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Editorial

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Milk and the National Defense

As we engage in the immediate task of strengthening our defenses, we may be inclined to think entirely in terms of airplanes, naval craft, guns, and other such armaments. Without doubt, these are necessary. But there are other weighty considerations about which we do not seem to hear much, if anything. These are concerned with the morale and strength of the people themselves.

An epidemic in a community may seriously handicap the defense program. The recent series of sit-down strikes demonstrated that when an important power house or some key plant that produced a necessary part was closed down, a huge industry stopped production. We recall the case where an epidemic in a manufacturing town necessitated the closing of a large factory because so many of the employees were sick. As new plants are erected in what are now open fields, as great numbers of workmen create correspondingly new communities, and as the tempo of production is stepped up, there must be an attendant creation of an adequate and safe food supply. As our Dr. Jones colorfully says, "Where they're rushing night and day turning out airplanes and ships and guns and food and what not—stuff that's needed to meet an emergency—well, it don't take any great imagination to see what a handicap it (an epidemic)'d be . . . An epidemic's an emergency in itself and one emergency at a time's about enough . . ."

Military centers themselves are one of best (!) breeders of epidemics. Dr. W. S. Leathers, President of the American Public Health Association, states, "The protective measures of general sanitation around army camps and cantonments can be greatly facilitated by thorough coordination between the state and local health agencies and the military population. Well prepared and experienced sanitation officers are required in the enforcement of regulations, in providing a pure water supply, in the proper supervision of milk, and in the safe disposal of sewage. A careful inspection of food supplies to prevent contamination, and general cleanliness of the vicinity in cooperation with the officials of military camps must be strictly enforced."

But the situation has still another important aspect. Dr. George W. Cox, Texas State Health Officer, says:

"Defense in its broadest sense means security. It is a great satisfaction to have a feeling of security against foreign aggression, internal revolution, unemployment,

need, hunger, and communicable disease. We treasure freedom of speech, freedom of action, and freedom to exercise initiative. The whole defense program means more than airplanes and armament—it includes man power whose development has been so guided that they can meet and survive the pressures brought upon them whether in war or peace. It includes food, clothing, shelter, drugs, and thousands of articles from mine and field. . . .

"Some of our statesmen fear disturbance from within our borders more than aggression from abroad. These statesmen are far-seeing men who have looked down into the mass of humanity and recognized these individuals who, through poor hygiene, disease, and undernourishment have become warped either mentally, emotionally, or physically. . . . If relatively simple diseases such as cold and hayfever may bring about bad tempers, how much more may chronic illness, malnourishment, and starvation lead the individual to react violently to his surroundings. We must maintain a high morale in our home population. . . .

But we have more than our present "home fires" to consider. New fires are being lighted as industrial centers spring up and flourish. Our former president, Mr. V. M. Ehlers, writes: "There is work to be done by the milk sanitarians—milk to be provided for the new centers of population that are being created, milk to be properly supervised to keep down disease, milk for the young, and milk for the building of man power."

These new centers of population, arising out of our increased defense program, will require the opening of new milk-producing territory, the employment of new help, the erection of new plants. When such enterprises are undertaken in a hurry, there is a tendency for important but unrecognized principles of public health to be neglected. A corollary of such a development would be the diversion of necessary milk supplies from a given community to some large cantonment or other newly constructed series of plants or community.

Will the new payrolls be used entirely for the purchase of new cars, new radios, and new clothes—or will the necessary part go to providing the family table with not only the proper quantity but also the proper quality and variety of food? Unless there is an effective educational program for these groups who have suddenly come into greater prosperity than they have heretofore enjoyed, it is probable that current nutritional deficiencies will not be corrected. With such a background to the faster tempo of living with its accompanying increased output of work, we might expect decreased resistance to disease, an undermining of morale, and a curtailment of production.

So we see that national needs are facing us. We should tighten our defenses against the inroads of epidemics. We should improve our nutritional health, so to speak. We should provide for a safe supply of milk to the new communities, and also to the present ones now well supplied. We should educate the public on the necessity of buying the proper food. In addition to these, we should provide the government with information as to what type of training should be given the officers of the sanitary service of the army and the other officials who will be responsible for administering the newly established communities.

To insure that the above aspects of the defense program get off to a good start with a minimum of misdirection, we suggest that the President of the INTERNATIONAL ASSOCIATION OF MILK SANITARIANS appoint a Committee on Defense. This committee would make the necessary contacts with governmental and other professional and civic agencies concerned, to the end of marshalling our technical knowledge for the prevention of epidemics from infected milk, for supervising the production of a safe supply, and for educating the public on the health value of an adequate diet.

J. H. S.

A Presumptive Test for the Oral Contamination of Drinking Utensils

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INTRODUCTION

For some time it has been obvious that a concerted effort to raise the general level of the sanitary conditions of public eating establishments would be in the best interest of the public. Not the least in importance among the many phases of this problem has to do with the cleaning and sterilizing of drinking utensils. This phase of eating establishment sanitation is of particular importance due to the direct oral contact between the glass and its user, and the possible spread of disease through the subsequent use of an unclean glass.

Little has been accomplished on the perfection of direct or even presumptive methods for the determination of oral contamination of drinking and eating utensils. That tests for specific pathogenic organisms have not been developed for a direct control procedure is not surprising. The examination for such organisms on drinking glasses is difficult, and as such, these procedures could not be used in a practical manner as an index of contamination. Inasmuch as a parallel condition probably exists between the problem of drinking-glass sanitation and presumptive or indirect tests for pollution of water, a presumptive test for oral contamination of drinking glasses would be of significance. For this reason the present investigation had as its object the development of procedures which might serve as indexes of oral contamination of drinking utensils.

PREVIOUS INVESTIGATIONS

The influenza epidemics that developed during the World War stimulated one of

the first investigations of the possible role of eating utensils as vectors in disease contamination. The reports of Cumming and Lynch (1) and Cumming (2) directed attention to the importance of adequate sanitization of eating and drinking utensils.

A sub-committee of the American Public Health Association was appointed in 1935 to study the problem of dishwashing. The report of this committee (3) contained recommendations for the inspection and also proposed a standard procedure for bacteriological examination of eating utensils. The proposed standard procedure limited the maximum number of bacteria to five hundred organisms per surface area of each utensil. Concurrent with this development, many state and city health departments have established various standards on the basis of the number of bacteria present (4). The lack of standardization of methods and unification of standards has contributed to the present confusion in the sanitary control of eating and drinking utensils. Attempts to standardize procedures and establish a uniform standard for an allowable number of bacteria per unit utensil area have met with little success, chiefly because the "total bacterial count" gives inadequate information from which to establish the sanitary condition of eating and drinking utensils.

Until the recent work of MacNabb (5) and Marden *et al.* (6) little attention has been given to qualitative bacteriological methods as indexes of the sanitary condition of eating utensils and

to methods of freeing such utensils from pathogenic microorganisms.

PROCEDURE FOR THE DETECTION OF ORAL CONTAMINATION OF DRINKING GLASSES

During the progress of the present investigation, the following procedure has been developed as a test for oral streptococci found on lips or on the rims of drinking glasses.

An ordinary test tube containing a small, tightly wound cotton swab made on a 7-inch wood applicator which extended through the cotton plug of the tube, was sterilized at 15 pounds pressure for 15 minutes. Prior to taking the sample, the swab may be moistened in sterile broth; however, if the glass to be examined is moist, this step can be eliminated. Samples were taken by swabbing three times around the inner and outer rim of the glass approximately one-half inch from the rim, using a slow rotary motion. The swab was then placed in a test tube containing an enrichment medium (10 ml. of 1 percent lactose veal infusion broth containing crystal violet in a concentration of 1-400,000, adjusted to pH 7.6), and incubated for 24 to 48 hours at 37° C.

The type of growth which is important was observed at the end of 24 hours incubation. A flocculent growth attached to the swab proved through many subsequent confirmations to be a routine indication of the presence of streptococci. For

confirmation, a microscopic preparation was made and stained by the Gram method. Tubes containing a Gram-positive streptococcus were plated on a veal infusion agar containing 0.8 percent horse blood, and incubated for 24 hours at 37° C. Typical streptococcal colonies showing alpha to gamma hemolysis were transferred into litmus milk or any other suitable stock medium and studied for fermentation characteristics.

Many tubes were found upon microscopic examination to contain Gram-negative rods. In such instances the incubated broth was plated on Endo medium as a confirmatory procedure. In some instances Gram-positive rods were found but no effort was made to determine the species to which they belonged.

Of particular significance in the incubation of swabs is the *type* of growth which appears when inoculations have been made from drinking glasses known to carry an oral contamination. The characteristic growth of streptococci in these tubes serves as a most acceptable presumptive index of the presence of oral streptococci.

THE PRESENCE OF *Streptococcus salivarius* ON HUMAN LIPS

Early in the investigation it was felt that if a study was to be made of oral contamination of drinking utensils, information should be available on the type of organisms which predominate on hu-

man lips, hence the lips of 100 individuals selected at random were examined. The outer portion of the closed lip was swabbed with moistened swabs and the organisms present were determined by the procedure outlined above. Of 100 individuals (Table 1) it was found that all carried streptococci on the outer portions of the lips. The 340 strains isolated from the various lips appeared to be of one type. All of the strains failed to produce acid from mannitol and sorbitol, and practically all reduced litmus subsequent to the curdling of the litmus milk. The fermentation of raffinose and trehalose was somewhat variable. With this information at hand, it would appear that these streptococci were all of the *Streptococcus salivarius* Andrewes and Horder type.

No information was obtained as to the relative numbers of *Streptococcus salivarius* on the individual lips. The use of the selective enrichment medium precluded making estimates of this type. From these data it is evident that the lips of practically all individuals carry streptococci identified as *Streptococcus salivarius* or closely related types.

THE DEPOSITION OF *Streptococcus salivarius* ON THE RIMS OF DRINKING GLASSES

The mere presence of *Streptococcus salivarius* on human lips is of little significance insofar as a study of contamination of drinking glasses is concerned unless they are deposited on the rims during the use of the glass. To determine whether these organisms are so deposited, a study was made (Table 2) of 100 controlled cases in which an examination was made of the glass before and after use. The procedure followed was to sterilize glasses, and offer the test subjects a glass filled with a sterile liquid. Swabs were taken of the lips prior to drinking and immediately following from the rim of the glass. The procedure is outlined above.

Without exception in these 100 controlled cases the user of the glass deposited streptococci on the rim. One hun-

dred and seventy-one strains of streptococci were isolated from the rims of these test glasses immediately following use. A study of these streptococci indicated that they were approximately of the same type (Table 2) as the streptococci which were earlier found during the survey of the human lips.

If, as would be indicated from the above results, all individuals carry *Streptococcus salivarius* on the lips, and if during the process of drinking some of these organisms are deposited on the rims of glasses, the presence of *Streptococcus salivarius* on drinking glasses would serve as a most satisfactory presumptive index of oral contamination. The use of such a test will not be concerned with the number of *Streptococcus salivarius* present but rather upon the significant fact that any demonstrable number of these organisms, even though few, indicates oral contamination.

THE SURVIVAL OF *Streptococcus salivarius* ON DRINKING GLASSES

If the presence of *Streptococcus salivarius* is to be used as an index of oral contamination of drinking glasses, it is important that definite information be available as to the length of time that this organism will survive on the rims of drinking glasses, providing the utensil has received no washing or sterilizing treatment. A study of 160 drinking glasses which received no treatment after use (Table 3) indicated that *Streptococcus salivarius* will usually survive on the rims of drinking glasses at least for 48 hours when held at room temperature (22° C.). This was particularly true when the glass was used for dispensing water. In some instances, however, when the glass was used for dispensing milk, the streptococci appeared to survive for a somewhat shorter length of time.

From these results it would appear that the presence of *Streptococcus salivarius* on the lips of drinking glasses would indicate that it has been orally contaminated within 48 hours and has received little or no washing or sterilizing treatment.

TABLE 1
Presence of streptococci on human lips

Number Examined	Number of strains isolated	Persons carrying streptococci		Percent of strains fermenting				Percent of strains which reduced litmus after curdling litmus milk
		Number	Percent	Raffinose	Mannitol	Trehalose	Sorbitol	
100	341	100	100	65	0	52	0	99

TABLE 2
Presence of streptococci on rims of drinking glasses after use

Number of tests	Number of strains isolated	Persons depositing streptococci on glasses		Percent of strains fermenting				Percent strains which reduce litmus after curdling litmus milk
		Number	Percent	Raffinose	Mannitol	Trehalose	Sorbitol	
100	171	100	100	58	0	57	0	99

TABLE 3
Survival of lip organisms on drink glasses held at room temperature (22°)
GLASSES USED FOR DISPENSING WATER

No.	No. of glasses	No treatment after use							
		Time in hours							
1	8	S	S	S,GN	S,M	S,M	M	S	S
2	8	S	S,M	S	S,M	S,GN	S	S	M,S
3	8	S	S	S	S	S	S	S	M,GP
4	8	S,M	S	S	S	S	GNS	S,M	S
5	8	S	S	S	S,GN	M,S	S,GP	M,GP	M
6	8	S	S	S,M	S	S	S,M	M,GN	
7	8	S,GN	S,GN	S	S	S	M,GN	S,M	GN
8	8	S	S	S,M	M,S	GN,S	S,M	GP,GN	M
9	8	S	S	S	GN,S	GN	S,GN	GN,S	GN,S
10	8	S	S	S	S	S	S,GN	M,GP	GP,GN
Total	80								

No.	No. of glasses	Rinsed with cold tap water after use							
		Time in hours							
1	8	S	S	S	S,GN	M	S,GN	M,S	M,GP
2	8	S,GN	S	M	S,M	S,GN	M,GP	GP,GN	S,M
3	8	S	S	S,GN	GN	S,M	S	M,GP	S,GN
4	8	S	S,M	S,M	GN,S	SN,M	M,GN	M,GP	M,GP
5	8	S	S	S	S,M	S,GN	S	M,SGP	GP,GN
6	8	S	S	S	S,GN,M	M,GN	GN,M	GN,GP	S
7	8	S	S	GN,S	S,GN,M	S,GP	S	S,M	M,GP
8	8	S	S	M,GN	S	S,GN	S	M,GP	GP,GN
9	8	S	S	S,M	M,GN	S,GN	M,GP,GN	S	S
10	8	S	GN,S	S	S,GN	M,GP	S	GP,M	GP,M
Total	80								

No.	No. of glasses	GLASSES USED FOR DISPENSING MILK							
		No treatment after use							
1	8	S	M,S	S,M	S	GN,S	S	M	M,GN
2	8	S	S,M	S	S	S	M	GP	M
3	8	S,M	S	M,S	S	M	S,M	M,S	M,GP
4	8	S	M,S	S,M	S	S	S	GN	
5	8	S	S	S	S	SN	S	M,S	
6	8	S	S	M,S	S	S	GN,SM	M,GN	
7	8	S	S	NG,S	M	S	M	M	
8	8	S,M	S,M	GN	M	GN,S	GN	M	
9	8	S	M,S	S,M	GN,S	S	S	M,GP	
10	8	S	S	S,GP	GP,GN	GN,M	S,M	GN	
Total	80								

No.	No. of glasses	Rinsed with cold tap water after use							
		Time in hours							
1	8	S	S	M,GN	S	M	GN,S	-----	-----
2	8	S	M,S	S	M	GP	S	-----	S
3	8	S	S	M	S	S,GN	S	M	-----
4	8	S	M,S	M,GN	S	S	M	-----	-----
5	8	M,S	S	S	M	GP	GN,M	M	-----
6	8	S	GN,S	M,GP	S	S,M	S	-----	-----
7	8	S	M,S	S	M	GP	S	S	-----
8	8	S	S	GN,M	M,GN	M	M,GN	-----	-----
9	8	M,S	M,S	S	GN,GP	S	GN,S	-----	-----
10	8	S	S	GP,GN	M,GP	GN,S	GN,S	M	-----
Total	80								

S=streptococci
GN=Gram-negative rods
M=micrococci (staphylococci)
GP=Gram-positive rods

TABLE 4

The effect of washing on survival of organisms on orally contaminated glasses.

Organisms on lip of glass	Percent of glasses showing organisms after treatment			
	Washed and rinsed in cold tap water	Washed in water at 120° F. and rinsed in water at 120° F.	Washed in soap water at 120° F. and no rinse	Washed in soap water at 120° F. with 5 min. rinse in water at 165° F.
Streptococcus	100	70	30	0
Micrococcus	33	35	30	0
Gram negative rods	16	10	0	0

Further studies (Table 3) on the effect of a rinse in cold tap water subsequent to use indicated that such a treatment may reduce in part the survival of *Streptococcus salivarius* but not the universal presence of the organisms on the glass following its use. A study of 160 cases in which both water and milk were dispensed from the glasses, indicated that *Streptococcus salivarius* could be found on drinking glasses immediately after such use but that the number of organisms began to decrease following 4 to 8 hours when held at room temperature. However, *Streptococcus salivarius* could still be recovered after 24 to 30 hours.

A study of the effect of a double rinse as well as washing in soapy water at 120° F. followed by various types of rinses indicated (Table 4) that when such procedures were used the number of instances in which *Streptococcus salivarius* could be recovered was materially reduced. It was found that in a series of examinations, washing and rinsing in cold tap water did not reduce the incidence of *Streptococcus salivarius* on the rims of drinking glasses when examined immediately following this treatment. However, in over 33 percent of the cases, micrococci were found following such a treatment, whereas in the case of the Gram-negative rods of the coliform type only 16 percent of those originally showing these organisms were still found to contain the coliform types of organisms following washing and rinsing in cold tap water. If subsequent to use drinking glasses were washed in water at 120° F. without soap and subsequently rinsed in water with the same temperature, 30 percent of the glasses which originally contained streptococci were found to be free of this or-

ganism following such a treatment. However, where soap was added to the original water (pH 8.6) at 120° F., 70 percent of the glasses on which streptococci were originally found proved to be free of this organism after such treatment. It is of particular interest also to note that washing in a soapy water at 120° F. followed by a rinse for five minutes at 165° F. completely removed, in all cases studied, streptococci, micrococci and Gram-negative rods from the rims of drinking glasses. This temperature is of interest insofar as glasses are concerned. By examination it was found that tap "hot water" rarely exceeds 165° F., while the report (1936-1937) of the sub-committee of the American Public Health Association suggests a rinse water of 170 to 180° F.

