplicate counts by a given method showed acceptable agreement by the standard plate count, even better agreement by the oval tube meth-

od, and generally good agreement among the six fields counted over the duplicate microplates.

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ASPECTS OF PESTICIDES AND ANTIBIOTICS AS THEY RELATE TO THE DAIRY INDUSTRY¹

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It is desirable and timely, in the light of the developments in statutory and regulatory procedures, and their application, in recent months, to take stock of the aspects of these agents as they relate to the dairy industry.

The effect of strict interpretation of the assignment of law as it applied recently to specific products such as cranberries, and to poultry, is all plain to see. It must not be forgotten that the application of the same basic principles of law to foods, and to milk, has been going on for a long, long time. Since 1896, over 20 acts have been stipulated by Congress dealing with various aspects of production, processing and distribution of foodstuffs. It may be presumed that these have had serious consideration in their enactment, and that the intent was for the public welfare. There have been, of course, multitudinous applications of law by the 48 states, among which the differences are indeed confusing in many respects.

For the moment, attention is being focused specifically on aspects of antibiotics and pesticides, since the application of the Amendment to the 1938 Food, Drug and Cosmetic Act has brought light on problems in their use.

Some consideration should be given to the status of need of both antibiotics and pesticides in their respective areas of application.

Mastitis, in all its implications, is probably as far from satisfactory control and elimination, as it ever has been in the history of modern dairying. There is ample reason to believe that the difficulty of control of mastitis has increased in recent years. Changes in farm dairying alone have altered conditions of cow care, and herd management from the individual personal care of a limited number of cows to industrialized care of several hundred of animals.

The number of farms with milking animals is constantly decreasing, by some 30 per cent in 15 years, and the amount of milk per cow, and per farm, has been increasing in like manner in a similar period. There exist fully industrialized, as well as privately farm operated milk producing centers.

For a period of years, diligent progress was made in the control of mastitis by good herd management, proper feeding, modern controlled milking techniques, and by sound sanitation procedures. Much of this seems to have been forgotten in the convenient adoption of the "miracle" drug, originally found successful in part, in treatment of streptococcal infections. But the control and elimination of mastitis has not been simplified, nor achieved by this trend,

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primarily because of the change in udder flora, and failure of the infection to respond to the treatment. Dairymen generally are becoming discouraged by the failure of antibiotics to solve their mastitis problems, and are turning with renewed interest to methods formerly taught, in prevention.

USE OF ANTIBIOTICS IN THE DAIRY INDUSTRY

The magnitude of the mastitis problem should be considered also in terms of the extent of use of antibiotics. It is reported that the total amount of antibiotics certified for use by FDA for oils and ointments in the first quarter of 1960 was equivalent to an annual rate of 2876 million units annually, or 510 units per quart of milk produced. Elsewhere it is reported that the quantity of combination penicillin and dehydrostreptomycin in batches certified in 1959 for use in dairy animals by injection (intramuscularly or intravenously) was 34.2 trillion units penicillin and 48.1 million grams dehydrostreptomycin, equivalent to a 1500 units per quart. Thus the total *disappearance* of antibiotics in the dairy industry is at the rate of 2000 units per quart. The dollar value of antibiotics used for mastitis treatment is reported at 27 million in 1958. The breakdown of antibiotic sales in 1958 is reported at 380 million dollars for human dosage, 35 million dollars for veterinary dosage, 30 million dollars for animal feed, and 2 million dollars for preservative and pesticide uses. On the basis of projected sales in the next decade it is anticipated the usage of antibiotics in the dairy industry will increase. It is of interest to note that the role of antibiotics in food production other than in dairying is increasing. The quantities used in animal field supplements has increased from 196,000 pounds valued at 17.5 million dollars in 1951 to 795,000 pounds valued at 31.3 million dollars in 1957. The fate of feed antibiotics in milk production is in great need of scrutiny by the dairy industry.

PESTICIDES

The utilization of pesticides is constantly increasing in the United States. Recent reports for one of the largest states indicate that the area of land treated with pesticides has increased from about 200,000 acres in 1946 to over 7 million acres in 1958. The production of all pesticides has increased from 400 million in 1950 to 800 million pounds in 1959. The use of insecticides has increased from 200 million to 400 million pounds annually. The number of registered trade mark economic poisons has increased from 10,000 in 1949 to 90,000 in 1959.

The economic advantage of pesticides is so vast in agriculture that it is hardly conceivable this system of augmenting food supplies would be abandoned.

The necessity of presence of pesticides in food products in our present system of economy is tacitly recognized in the application of the Amendments of the 1938 Food Drug and Cosmetic Act which provide for registration, review of technical data and establishment of tolerances for their presence in foodstuffs. It is variously estimated that without the uses of such pesticides, the production of certain crops periodically would reach zero, and of food crops in their entirety, would decrease 10-30 per cent. The monetary annual losses due to weeds are estimated at 5 million dollars, and to insects, 3 million dollars. The threat of new pest hazards is constantly in the picture, and new applications and new developments in pesticides will in all probability be essential to thwart the possible effects of such intrusions.

The increase in agricultural production over the past several decades is due primarily to intense mechanization, to agronomic developments and to the uses of pesticides and fertilizers. It has been reported to Congress that 40-50% of the increase since 1942 in agricultural production is due to the use of agricultural chemicals. The trend in agriculture is intensity of production: larger herds, larger farms, larger yields per acre, and per animal, larger units of machinery. Farmers have heavier investments in facilities of land, buildings, machinery, cattle, irrigation, and so forth. Thus the use of pesticides is not only in the sense of correcting an infestation of weed, fungus or insect, but also in the sense of insurance against a possible hazard, and to protect the investment.

DEFENSIVE PROBLEMS

The utilization of antibiotics and pesticides has resulted in difficult defensive relations problems for the dairy industry. The use of ingredients, and materials, from the moment of origin of milk, until its manufacture, and distribution, is not continuously under a given process control of the processor. The history of milk, with respect to antibiotics and pesticides, is not known, until subjected to test. The processor, through definition, is prohibited *both* from procuring, and distributing milk, which by definitive terms, is adulterated. The actual control of the biochemical status of the milk by the processor is extremely difficult. It would seem there is greater need for placing responsibility for the presence of adulterants in raw milk where it logically belongs, rather than by a system of indirect mechanics of convenience. The processing organization cannot know, except by test, of the presence of such adulterants, and these tests are at present, entirely too time consuming.

The increase in the intensity of dairy farming has

resulted in increased use of purchased grain, forage and supplement, grown elsewhere than on the dairy farm. The history of treatment of such feeds is in the hands of others who may never know the ultimate destination of their crops, whether poultry, animal or dairy farm. The dairy farmer, in turn, may never know the origin of the purchased feed, nor of its treatment. The dairy industry is in need of labeled forage and feed to better control the problem of transmitted pesticide. Even such identification may be without the desired effects. The identification of use of pesticides is not the whole story. Some pesticides carry over in soils for periods of several years; there may be accumulation, from a sequence of applications. Some chemicals degrade into forms even more toxic than their precursors. There are many forms of pesticides: nematocides, weedicides, fungicides, herbicides, insecticides, plus treatments such as defoliant, growth regulators, dessicants, and the like.