These results further would indicate that *Streptococcus salivarius* is an acceptable index for the oral contamination of drinking glasses and in addition that the use of soapy water for washing together with 165° F. rinse would serve as an effective procedure for eliminating this streptococcus.

ORAL CONTAMINATION OF DRINKING GLASSES AS ENCOUNTERED IN PUBLIC EATING ESTABLISHMENTS

On the assumption that the presence of *Streptococcus salivarius* is an index of the oral contamination of drinking glasses, a survey was made of about 200 (Tables 5 and 6) eating establishments to determine the presence of this organism as well as micrococci, Gram-negative rods and Gram-positive rods on the rims of drinking glasses. It was found that a wide variation occurred among various types of eating establishments (Table 5) and even

TABLE 5
Oral contamination of drinking glasses in public eating establishments.

Number of establishments	City	Percent of establishments with glasses showing				Percent sterile
		Streptococci	Micrococci	Gram-Neg. rods	Gram-Pos. rods	
50	A	39	39	81	12	8
50	B	38	64	30	24	8

between different municipalities. This variation obviously is to be expected because of a general lack of sanitary control over this matter in eating establishments.

A detailed study (Table 6) of 100 eating establishments indicated that the greatest oral contamination of drinking glasses in the cases studied was found in tap rooms and bars. In the 35 establishments of this type which were examined, only 3 percent were found in which glasses were free of streptococci. In 63 percent of the tap rooms and bars, indicated by the *Streptococcus salivarius* test, the glasses were found to have been previously subjected to oral contamination without subsequent washing or cleaning. Soda fountains were found to be the most acceptable insofar as oral contamination was concerned, as 20 percent of this type of establishment had glasses available upon the rims of which no organism could be found.

In the survey of 100 eating establishments it is interesting to note that roughly one-half of these establishments had drinking glasses available for public use which showed evidences of oral contamination not removed by subsequent washing or sterilizing.

The presence of micrococci and Gram-negative rods on a large percentage of drinking glasses in public eating establishments no doubt indicates contaminations other than that of an oral nature. If the control of drinking glasses is to be dependent entirely upon oral contamina-

tion, neither the presence or staphylococci (micrococci) nor Gram-negative rods will serve as an index. Micrococci may indicate excessive handling without subsequent washing and the same may be true of the presence of Gram-negative rods, although in such instances indications are that the presence of these organisms may be an indirect index of unsanitary contaminations.

CONCLUSIONS

1. A study of 100 individuals indicates that a streptococcus identified as *Streptococcus salivarius* could be recovered from the closed lips of all persons tested.

2. A study of 100 controlled cases indicated that without exception *Streptococcus salivarius* is deposited on the rims of glasses during use.

3. When not cleaned or sterilized, *Streptococcus salivarius* will survive on the rims of drinking glasses for at least 48 hours following use.

4. A cold tap water rinse following use will not remove *Streptococcus salivarius* from the rims of glasses.

5. The use of a double rinse of water at 120° F. is inadequate for removal of oral contamination from glasses.

6. A soap water wash (pH 8.6) at 120° F. following by rinsing for five minutes at 165° F. in clear water will remove traces of oral contamination provided the deposition made on the glasses during the act of drinking is not too great.

TABLE 6
Oral contamination of drinking glasses in various types of eating establishments

Type of establishment	No.	Percent of establishments with glasses showing				Percent of glasses showing no organisms
		Streptococci	Micrococci	Gram-neg. rods	Gram-pos. rods	
Hotel and restaurants	40	40	60	52	40	7.5
Soda fountains	25	20	64	48	20	20.0
Tap rooms	35	63	66	79	9	3.0
TOTAL	100	45	56	62	19	6.0

7. The presence of *Streptococcus salivarius* on the rims of drinking glasses is a presumptive index which indicates previous oral contamination of these utensils. A procedure for making such a presumptive test is proposed.

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"The paper is based upon a detailed study of over 400 samples of spray-dried milk powder, from 8 factories. About 10 per cent were whole milk samples, the remainder from separated milk.

"Plate counts at 37° C. of the reconstructed milk showed individual variations from 1,400 to 149,000,000 per gm., while the methylene blue reduction test figures varied from 3 to 14 hours. The mean figures calculated as reconstructed milk approximated to a plate count of 400,000 per ml. and compared unfavorably with good quality raw milk. Testing 1 ml. quantities of the reconstructed milk about 10 per cent gave positive results to the presumptive coliform test, but a material proportion was due to the growth of anaerobic spore-formers and not to coliform strains.

"Consideration of the findings from the different factories showed wide variations between different factories, and also marked differences within the individual factory, showing a complete lack of consistency in the day to day management of certain of the plants. No marked variations with season were found unless the general bacterial standard was normally low. The full cream samples were more satisfactory than the skimmed milk, both on counts and reduction times.

"One factor causing variation in the day counts of individual factories is the growth of thermophilic and thermoduric organisms as a

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day run may last 18 to 22 hours, shown by a definite deterioration in the bacteriological quality as the day's run continues.

"The majority of organisms which are present in spray-dried milk powder produce souring readily. Of the colonies on plates incubated at 37° C. more than 97 per cent which produced acid coagulation of milk were found to be heat-resistant streptococci. Of these 66 percent were 'viridans' type, 57 per cent *Str. thermophilus*, and 33 per cent enterococcus type. No *Streptococcus pyogenes* types found. The milk-curdling thermophilic organisms isolated from the 55° C. plates were all strains of *B. calidolactis*. The number of yeasts and moulds present was negligible.

"The keeping quality of the reconstructed milk when stored at 60° F. was usually 2½ to 3½ days. The flora of reconstructed milk did not increase as rapidly as that of raw milk. The reduction test was found to be the most sensitive indication of the additional ageing of the milk.

"The author emphasizes the need and value of grading spray-dried milk powder. Her figures show, however, that there is a marked reduction in viable bacteria with storage. With six months' storage in a refrigerator, the reduction was 13 per cent, at room temperature 82 per cent, at 22° C, 98 per cent, at 40° C. 99.9 per cent. Methylene blue reduction times roughly corresponded. The mean percentage reduction in count after 12 months' ageing at room temperature was 95 per cent. In formulating quality standards the approximate age of the powder at the time of testing would need to be formulated."

R. A. C.

The Early Detection of Bovine Mastitis by an Electrometric Method

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The detection of cows yielding abnormal milk presents a serious problem to the veterinary inspector whose duty it is to exclude all but "the lacteal secretion obtained by the complete milking of one or more healthy cows." (1).

Examinations for udder health should have two definite objectives. The first must be to protect the consumer against receiving the products of unhealthy udders. Milk inspection which does not include this service cannot be worthy of the name. The second objective should be to point out to the dairymen those cows whose presence in the herd constitutes a distinct menace to the udder health of the herd. Mastitis frequently has been shown, both by controlled experiments and by herd histories, to be an infectious disease. Evidence is accumulating rapidly to indicate that a virus, which alters the lacteal cells and lowers their resistance to the entrance and multiplication of microorganisms, may prepare the field for invasion by the more familiar *Streptococcus agalactiae* and related streptococci in the production of mastitis. (2)

Early diagnosis of infected animals, followed by their isolation or removal, and sanitary measures offer the best means of control. Merely replacing the grossly infected cows with susceptible heifers, without removing the chronic carriers of the infection, offers little promise of ultimate control.

The multiplicity of mastitis tests advocated by various research workers, the difficulties and uncertainties in the interpretation of these tests, together with the lack of agreement as to their accuracy,

have led to such general confusion that some health officials feel that extensive mastitis control measures in their milk-sheds must await more research work and the presentation of more lucid data. In the meantime they are unable to certify as to the wholesomeness of the milk which passes under their inspection, with the result that udder infections unchecked continue to spread through the producing herds.

The U. S. Public Health Service Milk Ordinance provides that, "Cows which show an extensive or entire induration of one or more quarters of the udder upon physical examination, whether secreting abnormal milk or not, shall be permanently excluded from the milking herd. Cows giving bloody, stringy, or otherwise abnormal milk, but with only slight induration of the udder, shall be excluded from the herd until re-examination shows that the milk has become normal. For other diseases such tests and examinations as the health officer may require shall be made at intervals and by methods prescribed by him, and any diseased animals or reactors shall be disposed of as he may require." (1).

Physical Examination. Experience has shown physical examination of udders by an experienced and competent veterinarian to be excellent (3). To date, there has been no test described that has given a higher degree of accuracy and as much protection to the consumers and satisfaction to the herd owners. Unfortunately, such examinations are time-consuming, and few milk inspection services have the funds or qualified personnel necessary to make a thorough examina-

tion of all producing cows at reasonably frequent intervals. When hurriedly done or by unqualified individuals, physical examinations are not very good and much harm is done by the false sense of security given.

Bacteriological Methods. Samples for bacteriological examination must be aseptically drawn, and examinations made in a well equipped laboratory by trained personnel. This limits the method to very valuable herds or to research work. The mastitis streptococci do not grow well on ordinary media, and an enriched medium usually must be employed. Changes due to the action of a virus are not detected by bacteriological methods.

Hotis Test. In many herds the Hotis test has given satisfactory results. The quarter samples are drawn into a solution of brom cresol purple and then incubated. Mastitis streptococci, if present, tend to agglutinate on the surface of the tube where they multiply to form yellow flakes which are readily visible on the purple background.

Indirect Tests. The so-called indirect tests for the detection of mastitis are in more general use. All are based upon the alterations in the milk which follow inflammatory changes in the udder. These changes approximately parallel the degree of udder involvement, although much controversy exists at present over which changes in mastitic milk may correctly be considered as diagnostic, always being present in cases of infection and never present in milk from normal udders. So far, no single test has been accepted universally. The following are those most used.

The Strip Cup. Mastitic milk frequently is stringy or contains solid clots. If the first stream of the fore milk is drawn through a black cloth or fine screen, clots or strings are readily recognized. The strip cup practically never gives false positives, and it can safely be left in the hands of conscientious milkers. However, it is of only limited value in detecting chronic cases of mastitis, and the use of the strip cup alone probably

will not make possible the elimination of udder infection from the herd.

Alkalinity or pH Tests. Normal milk, as drawn from the cow, is slightly acid. Inflammatory conditions in the udder result in a decrease in this normal acidity so that mastitic milk usually is less acid or even slightly alkaline in reaction. This change in the degree of acidity can be detected in any one of several ways. The most common is by the use of the dye, brom thymol blue, which is yellowish-green in the degree of acidity of normal milk and which becomes more intensely blue as the milk is less acid or alkaline. Brom thymol blue is used as a solution with color standards for comparison, or strips of filter paper are impregnated with the dye and the milk is placed directly on them. Considerable alteration in the milk must be present before the average observer can detect changes in the color of the brom thymol blue, so that while this method does not falsely pick many normal cows as mastitic, it does miss many cases of chronic udder infections.

The indicator dye, brom cresol purple, which is used in a similar way, picks most of the cows that have udder infections but, unfortunately, it also gives positive results for many cows that apparently are not infected.

More accurate determinations of the degree of acidity or alkalinity can be made by means of a potentiometer. This, however, involves the drawing and labeling of quarter samples and taking them to a laboratory, which greatly adds to the labor and expense.

Rennet Clotting Time. Partly because of the decrease in acidity, mastitic milk clots less readily with rennet. This test has not been extensively used as a method of testing individual quarter samples, probably because other methods are equally reliable and more rapid. It is used, however, in some cheese factories on the mixed milk from individual herds.

Leucocyte Determination. Mastitic milk contains more leucocytes or white blood cells than does normal milk. Smears of quarter samples are made on glass

slides, properly stained, and examined under the microscope. Some have considered the presence of over 50,000 leucocytes per cubic centimeter as an indication of udder involvement. Others have set the figure at 100,000, 500,000 or even 1,000,000. The first figure probably is too low, and would result in normal cows being regarded as mastitic, while the last figure probably is so high as to allow many mastitic cows to go undetected.

Sediment. Mastitic milk yields more sediment when centrifugized than does normal milk, probably due almost entirely to the greater number of leucocytes it contains.

Catalase. With an increase of leucocytes there is also an increase in the enzyme, catalase. This enzyme decomposes hydrogen peroxide with the liberation of oxygen gas. Field tests have been made with this method. A small amount of the milk sample and hydrogen peroxide solution are mixed on a piece of dark glass, and the intensity of the liberation of bubbles of oxygen observed. This test is so difficult to interpret quantitatively that it is but little used at the present time. More frequently, the milk sample is drawn into a test tube, hydrogen peroxide solution added, the tube filled to the top with water and stoppered with a one-hole stopper and then inverted. The oxygen gas liberated by the catalase collects in the upper portion of the inverted tube where the amount readily can be estimated. This method has given variable results. Some investigators have found it very sensitive in detecting obscure infections. Others have found it to give many false positives. Milk from normal udders shortly after parturition and near the end of lactation frequently gives positive results.

Chloride Content. Mastitic milk almost always is high in chlorides. The chloride content of milk may be measured by titration with silver nitrate solutions in the presence of potassium chromate or other indicators such as dichlorofluorescein.

This method is the most widely used of the indirect tests and has given satisfactory results, although it is known that the chloride content of milk from normal udders is very high during the first week following parturition. It then falls, only to rise gradually after the third month, normally being rather high after the eighth or ninth month in milk. Due to the absorption of some of the silver nitrate by milk constituents other than chlorides and the masking of the color changes by the opacity of the milk, the results given are high, usually by approximately 0.025 percent. Field modifications of this test also have been developed. They indicate whether the chloride content is above or below a predetermined amount.

Electrical Conductivity. Mastitic milk conducts electrical currents more readily than does normal milk. Direct current measurements of the electrical conductivity of milk are not practical because of the rapidity with which milk breaks down with the passage of a continuous electrical current. Until the present time, measurements with alternating current have not been used extensively for the detection of abnormal milk because samples had to be drawn, taken to a laboratory, remixed to distribute the cream, brought to a constant temperature, and then the conductivity determined by means of rather expensive equipment.

A Portable Electrometric Device. In order to provide a rapid, convenient, and reasonably accurate method for obtaining quantitative measurements of the degree of abnormality of milk, without the necessity of drawing samples into test tubes which must then be labeled, taken to a laboratory and the results known only at a later time, the writer has developed a portable electrometric mastitis detector (4). This is shown in Figure I. Essentially, this consists of a Wheatstone's bridge in which the milk cell forms one arm. The milk cell of the present model consists of a one-half inch hole in a block of the thermoplastic, "Plexiglas." The upper portion of the cell is enlarged

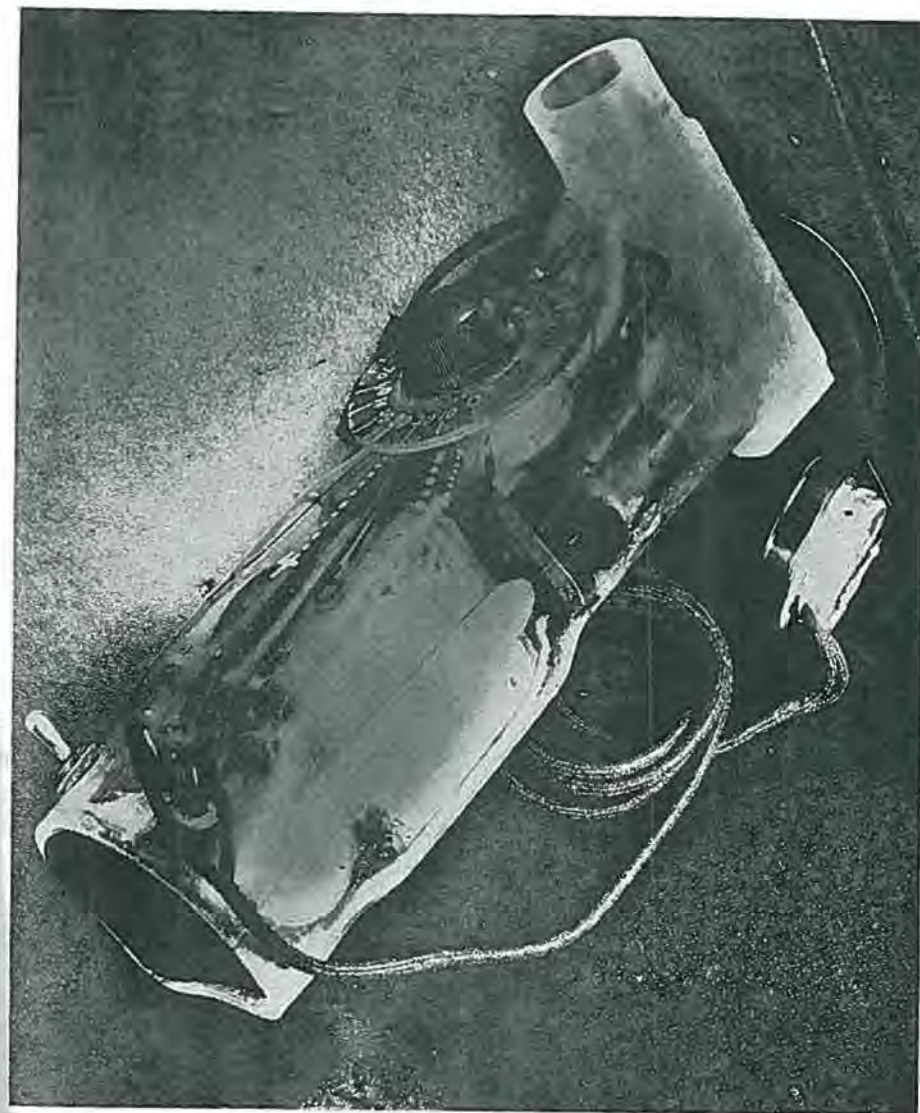


FIGURE 1. The electrometric mastitis detector.

to facilitate the drawing of milk into it. Two nickel electrodes pass through the lower portion of the milk cell. The other arms of the bridge consist respectively of a 200, a 200 and a 400 ohm resistor with a 200 ohm potentiometer. A diagram of the electrical circuit is shown in Figure II. The source of the alternating electrical current is an audio oscillator, which uses six "Penlight" cells as "B"

batteries and one flashlight cell as an "A" battery. The drain on the "B" batteries is almost negligible and the drain on the "A" battery is approximately one-fifth that of a flashlight. These batteries can be purchased for a few cents at any hardware or drug store. Radio earphones are worn to detect the current.