While there has been a considerable amount of research on the fate of chemical treatments of food crops used directly by humans, such as vegetable and fruit crops, there has been relatively much less on the fate of these applied to animal feeds, which represent a larger part of total agricultural production. The dairy industry needs to be cognizant of both areas of treatments, since fruits, nuts, and natural agents are used in frozen desserts and cheese. It should be noted that other branches of the food industry have had to deal with similar problems and have done so in a positive and forthright manner, e.g., the infant food business. In such products the presence of incidental chemical factors is as much, if not more of an anathema, as in the dairy industry. The fruit and vegetable industries, producing products destined for infant uses for example, do have specific and highly controlled agricultural arrangements for uses of chemical agents on contracted crops. These industries also have conducted intensive investigation of the effects of pesticides not only in terms of freedom from pests, but also in terms of yield, surface effects, effects on storage life, on color, flavor, fibrosity tenderness, deterioration during processing, composition of product, container corrosion, fermentation, drained weight, and so on. The extent of related effects of the agricultural chemicals on dairy products seems not to have been as completely investigated.

ZERO TOLERANCE

Administratively the philosophy prevails that milk and its products must be "analytically free" of pesticides and antibiotics. There is a real need for a hard realistic fast look at the concept of "zero tolerance" for milk and its products. It is becoming increasingly

apparent that in today's scheme of technology, and agriculture, and with increasing levels of sensitivity of analytical methods, there is no such thing as "zero" free milk. There is evidence that "zero" free levels cannot be found or applied to modern day mother's milk.

The sensitivity of methods of analysis for chemical agents in foods is obviously increasing. The necessity of increased control of the agents in the dairy world obviously will also have to increase. The matter of desirability of tolerances of antibiotics and pesticides in milk and dairy products has been and will be a subject for consideration for some time to come. If the dairy industry believes it wants to move in the direction of tolerances, it will of necessity have to establish better systems of complete reliable control of use of antibiotics and pesticides than it now has. This will be necessary to provide assurance not only to Food and Drug Administration, but to the public as well. Few people in the food industry realize the destructive effect on the cranberry industry of the seizure and condemnation of only a very minor part of the total cranberry crop. How then, will the assurance to the public develop? How seriously will the industry prosecute violations in order to retain public confidence? Cognizance should be had that there are problems in complying with the "pass or fail" values of a tolerance as well as with a zero tolerance. Levels which are below tolerance and acceptable may become objectionably above tolerance through uncontrollable variables: through transfer from change of feeds, through change in rations of the cows, through change in production of the cow, and so on. Moreover, there will be changes in composition of the milk: separation of fat (some agents may tend to follow the fat, or the serum), blending, standardization, concentration, and so on. These may change the status of acceptability on a tolerance basis. This, however, is no different a problem than already faced by other food manufacturers such as of soups, fruit purees, jams and jellies, and so on.

In many food operations, the useable fraction may be as little as 40-60% of the raw harvest product. With milk, the utilization is almost complete, except for mechanical losses. Thus while some industries may separate through waste, occluded chemicals, that in milk will be processed and perhaps even fraction-concentrated in product. In this connection, the waste of some foods (apple and citrus pulp) may be processed by concentration into animal feeds. The concentration of applied pesticides may render their use for dairy feeds questionable.

ASSAY COSTS

There is great concern that the necessity of fre-

quent appraisal of the presence of the antibiotic and pesticide residues will become a costly burden. Many of the analytical procedures thus far developed are specific, and require extensive and expensive laboratory facilities and personnel. There are in use many pesticide agents, many of which may become adulterants; the use of antibiotics in feeds is increasing. Some practical means must be found to both reduce, and properly assign, the cost of these appraisals, and the supervision in control of use of the agricultural chemicals.

COTERIE OF THOUGHTS

Finally, in this coterie of thoughts, it may be wise

to reflect that the problems of use of antibiotics will not become simpler by legislative fiat. The population of the world is increasing; the sobering thought is that surpluses exist only in the western world, and food deficiency is possible even in a foreseeable future. Man and his cultivated food supplies have many enemies: diseases, pestilence, insects, weeds. It has been stated that the effectiveness of one man in a chemical factory is equivalent in agricultural productivity to the work of 4,000 men armed with hoes. Thus, in the foreseeable future, the uses of agricultural chemicals, begun in France only some 20 years ago, will probably be with us a long time to come.

THE INCIDENCE OF POTENTIALLY PATHOGENIC STAPHYLOCOCCI IN DAIRY PRODUCTS AT THE CONSUMER LEVEL

I. FLUID MILK AND FLUID MILK BY-PRODUCTS¹

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Two hundred and seven samples of pasteurized dairy products obtained from consumer marketing channels were analyzed for the presence of staphylococci. Potentially virulent coagulase positive staphylococci were isolated from 3.4 percent of the samples examined. All of the various classes of products studied contained staphylococci, however, not all samples within a class contained the organism. The presence of coliform organisms and staphylococci did not correlate positively, leaving some question as to the source of contamination.

Research findings have shown a trend toward the development of antibiotic-resistant pathogenic staphylococci in dairy cattle undergoing mastitis therapy (11, 12, 13). McCoy (9) suggests the danger of the staphylococci developing resistance to some antibiotics and thereby limiting their use in treating human and animal infections. Numerous reports have indicated that antibiotic-resistant staphylococci have caused infections in hospital surgical patients and infants in nurseries throughout the country (4). Aside from

this potential danger, is the ever-present menace of staphylococcal food poisoning. Increased incidence of staphylococci in the udder of dairy cattle has added significance to this problem (9). Recent reports incriminating dairy products in outbreaks of staphylococcal food poisoning have focused attention on the need for research in this area (2, 3, 7).

Increased importance of the problem and relatively little information available on it, prompted this survey of the incidence of potentially pathogenic staphylococci in dairy products at the consumer level. This paper reports results of studies on fluid milk and fluid milk by-products in original containers at the consumer level. Subsequent papers will report the results of similar examinations of powdered milk and a wide variety of cheese and frozen dairy products.

EXPERIMENTAL PROCEDURE

Samples.

Two hundred and seven samples of pasteurized dairy products processed in 42 plants were obtained during June and July, 1959, from retail outlets throughout Kansas. The samples included the following products: milk, low-fat milk, chocolate drink or chocolate milk, cultured buttermilk, half and half, coffee cream, and whipping cream.

The samples were held under refrigeration (35°F.)

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from the time of collection until analyses were started. Total elapsed time never exceeded 12 hours.

Bacteriological Examination.

Presumptive examination for staphylococci. Both Tellurite - Glycine (TG) agar recommended by Zebovitz *et al.* (15) and Staphylococcus Medium No. 110 (S-110) (5) were used for the initial isolation of staphylococci.

A 0.25-ml. volume of each sample of dairy product was aseptically transferred to plates of TG and S-110 media. The surface plating technique of Snyder (14) was used to spread the sample over the plates. After inoculation, the TG plates were incubated at 37°C. for 24 hours, and the S-110 plates were incubated at 37°C. for 43 hours.

In addition, an enrichment procedure was used in which 1 ml. of each sample was transferred to 10 ml. of enrichment broth consisting of Staphylococcus Medium No. 110 minus the gelatin and agar. The broth culture was incubated at 37°C. for 24 hours after which aliquots were transferred to and incubated on TG and S-110 plates as described. After the TG and S-110 plates were inoculated with the broth culture, they were designated TGE and S-110E to differentiate between organisms obtained directly from the dairy product and organisms obtained from the product via the enrichment.