Testing by means of this device is very rapid. The operator dons the earphones,

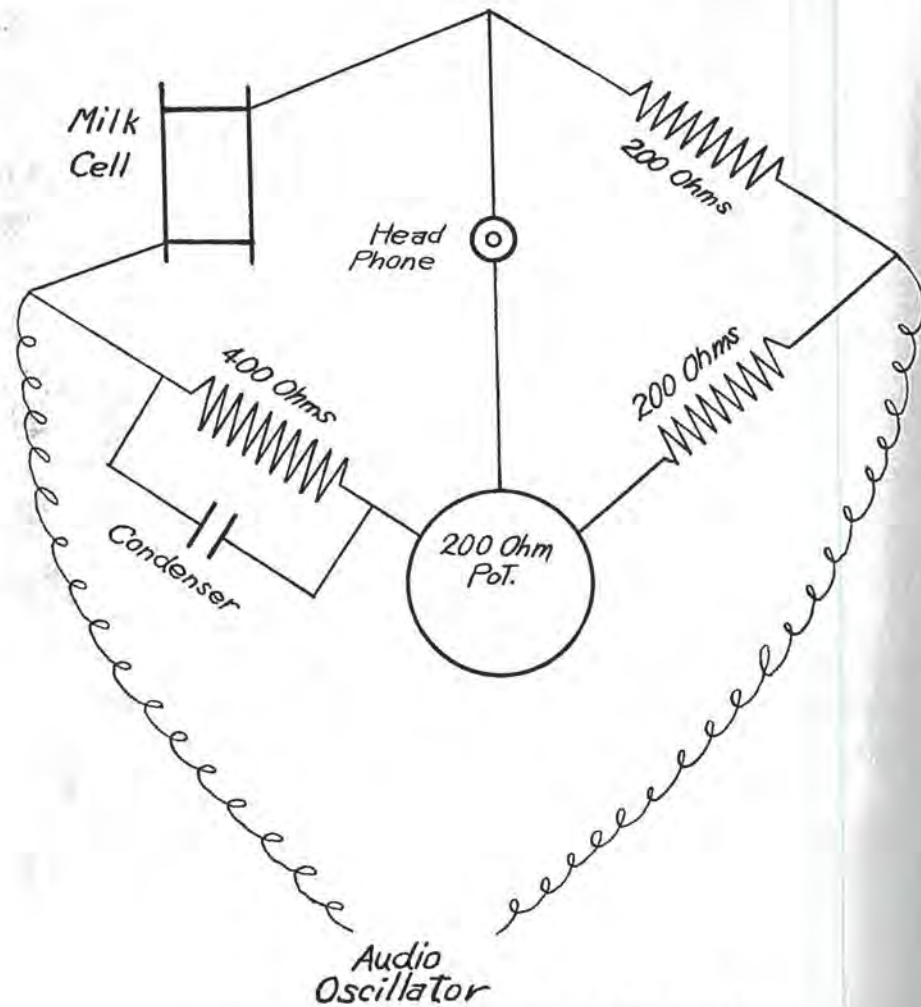


FIGURE 2. Diagram of electric circuit of electrometric device.

turns on the operating switch, and assumes the milking position. If the fore milk has not been removed previously by the milker or assistant, the fore milk is milked into a bucket. The device, which weighs approximately two pounds, is held in the left hand, with the milk cell tilted slightly down. A single stream of milk is milked into the cell and the device tilted so that the cell is slightly higher than the rest of the machine. This closes the mercury switch, and a signal is instantly heard in the headphones. The

dial attached to the potentiometer is then turned until the signal ceases, at which point the electrical circuit has been balanced. The conductivity, and hence the degree of udder involvement, is read directly from the dial. The cell is emptied by turning the device over, and the process repeated.

Quarter samples can be made on 30 cows in an hour without undue haste. Unless the previous reading was unduly high, there is no necessity for rinsing the milk cell. If rinsing is necessary, a sin-

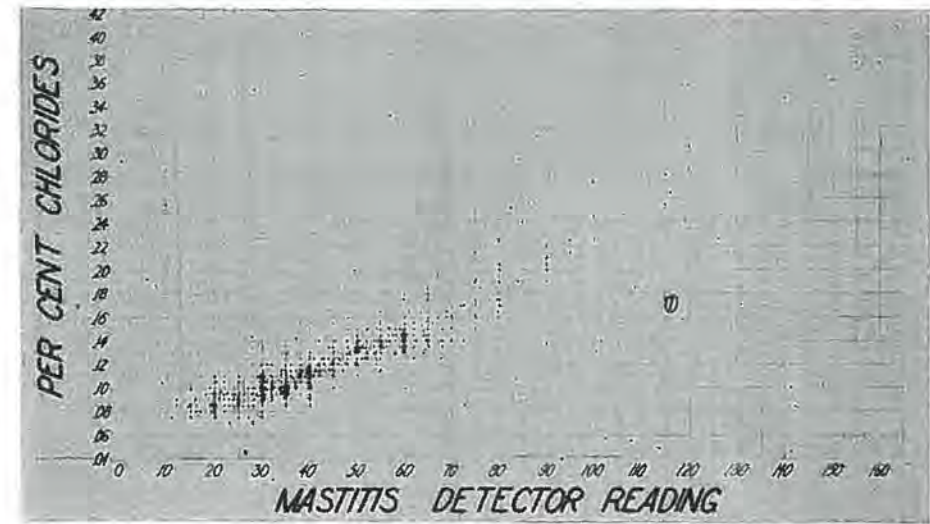


FIGURE 3. The correlation between electrometric readings and results of titration for chlorides (The reading marked 1 was positive to catalase and pH tests.)

gle stream from the next quarter to be tested suffices. Bacteriological sterility is not necessary.

Since the results are known immediately while the examining veterinarian is seated at the cow, opportunity is given for confirmation of high readings by physical examination. Samples of milk also can be drawn for such other tests as may seem advisable. Since thorough physical examination and other tests need be made only on those quarters showing high conductivity, the examination of the herd can be made in much less time. Also, the inspector is in a position to supervise the isolation or removal of the animal and to suggest such sanitary measures as he may deem necessary.

The accuracy of the device, as compared with the estimation of the chloride content of the milk by means of a silver nitrate solution in the presence of potassium chromate as an indicator, is shown in Figure III. Those samples, showing high chloride content in relation to the electrical conductivity, are, for the most part, from cows just fresh or in the latter stages of lactation. To date, approxi-

mately 5,000 quarter samples have been compared.

Like all of the previously described methods, high readings are obtained on milk from normal udders immediately following parturition and during the latter stages of lactation. A correction factor for this is provided on the dial.

In order to prevent unethical exploitation of this method, patent rights have been applied for and assigned to the Research Foundation of the State College of Washington.

The writer wishes to express appreciation to the National Youth Administration for student help in collecting samples.

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A Modified Resazurin Test for the More Accurate Estimation of Milk Quality*

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Among its other attributes, good milk should be the product of healthy udders and relatively free from contaminating bacteria. While the desirability of a minimum bacterial content is generally accepted, less attention has hitherto been given to the udder condition. It is well known that many herds contain cows with udders seriously affected by mastitis. Milk from such udders is generally changed in composition and contains large numbers of leucocytes. Studies on the leucocyte content of normal milk have shown (3, 6, 15) that this rarely exceeds 100,000 per ml., while counts in excess of 500,000 per ml. indicate that the milk has come from an udder containing an appreciable amount of fibrosis (6). The leucocyte content, as indicated by either the direct microscopic count (1) or the catalase test (6, 8, 18), therefore furnishes a useful indication of the degree of udder abnormality in a herd. Where this is high, the milk can scarcely be regarded as an entirely acceptable product for human consumption.

If a routine test were available which in addition to reflecting the bacterial content also placed these "abnormal" milks in a lower grade, there would be a stronger stimulus to the dairyman to eliminate advanced cases of mastitis. While the direct microscopic count (1) is very useful here, many who have had experience with it prefer to use one of the simpler dye reduction tests for routine grading, especially where large numbers of samples must be examined. The most

widely used method, the methylene blue (M. B.) reduction test (1), is of little value in detecting mastitis when applied to herd milks (13). The newer resazurin "one-hour" test is more sensitive to both physiologically and pathologically abnormal milks (2, 5, 11, 16, 17). Nevertheless, a fair proportion of milks with high leucocyte counts fails to cause an appreciable color change within one hour (10, 16) while high counts of dormant bacteria frequently escape detection (10).

The accuracy of the resazurin test as an index of bacterial numbers is considerably improved by continuing incubation to the pink end-point (10). However, the resazurin "pink" test is less effective than the "one-hour" test in the detection of "abnormal" milks. Such milks usually show considerable color change during the first few hours, after which the rate of change is so slow that, in the absence of sufficient bacterial activity, the pink stage may not be reached within the time limit adopted. The following data (Table 1) illustrate the difference between color changes with such milks and with those where changes are mainly due to bacterial activity.

Our studies, supplemented by plant experience, indicate that the greatest amount of information is obtainable where the color number of each sample is recorded hourly. This, however, is impracticable in the routine testing of large numbers of samples. We have therefore subjected our data¹ on 279 market milks to addi-

¹ These data include direct microscopic count of individual bacteria and leucocytes (1), modified methylene blue reduction time (9, 10, 21), resazurin "pink" reduction time (10), and resazurin color number at each hour until complete reduction.

Sample No.	Breed count (thousands)		Resazurin color no. ¹ after hours								Reduction time Modified M.B. Test
	Bacteria	Leucocytes	1	2	3	4	5	6	7	8	
1	64	1,100	6	9	10	10	11	12	22	24	8¾
2	413	169	1	2	6	8	9	13	24		8¼

tional careful study to determine whether it would be possible to develop a simpler method for routine grading which would combine the sensitivity of the "one-hour" test for "abnormal" milks with the greater accuracy of the "pink end-point" for bacteria.

Table 2 shows the distribution of samples on the basis of color number after 1, 2 and 3 hours, the samples being grouped according to bacteria and leucocyte counts. It will be observed that within each bacteria count grouping the higher leucocyte counts are reflected in a higher color number. The number of high-count samples showing little change after one hour should also be noted. As the incubation period is lengthened, the proportion of such discrepancies decreases until by the third hour these are relatively few. The value of further observations beyond the first hour has been recognized by Ramsdell *et al.* (16) and by Keenan (12).

Just where the leucocyte count limit for herd milk should be set is a problem with which we are not particularly concerned. The important thing is that the degree of color change with resazurin, especially by the third hour, is closely related to the leucocyte count. If a color number not greater than 8 at three hours is taken as the limit for a first grade milk, it will be seen from Table 2 that the great majority of milks with high leucocyte counts fails to meet this standard. This is true even where the bacteria count is low. The color limit indicated also reflects the bacteria count very satisfactorily.

¹ Color numbers range from 0 for initial color to 16 for full pink, and 24 for decolorized. Changes from the initial blue color are quantitatively related to the action of bacteria, or to that of leucocytes or reducing substances present in abnormal milk.

These results suggested the possibility of using this same color reading at one, two, and three hours as a simple method of grading a series of shippers' milks into four grades. In the proposed method, all samples showing a color number in excess of 8 at 1 hour would go into 4th grade; those showing a similar change by the 2nd hour would be 3rd grade; those doing the same by the 3rd hour would be 2nd grade; while the remainder would constitute the 1st grade. In passing, it should be noted that the "creaming error" is minimized by inverting the tubes after each hourly reading (9, 10).

To compare the grading by the proposed "triple reading" method with that by several other methods, we have prepared Table 3 in which are shown the percentages of samples in the various classes placed in the different grades by each method. For the modified methylene blue reduction test (10) we have taken 2, 4 and 6 hours as the class limits; for the resazurin "pink" test, 1½, 3 and 4½ hours (10); and for the resazurin "one-hour" test, the color limits suggested by Ramsdell *et al.* (16) but expressed in terms of the color notation employed by us.

While with the low bacteria count milks the resazurin "one-hour" test is more sensitive to high leucocyte counts than are either the resazurin "pink" or M. B. tests, it is much less so than is the "triple reading" method. Furthermore, the "one-hour" test, using Ramsdell's standards, places an unduly large percentage of high bacteria count milks in the first and second grades, there being practically no differentiation between bacteria counts below 2,000,000 per ml. The triple reading method reflects the bacterial and leucocyte contents of the milk much more satisfactorily than any of the other three methods.

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TABLE 3
Comparison of Results of Grading 279 Market Milks by 4 Different Methods

Bacteria (thousands)	Leucocytes (thousands)	No. of samples	Resazurin "Triple Reading"				Resazurin "One-Hour" Test				Resazurin "Pink" Reduction Time				Modified Meth. Blue Reduction Time			
			1st %	2nd %	3rd %	4th %	1st %	2nd %	3rd %	4th %	1st %	2nd %	3rd %	4th %	1st %	2nd %	3rd %	4th %
<100	<250	15	100.0				93.3	6.7					86.7	13.3				
	250-500	27	81.4	18.6			96.3	3.7					77.8	22.2				
	>500	62	29.1	43.5	24.2	3.2	54.8	45.2					74.2	22.6	3.2			
101-500	<250	7	85.7	14.3			100.0						71.4	14.3	14.3			
	250-500	25	56.0	28.0	16.0		92.0	8.0					52.0	36.0	12.0			
	>500	53	22.2	25.9	42.6	9.3	56.6	43.4					47.2	43.4	7.5	1.9		
501-2000	<250	3	66.7	33.3			100.0						33.3	66.7				
	250-500	12	33.3	50.0	16.7		83.3	16.7					50.0	41.6	8.4			
	>500	33	5.0	18.7	70.0	6.3	42.4	57.6					24.2	54.6	21.2			
>2000	<250	6		33.3	16.7	50.0	33.3	33.3	16.7	16.7			16.7	50.0	33.3			
	250-500	12	25.0		25.0	50.0	25.0	75.0					16.7	8.4	33.3	41.6		
	>500	24	4.0	16.0	16.0	64.0	16.7	50.0	20.8	12.5			12.5	16.7	20.8	50.0		
Grade Standards			Not >8 at 3 hrs.	Not >8 at 2 hrs.	Not >8 at 1 hr.		1-4	5-15	16-23	24			>4.5 hrs.	>1.5 hrs.	<1.5 hrs.	>6 hrs.	>4 hrs.	>2 hrs.

superior to any other type of daylight lamp we have tried.

It may be objected that the grade standards selected in these studies are too high for use in some communities. [The same objection holds true in regard to the recently suggested standards for the M. B. reduction test (1).] In such cases the same color standard (No. 8) may be used and readings made at shorter intervals until the quality of the supply has improved to the point where the suggested standards can be introduced.

SUMMARY

Using a single color standard (P 7/4 Munsell notation) approximately half way between the initial blue and the full pink color, and making hourly readings up to the third hour, milk may be graded simply and accurately by means of the resazurin test.

The "triple reading" test is superior to the "one-hour" resazurin test in the detection of high bacteria and leucocyte counts, and reflects high leucocyte counts much more definitely than do either the resazurin "pink" or M. B. reduction tests.

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Mr. Jennings Now in Health Work

The many friends of Mr. J. R. Jennings will be glad to know that he is back in the field of public health. He is now in charge of the supervision of milk sanitation for the Iowa State Department of Health at Des Moines. For two years he has been associated with one of the commercial companies which sold supplies in the public health field.

Mr. Jennings has been an active member of the International Association of Milk Sanitarians for many years. He has regularly attended the meetings of the Association, and has actively contributed to the advancement of milk sanitation. We are glad to see him back in the field of public health where he can devote his full time and capabilities to this field of public need.

Factors Affecting the Survival of *Streptococcus pyogenes* in Cheese *

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INTRODUCTION

Milk-borne outbreaks of septic sore throat and scarlet fever are most frequently traced to raw milk. Hucker and Marquardt (1) pointed out that inasmuch as a large percentage of cheese is made from raw milk from supplies which are subjected to little or no sanitary supervision, these milk sources are as liable to contamination with *Streptococcus pyogenes* as raw milk supplies that are used for fluid consumption. They were the first to study the possibility of cheddar cheese being incriminated in septic sore throat epidemics, presenting their work at the Schenectady meeting of the New York State Association of Dairy and Milk Inspectors in 1936.

These authors found in a brief study that *Streptococcus pyogenes* could live in cheddar cheese for more than 160 days when cured at 40° F. (4.4° C.) and for more than 85 days when cured at 60° F. (15.5° C.). In addition, an examination of cheese known to have been made at a factory from milk containing *Streptococcus pyogenes* showed that this organism was present in the cheese.