Proof of isolation. Isolates from the TG, S-110, TGE and S-110E plates were examined for morphological characteristics, Gram stain and anaerobic growth in the lower portion of a deep shake tube of glucose yeast extract agar.

Determining potential pathogenicity. Potential pathogenicity was determined on the basis of accepted criteria, that is; pigmentation, gelatinase activity, mannitol fermentation, coagulase production (citrated

human plasma) and hemolysins on sheep blood agar.

Antibiotic sensitivity. Sensitivity to antibiotics was determined using antibiotics commonly used in mastitis therapy and antibiotics used in human therapy of staphylococcal infections (Antibiotic Sensitivity Disks, Difco).

Bacteriophage typing. Bacteriophage patterns were determined on coagulase positive cultures. All bacteriophage typing was done at the Regional Bacteriophage Typing Center, Kansas State Board of Health Laboratories, Topeka. This laboratory follows the recommendation for bacteriophage typing as established by the National Reference Laboratory, Laboratory Branch, Communicable Disease Center, U.S.P.H.S., Chamblee, Georgia.

Coliform counts. Coliform counts were made with Violet Red Bile agar (V.R.B.) (Difco) to establish whether a correlation existed between the number of coliform organisms in a dairy product and potentially pathogenic staphylococci.

RESULTS

The data showing incidence of staphylococci in the dairy products analyzed are presented in Table 1. All of the classes of products analyzed contained staphylococci. However, not all of the samples within each individual class contained the organisms.

Six of the seven classes of products examined contained *Staphylococcus aureus* and all seven classes contained *Staphylococcus epidermidis*. Twenty-five potentially virulent coagulase positive cultures were isolated from four of the seven classes of products studied. These cultures were obtained from seven product samples produced in seven processing plants.

A chi square test indicated ($.50 < P < .70$) that

TABLE 1—ISOLATIONS OF STAPHYLOCOCCI FROM DAIRY PRODUCTS IN CONSUMER MARKETING CHANNELS

Product	No. samples examined	Samples containing staphylococci ^a		Samples containing <i>Staph. epid</i> ^b		Samples containing <i>Staph. aureus</i>		Samples containing both <i>Staph. epid.</i> and <i>Staph. aureus</i>		Samples containing coagulase positive staph.	No. of coagulase positive cultures isolated
		(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)		
Past. milk	86	23	26.7	17	19.8	12	14.0	6	7.0	1	1
Low fat	28	12	42.9	10	35.7	3	10.7	1	3.6	0	0
Choc. milk	10	1	10.0	1	10.0	0	0	0	0	0	0
Buttermilk	15	3	20.0	1	6.7	2	13.3	0	0	1	4
Half & Half	35	10	28.6	5	14.3	6	17.1	1	2.9	3	15
Goffee cream	6	2	33.3	1	16.7	2	33.3	1	16.7	0	0
Whipping cream	27	11	40.7	10	37.0	2	7.4	1	3.7	2	5

^aStaphylococci differentiated from micrococci on the basis of anaerobic growth in glucose medium.

^b*Staphylococcus epidermidis* differentiated from *Staphylococcus aureus* on the basis of mannitol fermentation.

the level of staphylococcal contamination was essentially the same for all classes of dairy products analyzed. A chi square test also indicated ($.20 > P > .10$) that the level of contamination was essentially the same in the products processed in the various plants.

The pigmentation, hemolysins, bacteriophage patterns and lytic groups of the coagulase positive organisms are presented in Table 2. None of the coagulase positive organisms was resistant to any of the eleven antibiotics tested. However, twelve of the coagulase negative *Staphylococcus aureus* and eight *Staphylococcus epidermidis* cultures showed resistance to one or more antibiotics. Seven cultures of coagulase negative *Staphylococcus aureus* and ten cultures of *Staphylococcus epidermidis* were hemolytic. Data on the antibiotic resistance and hemolytic patterns of the coagulase negative organisms are presented in Table 3.

In comparing the relationship between coliform organisms and staphylococci, contingency chi squares

were nonsignificant, indicating that the presence of coliform organisms and staphylococci were the result of chance.

DISCUSSION

Coagulase positive staphylococci were isolated from 3.4 percent of the products examined. That potentially virulent organisms were present in even a low percentage of the products warrants the attention of the dairy industry. Improper refrigeration of the products before they were consumed could result in high populations of staphylococci with accompanying enterotoxin formation. There is no implication that enterotoxin was present in samples examined, but potentially pathogenic organisms were isolated and their virulence determined according to generally accepted criteria. Analysis for staphylococcus enterotoxin was not made because of the lack of an acceptable laboratory test.

Three of the seven products containing coagulase

TABLE 2—CHARACTERIZATION OF COAGULASE POSITIVE STAPHYLOCOCCI ISOLATED FROM MARKETED DAIRY PRODUCTS

Type product	Product sample No.	Culture number (s)	Isolation media	Pigmentation	Hemolysins	Bacteriophage pattern		Lytic group
						CTD ^a	Concentrated ^b	
Milk	194	229 ^c	TGE	white	—		N.T. ^d	
Buttermilk	35	72, 63, 71	110E, TGE	orange	<i>a</i>	42D, 81		IV. and Uncl ^e
Buttermilk	35	64	TGE	white	<i>a</i>	42D, 81		IV. and Uncl
Half & Half	39	25	TG	orange	<i>a</i> & <i>β</i>	42D, 81		IV. and Uncl
Half & Half	75	78, 80, 90, 96, 103	110, TG	orange	<i>a</i>		44A	Ur
Half & Half	75	94	TG	orange	<i>a</i>		79, 7, 44A	I, III. and Uncl
Half & Half	179	236	110E	white	<i>a</i> & <i>β</i>	42D		IV.
Half & Half	179	196	TG	orange	<i>a</i> & <i>β</i>	42D		IV.
Half & Half	179	190, 239	110, 110E	orange	<i>a</i> & <i>β</i>		44A	Uncl
Half & Half	179	188, 189	TG	orange	<i>a</i> & <i>β</i>		44A	Uncl
Half & Half	179	224	TGE	orange	<i>β</i>	42D		IV.
Half & Half	179	225	TGE	white	<i>a</i>	7, 42E, 83(VA4)		III. and Uncl
Whipping cream	201	231	110E	orange	<i>β</i>	7, 42E, 83(VA4)		III. and Uncl
Whipping cream	201	201	TG	white	—		N.T.	
Whipping cream	201	202	TG	orange	—		N.T.	
Whipping cream	201	212	110	orange	<i>a</i> & <i>β</i>	3B, 42E		II., III.
Whipping cream	207	193	TG	orange	—		N.T.	

^aCritical Test Dilution

^bCritical Test Dilution x 1,000

^c*Staph. epidermidis*

^dNon-typable

^eTypable but have not been grouped

a = Alpha hemolysins

β = Beta hemolysins

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HOTEL MORRISON, CHICAGO, ILL.

OCTOBER 26, 27, 28, 29.

THE CONTROL OF ANTIBIOTICS IN MILK THROUGH A SOUND TEST PROGRAM¹

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(Received for publication April 17, 1960)

The application of a steady field test program along with proper fieldman supervision markedly reduced the antibiotic incidence in milk at one large New York State fluid milk and cream dairy firm. Using the simple Cornell field antibiotic test kit much of the time, approximately 10,000 milks were tested over a four-month period on about 4,000 farms. The incidence in milk from producer can farms dropped from 6.5 per cent to 0.45 per cent during this period. On producer bulk tank farms the rate of incidence dropped from 5.1 per cent to between 0 and 0.5 per cent. As a result of this significant drop, no over-the-road tanker of milk destined for metropolitan areas contained antibiotics within the sensitivity limits of the tests employed.

Control of the antibiotic contamination of milk apparently is only as effective as the testing program. Because cows are constantly being treated with antibiotics the incidence of these drugs in milk will be a reflection of the constancy of testing.

Ten years ago a survey of New York State milks indicated an incidence of antibiotic contamination of six per cent (4). A later nationwide survey in 1956 (7) showed a similar incidence. Relatively little progress has been made in the intervening decade toward significantly lowering the rate of incidence for a number of reasons.

One was the prevailing attitude that public health officials were not unduly concerned about the problem and were not insisting upon enforcement of adulteration laws concerned with chemical additives. This attitude underwent considerable revision when the position of Federal regulatory officials became better known to the industry in November, 1959 (2). Another equally important reason was that the dairy industry considered itself inadequately prepared to solve the problem. Education alone, through the written word to milk producers, was not accomplishing its objectives and direct testing of milk supplies was generally considered impractical. A quick detection test for antibiotics in milk was thought essential for control and no rapid test existed.

This writer has always considered the latter hypothesis invalid (3) because analytical laboratory methods currently available were sensitive and reliable, despite their three to six hour requirement for

completion. What was needed was the wide application of testing regardless of the time element, and a strong follow-up program by field inspectors or supervisors. Actually, some dairy laboratories and health officials, in a limited way, for some time have followed this course of action to their satisfaction.

Some months ago an opportunity was presented to test this point on a large scale and with new and extremely simple test methods. A field antibiotic test kit developed at Cornell University and requiring three to six hours for completion was supplied in large numbers to one fluid milk and cream New York State dairy firm in an attempt to eradicate its antibiotic problem. The principle of this test was recently published (5). Detailed records were maintained of about 4,000 farms and approximately 10,000 samples of milk were tested during this period. The present paper deals with the results of this carefully observed study and the efforts to reduce the incidence of antibiotics.

Methods

Analytical methods for antibiotic detection in the present study included mainly the Cornell field test, but at times when supplies of such tests were exhausted the standard disc assay laboratory test using whey agar (1) was substituted. In mechanical operation the two methods have much in common. Both use *Bacillus subtilis* spores as test organisms and both depend on clear zones around discs for evidence of positive inhibition. Finally, both exhibit the same degree of sensitivity, 0.05 I. U. penicillin per ml. or lower. To insure that positive zones were in fact related to pharmaceutical antibiotics, all milks tested were heated to 180° for 5 mins. according to earlier recommendations (6). As an additional check for the presence of penicillin, frequent use was made of penicillinase discs on positive cases. Milks generally were brought to central testing sites and during transit were maintained cold.

Farmers delivering milk to this large New York dairy firm were instructed through printed notices delivered to them and posted on the barn to retain all milk from treated quarters of the animal and not allow any to enter the general milk supply.

¹This work was supported in part by U. S. Public Health Grant No. E-2078.

Field inspectors for the company worked closely with the testing centers. Positive evidence of the existence of an antibiotic in a milk producer's supply initiated a visit by the field inspector to that farm within 24 hours. The test result was shown to the farmer and an explanation was asked regarding his positive milk. Other questions raised concerned how the cow or cows were treated, time of treatment, number of units in treatment and time when first milking from treated cow was put into the milk's supply. The farmer was informed of the consequences of antibiotic contaminations and precautions for avoiding future adulterations were carefully explained. A second offense carried with it the penalty of a three-day expulsion from the milk shed.

Results

A preliminary survey of a limited nature taken during the first period, November, 1959, showed an incidence of 6.5 per cent for producer can dairies and 5.1 per cent for producer bulk tank dairies, Table 1. This incidence was about normal for the United States in recent years.

In the second period of testing—January 1 to February 1—activities were resumed on a larger scale. A total of 1634 producer can dairies showed an incidence of 4.0 per cent and 158 producer bulk tank farms displayed 1.3 per cent, Tables 1 and 2.

Between the beginning of the first period and the end of the second period the field inspector had paid many visits to farms and had attracted the notice of a large segment of the producers in this dairy's milk shed. Through such visits and by word of mouth, producers were aware that antibiotic testing was now a steady program of this company.

The surveys for the third and fourth periods in the months of March and April were far more ambitious than for the previous two periods. All producers' milks were checked, at least once. The results for these periods showed a significant drop of positive samples. Of 3,054 producer can dairies tested in March, only 14 or 0.45% showed any evidence of antibiotics, Table 1. The concentrations of these positive dairies, milks were low, on the order of 0.05 I. U. penicillin per ml. milk. No positives were found among the 372 producer bulk tank farms, Table 2. On 3,491 producer can farms checked in April, a similar low incidence was observed; but two positive milks were found among 382 bulk farms. This was still tenfold lower than the percentage of positives at the beginning of the study.

Significant changes were also noted in the results from over-the-road tank truck milk destined for large metropolitan areas and from bulked tank milk coming from the farm, Table 3. After the testing program was in effect two months, only one bulk tank from

TABLE 1—THE INCIDENCE OF ANTIBIOTICS IN MILK FROM 8,379 NEW YORK PRODUCER CAN DAIRIES OVER A FOUR-MONTH PERIOD

Period (1959-60)	Number of farms tested	Number milks positive for antibiotics	Per cent milks positive for antibiotics
Nov. 4 - Jan. 1	200	13	6.5
Jan. 1 - Feb. 1	1634	66	4.0
Feb. 1 - Mar. 1	3054	14	0.45
Mar. 1 - April 1	3491	17	0.52

TABLE 2—THE INCIDENCE OF ANTIBIOTICS IN MILK FROM 1,050 NEW YORK PRODUCERS, BULK TANK FARMS OVER A FOUR-MONTH PERIOD

Period (1959-60)	Number of farms tested	Number milks positive for antibiotics	Per cent milks positive for antibiotics
Nov. 4 - Jan. 1	138	7	5.1
Jan. 1 - Feb. 1	158	2	1.3
Feb. 1 - Mar. 1	372	0	0.0
Mar. 1 - April 1	382	2	0.5

TABLE 3—ANTIBIOTICS IN MILK FROM 279 BULK TANK TRUCKS IN NEW YORK STATE OVER A THREE-MONTH PERIOD

Period (1960)	Number bulk tank trucks tested	Number milks positive for antibiotics	Per cent milks positive for antibiotics
Jan. 1 - Feb. 1	78	4	4.1
Feb. 1 - Mar. 1	93	0	0.0
Mar. 1 - April 1	108	1	0.7

TABLE 4—ANTIBIOTICS IN MILK FROM 256 "OVER-THE-ROAD" NEW YORK TANKERS DESTINED FOR METROPOLITAN AREAS DURING A THREE-MONTH PERIOD

Period (1960)	Number of over-the road tankers tested	Number of milks positive for antibiotics	Per cent milks positive for antibiotics
Jan. 1 - Feb. 1	53	2	1.9
Feb. 1 - Mar. 1	92	0	0.0
Mar. 1 - April 1	111	0	0.0

211 tested was positive, while milk from 203 large over-the-road milk tankers was negative to antibiotics during this same period.