Information on cheese-borne epidemics is meager. Swanner (2) summarized 31 cheese-borne epidemics reported in the literature which occurred throughout the world between 1883 and 1939. While none of these are reported as due to streptococci, this does not constitute positive proof that cheese is never responsible for

outbreaks of septic sore throat or scarlet fever. Due to the wide distribution of some types of cheese and to the varying lengths of time which may elapse before consumption, cheese-borne outbreaks are more difficult to discover than are milk-borne outbreaks.

Results obtained with one variety of cheese are not applicable to all. In the present investigation, cottage, limburger, and cheddar cheese were selected to represent soft, semi-soft, and hard varieties.

METHODS

Raw milk from the mixed supply of the Station herd was divided into equal lots. One lot of milk was made into control cheese and the other, after inoculation with one percent of an 18 hour milk culture of *Streptococcus pyogenes*, into experimental cheese. Cheese color was added to the inoculated milk to facilitate distinguishing the cheeses containing *S. pyogenes* from the controls. Manufacturing methods were similar to those used by the industry, with the exception that they were carried out in the laboratory on a much smaller scale.

For bacteriological analysis, the cheese was sampled with a sterile trier, and the trier holes filled with melted paraffin. After discarding the surface of the plug, one gram was weighed on wax paper and transferred to a sterile mortar. The cheese was then ground with 9.5 ml. of 2 percent aqueous sodium citrate in the preparation of the 1:10 dilution.

Due to the high total bacterial content of cheese, satisfactory counts of beta hemolytic colonies of *Streptococcus pyogenes* could not be made using standard veal infusion blood agar. Addition of

crystal violet in a concentration of 1:500,000 as employed by Edwards (3) for inhibition of staphylococci improved results. However, blood agar plates were frequently overgrown with other types of bacteria and valueless in spite of all precautions to the contrary.

SOURCE AND VIRULENCE OF CULTURES

Two cultures of *Streptococcus pyogenes* isolated from the diseased quarters of cows concerned in two septic sore throat outbreaks were furnished by the New York State Department of Health.* The first culture was isolated from milk collected in March, 1938, during an outbreak of 59 cases of septic sore throat in Minetto, New York (4). The second culture was isolated from milk collected in July, 1939, during an outbreak of 100 cases of severe sore throat at Medina, New York (5). Both of these cultures belonged to Lancefield's Group A.

One of the objectives of the study was to determine whether there was any loss in virulence of *Streptococcus pyogenes* organisms during their existence in cheese. The majority of strains of *S. pyogenes* when freshly isolated from milk associated with outbreaks of human infection are virulent as shown by Coffey (6). However, no information was available as to the virulence of the Minetto and Medina strains upon original isolation. Intraperitoneal injection into white mice of 0.1 cc. of 18 hour broth cultures failed to kill the mice which indicated that the Minetto and Medina cultures, which were several months old when tested, were lacking in virulence at the time of their introduction into the experimental cheeses.

Another approach was made to the virulence problem by manufacturing cheeses from milk inoculated with the milk of a cow responsible for an epidemic. On November 10, 1939, Dr. Graves obtained about 120 ml. of milk from one quarter of a cow concerned earlier in the month in an outbreak of 18

cases of scarlet fever at Hornell, New York (7). This sample had a beta hemolytic streptococcus colony count of 5,700 per ml. While the beta hemolytic culture belonged to Group A and was virulent for white mice, experiments with cheddar, brick, and limburger cheese failed because the bacteria in the milk were so few that beta hemolytic colonies were not recoverable from the freshly made cheese.

These difficulties caused further studies of virulence to be discontinued and work with the laboratory cultures of the Minetto and Medina strains restricted to studies of the number of survivors.

DATA

Twelve experimental trials with cheese made from milk inoculated with *Streptococcus pyogenes* included three with cottage cheese, six with limburger cheese, and three with cheddar cheese. Six of the twelve trials yielded cheese satisfactory for study. Failures were due in four cases to insufficient inoculation with *S. pyogenes*, and in two cases to cheese so defective in quality that they were not representative. The study was continued until two independent and satisfactory experiments had been made with cottage, limburger, and cheddar cheese each.

SURVIVAL IN COTTAGE CHEESE

Considerable quantities of cottage cheese and related unripened soft types such as pot and bakers' cheese, are made from raw skim milk. These cheeses are usually consumed within a short period following manufacture.

In a preliminary study, cottage cheese was made from pasteurized skim milk using five percent lactic starter in the control lot and four percent lactic starter in the inoculated lot. One percent of an 18 hour *S. pyogenes* (Medina strain) milk culture was added to the latter. While the inoculated milk contained 213,000 beta hemolytic colonies per gram, none were recovered six hours later from the curd at the time of dipping. The lowest dilution used was 1:10,000, since the rapid decrease in numbers was not anticipated. No beta hemolytic colonies were recovered from the 1:10,000 dilution.

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TABLE 1
Survival of *Streptococcus pyogenes* in Cottage Cheese made with 1, 5 and 10 percent Lactic Starter

Sample Source	Experiment 1				Experiment 2			
	Hours after inoculation	Lot A 1% lactic starter <i>S. pyogenes</i> colonies per gm.	pH	Lot B 5% lactic starter <i>S. pyogenes</i> colonies per gm.	pH	Lot C 1% lactic starter <i>S. pyogenes</i> colonies per gm.	pH	Lot D 10% lactic starter <i>S. pyogenes</i> colonies per gm.
Uninoculated milk	0	<10	6.60	<10	6.60	<10	6.58	<10
<i>S. pyogenes</i> culture	0	86,000,000	5.18	86,000,000	5.18	102,000,000	5.90	102,000,000
Inoculated milk	1	990,000	6.20	1,480,000	6.10	1,130,000	6.45	790,000
Milk	3	1,230,000	6.17	1,030,000	5.23			
Milk (A, C); Curd (B, D)	6	1,480,000	5.70	300,000	4.75	520,000	6.25	<10
Curd	24	<100	4.57	<100	4.47	<10	4.32	<10
Reinoculated curd*	28					80,000		182,000
Reinoculated curd	48					<10	4.63	<10

* Fifty grams of the salted curd inoculated with 25 ml. of pasteurized whole milk containing 670,000 *S. pyogenes* colonies per ml.

the salted curd at 24 hours of age. The pH value of the curd at this time was 4.5 and the moisture content 78.9 percent.

In the commercial manufacture of cottage cheese, the amount of lactic starter usually varies from 1 to 10 percent. The length of survival of *S. pyogenes* was compared, using one percent and five percent of starter (Table 1). One percent of a milk culture of *S. pyogenes* (Medina strain) was used for inoculation. The milk with one percent starter was held at 70°F. (21.1°C.) for 24 hours, and that with five percent starter at 86°F. (30°C.) for six hours when the curd was ready for cutting.

In the case of lot A containing one percent starter, the number of beta hemolytic colonies one hour after inoculation was 990,000 per gram compared to 1,480,000 per gram at six hours. In lot B, containing five percent starter, the count decreased appreciably from 1,480,000 per gram at one hour to 300,000 per gram at six hours. At the end of 24 hours, no beta hemolytic colonies were recovered in the 1:100 dilution from either lot A or B. The pH values at the end of six hours were 5.70 for lot A and 4.75 for lot B, indicating that the more rapid acid development in lot B was responsible for the decrease in numbers of beta hemolytic colonies. The pH values at the end of 24 hours were 4.57 for lot A and 4.47 for lot B.

In a second experiment, cottage cheese was made with one percent and 10 percent amounts of lactic starter. In lot C made with one percent starter, the beta hemolytic colony count six hours after inoculation with *S. pyogenes* was 520,000 per gram. The milk in lot C had a pH value of 6.25 at this time. Lot D made with 10 percent starter yielded no beta hemolytic colonies at the end of six hours when the curd had a pH value of 4.56. No beta hemolytic colonies were recovered from lot C at 24 hours when the pH value of the curd was 4.32. The above results indicate that the more rapid the acid development in cottage cheese, the sooner *S. pyogenes* is destroyed.

Light cream is frequently added to cot-

TABLE 2
Survival of *Streptococcus pyogenes* in Limburger Cheese of Low Moisture and of High Moisture Content*

Sample Source	Lot A (Low Moisture)		Lot B (High Moisture)	
	<i>S. pyogenes</i> Colonies per gram	pH values	<i>S. pyogenes</i> Colonies per gram	pH values
Uninoculated milk	<10		<10	
<i>S. pyogenes</i> culture	46,000,000		15,000,000	
Inoculated milk	560,000		300,000	
Curd at dipping	3,190,000		1,500,000	
Cheese, 1 day	3,000,000		13,900,000	
Cheese, 3 days	Not sampled		100,000	
Cheese, 7 days	14,400,00		Not sampled	
Cheese, 9 days	Not sampled		40,000	
Cheese, 14 days	4,150,000		<1,000	
Cheese, 21 days	Not sampled		<1,000	
Cheese, 28 days	390,000		<1,000	
Cheese, 35 days	Not sampled		<1,000	
Cheese, 51 days	<10		Not sampled	
Cheese, 103 days	<10		Not sampled	

* Moisture content, Lot A was 42.8 percent, Lot B, 49.3 percent. Salt content, Lot A, 1.7 percent, Lot B, 2.8 percent.

tage cheese before packaging. If raw milk or raw cream is used, there is a possibility of introducing *S. pyogenes* into the cheese at this stage. The probable length of survival of *S. pyogenes* under such conditions was studied by adding to the 28 hour old curd, pasteurized milk inoculated with one percent of an *S. pyogenes* culture.

The freshly reinoculated curd had beta hemolytic colony counts of 80,000 per gram in lot C and 182,000 per gram in lot D. Twenty hours later (48 hours from the start of the experiment) no beta hemolytic colonies could be recovered. The pH values were 4.63 and 4.80 respectively at this time. These results indicate that it is unlikely that cottage cheese made from infected raw milk contains *S. pyogenes* at time of consumption due to a lack of tolerance of *S. pyogenes* for the high hydrogen ion concentration.

LIMITING HYDROGEN ION CONCENTRATION

Strains of *S. pyogenes* from human sources have a limiting hydrogen ion concentration in glucose broth of pH 4.8-6.0 with the majority of strains ceasing to grow below about pH 5.5 (8, 9, 10). The limiting hydrogen ion concentration in milk for the Medina and Minetto

strains of *S. pyogenes* was found to be pH 5.3 and pH 5.9 respectively. This was determined after incubation for three days at 37°C. in sterile skim milk.

SURVIVAL IN LIMBURGER CHEESE

Limburger cheese is made almost entirely from raw milk in New York and Wisconsin, the two states which manufacture 95 percent of this cheese. A good but mild type of limburger can be manufactured from pasteurized milk, as has been demonstrated commercially (11) and experimentally (12). Since this type of cheese has a higher moisture content than cheddar and is surface ripened, whereas cheddar is not, it was selected as a type for study.

Two of the six experimental trials yielded limburger cheese of representative quality and with sufficient numbers of *S. pyogenes* to render the cheese satisfactory for study (Table 2). Lot A consisted of 3 one-pound cheeses made from 20 pounds of raw Station milk inoculated with one percent of the Minetto culture. Lot B consisted of 5 one-pound cheeses made from 35 pounds of raw Station milk inoculated with one percent of the Medina culture. No lactic starter was added and the cheese was made according to the method commonly followed in this

state. Control lots of cheese were made from equal amounts of uninoculated milk. The cheese was held at 60° F. (15.5° C.) for 14 days for development of a smear, then wrapped and placed in storage at 50° F. (10° C.).

The inoculated milk in lot A contained more beta hemolytic organisms than that in lot B, 560,000 per ml. compared to 300,000 per ml. This relation also held true for the curd at dipping, beta hemolytic colony counts being 3,190,000 per gram in lot A compared to 1,500,000 per gram in lot B. The maximum number of beta hemolytic colonies in the cheese of lot A, 14,400,000 per gram, was obtained on the seventh day while the maximum number in lot B, 13,900,000 per gram, was obtained when the cheese was but one day old.

S. pyogenes survived considerably longer in the cheeses of lot A than in those of lot B, beta hemolytic colonies being recovered from lot A at 28 days but not at 51 days and from lot B at 9 days but not at 14 days. The more rapid increase in numbers in lot B than in lot A during the first 24 hours suggested that *S. pyogenes* might die off at an earlier age in lot B which proved to be the case.

The hydrogen ion concentrations of the first 5/16th inch of cheese beneath the surface and of the center portion were determined in lot B. Since in limburger and other surface-ripened types of cheese there is a more rapid shift in pH toward neutrality near the surface than at the center, there is a possibility that *S. pyogenes* might survive longer in one part of the cheese than in another. Comparative blood agar plate counts of outer and inner portions showed no significant differences in count. The pH values (Table 2) are in line with those reported for commercial limburger by Kelly and Marquardt (13), and dropped as low as pH 4.75 in the interior of the cheese. These values are not as low as those for cottage cheese (Table 1), which dropped to pH 4.32 in one instance.

Lot A contained 42.8 percent moisture and 1.7 percent salt, and lot B 49.3 percent moisture and 2.8 percent salt. These

differences in composition probably afford the chief explanation for the longer survival time in lot A than in lot B. It is reasonable to expect that *S. pyogenes* would die off more rapidly in the high moisture cheese with more lactose present to be converted into acid resulting in the limiting hydrogen ion concentration being attained more quickly.

Since the moisture content of commercial limburger falls within the above range of 42.8 to 49.3 percent and since pH values vary considerably in market cheese, *S. pyogenes* when present may survive considerably longer under some conditions than under others. These experiments indicate that *S. pyogenes* will not survive longer than five or six weeks in limburger cheese. Since the great bulk of commercial limburger is 6 to 12 weeks of age when purchased by the consumer, it appears that the chance of septic sore throat being transmitted through limburger is negligible.

SURVIVAL IN CHEDDAR CHEESE

Only a small percentage of cheddar cheese is made from pasteurized milk in this country. While pasteurization of milk for cheddar cheese making is increasing each year, it is being accepted very slowly in leading cheese-producing states such as Wisconsin and New York. Much of the high-moisture, quick-curing type of cheddar cheese made from raw milk is placed on the market when only about five weeks of age*.

Two lots of cheddar cheese were made from raw Station milk (Table 3). Lot A was made from 157 pounds of milk inoculated with one percent of a lactic starter and with one percent of the Minetto strain of *S. pyogenes*. Lot B was made from 55 pounds of raw Station milk inoculated with one percent of lactic starter and one percent of the Medina strain of *S. pyogenes*. Controls were made using equal amounts of milk. In the case of lot B, this control was made by a separate worker in a separate room to avoid

* Based on a statement by an experienced cheese buyer to the effect that about 80 to 85 percent of the cheddar cheese handled by chain grocery stores now has an age of not over five weeks.

TABLE 3
Three Different Temperatures*
Survival of *Streptococcus Pyogenes* in Cheddar Cheese Cured at

Sample Source	Lot A		Lot B	pH values
	45° F. (7.2° C.) <i>S. pyogenes</i> colonies	62° F. (16.6° C.) <i>S. pyogenes</i> colonies	50° F. (10° C.) <i>S. pyogenes</i> colonies	
Uninoculated milk	<10	<10	<10	6.62
<i>S. pyogenes</i> culture	152,000,000	152,000,000	12,500,000
Inoculated milk	500,000	500,000	280,000	6.62
Whey at cheddaring	6,800	6,800	2,800
Curd at cheddaring	400,000	400,000	2,000,000	5.34
Cheese, 2 to 3 days	560,000	560,000	160,000	5.02
Cheese, 1 week	350,000	129,000	22,000	5.07
Cheese, 3 weeks	251,000	34,000	145,000	5.05
Cheese, 5 weeks	92,000	10,300	129,000	5.07
Cheese, 7 weeks	29,500	28,000	5.10
Cheese, 9 weeks	28,000	14,000	17,000	5.11
Cheese, 11 weeks	6,800	<10	18,000	5.16
Cheese, 13 weeks	1,550	<10	20,000	5.18
Cheese, 18 weeks	2,400	<10	<100	5.21
Cheese, 39 weeks	<10	Not examined

* Moisture content, Lot A, 37.0 percent, Lot B, 35.0 percent.
Salt content. Lot A, 1.7 percent, Lot B, 1.8 percent.

any possibility of accidental inoculation with *S. pyogenes*. The cheeses were made as Young Americas and weighed about 5.5 pounds each. In addition, some of the cheese in Lot A was canned. Results obtained from the canned cheese (vented cans) are similar to those for the Young Americas and are not reported for this reason.

One of the Young Americas of lot A was cured at 45° F. (7.2° C.), the other at 62° F. (16.6° C.) while the Young America in lot B was held at 50° F. (10° C.) *S. pyogenes* survived for over 18 weeks in the cheddar cheese held at 45° F., but could not be detected in the cheese held at 62° F. at the end of 11 weeks. *S. pyogenes* died within 18 weeks in the cheese of lot B cured at 50° F. This indicates that the temperature of curing is an important factor affecting the length of survival of *S. pyogenes* in cheddar cheese. The maximum count of *S. pyogenes* in lot A was 560,000 per gram in the two day old cheese, while the maximum count in lot B of 2,000,000 per gram was obtained from the curd at cheddaring.

Hucker and Marquardt (1) found maximum counts at 160 days and 85 days in the case of two lots of cheddar

cheese ripened at 40° F. (4.4° C.). Since *S. pyogenes* does not grow at this temperature (14, 15), it should be pointed out that apparent increases in counts were probably due to limitations of sampling and plating methods rather than to growth of the organisms.