DISCUSSION

It has been particularly gratifying to all persons involved in this study to note the marked improve-

ment in the eradication of antibiotic contamination among milk supplies after the installation of a basic testing program coupled with field inspector or supervisor follow-up.

This sharp reduction was made in the face of the facts that cows in this milk shed received antibiotics through a variety of channels, udder insertions, intramuscular injections, and through direct feeding. Also equally striking is the point that the incidence dropped more than fifteenfold despite the fact that the farmers were directed to retain milk only from treated quarters. Transfer of antibiotics from a treated to an untreated quarter may or may not be definitely proved from current research under way, but for this dairy firm such transfer, if any occurred, was not an important impediment to obtaining a good record.

Naturally, the present study has not indicated that this company's problems are solved. As long as cattle diseases are treated with antibiotics contamination problems will exist. Also, it is possible with new and more highly sensitive field tests now under development in the writer's laboratory to show an even greater incidence in this company's milk supply. But the survey has shown that through a proper test program, not necessarily dependent upon rapid tests, it was possible to reduce the problem significantly. Testing, however, must be steady and periodic, for as soon as testing stops the incidence will rise again.

Another factor for the marked success obtained by the present dairy company was the indirect psychological effect upon farmers and even upon suppliers of antibiotic preparations. Word of a steady test program in rural areas evidently did much to restrain producers from infecting their milk supplies with milk from treated udders and it even had a salutary effect upon indiscriminate sales of antibiotic prepara-

tions. One large feed dealer, correctly or incorrectly, withdrew all of his antibiotic-treated feeds during the period of testing.

Interestingly enough, the type of testing employed did not place any excessive burden on laboratory personnel engaged in testing. With simple field test kits no extra personnel was required as 400 to 600 samples of milk could be tested daily. Obtaining milk samples and their required heating were delaying factors in testing but heating of milks removes many false positives and thus is an extremely valuable adjunct to testing. This point was first brought up for attention in 1952 (6).

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NEWS AND EVENTS

QUESTIONS AND ANSWERS

Note: Questions of technical nature may be submitted to the Editorial Office of the Journal. A question in your mind may be in the minds of many others. Send in your questions and we will attempt to answer them.

QUESTION:

Can the membrane filter technique be used for the estimation of coliform bacteria in ice cream?

ANSWER:

According to Nutting, Lomot and Barber (Appl. Microbiol. 7: 196-199, 1959) membrane filtration, in conjunction with the use of warm dilute Triton X-100, provides a direct one day method for the enumeration of "true" coliform bacteria in ice cream. They also reported that this method eliminates interference from sucrose and false positive reactions.

QUESTION:

Is there a rapid test for detecting *Staphylococcus aureus* in food?

ANSWER:

A method has been developed which allows detection of *S. aureus* in food within a 7 to 9 hour period. Wilson, Foter and Lewis of the Robt. A. Taft Sanitary Engineering Center, U.S.P.H.S. Cincinnati, have described a method whereby a selective culture technique in brain heart mannitol salt broth has been combined with the growth accelerating effect of agitation in air during incubation. Following incubation of 4-6 hours at 35°C. on a shaking machine, the broth culture is used for a gram stained preparation and for the coagulase test. Experimental data indicate that this rapid test permits detection of *S. aureus* well below the level cited as the threshold for the production of enterotoxin to induce symptoms in man.

QUESTION:

How does ultrasonic cleaning work?

ANSWER:

Electrical energy is converted to ultrasonic energy by passing alternating current around a stack of thin nickel sheets, or a transducer. The transducer vibrates mechanically in sympathy with the electrical impulses to produce ultrasonic energy, or sound too high in frequency to be audible. These silent sound waves are directed into a liquid detergent bath where they cause activity known as cavitation or "cold boiling." Tremendously effective cleaning power comes from energy released by the implosion of millions of tiny bubbles as sound waves pass through the bath. Contaminants are literally torn from surfaces, as the violent internal scrubbing motion of the bath reaches into microscopic holes and recesses.

WISCONSIN REGULATION ON GRADE A NON-FAT MILK SOLIDS IN EFFECT

On December 1, 1959, Order Ag. 86, promulgated by the Department of Agriculture, became effective. This Order is the Supplement No. 1 to the 1953 Recommendations of the Public Health Service Milk Ordinance and Code, for Grade A powder used in Grade A pasteurized milk products. This program is similar to the Grade A fluid milk program as far as the supervision and the certification are con-

cerned. Before a milk plant can be certified as meeting Supplement No. 1, a structure of supervision will have to be provided. The Department of Agriculture has provided this structure of supervision for milk plants that are interested in going on the program. When the minimum requirements have been conformed to, a survey for certification is conducted by the State Board of Health to determine the effectiveness of enforcement on the official and plant level. To date, four milk plants have been certified as complying substantially with the requirements of Supplement No. 1 to the Milk Ordinance and Code, 1953 Recommendations of the Public Health Service for Grade A non-fat solids used in Grade A pasteurized milk products. The plants that have presently been issued Wisconsin Grade A permits for non-fat solids are as follows: Turtle Lake Coop. Creamery Assn., Turtle Lake, Wisconsin-P1. Consolidated Badger Coop., Appleton, Wisconsin-P2. Galloway West Company, Fond du Lac-P3. Pabst Farms Incorporated, Oconomowoc, Wisconsin-P4.

In view of the fact that adequate Grade A non-fat solids for pasteurized milk products are now available, the State Board of Health, in its certification program, will debit the enforcement agency and the pasteurization plant where other than Grade A non-fat solids are being used, as of September 15, 1960. All official agencies in Wisconsin are being given notice of this through an official memorandum. The purpose of the Grade A program in fluid milk and powder is to provide the best possible product which can be afforded to the consuming public.

FDA ISSUES NEW ORDER ON ICE CREAM COMPOSITION

Federal standards for the composition and labeling of ice cream and other frozen desserts were announced recently by the Food and Drug Administration. The standardized products are: regular ice cream; egg-containing ice cream sold as frozen custard, french ice cream, or french custard ice cream; ice milk often sold as soft ice cream; fruit sherbets; and, water ices.

The standards, FDA said, should assure that the purchaser will be able to select the product of choice. They establish firm minimum requirements and provide a sound basis for proceeding against products cheapened by the omission of expected ingredients or the substitution of inferior ingredients.

The standards provide safeguards against practices which would result in adulterated products, and permit only ingredients of generally recognized or proven safety, FDA said.

In addition, the standards for ice milk, fruit sherbets and water ices should prevent these products from being represented to consumers as ice cream, or as having greater food value than they actually have, the agency said.

For assurance of nutritive quality, the standards require (with certain specified exceptions) that ice cream and french ice cream contain at least 20 percent by weight of milk solids, of which at least 10 percent must be milk fat. Frozen custard or french custards or ice creams must also have a specified amount of egg yolk solids. Parallel requirements for the other frozen dairy products are:

Ice milk—Not less than 11 percent of total milk solids, of which not less than 2 percent and not more than 7 percent are milk fat.

Fruit sherbets—Not more than 5 percent total milk solids content, of which not less than 1 percent and not more than 2 percent are milk fat.

For protection against cheapening ice cream, the standards restrict the addition of air and water to the ice cream mix. To prevent excessive aeration, ice cream is required to weigh at least 4.5 pounds per gallon. To prevent adulteration by addition of water, the standards require ice cream to have a total solids content of not less than 1.6 pounds per gallon. To prevent possible adulteration with permitted stabilizers, the standards restrict the use of any one or a combination of stabilizers to not more than ½ of one percent of the finished ice cream. (Stabilizers, in addition to retarding formation of ice crystals in stored ice cream, also affect the capacity of a mix to hold air, make ice cream stiffer, drier, and slower melting, and provide smoothness.)

The standards do not permit neutralizing agents which would make it possible to use sour dairy ingredients.

The new order conforms with the tentative ice cream standards published March 26, 1958, with these principal exceptions:

USE OF ARTIFICIAL FLAVORINGS

The flavor of vanilla ice cream may be supplied by natural vanilla, artificial vanilla (vanillin), or a blend. The proposed standards published in March, 1958, did not provide for the use of artificial flavoring in ice cream characterized by vanilla beans or vanilla extract. This was a major basis of industry objections.

The addition of natural or artificial flavors to fruit sherbets is also permitted by the order, although not contemplated by the former proposal. Whether or not artificial flavor is used, the fruit sherbets must contain specified proportions of fruits.

The package labels of ice cream and fruit sherbets

are required to inform consumers of the use of any added flavorings whether natural or artificial, except that where a mixture of both natural and artificial flavors so used, and the artificial flavor predominates, only the artificial flavor may be declared.

NEW PERMITTED INGREDIENTS

Casein and casein compounds made from skimmed milk have been used to avoid "sandiness" caused by the crystallization of milk sugar in high-solids ice cream products. The current order permits their use on condition that they do not replace any part of the required minimum of 20 percent total milk solids in ice cream.

While not a part of the published standard, the agency also announced simultaneously a new regulation under the Food Additives Amendment to the Federal Food, Drug, and Cosmetic Act authorizing the use of two polyoxyethylene type emulsifiers in ice cream. Based on the record of the ice cream standard hearings held in 1951 and 1952, FDA originally opposed use of these emulsifiers on the ground that their safety had not been established. FDA said, however, that extensive data submitted in petitions under the Food Additives Amendment are convincing that these two ingredients will be safe in the amounts permitted.

Persons adversely affected by the regulation allowing the use of these emulsifiers have 30 days after publication in which to file objections and request a public hearing, the agency noted.

Today's standards for frozen desserts go into effect on October 25, 1960, unless stayed by Court action.

PENNSYLVANIA COMMITTEE SETS FORTH SOME GUIDE LINES ON PLASTIC HOSE

At a recent meeting of the Pennsylvania Dairy Sanitarians Association, the Farm Practices Committee gave a report on the use of Plastic hose used in handling milk on the farm. The Committee's recommendations and comments were made after a study of replies on this subject received from various colleges, agencies and manufacturers in the Northeast.

From the New York State Association of Milk Sanitarians who studied this subject in 1959, the following recommendations were stated by the Pennsylvania group.

Non-rigid transparent plastic tubing cannot be expected to last indefinitely. The user should be aware that ultimately the tubing must be replaced.

Accepted, transparent tubing may be used to transport milk to the milk house. The tubing must be washed in the milk house and this system must meet

applicable requirements (for the cleaning of) auxiliary equipment.

Applicable items under cleaning and procedure shall apply to non-rigid pipelines. In addition, means must be provided to render the tubing essentially dry during storage. Such lines need not meet the requirements for hangers and slope but must be protected during use and storage from interior contamination and must not contact the floor.

The Pennsylvania Committee followed in its report by giving the following information.

Plastic tubing is judged for acceptability by the following criteria:

1. All surfaces must be visible for inspection
Reason: (a) Burrs may not be used because of possible damage to interior surface.
(b) Darts cannot effectively remove hard deposits for inspection.
(c) It is not practical to inspect long sections of flexible tubing by sighting through the bores.
2. Opaque tubing must be rendered transparent for inspections. Physical treatment may restore some opaque plastics to transparency. Such plastic, if its surfaces are essentially smooth and free from crevices, shall be acceptable. If transparency cannot be restored and/or the inside surfaces are visibly cracked, roughened, softened, or coated, the tubing shall not be used to transport milk. Tubing is considered transparent when the outline of of a pencil or other small object can be clearly discerned through the diameter of the tubing.

In order to obtain information on the use of plastics a questionnaire was sent to the members of the Farm Practices Committee.

A summary of the findings from the 39 questionnaires is as follows:

1. Plastic hose is permitted to be used in handling raw milk in the various areas of Pennsylvania.
2. In every instance plastic hose for handling raw milk may be under ten feet. There were no replies showing longer lengths were permitted.
3. Thirty-three of the questionnaires revealed plastic hose used on the milking machine while the remaining 6 had plastic on the milking machine and conveyors.
4. In response to cleaning the non-rigid line, 3 answered, circulation cleaning; 33 by brushing; 2 by rodding and, one by rinsing.
5. In sanitizing, 9 used quaternaries, 9 used chlorine and 21 used iodine compounds.
6. The inspection for cleanliness was a combination of transparency, 27; appearance 39 and smell, 13.

7. In seeking the answer to the question "How long has the plastic hose been in use on the farm," 8 replied under 6 months, 27 answered 6 months to one year, and 3 gave the reply, one to two years and one answered over two years.

8. Selected comments that were offered on the use of plastics are as follows:

More acceptable in warm than in cold weather. Transparency difficult to maintain over a long period of time.

Iodine stained heavy hoses need to be replaced. Circulation cleaning is preferred.

High temperatures, over 160°F. and detergent sanitizers will discolor plastic.

Detergent and sanitizer stains may be bleached by storing hose in sunlight.

Plastic air hose gives indication of milk in vacuum lines.

Plastic ends begin to crack sooner in cool weather.

Have difficulty in assembling and disassembling unit with flange on milker pail cover and claw.

Plastic is subject to 'stone' formation when sodium hypochlorite is used as a sanitizer. Transparency, while it lasts, appears to be an advantage.

Plastic is non-porous, resists the action of fats and wears longer than rubber hose.

The commentary contained in this report may stimulate others to add to the findings. Any of our members, having had experience with plastic hose used in farm milk production, are invited to write the editor or to contact the Secretary of the Pennsylvania Association.

RESEARCH SHOWS BENEFIT OF FLUORIDATED MILK

A University of Louisiana research team has found that fluoridated milk, like fluoridated water, will help prevent tooth decay in children.

Dr. Louis L. Rusoff, a nutritionist, told the International Congress of Nutrition that youngsters who drank fluoridated milk had seventy-six per cent fewer cavities than those who drank the non fluoridated product. The benefit showed in the permanent teeth which erupted after the children began drinking the fluoridated milk. The addition of the chemical, in very minute amounts, doesn't change the taste, color or odor of the milk.

Dr. Rusoff reported that fluoridated milk could be useful in areas not having fluoridated water supplies although it is not advocated as a substitute for the fluoridation of public water supplies.

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PROPOSED MODEL ACT FOR THE REGISTRATION OF SANITARIANS

Elsewhere in this issue of the JOURNAL, will be found a proposed sanitarian's registration act. This proposal is the result of deliberations of the *Sanitarian's Joint Council*. After a period of three years and numerous revisions, the Council reached agreement on this Act at its meeting in Washington, June 18, 1960.