The pH values for lot B were normal and did not drop below pH 5.02, which was obtained at three days. Although the hydrogen ion concentration increases more rapidly during the first few hours in cheddar cheese than in limburger, pH values do not drop as low as they do in the case of limburger and remain at pH 5.0 or above unless the cheese is abnormally high in acid.

CONFIRMATION AS PYOGENES

Broad zone beta hemolytic streptococci which do not belong to Lancefield's group A are sometimes present in raw milk (16, 17). Lancefield (18) found that strains of hemolytic streptococci which Avery and Cullen (8) isolated from cream cheese belonged to group D. Obviously, beta hemolytic colonies on blood agar plates prepared from experimental cheese need confirmation before acceptance as *S. pyogenes*.

The raw milk from the Station supply

used in most of the experiments was free from broad zone beta hemolytics in the 1:10 dilution and usually developed only a few narrow zone beta hemolytic colonies.

The presence of broad zone beta hemolytic colonies on plates from cheese made from milk inoculated with *S. pyogenes* and absence of such colonies on plates from control cheese were accepted as presumptive evidence that the colonies were *S. pyogenes*. Several broad zone beta hemolytic colonies were picked from plates during the course of each experiment, purified by replating on blood agar, and picked again into veal infusion broth. If growth and morphology were typical of *S. pyogenes*, one culture was selected for Lancefield grouping with group A serum*. Both the Lancefield (18) and Coffey (19) technics employing this serum were used with satisfactory results.

In the case of the cottage cheese, confusing types of beta hemolytic colonies were absent, but this was not true of some of the experiments with limburger and cheddar cheese. Beta hemolytic colonies of gram negative rods were found on plates prepared from limburger cheese, lot B, at 28 and 35 days of age.

In the case of the cheddar cheese, the type of beta hemolytic colony most easily confused with colonies of *S. pyogenes* was a streptococcus which increased in numbers during the curing of the cheese, first appearing on blood agar plates when the cheese was about three weeks old. In pure culture, colonies of this streptococcus could not be distinguished from colonies of *S. pyogenes* on blood agar plates. However, it was easily distinguished by its dense uniform growth in veal infusion broth and by its morphology, since it grew mostly in pairs with only a few very short chains. This streptococcus did not belong to group A. It coagulated milk and grew well at both 10° C. and 45° C., corresponding in these characters to enterococci in group D. Beta hemolytic colonies which appeared on

control plates (1,000 to 5,000 per gram) were usually of the above type.

DISCUSSION

There appear to be many factors which influence the length of survival of *Streptococcus pyogenes* in cheese such as the original number of *S. pyogenes* organisms in the milk, the limiting hydrogen ion concentration of the strain of *S. pyogenes*, the type of cheese, and the curing temperature. Cheese is subject to great variation in manufacturing methods and in composition so that it is reasonable to assume that there is considerable variation in the length of time during which *S. pyogenes* will survive in the cheeses of any given variety. Moreover, abnormal cheeses of any variety are sometimes manufactured which do not show normal acid development. The length of survival of *S. pyogenes* in cheese of abnormal quality may be quite different from that in cheese of normal quality.

Not all cheese which is consumed passes through regular trade channels. Fresh curd is frequently eaten by cheesemakers and there is a limited practice of selling curd only a few hours old to customers who bring their own containers. Young cheeses which may be only a few days old are regularly examined at factories by inspectors, buyers and cheesemakers who taste the cheese as part of the scoring procedure.

There is also a limited amount of farm cheesemaking, where the raw milk used comes from single herds. If infection of the milk supply should occur, greater numbers of *S. pyogenes* would likely be present under farm than under factory conditions due to greater dilution in the latter case with milk from non-infected herds.

The important question whether *Streptococcus pyogenes* retains its virulence and pathogenic properties during its existence in cheese remains to be answered. Just as cultures of *S. pyogenes* lose virulence under laboratory conditions, so *S. pyogenes* organisms in cheese may gradually lose their virulence. This is likely in view of the unfavorable acid condition

of many cheeses. Furthermore, no cheese-borne outbreaks of septic sore throat or scarlet fever are reported in the literature.

Cottage cheese and limburger cheese made from raw milk appear to be safer from the standpoint of transmitting *S. pyogenes* than does cheddar cheese. *S. pyogenes* may survive in cheddar cheese as long as 18 weeks if the cheese is cured at a low temperature. This confirms results previously obtained by Hucker and Marquardt (1).

SUMMARY

Streptococcus pyogenes Rosenbach was not recovered from a high-acid, non-Genet type of cottage cheese several hours after manufacture. Lots of cheeses were made using 1, 5, and 10 percent amounts of lactic starter. When the curd was reinoculated, as might occur in the creaming of cottage cheese with raw cream, *S. pyogenes* again died off rapidly due to the high hydrogen ion concentration. Minimum pH values in the different lots of cottage cheese were about pH 4.5.

S. pyogenes survived between 28 and 51 days in one lot of limburger cheese with a moisture content of 42.8 percent and between 9 and 14 days in another lot with a moisture content of 49.3 percent. The minimum pH value as determined for the second lot of limburger was pH 4.75.

S. pyogenes survived for over 18 weeks in cheddar cheese cured at 45° F. (7.2° C.) and between 9 and 11 weeks in a duplicate cheese cured at 62° F. (16.6° C.). In a second lot of cheddar cheese, *S. pyogenes* survived less than 18 weeks when the cheese was cured at 50° F. (10° C.). The pH values as determined for the second lot of cheddar cheese did not drop below pH 5.0.

The variety of cheese, its moisture and salt content and the curing temperature are some of the important factors affecting the length of survival of *S. pyogenes* in cheese.

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* The streptococcus grouping serum was furnished through the courtesy of the Lederle Laboratories, Pearl River, New York.

Application of the Resazurin Test in Determining the Quality of Pasteurized Cream

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INTRODUCTION

Resazurin has been used in quality tests by the United Farmers' Co-operative Creamery Association, Inc. laboratories for nearly three years and has proven very useful.

The results discussed in this paper were secured by the Quality Control Department of United Farmers' in its laboratory in Boston, Massachusetts.*

The data were obtained from the daily routine samples of pasteurized cream received by truck and rail from Vermont plants.

PURPOSE OF THE INVESTIGATION

The purpose of carrying on further work with resazurin dye as an indicator in quality tests with cream was:

(a) To compare the resazurin test with the incubation test and the standard plate count as a rapid method for determining the quality of pasteurized cream.

(b) To ascertain whether or not the resazurin test alone could be used as an index to (1) initial quality, and (2) keeping quality, in order to secure the information within a few hours following processing and before the cream is shipped, or to enable the city plants to quickly evaluate the quality of cream they receive from outside plants.

HISTORICAL

Although several investigators, both institutional and commercial, have studied resazurin in comparison to methylene blue, plate counts, and microscopic examinations on raw milk and cream, we are aware of only one report of work with pasteurized cream. This work was reported by Jenkins (2) at the Dairy

Science Convention held at Columbus, Ohio, in June, 1938. Jenkins concluded that resazurin was very useful as an indicator in quality studies of pasteurized cream.

METHODS

(1) The strength of resazurin used was 0.005 percent aqueous solution as suggested by Moldavan (3), and used by Collins, White, Chilson, Turner and Rice (1) in their work on the resazurin test as applied to raw milk and cream.

(2) The end point read was a uniformly pronounced pink color. The end point rather than a set time was the standard.

(3) The incubation test comprised (a) the initial acidity (made with a 9 ml. sample of cream diluted by means of 9 ml. of water and titrated against N/10 NaOH using 5 to 6 drops of phenolphthalein as an indicator) when the fresh cream reached the city, (b) incubation at a constant temperature of 72° F. for 15 hours, after which time the second acid titration was made. A rise of 1/100 percent in acid is discussed as a one-point rise.

(4) The plate count was made with tryptone-glucose-skim milk agar, according to Standard Methods of Milk Analysis, the samples being incubated at a temperature of 35-37° C. instead of 32° C. as is now the practice in Boston. The detailed procedure of the resazurin test was as follows:

Ten milliliter quantities of cream were placed in sterile test tubes and 1 ml. of 0.005 percent resazurin solution added. The tubes were then tempered to approximately 98° F. The resazurin dye solution was made up weekly and stored

TABLE 1
The Resazurin Test Compared to the Incubation Test and the Standard Plate Count for Pasteurized Cream
(Summer Season)

Reduction Number	Time Of Resazurin Samples	Acid Rise During Incubation						Standard Plate Counts					
		0.00 to 0.02		0.03 to 0.10		0.11 or over		40,000 or under		41,000 to 100,000		over 100,000	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
A- 6 hrs. or more	46	42	91.3	4	8.7	0	0	34	73.9	5	10.9	7	15.2
B- 5 to 5¾ hrs.	76	69	90.8	6	8.0	1	1.2	52	68.5	17	22.4	7	9.1
C- 3 to 4¾ hrs.	57	38	66.7	16	28.0	3	5.3	27	47.4	16	28.1	14	24.5
Total	179												

Note. Initial tests were made after cream had been held for 36-48 hours at 40° F. including transit.

in brown glass bottles. The samples were prepared and read in a medium light, but away from direct or reflected sunlight. Observations for color changes were made at fifteen minute intervals. If the reduction appeared to be at the pink end point but the color had developed irregularly from top to bottom, the tube was inverted once to bring the entire contents to a uniform color before recording the final reading.

RESULTS

Initial Quality of Fresh Pasteurized Cream

The first studies were made on 20 or 40 percent cream pasteurized during the months of June, July, and August, 1939. These shipments were at least 36 hours old when they were sampled in the plant at Boston where all the tests were made. The results secured are recorded in Table 1, using the resazurin reduction time as the standard for comparison. Routine work in United Farmers' laboratories for several months prior to June, 1939, had indicated that pasteurized cream which would not reduce 0.005 percent resazurin to pronounced pink within six hours had exceptionally fine keeping quality. An acid rise of 0.02 percent has been accepted as appreciable and one which can be read accurately. A plate count not exceeding 40,000 bacteria per ml. has been accepted as the top standard because it is the current maximum set by the

Boston Board of Health for pasteurized cream.

The data in Table 1 shows that 122 of the 179 samples had resazurin tests of five or more hours and 111 of these gave not more than 0.02 percent acid increase in incubation. Also 86 of the 122 samples had plate counts not exceeding 40,000 bacteria per ml. There are sufficient samples in both A and B to give a fairly true picture and since there was little difference in the relationship of resazurin tests to incubation tests or plate counts between the two groups, we may conclude that the cream in Group B was almost as good as that in Group A and that a five hour resazurin-pink test indicates a good quality cream and one which will conform to the new Boston plate count standards at least seven times in ten. Twenty-two samples of the 122 had plate counts above 40,000 but under 100,000 which, until January 1st, 1940, was the Boston Board Department maximum. In Group C, we find a closer correlation between the resazurin time and the plate count than between the resazurin time and the incubation test. Additional results taken on winter shipments are recorded in Table 2.

The data in Table 2 show that practically all the winter samples fell into Group A having resazurin tests above six hours. However, it is significant that in this table also, all the samples in group B (5-5¾ hrs.) showed a marked

* Paper completed in June, 1940.

TABLE 2
The Resazurin Test Compared to the Incubation Test and the Standard Plate Count for Pasteurized Cream (Winter Season)

Reduction Number	Time Of Resazurin Samples	Acid Rise During Incubation						Standard Plate Counts					
		0.00 to 0.02		0.03 to 0.10		0.11 or over		40,000 or under		41,000 to 100,000		over 100,000	
	of	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
A- 6 hrs. or more	440	433	98.8	7	1.6	0	0	352	80.0	77	17.5	11	2.5
B- 5 to 5¾ hrs.	10	10	100.	0	0	0	0	9	90.0	1	10.0	0	0
C- 3 to 4¾ hrs.	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	450												

Note. Initial tests were made after cream had been held for 36-48 hours at 35-40° F. including transit.

correlation with those in Group A (6 hours or over). More than 80 percent of the 450 samples complied with the Boston Board of Health standard of 40,000 or less bacteria per ml. Seventy-eight samples which had counts above 40,000 came within the 40,000-100,000 class, while only 11 of the 450 samples had plate counts above 100,000 per ml. The results in Table 2 lend support to the statement regarding Table 1 that a five hour or longer resazurin test on pasteurized cream indicates good initial quality and the ability to remain sweet through the fifteen hour incubation period at 72° F.

Summarizing the results recorded in Tables 1 and 2, we note that of a total of 572 samples, which had resazurin tests of five hours or more, 451 (78.8 percent) gave less than 2 points (0.02 percent) acid rise on the incubation test and 547 (95.6 percent) complied with the Boston Board of Health standards (100,000 or less bacteria per ml.) which were in effect for pasteurized cream when the samples were studied. Four hundred and forty-seven of the 572 samples (78.1 percent) conformed with the new Boston Board of Health standards (40,000 or less bacteria per ml.) which became effective early in 1940. From the foregoing results shown in Tables 1 and 2, it would seem justifiable to conclude that a reduction time to pronounced pink of 0.005 percent resazurin solution in five hours

or longer is a satisfactory criterion of good quality pasteurized cream.

Initial and Keeping Quality of Pasteurized Cream

The relationship of the resazurin test to the incubation test and standard plate count on pasteurized cream stored at 40-45° F. for about four days was studied with 75 samples of 20 or 40 percent cream, first sampled at from 36 to 48 hours and on two successive days. Large samples were taken from the 40-quart cans when they arrived in the city and these were held in a refrigerator regulated between 40 and 45° F. All the samples were from 84 to 96 hours old when the last tests were made. This meant that the cream was at least 100 hours old at the end of the third incubation test.

The samples were divided into two classes on the basis of incubation tests and these two divisions in turn were divided on the basis of the resazurin reduction time, with 6 hours again being chosen as the standard. The classification is summarized in Table 3.

Attention is drawn to the fact that 69 of 75 samples (92 percent) failed to reduce resazurin to pink in less than 6 hours and of these 69 samples, 47 or 62.6 percent of the total number, showed plate counts of 40,000 or less through the fourth day.

Summarizing the results secured on the 69 samples in more detail, there were 207

plate counts made during the three days. Fifty-seven of the 69 initial counts (36-48 hour old cream) were 40,000 or less: fifty-seven of the counts made on the 60-72 hours old were 40,000 or less, and fifty-two samples conformed to the standards at ages of 84 to 96 hours. That is, 52 of 69 samples (79.7 per cent) aged four days conformed to the latest standard of the Boston Board of Health. All these samples had resazurin tests above six hours. A summary of the 22 samples which had plate counts above 40,000 is included at the bottom of Table 3 and the results of tests on these samples are discussed in the following paragraphs.

The data on the 22 samples indicate clearly that only seven of these samples showed an appreciable increase in count between the 2nd and 4th days (between the first and third tests). In five of these seven samples the resazurin reduction time was lowered sufficiently to indicate a definite change in quality. On the contrary, with two samples the change would not have been predicted from the resazurin tests. Six samples had relatively high counts, ranging from initial counts of 80,000 to 120,000 and final counts of 72,000 to 120,000 bacteria per ml. through the three days. None of these samples showed appreciable increases between the first and third tests, while some of the counts were reduced. Microscopic

examinations of these samples revealed appreciable numbers of thermophilic organisms. Also, it is significant that only one sample did the resazurin show an appreciable reduction. It would seem reasonable to conclude that in view of the long reduction times of resazurin and the relatively unimportant changes from the initial count during the 48 hour storage period, the types of bacteria constituting the high counts did not possess strong reducing powers and probably were types which would not increase in numbers significantly at a low temperature, although they were largely responsible for the high counts at the 37° C. incubation temperature.

In order to secure more information about the relationship of resazurin reduction times to both incubation tests and standard plate counts, it was decided to hold samples of pasteurized 20 or 40 percent cream at rather high cold storage temperatures (45-50° F.) until incubation tests on these samples showed an acid increase over the initial titration of more than two points (0.02 percent). The final resazurin tests and plate counts recorded were on the samples which, on the corresponding incubation tests, had shown more than two points acid rise. The results from the examination of these samples are tabulated in Table 4.

The data in Table 4 show that only 3

TABLE 3
Relationship of the Resazurin Test to the Incubation Test and Standard Plate Count on Pasteurized Cream

Number Of Samples	(Stored at 40° to 45° F.)			
	No. Showing Not More Than 0.02% Acid Rise On Incubation Through The Fourth Day		No. Showing More Than 0.02% Acid Rise On Incubation Through The Fourth Day	
	Resazurin pink under 6 hours	Resazurin pink 6 hours or more	Resazurin pink under 6 hours	Resazurin pink 6 hours or more
75	3	69	0	3
	Standard Plate Count	Standard Plate Count		Standard Plate Count
	40,000 or less	over 40,000	40,000 or less	over 40,000
	1	2	1	2
	Plate Count Exceeding 40,000			
	A—Over 40,000 on 4th day, less on 2nd and 3rd days		B—Over 40,000 on 4th and 3rd days, less on 2nd	
	6		1	
	On One Or More Days Over 40,000 on one day but not included in A. or B.		Over 40,000 on all days	
	6		9	

* On all counts through the fourth day.

of 19 samples gave more than two point acid rises on incubation until the end of the fourth day. At the corresponding ages, 10 of the remaining 16 samples showed appreciable drops in reduction times of resazurin while 11 of the 16 samples appeared definitely inferior on the basis of the plate count. These results correspond with the agreements shown in the last column of the table. This column gives our conclusion as to which two tests agree most closely in picking out definite quality deterioration. Only in sample 10 did it appear that the seven point acid rise on the fourth day predicted more truly the high count than did the 5 1/4 hour resazurin test and agreed more closely with the plate counts. Samples 1, 3, 12, 15, 17 and 18 are the best examples of close agreement between the resazurin test and the plate count.