The membership of International should read and study this Act. The Council believes it incorporates desirable features to be included in an act of this kind. It is recognized that certain changes and additions may have to be made within the frame work of the laws of various states. However, the act in question should serve as an excellent foundation upon which to build and it can be used as a guide.

Some eighteen states now have legal registration for sanitarians and undoubtedly in other states whose legislatures will meet in 1961, there are Sanitarian Associations who may wish to introduce such an act. With proper legal guidance the proposed model should form the basis for a movement toward registration in those states not now having this legislation.

At the forthcoming annual meeting of International to be held in Chicago in October, the membership will be asked to approve this Act as the one to be recommended to affiliates and others who may be interested.

CONGRESS MAKES APPROPRIATION FOR EXPANSION OF PUBLIC HEALTH TRAINING

During the congressional session just closed, an appropriation of two million dollars was made to enable the Surgeon General of the Public Health Service to make project grants to schools of public health, and to those schools of nursing or engineering which provide graduate or specialized training in public health for nurses or engineers. The purpose of the appropriation and subsequent grants from it will be strengthen or expand graduate public health training through such authorized schools.

The appropriation in question is over and above monies appropriated in 1956 as an amendment to Title I of the Public Health Service Act which set up funds for the graduate training of public health personnel, particularly as a stimulus for new people to enter the field.

The new appropriation will enable the schools in question to expand their academic and training program by appointing new personnel, expanding teaching facilities and laboratories, exploring new areas for public health training, engaging specialists and consultants and otherwise improving curricula to meet new and expanding public health needs.

All projects submitted are subject to review and evaluation by the National Advisory Committee on Public Health Training. Meeting periodically in Washington, this Committee is empowered to make recommendations to the Surgeon General on the soundness and practical value of each project and only those which are found to meet certain specified criteria will be certified to the Surgeon General. Currently on hand are eighty-two proposals which will be reviewed late in September by the Advisory Committee.

THE UNIVERSITY OF GEORGIA ADDS CURRICULUM IN SANITARY SCIENCE

A new four-year course designed to train personnel for the fields of milk, food, environmental and general sanitation is being offered at the University of Georgia. It is a revision of a former program followed by the majority of those going into the positions of sanitation or sanitary engineer in the area. More subjects dealing specifically with sanitation are included, and at the same time a wider choice of sup-

porting science courses from many parts of the institution is allowed.

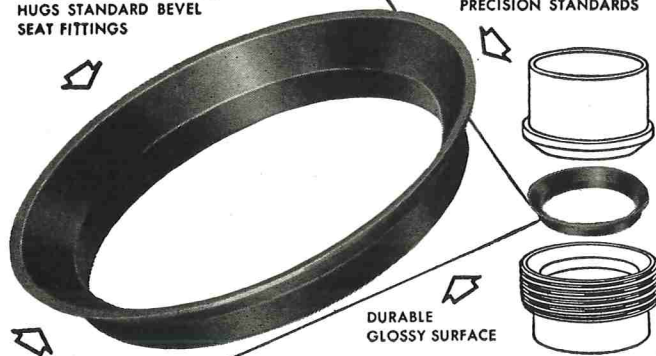
The first two years include largely the subjects which are more or less standard in junior colleges and universities. Included are inorganic and organic chemistry, botany, zoology, English, physics, algebra, American history, physical education, economics and agricultural electives.

The required studies in the third and fourth years include physiological chemistry, dairy chemistry, general microbiology followed by dairy, food, and pathogenic bacteriology, immunology, milk and dairy products technology, general entomology, medical entomology, statistics, dairy plant management, engineering drawing, public administration, personnel administration and administrative law. Electives may be chosen from many parts of the University; such as, milk and food sanitary regulations, food processing, health education, foods and nutrition, institutional management, heating and refrigeration, food plant engineering, environmental sanitation and water supply, soil and water engineering, surveying, veterinary public health, virology, veterinary hygiene, veterinary micro-

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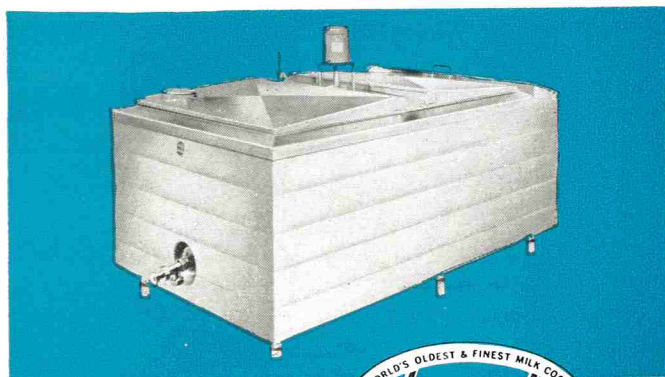
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ALDO E. NESSLER

Word has just been received of the death on September 1 of Al Nessler, Kraft Foods Company, who was a prominent member of the 3-A Sanitary Standards Committees. In view of Al's long and noteworthy participation in the 3-A work, it seems highly appropriate that we pause to reflect on his passing.

Al had represented the National Cheese Institute on the Sanitary Standards Subcommittee of the Dairy Industry Committee since the very early formative days of the 3-A program. In addition he was representative of the Dairy Industry Committee on the 3-A Symbol Administrative Council.

Probably Al Nessler is best remembered as a strong pillar of the User group. Not a highly vocal participant, Al was characterized by terse but quiet statements which commanded the attention and respect of all. He frequently did not inject himself until discussion seemed at an impasse, at which time a few words from Al seemed to throw a whole new light on the subject. To say that he will be missed from future gatherings of the 3-A group is but an inadequate expression of his importance to the program.

There was a request for no flowers at Al's services. His family has indicated that those wishing to do so may make contributions to St. Mathews Episcopal Church, Evanston, Illinois, in his memory.

BACK SIPHONAGE DEMONSTRATION PLANNED FOR ANNUAL MEETING

Jim Meany of the Chicago Board of Health has scheduled three lecture demonstrations on "Back-Siphonage" on plumbing fixtures and farm water supplies. These will be at the Chicago Plumbing Laboratory on Tuesday the 25th at 10:00 A.M., on Wednesday the 26th at 9:00 A.M. and 11:00 A.M. At the time of registration a card will be given showing location and how to proceed.

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WORLD HEALTH ORGANIZATION NOW IN TWELFTH YEAR

The First World Health Assembly met in Geneva, Switzerland in June 1948. The creation of WHO in 1948 was the culmination of a long series of efforts made over the centuries to prevent the spread of disease from one continent to another and to achieve international cooperation for better health throughout the world. During the latter part of the 19th century, the United States of America began to participate in international programs directed primarily at reaching agreements on methods of preventing cholera and other plagues from spreading from nation to nation. These meetings culminated in a meeting in Paris in 1903 where a convention was drawn up which consolidated earlier agreements and set up the first effective and comprehensive international pattern for foreign quarantine. The concept of international cooperation in controlling and preventing diseases grew out of these early sanitary conferences. Gradually these international health programs were broadened, and the International Office of Public Health was established in Paris in 1907.

The first major step toward international cooperation in the field of health came in 1920 when the League of Nations first created an Epidemic Commission and, subsequently, a health organization that concerned itself not only with major diseases but also with studies on nutrition, housing, and the standardization of therapeutic substances.