Considering again the relationship between five or six hour resazurin tests and corresponding plate counts, it would appear from samples 13 and 14 that when cream is stored below 40° F., the resazurin may be less sensitive to the deterioration in quality than is the plate count. More samples with six hour resazurin tests had plate counts over 40,000 than in previous tables. This can be explained no doubt by the 32° C. incubation temperature which is known definitely to increase the plate count over the 37° C. temperature.

We may conclude from the foregoing discussion on Table 4 that there is a closer correlation between the time of reduction of resazurin to pink and the standard plate count than there is between the acid rise on samples incubated at 72° F. for 15 hours and the standard plate count.

DISCUSSION OF RESULTS

It is readily understood why creamery organizations in Vermont which are processing cream for the large urban centers of population such as Boston, Worcester, Providence, etc., should seek a quality test which will give reliable information within a few hours after pasteurization. We believe that the resazurin test as dis-

cussed in this paper fulfills this purpose. Cream pasteurized in the Vermont plants of the United Farmers' Co-operative Creamery Association, and no doubt the same applies to that processed in other commercial plants, is aged at approximately 40° F. for several hours before shipment. Even a six hour holding period in country plants enables the laboratory to send the resazurin results with the shipment to the city customers or distributors.

Any batches of cream which are not sold immediately upon arrival in the city are sampled at once and again checked by the resazurin test, incubation test, and plate count. Since most of the cream is distributed the first day after arrival, it is not possible to wait for an incubation test or plate count. Here again the resazurin test gives very valuable information in as little as three hours and dependable information within six hours.

The results in the first four tables were secured before the Boston Board of Health recommended that 32° C. be used as the standard incubation temperature for all plate count work. However, the tryptone-glucose-skimmilk agar was used throughout. The plate counts recorded in Table 4 were secured at an incubation temperature of 32° C.

It should be mentioned also that the plate counts recorded in Tables 1 to 3, inclusive, were made while the Boston Board of Health Standard for pasteurized cream was less than 100,000 bacteria per ml. Nevertheless the plate counts were classified and discussed from the standpoint of the new regulations (40,000 or less) which became effective January 1, 1940.

There was some question as to what acid rise should be considered the standard. In this work we have called a two point (0.02 percent) acid rise significant although it is questionable if this much increase during a 15 hour incubation test at 72° F. is sufficient to predict poor keeping quality. Although the results have shown that a resazurin reduction time to pronounced pink of more

TABLE 4
A Comparison of the Resazurin and Incubation Tests in Relation to the Standard Plate Count when Used on Pasteurized Cream.
(First group stored at 45-50° F.)

Sample Number	Before incubation	(A) Acidities		(B) Resazurin Tests		* (C) Standard Plate Counts		Closest agreement between	
		Significant tests after incubation**	Initial in hours	Corresponding to incubation tests	Initial at 36-48 hrs.	Corresponding to incubation tests			
1	0.115	0.135 (6)	7 1/2	5 1/4 - 1 1/4	82,000	423,000	3,130,000	B & C	
2	0.12	0.125 (5)	8	6 3/4 - 5	39,000	98,000	279,000	B & C	
3	0.09	0.11 (4)	8	4 3/4 - 3 1/2	9,000	52,000	186,000	B & C	
4	0.10	0.12 (5)	7 1/2	6 - 5	20,000	138,000	308,000	Equal	
5	0.09	0.105 (4)	7 1/4	5 1/4 - 4 1/4	16,000	25,000	76,000	Equal	
6	0.09	0.10 (3)	7 1/4	6 1/4 - 5 3/4	60,000	51,000	104,000	B & C	
7	0.095	0.105 (5)	8	7 1/2 - 5	11,000	88,000	850,000	Equal	
8	0.095	0.105 (4)	7 1/2	7 1/2 - 5 1/2	22,000	40,000	140,000	B & C	
9	0.11	0.12 (3)	7 1/2	6 3/4 - 6	15,000	21,000	40,000	B & C	
10	0.10	0.115 (3)	7 1/2	6 1/2 - 5 1/4	13,000	14,000	250,000	A & C	
11	0.09	0.10 (4)	7 1/2	6 3/4 - 5 1/2	18,000	16,000	89,000	B & C	
12	0.12	0.13 (5)	7 1/2	6 - 3 1/2	40,000	297,000	1,300,000	B & C	
(Second Group stored at 38-40° F.)									
13	0.115	0.125 (5 1/2)	8	8 - 6 1/2	35,000	79,000	1,500,000	Equal	
14	0.10	0.11 (5 1/2)	8	8 - 6 1/2	22,000	32,000	1,500,000	B & C	
15	0.10	0.11 (5 1/2)	8	8 - 3 3/4	21,000	103,000	4,400,000	B & C	
16	0.10	0.12 (4 1/2)	8	7 1/4 - 3 1/2	7,000	75,000	260,000	B & C	
17	0.09	0.105 (4 1/2)	8	7 1/2 - 3 1/2	25,000	98,000	151,000	B & C	
18	0.10	0.115 (4 1/2)	8	7 1/2 - 3 1/2	65,000	230,000	355,000	B & C	
19	0.12	0.14 (5 1/2)	8	8 - 5 1/4	13,000	32,000	184,000	B & C	

** Figures in parenthesis indicate age of cream in days.

* Plates incubated at 32 degrees C.

than five hours indicates good quality cream, we feel that it would be wiser for commercial plants to accept six hours as the minimum standard for "top grade" quality, especially for 20.0 percent pasteurized cream.

SUMMARY AND CONCLUSIONS

1. A six hour reduction time of 0.005 percent resazurin dye to a pronounced pink color is a criterion of fine quality in pasteurized 20 or 40 percent cream. Such cream will generally show not more than 2 points (0.02 percent) acid rise upon incubation and standard plate counts not exceeding 40,000 bacteria per ml. when aged four days at 40-45° F.

2. A five hour resazurin test indicates an initial quality and a keeping quality which are slightly inferior to that shown by a six hour resazurin test when both are compared to incubation tests and standard plate counts on pasteurized cream.

3. A reduction time of five hours for 0.005 percent resazurin to pronounced pink is a more accurate criterion of satisfactory quality in pasteurized 20 or 40 percent cream than is a 0.02 percent acid rise upon incubation at 72° F. for 15 hours.

4. A reduction time of over five hours for 0.005 percent resazurin used on pasteurized 20 or 40 percent cream indicates a standard plate count of less than 100,000 bacteria per ml. in 9 of 10 samples

and of 40,000 or less in at least 7 of 10 cases.

5. When the pasteurized cream was stored for several days at less than 40° F., the resazurin reduction time did not indicate fully the increase in plate counts which occurred.

6. There was a much closer agreement between the resazurin test and the standard plate count than between the incubation test and the standard plate count in the indication of poor quality.

7. The resazurin test as applied in the work reported herein is a satisfactory and rapid criterion of both initial and keeping quality of pasteurized 20 or 40 percent cream. However, it cannot be depended upon always to pick out high counts in cream that has been stored at 40° F. or lower for four or more days.

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Hemolytic Streptococci in Raw Market Milk. J. B. Gunnison, M. P. Luxen, M. S. Marshall, and B. Q. Engle. *J. of Dairy Science*, Vol. XXIII, No. 5, May 1940, pp. 447-455; *Pub. Health Engin. Abs.* xx, Mi. 35.

"A total of 444 samples of raw market milk from as many different dairy farms was examined for the presence of hemolytic streptococci of both wide-zone and narrow-zone types. Such streptococci were found in 134 of these samples, and were distributed among the Lancefield serologic groups as follows: group A in three samples, group B in 125, group C of the animal type in one, group C of the human type in two, group D in two, group E in two, group G in six and group H in one. Only four samples

contained streptococci which could not be assigned to a group. Nineteen samples of raw cream were tested and group B streptococci were found in five of them. Approximately half of the group B streptococci produced double zones of hemolysis in rabbit blood agar.

"The fact that high grade milk produced under rigid inspection and, so far as the plate count is concerned, above the standard set for certified milk, may contain hemolytic streptococci of human origin is a strong argument for universal pasteurization even though the number of instances in which such organisms are found is extremely low. In this city, San Francisco, California, all milk including the certified grade must be pasteurized." R. A. C.

The Leucocyte Count and the Chloride Content of Milk from Bovine Udders with Mild Streptococcic Infections *

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INTRODUCTION

The general practice, in using the chloride content and the leucocyte count of milk as indicators of mastitis, is to establish some arbitrary level for each test as a standard for interpreting the results. The standards, as used by different investigators, have varied widely. In a brief review of the literature, Little (4) pointed out that one group of investigators recommended as a criterion of mastitis a leucocyte count of 3,000,000 per ml. or more, whereas another group of investigators considered a count of 100,000 per ml. as indicating mastitis. In the case of direct titration values for chlorides, the usual criterion employed is 0.14 percent.

Little (4) reports an experiment in which 95 percent of 1,010 quarter samples of foremilk from 8 young cows contained fewer than 300,000 leucocytes per ml., and 99.3 percent of the 1,010 samples contained less than 0.135 percent of chlorides. The 8 cows in the experiment were then exposed to or inoculated with a hemolytic streptococcus. Of 2,163 samples from quarters that developed subclinical mastitis, 94.5 percent contained leucocytes in excess of 300,000 per ml., but only 34.2 percent gave chloride titration values greater than 0.14 percent. Similar though generally less clear-cut results have been obtained in this laboratory and by other investigators.

It is generally recognized that the normal chloride content and leucocyte count of milk from different cows may differ widely. Some of the factors influencing

these values are breed, age, and stage of lactation of the cow. These wide normal variations probably account for the poor definition of results that are obtained when one is dealing with a mixed herd of all ages and in various stages of lactation. In the mastitis investigations in the Bureau of Dairy Industry herd at Beltsville, Maryland, such a problem exists. In this work individual quarter samples are taken at intervals of two or three weeks. A comparison of the results from each quarter sample with the previous history of the quarter and also with results from other quarters of the same cow on the same day has proved to be of more value than arbitrary standards. The purpose of this paper is to show the results of such a comparison.

METHODS

Strip-cup tests are made daily on all of the cows. Samples for laboratory examination are collected at intervals of two or three weeks. Sampling is done in the afternoon just prior to the milking of each cow. Each teat is wiped off with a pledget of cotton saturated with alcohol. After the first two streams of milk are discarded, about one-fourth pint is milked into a sterile, half-pint, screw-cap jar. These jars are collected in insulated shipping cases and iced for transport to the laboratory, where the samples are examined on the following day.

The examination consists of the Hotis test, as modified by Cone and Grant (1); plating 0.1 ml. in Edwards' (2) aesculin blood agar; leucocyte counts by direct microscopic examination of Breed smears stained with Newman's (5) stain; and

* Presented at the joint meeting of the International Association of Milk Sanitarians and the New York State Association of Dairy and Milk Inspectors, New York City, October 17-19, 1940.

TABLE 1
Chloride titration values and leucocyte counts before and after infection

Cow and quarter	Lactation period prior to infection				Samples prior to infection in the same lactation period				Samples following infection in the same lactation period					
	No. of sam- ples	Per- cent Cl. (Ave.)	No. of leuco- cytes (Ave.) (000 omitted)	No. of sam- ples	Per- cent Cl. (Ave.)	No. of leuco- cytes (Ave.) (000 omitted)	Per- cent Cl. (Ave.)	No. of sam- ples	Per- cent Cl. (Ave.)	No. of leuco- cytes (Ave.) (000 omitted)	Per- cent Cl. (Ave.)	No. of sam- ples	Per- cent Cl. (Ave.)	No. of leuco- cytes (Ave.) (000 omitted)
1234 LR	5	0.113	164	3	0.105	127	0.105	127	0.112	181	0.119	10	0.119	294
1235 RR	17	0.136	117	0.253	14,900	0.230	12	0.230	9,270
1235 LF	17	0.131	88	0.232	8,090	0.192	12	0.192	5,703
1235 LR	4	0.223	1,730	0.141	182	0.141	17	0.141	195
1278 RR	2	0.117	317	0.112	979	0.122	15	0.122	1,020
1278 RF	2	0.109	64	0.148	3,770	0.129	16	0.129	1,630
1278 LF	5	0.107	98	0.115	863	0.121	11	0.121	1,510
1278 LR	5	0.117	231	0.107	326	0.113	13	0.113	310
1285 RF	20	0.112	94	0.150	3,860	0.150	6	0.150	5,600
1285 LR	19	0.107	135	0.196	8,940	0.189	6	0.189	8,460
1411 RF	6	0.111	64	0.114	66	0.120	931	0.136	7	0.136	6,690
1411 LR	10	0.150	2,300	0.133	3,755	0.107	5,300	0.165	3	0.165	4,660
1441 RR	11	0.102	99	0.111	5,120	0.102	8	0.102	6,360
1441 LF	11	0.115	353	0.109	661	0.104	8	0.104	7,130
1441 LR	11	0.121	510	0.131	4,080	0.133	12	0.133	2,610
1445 RF	17	0.098	153	0.103	447	0.103	13	0.103	254
1469 LF	6	0.095	4,260	0.095	136	0.119	962	0.120	19	0.120	2,540
1472 RF	3	0.090	284	0.090	284	0.121	3,140	0.128	17	0.128	3,970
1472 LF	5	0.099	421	0.104	671	0.111	640	0.122	16	0.122	1,480
1472 LR	6	0.097	30	0.099	24	0.208	4,150	0.245	13	0.245	10,900
A71 RF	8	0.192	2,170	0.180	1,290	0.168	12	0.168	1,670
859 LF	8	0.137	43	13
Total	148	53	254
Weighted average	0.124	206	0.114	1,035	0.107	581	0.143	3,340	0.143	3,320

direct titration for chlorides with silver nitrate according to the method of Ham-mer and Bailey (3).

RESULTS BEFORE AND AFTER INFECTION

Eleven cows have developed subclinical streptococcic infections in 22 quarters while under observation. For 12 of these quarters complete or partial histories are available for the lactation period prior to the one in which infection occurred. Eleven quarters were found to be infected at the time of the first sampling after calving. The other 11 quarters developed infections several weeks after calving and after at least 2 samples had been taken in that lactation period. The data obtained before and after the beginning of infection are shown in Table 1.

With 2 exceptions, the average of the leucocyte counts obtained after the onset of the infection is significantly higher than the average of those obtained before infection. The four samples from the left rear quarter of cow 1235 taken before the infection started were obtained at the end of a lactation period. At that time all quarters of the cow were giving milk high in chloride and leucocyte content. Since no infection occurred in the other 3 quarters in the next lactation period, concurrent figures for those quarters do not appear in Table 1. In the case of the left front quarter of cow 1469, the first 2 of the 6 samples taken before the beginning of the streptococcus infection gave leucocyte counts that were inexplicably high — 22,500,000 and 2,600,000 per ml., respectively. In this case, however, the corresponding chloride values were low, and the average leucocyte count of the last 3 samples before the beginning of infection was significantly lower than the average for the infected samples.

In general the average chloride content of the samples taken before the onset of infection is lower than the average for the samples taken after the beginning of infection. There are some exceptions, however, and in addition the

the case of the leucocyte counts. This probably is due to the greater tendency for the chloride content to change as the result of factors other than infection.

When infection takes place the rise in the chloride and leucocyte values is usually rather abrupt. This is shown when the results of the last 3 samples before infection are contrasted with the results from the first 3 samples after the infection has become established. The average of 3 samples rather than the results of a single sample has been used in each case in this comparison to rule out possible aberrant results that occasionally occur with a single sample. In the eleven comparisons shown, there was always a rise, usually marked, in the leucocyte count. With one exception the chloride values also increased at the beginning of infection.

COMPARISON OF RESULTS FROM SAMPLES TAKEN AT THE SAME TIME FROM INFECTED AND UNINFECTED QUARTERS

In a comparison of the uninfected quarters with the quarters of the same cow that were infected with streptococci, it seemed advisable to eliminate from the study all samples that were taken in acute stages of the disease. No sample from any quarter that showed positive strip-cup reactions within one week of the sampling date was included. Furthermore, all samples having a leucocyte count of 10,000,000 or more per ml. were arbitrarily excluded. A total of 954 samples from 19 cows are included in this study. The results are shown in Table 2.

Without exception, the average leucocyte count of the samples from infected quarters was higher than that from uninfected quarters of the same cow. Seventeen cows show a higher average chloride content in samples from infected quarters than in those from the uninfected quarters. In the other 2 cases the chloride values in the uninfected quarters were higher.

The 19 cows listed in Table 2 were sampled a total of 198 times. With each sampling the results of the infected

TABLE 2
Chloride titration values and leucocyte counts from infected and uninfected quarters of the same cow

Cow No.	Uninfected quarter samples			Streptococcus infected quarter samples			No. of instances in which uninfected quarter samples were higher than infected quarter samples in:	
	No. of samples	Percent Cl. Ave.	Leucocytes Ave. (000 omitted)	No. of samples	Percent Cl. Ave.	Leucocytes Ave. (000 omitted)	Cl.	Leucocytes
1234	10	0.126	100	10	0.119	294	9	1
1235	78	0.152	396	51	0.173	2,290	17	4
1269	5	0.118	105	3	0.124	483	0	0
1273	12	0.139	150	4	0.146	159	1	2
1274	30	0.107	20	10	0.110	286	2	0
1285	14	0.111	28	12	0.169	5,900	0	0
1289	8	0.127	122	4	0.161	3,930	0	0
1411	7	0.120	176	8	0.139	1,820	0	0
1434	9	0.124	32	3	0.178	4,220	0	0
1445	66	0.104	61	30	0.147	2,200	0	0
1469	9	0.098	69	9	0.103	259	3	1
1472	37	0.103	49	56	0.123	1,200	0	0
A71	14	0.174	1,331	17	0.198	5,070	1	1
811	15	0.150	229	5	0.187	371	0	2
815	13	0.220	318	23	0.188	2,220	13	0
859	12	0.142	103	22	0.167	1,740	0	0
1071	29	0.138	144	10	0.145	1,310	7	1
1078	4	0.112	68	2	0.183	4,730	0	0
1089	2	0.106	281	1	0.120	798	0	0
Total	374			280			53	12
Weighted ave.	0.130	205	0.153	2,050

of the uninfected quarters. In all but 12 of these 198 comparisons, the counts were higher in the infected quarters than in the uninfected quarters. In 11 of the 12 aberrant cases, the leucocyte counts of the infected samples were less than 110,000 per ml. In the other case the leucocyte count of the sample from the infected quarter was 453,000 per ml., and the counts for the 3 quarters classed as uninfected were 163,000, 163,000, and 1,960,000, respectively.

In the comparison of the chloride values, the samples from the uninfected quarters gave higher results than the samples from the infected quarters in 53 of the 198 comparisons. Fifty-one of the 53 instances are attributable to 6 cows. The average chloride values for the infected samples from each of 3 of

these 6 cows were significantly lower than 0.14 percent. In the other 3 cases the average chloride values of the uninfected samples were 0.138 or higher.

Thus in almost every instance in which this sort of comparison failed to reveal the infected quarters, the commonly employed arbitrary criteria based on the chloride titration and the leucocyte count would also have failed to distinguish between infected and uninfected quarters.

DISCUSSION

Most of the infections considered in this study are obviously very mild. A large number of them yielded average chloride values lower than the usually accepted standard of 0.14 percent. That is true of 13 of the 22 infected quarters listed in Table 1, where the

weighted average for all samples from all infected quarters was only 0.143 percent. It is true also for the infected quarters of 7 of the 19 cows listed in Table 2. Also, a few of the infections would consistently have been missed on the basis of leucocyte counts if one considered a count of 300,000 per ml. as indicating mastitis.

On the other hand a large number of the uninfected samples would have been included with the mastitis group if they had been considered in comparison with the arbitrary standards commonly employed. Three of the 22 uninfected quarters listed in table 1 showed average chloride values greater than 0.14 percent and two others gave values greater than 0.135 percent. The samples from uninfected quarters of 5 of the cows listed in table 2 gave average chloride titration values greater than 0.14 percent and the uninfected samples of two other cows gave average chloride values greater than 0.135 percent.

The average leucocyte counts of uninfected samples from 8 of the 22 quarters listed in table 1, and the average counts for the uninfected quarters of 3 of the 19 cows listed in table 2, were greater than 300,000 per ml.

The results show clearly that in an unselected group of cows, such as the one reported here, no arbitrary values can be selected for the chloride and leucocyte tests that will distinguish with any degree of reliability between samples from infected and those from uninfected quarters. Comparisons of the results from any quarter with the previous history of that quarter, or with the results from other quarters of the same cow, usually give a fairly reliable clue to the condition of the quarter. But neither of these indirect tests is as reliable as an index of infection as bacteriological tests. When used in conjunction with bacteriological tests they add supporting information.

It can hardly be said that such mild infections have no significance, especially where an effort is being made to eliminate streptococcal mastitis by segregation

of the infected animals. These mild cases, if left in an otherwise clean herd, might serve as foci for spreading the infection to other cows. It is possible, too, that the long continued, though slight, inflammation will adversely affect the production of the involved quarter.

CONCLUSIONS

1. In a group of cows of different breeds of various ages and in all stages of lactation, the chloride content and the leucocyte count of the milk failed to distinguish reliably between quarters with very mild streptococcus infections and the uninfected quarters.

2. In conjunction with bacteriological tests a comparison of the chloride content and leucocyte count of the samples from different quarters of the same cow gave valuable information in support of the culture methods.

3. When a cow is being tested periodically, an abrupt rise in the chloride content and leucocyte count in the milk strongly indicates the beginning of infection, even though these values may not exceed the arbitrary values usually accepted as indicating mastitis.

4. The leucocyte count is a more reliable index of infection than the direct chloride titration values, probably because of the greater tendency for the chloride content to be affected by factors other than infection.

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Determining Riboflavin in Dried Milk Products

II. Seasonal Variations

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The average amount of riboflavin in various dried milk products has been reported (1) and slight differences in the riboflavin content of liquid milk from various breeds of cows has been noted (2, 3). In one locality it was found that summer milk contained more riboflavin than winter milk (2) while in another locality no difference was observed between milk obtained in May and that obtained in November (4). A study in one state of commercial milk as compared with that obtained from the experimental station farm, has demonstrated a 20 percent seasonal variation for the former as compared with a 13 percent variation for the latter (3). This has been attributed to differences in the silage fed, a factor which has already been shown to be of great importance in controlling the concentration of riboflavin (5).

Since dried milk products are among the most important sources of riboflavin for animal and poultry feeding, the question of a seasonal variation in concentration is of considerable practical significance. Before much of the above information became available, it was decided in the early part of 1937 to carry on an extended study of this problem. Instead of limiting the observations to a single herd of cows or even to an isolated area, a survey was made of several different states which were representative of the main sources of dried whey. The results would therefore indicate whether, throughout the country, there was sufficient seasonal variation in this factor to influence the value of milk products as feed supplements.

The material most readily available for

this study was dried whey from cheese. Although the results so obtained could not be interpreted directly in terms of liquid whole milk, any changes which were observed in dried whey would be an indication of corresponding changes in the original milk. The validity of such an interpretation is based upon the common observation that the major portion of the total riboflavin content of whole milk is found in the whey after the cheese-making operation.

EXPERIMENTAL

Six drying plants were selected for this study, and these were located in five different states. It was thought that in this manner the influence of any unusual local climatic conditions would be eliminated. Several of these plants combined their whey with that from other nearby cheese factories, so that the final product represented a composite sample from a very large number of dairies.

Bi-monthly samples were obtained from these plants over a period of two years beginning in July, 1937. The volume of milk which these individual samples represented may be judged from the total annual production of 5,300 tons of dried whey from these six drying plants.

The riboflavin concentration of the dried whey samples was determined by the photometric procedure which was described in the first paper of this series (6). The method is based upon the extraction of riboflavin with acid-acetone and the destruction of colored impurities by mild oxidation. After filtration, the riboflavin concentration is measured by the determination of light absorption be-

fore and after reduction to the leuco form. It was shown that when this method is applied to dried milk products, the results are reproducible to within one microgram per gram.

Each sample was run in duplicate and the average of several readings was taken. In Table 1 each horizontal row represents the values obtained from a single plant at three-month intervals. The mean value for each season together with its probable error, is given at the foot of the table.

TABLE 1

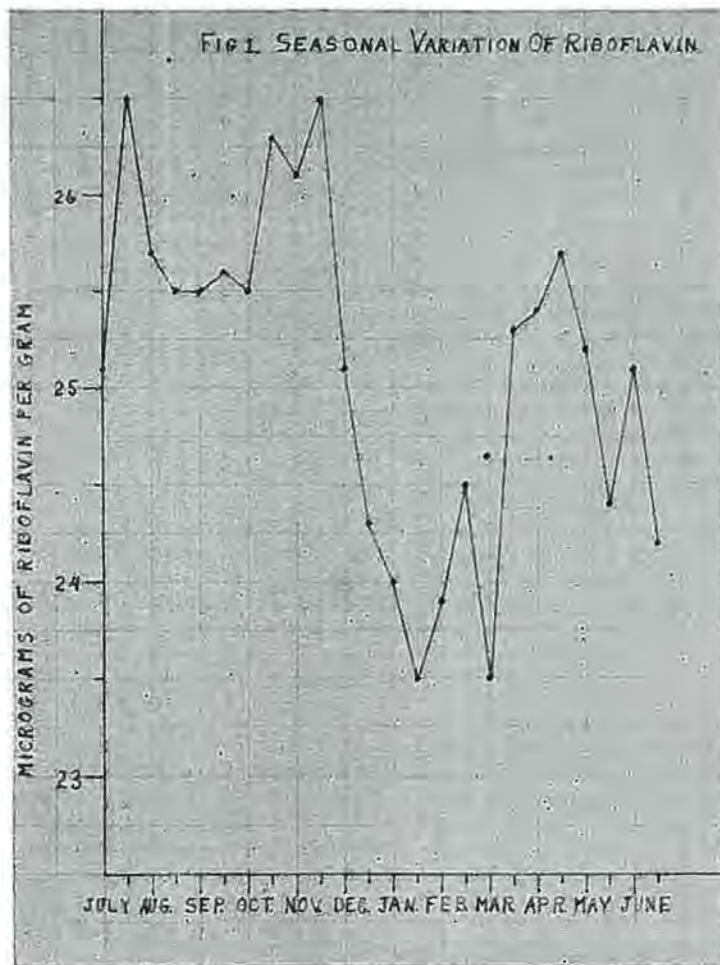
Riboflavin concentration of bi-monthly samples of dried whey obtained from five states over a two year period.

Summer	Fall	Winter	Spring	Summer	Fall	Winter	Spring
23.4	25.6	23.6	29.8	29.4
.....	24.7	23.0	25.9	30.6	22.5
23.1	26.2	24.0	26.3	27.0
23.3	26.2	23.2	27.3	23.2
25.9	23.5	21.1	21.4	24.4	26.5	26.6
26.5	25.0	23.0	23.6	28.2	22.5	24.6	26.2
26.3	26.4	20.3	25.6	25.4	22.6	24.3	28.0
24.3	25.5	22.3	25.4	26.8	27.3	23.7	26.7
24.6	25.6	22.2	24.2	29.2	24.8	26.9
24.0	23.5	20.9	22.2	26.3	24.5	26.3
24.2	23.0	19.6	22.7	26.5	23.3	24.1	28.6
24.6	20.5	23.0	25.1	25.7	22.5	25.1
22.0	27.2	20.4	25.7	26.8	29.4	26.0	23.3
22.6	27.3	24.3	26.6	26.1	27.3	26.0	24.0
24.7	22.6	21.7	27.8	24.2	25.8	30.0	22.8
.....	24.0	23.1	26.0	23.5	23.4	20.3	24.8
29.0	22.7	22.9	26.5	25.4	25.7	19.3	24.1
28.1	20.5	27.5	27.5	25.4	24.4	19.8	22.4
25.4	23.4	25.2	24.7	22.9	23.0
27.0	30.6	24.3	24.7	23.7	22.3	20.5	25.6
28.4	28.2	22.6	25.9	24.7	22.2	24.5	26.4
27.9	26.8	25.3	23.5	24.9	22.6	19.3	21.5
26.4	25.1	25.4	24.1	26.3	23.4	20.5
.....	25.1	26.2	22.6	22.6	21.2	20.4	23.2
24.1	24.2	28.3	28.4	22.3	23.1	20.7	22.5
25.7	27.9	28.6	28.3	21.3	21.6	22.2	23.6
24.5	26.5	29.0	28.8	21.6	20.1	20.3
24.6	23.9	29.5	27.9	MEAN			
28.8	28.9	27.2	28.9	25.6±0.27	25.6±0.32	23.8±0.39	25.0±0.29
25.6	27.6	27.1	The only significant differences which were observed were for the mean value for winter as compared with those for the other three seasons. Even in this case the average value for winter was only seven percent lower than that for summer. This is in good agreement with the value of thirteen percent for an individual herd and considerably better than the twenty percent variation found in one locality for commercial milk (3).			
31.6	29.3	24.1				
27.2	24.5				
28.3	28.7	23.1				
.....	31.0				
24.6	27.1	26.7				
27.8	26.4				
22.0	25.2	28.0				
28.2	27.6				
26.6	28.4	26.2	25.0				
27.8	29.8				
23.4	26.3	23.1	23.4				
25.7	24.2	26.3				
27.6	27.6				
30.1	29.1				

25.6±0.27 25.6±0.32 23.8±0.39 25.0±0.29

The only significant differences which were observed were for the mean value for winter as compared with those for the other three seasons. Even in this case the average value for winter was only seven percent lower than that for summer. This is in good agreement with the value of thirteen percent for an individual herd and considerably better than the twenty percent variation found in one locality for commercial milk (3).

In Figure 1, the results from the two-year survey have been averaged in order to demonstrate the general trend of the riboflavin concentration throughout the year. Several factors appeared to have influenced the riboflavin concentration. The most obvious one was the effect of green pasture in the spring. Since the concentration did not continue to increase uniformly up to its maximum value, it may be concluded that for a time the volume of milk produced was increasing too rapidly for the riboflavin to keep pace. In confirmation of this explanation, it should be pointed out that a negative correlation between riboflavin concentra-



tion and milk production has already been reported (3).

In the late summer and early fall there was a slight decrease in riboflavin concentration followed by an abrupt increase. This may have been due to a combination of two factors: the availability of green pasture in the early fall and the feeding of fresh silage in the late fall. As was anticipated, the lowest concentration of riboflavin occurred during the winter months when the feed that the cows received was relatively deficient in this factor.

SUMMARY

A two-year survey has been made of

the riboflavin content of dried whey from cheese. A total of 244 samples, collected from five different states, was analyzed by a photometric procedure. The average concentration of all samples was 25.1 micrograms of riboflavin per gram, a value which is in excellent agreement with the published value of 25 for dried whey from cheese (7). A statistically significant difference was found between the mean value for winter and that for any other season of the year. Contrary to the belief of many people, the maximum deviation amounted to only seven percent of the total concentration. This small seasonal variation would therefore

be insignificant for practical feeding purposes. The graphical representation of the results gave indications that the seasonal variation was not a simple phenomenon but depended upon several factors.

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Mere Possession of Unwholesome Food Not Illegal

Conviction of violation of city sanitary code in keeping unwholesome canned grapes reversed.—(New York Court of Appeals; *People v. Wallace & Co.*, 26 N.E.2d 959; decided April 16, 1940.) The defendant company was convicted of a violation of section 163 of the New York City Sanitary Code. This section provided, among other things, that no vegetables not being then wholesome or safe for human food should be brought into the city or kept, offered for sale, or sold as such food, or kept or stored anywhere in the city, and that any vegetables packed in cans, the contents of which had become fermented as evidenced by swelling or bulging, should be deemed not wholesome or safe for human food. The term "vegetables" included any article used as and for human food other than milk or meat. The case against the defendant, a candy manufacturer, was that a health department inspector had found, in a storeroom of defendant's factory, 12 cans that were swollen and bulging and which contained grapes that had become unwholesome. These cans had been in the storeroom for at least a month. In defense the proof was that the defendant made no use of the foodstuffs kept under lock in its storeroom without first inspecting them and that any article found

on inspection not to be wholesome was put aside for return to the seller. There was no proof in respect of the time when the grapes had become fermented, nor was it shown that anything in respect of the time of fermentation could have been validly inferred from the swollen and bulging shape of the cans.

In considering the case on appeal by the defendant, the court of appeals said that, on such record, the judgment of guilt must mean that the defendant's mere possession of the containers made it answerable as for a crime once the ensealed grapes became unwholesome, and that the broad text of the section—that no unwholesome food should be "kept or stored anywhere in the said city"—appeared to go a long distance in that direction. "But a penal statute," stated the court, "is not necessarily to be liberally applied in all circumstances." It was said to be the court's best judgment that no considerations of expediency required such unfairness as would result were section 163 to be so freely construed as to force its application to the facts which in the instant case were found below. The judgments were reversed and the information dismissed.

* Pub. Health Reports, September 20, 1940, p. 1744.

Dairy Herd Management Practices Affecting the Quality of Milk *

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A successful sanitation program calls for the coordinated efforts of many individuals, officials, and groups. The chief objective is safeguarding the public health, with the economic problems of the milk producer ordinarily receiving only incidental consideration. Some may disagree, but it is my judgment that it has been and is the inclination of public health officers, milk inspectors, and local officials to place reliance chiefly on forcing compliance by threatening to or actually degrading or revoking the permit of the producer. From many viewpoints it is difficult to find fault with such a program. In the final analysis, however, the role of the milk producer or herdsman in providing a safe milk supply, his viewpoint and methods, have a very direct and significant influence upon the *quality* of the milk, and the goal of the good inspector is to arouse his interest and secure his active cooperation.

The chief responsibility for the care and management of the dairy herd and the care of the milk produced rests first and foremost on the farmer or herd manager. Whether a small producer where all the work is done by the owner and members of his family, or a larger producer where hired help is employed, every operation is generally under the immediate supervision and control of the owner or herd manager. The economic success of his business is of paramount importance to him. He, too, is greatly concerned about the quality of the product he produces. I first heard the state-

ment from a *milk producer*, that "A dairy barn is a place in which human food is produced—not just a place in which to keep cows." The intelligent owner or herdsman knows that without a high quality product that commands a good price in a competitive market, his chances of economic success are reduced. Where is there a good dairy farmer who does not take satisfaction and pride in building up a good herd and marketing a healthful product of high food value? It is not natural for a man to do a good job of breeding, feeding, and managing his herd and who is economically successful to be indifferent and careless about milking and caring for the milk of the cows under his supervision. He has a right to expect an intelligent understanding of his problems and his business on the part of public health officials.

Good quality milk must be clean, free from pathogenic bacteria, high in food value, have no unpleasant odors or tastes, and have good keeping qualities. Milk of poor sanitary or nutritive qualities results from:

1. Disease organisms transmitted to the milk from cows infected with certain diseases, the most important of which are mastitis, Bang's disease, and tuberculosis.

2. Disease organisms introduced by persons handling the milk or from other sources, among the more common of which are typhoid and paratyphoid fevers, streptococcus infections such as septic sore throat and scarlet fever, diarrhea in children, and diphtheria.