When the Charter of the United Nations was drawn up in San Francisco in 1945, Brazil proposed that *health* be included in the United Nations charter as one of the essential factors for international peace and stability. An international health conference held the following year in New York drew up a constitution for the new organization. Before the interim commission that launched the work of the WHO could be replaced by a permanent organization, it was necessary for 26 member nations of the United Nations to approve the constitution. It was ratified by the required number of countries on April 7, 1948. That date, on which the WHO constitution came into force, and which established WHO as a permanent agency within the United Nation's orbit, has since been observed throughout the world as World Health Day.

Size Of Problem

Of the estimated 2,500,000,000 people alive in the world today, about two-thirds are struggling under conditions that make sickness and undernourishment the normal state of life. Much of this illness is chronic, cumulative, and progressive.

The per capita income of people in the economical-

ly under-developed areas of the world averages one-tenth that of income in developed countries and their expectation of life is only 30 as against approximately 70 years. Death rates in economically under-developed countries are estimated to be as high as 30 deaths per 1,000, whereas in the economically more developed nations they have been reduced to 10 per 1,000 or below.

The number of sufferers from some of the most common, controllable diseases approaches and even exceeds the total population of the United States of America. For example it is estimated that:

- . . . 600 million people are chronically infested with round worms
- . . . 200 million people were stricken with malaria as late as 1958
- . . . 100 million have schistosomiasis — a disease spread by snails
- . . . 80 million people have yaws — open sores that cover the body
- . . . 5 million people die each year of tuberculosis

These and many other preventable diseases exact a fearful toll in human lives, suffering and economic loss. More and more governments are realizing that the loss and misery need not continue and are turning to the international agencies for assistance in building efficient health services.

Some Basic Functions

WHO'S most important function is to provide advisory services that help countries strengthen and improve their own health services through assistance in 1) *attack on specific diseases*, 2) *strengthen of national health services*, and 3) *training of professional personnel*.

Provision of international aid rests on three conditions:

- 1) that the government of a country shall itself ask for help
- 2) that work started with international cooperation shall be continued nationally; and,
- 3) that the aid requested can be fitted into the programs of the international agencies.

WHO also provides essential Central Technical Services from which all countries benefit. These Central Technical Services include among many other services 1) *epidemiology and health statistics*, 2) *standardization of drugs*, 3) *promotion of research*, and 4) *reference services*.

Education And Training Stressed

The great need of the economically under-developed countries is for more health workers of all kinds. In one country, with a population of 3,500,000, there are only six persons trained in public health work. In India there are three hospital beds available per 10,000. In Burma, the ratio of inhabitants per physi-

cian is about one physician per 10,000 persons, while in French West Africa and Indonesia it is one physician per 50,000 persons.

Shortages of health personnel — whether doctor, nurse, sanitary scientist, laboratory workers, or others — exist in every country in the world. WHO helps countries develop their training schools and teaching staffs.

Most of the economically under-developed countries do not have the facilities for training health personnel. From a long-range viewpoint, it is most practical and economical to help these countries develop their own national or regional training centers, but until this can be accomplished, the under-developed nations use the educational facilities of other countries. WHO has awarded more than 5,000 fellowships for international study to health personnel from 70 countries. Currently about 900 are trained annually.

On-the-job training is another device that has been used extensively in international health programs. This method enables trainee to work side by side with WHO experts and thereby insures the continuance of the programs after the WHO representatives have been reassigned to other projects.

What has happened in India is an illustration of the medical usefulness of this program of cooperation of international agencies with the various nations. In 1946 there was one doctor to 30,000 in towns and cities and one doctor to 75,000 people in the rural areas. In 1954 the number of medical colleges had increased from 16 to 34 and the number of students and health personnel had increased from 1,800 to 3,000. Instead of 1,000 nurses a year, the number trained rose to 1,650.

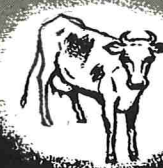
Health And World Stability

The present unbalance between prosperity and improverishment is a constant source of human suffering; it is also a threat to the stability of the world. Through active participation in international health activities, the nations of the world are promoting peace and strengthening their own economics. International health also contributes directly to political stability, an important factor in the ready access to critical materials from the economically under-developed nations.

The benefits of world health in the advance of mankind are not confined solely to the economically under-developed countries. As the less favored nations begin to raise their standards of living so will the market for the goods of the technically advanced communities steadily expand.

The strengthening of the economics of these nations also represents a material saving to the taxpayers of the more favored nations. The control of malaria, is an example. The hidden malaria tax on such items as coffee, tea, tin, rubber, and oil is enormous. With the use of new insecticides and other malaria control measures costs steadily decline.

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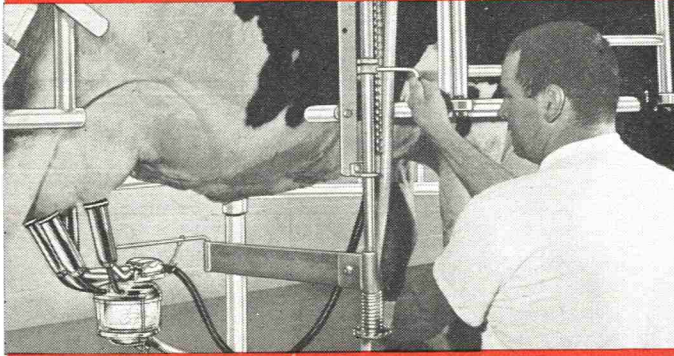
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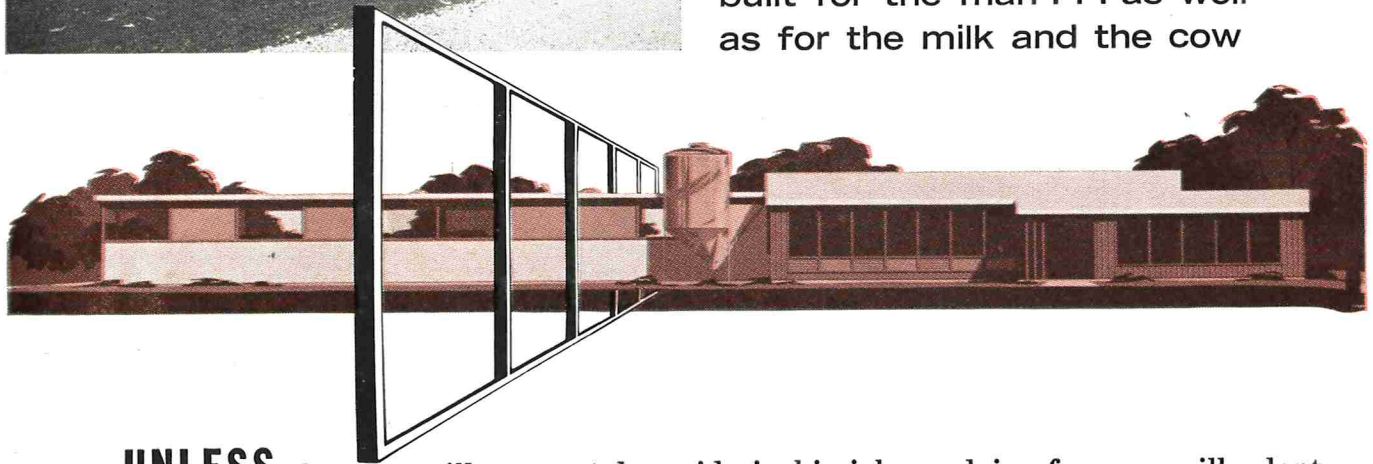
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