3. Milk utensils and equipment of poor construction or not properly cleaned and sterilized.

4. Infected water supply or sewage contamination.

5. Barn and milk rooms poorly located or constructed and inconvenient in arrangement.

6. Improper feeding, herd management, and unsanitary practices.

The six points just named may usually be controlled in such a manner as to insure high quality milk and milk products where there is:

1. An intelligent understanding and viewpoint on the part of the herd manager, assuring his economic success.

2. An intelligent understanding and common sense viewpoint on the part of the inspector and public health officials, particularly their showing ability to secure cooperation in all real essentials.

3. Cooperation, careful planning and educational work on the part of milk producers, and public health and local officials in presenting the superior food values of milk and its products for gaining consumer confidence.

DISEASE CONTROL IN THE DAIRY HERD

Tuberculosis, Bang's disease, mastitis, and occasionally other diseases and ailments of dairy cattle present problems that tax the ingenuity of science and especially of the bacteriologist, veterinarian, and herdsman.

The widespread public understanding of at least the general nature and dangers of tuberculosis and the very efficient testing and slaughter plan for the control of bovine tuberculosis has reduced its incidence among dairy cattle so that its public health and economic importance needs relatively less stress today. It is significant, however, that while glandular, bone, and abdominal tuberculosis in humans, to a large extent of bovine origin, increased slightly from 1900 to 1917 when little testing was done, it decreased by 76 percent from 1917 to 1936 when the tuberculin testing of cattle jumped from 20,000 to 25,000,000 annually. This compares with a decrease of 30 percent in respiratory tuberculosis, chiefly of human origin, during the early years, and 59 percent during the last years mentioned.

In other words, the tuberculosis eradication program in cattle once it got under way on a large scale has proven more effective than the best that medical science has been able to do in the case of tuberculosis of human origin.

Bang's disease, because of its relation to undulant fever in man and because of its great economic importance to the dairy farmer, needs special consideration. Fortunately, it is possible to detect animals infected with this disease by means of the "blood test" with a high degree of accuracy, especially when regular tests are conducted at short intervals. The economic aspects of this disease have special appeal to the farmer. For example, in infected animals (a) the milk yield is reduced 20 to 25 percent, (b) the loss of calves averages 30 to 40 percent greater than in healthy animals, (c) the calving interval is increased from a normal of 12 months to approximately 20 months, (d) one out of every five aborting cows becomes sterile and replacements in the herd are increased by 30 percent. Control measures consist chiefly of regular blood tests, removal of infected cows, and a carefully planned sanitation program.

Mastitis is a major dairy problem from both the economic and hygienic standpoints. Methods of detecting this disease and control measures are not so well worked out as in the case of tuberculosis or Bang's disease. Nevertheless, reasonably efficient control is possible with the help and cooperation of skilled bacteriologists, veterinarians, and herdsman. The careful bacteriological examination of milk, use of the Hotis test, determination of leucocytes, titration for chlorides, and the catalase test give the bacteriologist a reasonably satisfactory means of diagnosis. Likewise, a physical examination of the udder by an experienced veterinarian gives fairly satisfactory results. Watching the herd closely for injured and abnormal udders and abnormal milk, the use of the strip cup and regular use of one or more of the bacteriological or chemical tests just mentioned, coupled with the physical examination, gives the herd

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manager the principal information he needs for establishing management practices which lead to the elimination of the disease. Isolation of infected cows, milking them last or better still by a different milker and by hand is suggested. A careful and strict sanitation program is always essential.

DISEASES TRANSMITTED BY MAN AND MISCELLANEOUS SOURCES

When the herd manager understands the importance of personal hygiene, that no person who is sick or just recovering from any disease should act as a milker, care for the milk or handle milk utensils, the danger of introducing disease organisms is reduced to a minimum. His cooperation in proper cleansing and sterilization of all utensils and equipment, control of flies and their exclusion from milk and milk utensils, and avoidance of sewage contamination of the water supply eliminates the last possibility of introducing harmful organisms.

FEEDS, FEEDING AND MANAGEMENT

Nutritive Value and Color of Milk. The kind of feed is important in supplying the vitamin A and D requirements of cattle and influences the vitamin content and color of the milk. The relationship of feed to the vitamin A content of the milk deserves special mention. All green pasture crops, green colored, properly cured hays, and properly preserved fresh green silage crops provide liberal amounts of vitamin A. Much of the carotene and vitamin A value of hay is destroyed by oxidation from excessive exposure to sunlight and weathering in field curing. Consequently, hays that are cut in the early bloom stage, cured without exposure to rain or too much sun, retain a larger proportion of their carotene content than those cut in the later stages and exposed for long periods to sun or rain. If hay heats severely in the mow, there is also much loss of carotene. In the artificial curing of hay there is only a slight loss of carotene and consequently it is high in vitamin A value. In general,

the amount of green color in hay is in direct relation to the amount of carotene. Among foods of animal origin, cod liver oil, other fish oils, egg yolk, and milk fat are ordinarily rich sources of this vitamin.

Among cattle there are marked breed differences in their ability to convert carotene into vitamin A. This is reflected in the color of the body fat and milk fat of the several breeds. It is important to note that the amount of carotene, and consequently yellow color, present in the milk is not a true measure of its vitamin A value since it does not give a measure of the vitamin present as such. For example, in the milk of Guernseys and Jerseys most of the vitamin A value is due to carotene, whereas Holstein, Brown Swiss, and Ayrshire milk contains less of the pigment and more of the colorless vitamin A as such. As a result, cows of the various breeds, when fed the same rations, may produce milk and fat of equal vitamin A value although differing in yellow color. The extent of the conversion of carotene into vitamin A also varies with individuals and the species. In fact, the occurrence of carotene in milk is limited primarily to the bovine species. The milk of the goat and of women, for example, is nearly colorless because of the very complete conversion of carotene into vitamin A, and thus the vitamin A value of their milk may be high even though no color is present.

Flavor and odor of milk. Fresh clean milk has a rich and pleasing taste. Slight defects in flavor may prevent full enjoyment of milk and thus curtail consumption. During the spring months in particular when the cows are on pasture, complaints concerning undesirable flavors in milk and its products are most common.

Flavors in milk may originate at various stages in its production and handling. Bad flavors are not necessarily associated with the safety of the milk for food purposes nor is its bacterial content always high. Certain flavors are present when the milk is drawn and these are largely dependent upon two factors—the physical

condition of the cow, and the kind of feed consumed. Abnormal conditions of the udder may cause salty or bitter milk. "Off" flavors also frequently occur late in the lactation period. Feed and weed flavors are imparted to milk mainly by way of the blood coursing through the udder. In general, common dry feeds do not contain highly flavored constituents, but in contrast, many of the more succulent green feeds have strong flavors which are transmitted to milk. A change from dry feeding to grass always results in a different flavor appearing in the milk, which sometimes causes complaints from customers. These flavors can be prevented by following a well planned routine of feeding. Strong flavored feeds should be fed either several hours before or directly after milking. When fed at milking time, or even one hour before, such feeds as silage, green alfalfa, sweet clover, green rye, cabbage, and turnips affect the flavor and odor of milk. Wild onions, garlic, and bitterweed affect the flavor as soon as eaten and continue to do so for at least 4 to 7 hours. In early spring and particularly when pastures are short and weed-infested, weed flavors are quite common since cows are forced to eat herbage they might otherwise refuse. The various flavors imparted by weeds can be held to a minimum by removing the cows 3 to 6 hours before milking time.

The exposure of milk to the rays of the sun seriously affects its flavor. The presence of iron or copper salts causes a speedier action of sunlight than would otherwise occur. The "off" flavor developing in this instance is usually described as "tallowy", "cardboard", "metallic", or "astringent". The use of poorly tinned milk cans, buckets, coolers, vats, etc. is responsible for many "off" flavors, and also adds copper and iron salts to the milk. This aids in developing the flavors associated with exposure to sunlight.

Washing compounds and chemical disinfectants, if carelessly used, may be responsible for the addition of foreign

flavors. Only readily soluble cleaners free from odors should be used for milk utensils. Chemical disinfectants such as chlorine solutions used according to directions cause little trouble. Poor draining of cans and utensils after sterilization, or use of excessive amounts of this agent may, however, cause trouble.

After the milk is drawn, unless the utmost care and cleanliness is exercised, it may acquire various "off" flavors. Odors absorbed from the barn, milk house, and general surroundings often affect the flavor and destroy the pleasing taste of milk. Oils, fly sprays, and medicines used about the barn often impart flavors. Removing milk from the barn immediately after it is drawn, prompt cooling to 50° F. or lower, and holding it at this temperature in a clean place, largely eliminates the dangers from "off" flavors of the absorbed type.

In conclusion, may I point out that compared with other types of businessmen, dairy farmers are efficient. The percentage of failures falls well below the national average for all business. Education on the dairy farm is progressing. The successful milk inspector, health officers, and others who occupy places of public responsibility must really know their subject. They should know more than the average person with whom they work—yes, they must stand out above the crowd. The more the milk inspector uses the educational method and the less conspicuous he makes his authority, the more he believes in the value of milk and its products, the greater his enthusiasm and the better his qualities of salesmanship, the more likely he is to succeed. We need ever-increasing intelligence, understanding, better planning, and team-work on the part of the milk producer—yes—and likewise on the part of public health officials. Let the milk inspector and public health officials really join forces with the strong educational agencies now working with dairy farmers, as educators, if you please, and the public will be assured of a safe milk, high in nutritive value, and pleasing to the taste.

The Need of Milk in the South *

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Approximately 28 percent of the population of the United States live in the 13 states called Southern. In this section, there are 35 percent of the children under 15 years of age. Moreover, in their search for jobs the productive middle age groups leave the South in greatest numbers. This exodus tends to make this section the land of the very old and very young. Now both of these groups, the old and young, are the greatest users of milk. It is the first food for the young and the last for the old. The U. S. census for 1930 shows that 16,666,668 cows were milked daily with a yearly production of 11,053,034,357 gallons; but here in the South where we find 35 percent of the children, there are 22 percent of the cows and 17 percent of the milk. This comparison can be carried still farther. In Florida according to the same census there are 1,468,211 people and an annual production of 26,283,944 gallons of milk. If everyone in the state had a quart of milk a day the supply would last only 72 days, if a pint of milk, 144 days and if a half pint, the amount Streibling puts in the lowest cost protective diet for adults, then there would still be 77 milkless days. It is true of course that many farms did not report on milk production. Moreover, considerable evaporated and dried milks and also ice cream are used, but evaporated milk is used mainly in infant feeding and in seasoning, very little is used as a beverage, while ice cream is still considered a luxury.

If we examine the people of this state, what evidence is found that indicates that milk consumption is too low? In other words, can specific conditions be traced to a deficiency of milk?

Before determining this, it is necessary to know what milk contributes to the

diet and the effect of a lack of it on the health of the people. Milk furnishes proteins of high quality, a carbohydrate, lactose, and butter fat, an easily emulsified fat. But the outstanding nutritional advantage of butter over other fat lies in the fact that butter fat is associated with the fat soluble vitamin A. Milk is also an important source of vitamin G, and if handled so as to conserve its vitamin C value, is a good source of this vitamin.

In addition to these constituents, milk contributes a very well balanced mineral mixture to the dietary. This is especially true in regard to calcium. As a rule the calcium content of the diet depends mainly upon the amount of milk used. In family dietaries where limited amounts of milk are used, the diet is more often deficient in calcium than in any other element. A liter of milk furnishes approximately one-half the daily calcium requirement. Milk also furnishes phosphorus compounds, and while the amount of iron in milk is very small it appears to be well utilized. But of all these constituents the chief value of milk appears to lie in its protein, calcium, and vitamin A.

What effects then would a lack of these food factors have upon the health of the individual? In young children milk furnishes the greater part of the protein, and in the absence of it the protein is apt to be too low for optimum growth. The addition of meat or vegetable protein in amounts to furnish adequate protein often brings digestive disturbances. On the other hand, milk protein is particularly adapted for growth and also for the repair and maintenance of adult tissues. The proteins of milk and eggs are well suited for conversion into body proteins, and for that reason should be used extensively in the diet of children and any one who needs to be built up.

In the absence of adequate protein, growth would be retarded. Many investigators have shown the marked influence of milk upon the growth of children. They found that children getting milk showed more of that sleekness peculiar to well nourished animals, greater alertness and buoyancy of spirits, and greater height.

To be normal and healthy the full grown human body must be richer in calcium than in any other mineral element, yet every one is born calcium poor. This calcium poor conditions of an animal at birth is due to the fact that the bones are soft and this facilitates birth. Therefore, after birth the diet should contain an abundance of calcium to insure bone and tooth development. During pregnancy and lactation a mother's diet should be high in calcium else her bones and teeth will be sacrificed to supply this essential element. Then with a deficit of calcium, carious teeth and poorly formed bones would probably result. According to Sherman an absence of calcium or phosphorus in the diet of the young is apt to result in a permanent loss of the long, lithe form, the skeleton becoming unduly stocky, if not actually distorted.

As mentioned before, whole milk is one of the best sources of vitamin A. While green and yellow vegetables and fruits are high in carotene, the precursor of vitamin A, and egg yolk is a good source of vitamin A, it is the experience of many that a diet low in milk and butter means a diet low in vitamin A. The clinical symptoms in children attributed to a lack of vitamin A are retarded growth, eye and ear defects, conjunctivitis, dry hair and skin, and increased susceptibility to infections; in the adult, dry hair and skin, and eye defects. It would then appear that a deficit of protein, calcium, and vitamin A would all tend to inhibit growth. Nutritionists now recognize the difference between adequate and optimum nutrition just as physicians consider good health as more than freedom from disease.

Now let us examine the health records and see if we can find conditions which might indicate a lack of these constituents. In a nutritive study of approximately 4000 white rural school children in Florida, it was found that the average 8-year old girl was 2½ inches shorter than the age-height standard given by Rose, and 5 inches shorter than the standard of Holt, while the girl at 13 was 3 inches shorter than either standard. Similar observations were made on the boys. The 16-year old boy was 3 inches shorter by Rose's standard and 4 inches by Holt's.

In later nutritional studies of approximately 5000 children, the prevalence of vitamin A deficiency among a large number of children was established by blood studies and clinical examination. Recently studies were made on University students. Only those students using neither milk nor butter in the diet were examined. In every case symptoms of vitamin A deficiency were found. More recently examinations of women on reducing and restricted diets showed symptoms of vitamin A deficiency. In the women and students the outstanding symptoms were red, itching, and burning eyes, and dry hair and skin. Most of the women and about half the students wore glasses but in spite of this the eye defects prevailed. Among the children, retarded growth, dry skin and hair, and conjunctivitis were noted. That the diagnosis was correct was confirmed by giving large doses of vitamin A to selected subjects. In 4 to 6 weeks the gross symptoms of avitaminosis A had disappeared and in a few more weeks the blood picture returned to normal. Of course during that time no changes in growth were noted. After this intensive treatment the students and children were given one quart of whole milk per day and urged to eat large amounts of butter. The women on the reducing diets, however, continued to take vitamin A concentrates.

In the study of defects of 4000 rural school children, carious teeth were found in 45 percent of them. In many cases

* Presented at the meeting of the International Association of Milk Sanitarians, Jacksonville, Florida, November, 1939.

the incisors were carious and the 6-year molars were decayed before the 12-year molars erupted. A more or less cursory examination was made of the teeth of rural women, especially the mothers and expectant mothers. The entire absence of teeth, the absence of the upper front ones, the absence of jaw teeth, the presence of many carious teeth and malocclusions all gave evidence of poor tooth structure due for the most part, no doubt, to inadequate diets, perhaps calcium. And in the case of these women the old saying, "Every child takes a tooth" is an actuality. Later a study was made of the relation of diet to teeth. In this study 406 children were examined. It was found that 33 percent of the children using milk had teeth which were not defective, while a little more than 10 percent of those not using milk had good teeth. The incidence of carious teeth was twice as common for the children who did not use milk as for those who did. Moreover, chalky teeth were also more prevalent in the former group. Of late many investigators have shown that dental caries has been reduced by improving the diet, and an increased use of milk is a part of the dietary regime.

While the effects of parasitic infestation especially hookworm on the physical development of children is not to be minimized, in the study of hookworm in man it has been noted that a well-nourished adult may often have a heavy infestation of hookworm and show neither a reduction in hemoglobin or other noticeable clinical symptoms. Investigations conducted in Brazil and in this laboratory on hookworm infested subjects show the beneficial effect of milk. Smillie found that a group of hookworm infested milkers who drank large amounts of milk were better nourished and showed fewer symptoms of hookworm infestation than workers of the same age infested with a comparable number of hookworms but who did not use milk. In this laboratory a study of the effects of diet on children with a moderate number of

worms, both hookworm and ascaris, showed that the symptoms usually associated with hookworm infestation were alleviated if large amounts of milk were included in the diet. Moreover, ova counts made throughout the experimental period showed a reduction of 100 percent for ascaris and a trend towards reduction of hookworm. From this work it was concluded that in any community where parasitic infestation was endemic and where reinfection was common, the maintenance of children on a high plane of nutrition was imperative.

Several months ago in making recommendations for the food supply for humans and livestock in the state it was brought out that in estimating the amount of corn necessary to keep a horse or cow, double the necessary amount must be raised because the weevil ate half. Therefore, in planning the milk supply for Florida children, it is evident that after giving the child his quart, enough more should be allowed to take care of the hookworm.

SUMMARY

Data have been presented on 10,000 school children, approximately 50 university students and 400 women. Among these subjects, abnormal height-age relationships, vitamin A deficiency, and carious teeth were the symptoms which may be related to a lack of milk in the diet. Moreover, it has been shown that milk production and milk consumption in the state is much below even the lowest amount recommended as barely protective. Even with only one-half pint a day for men, women, and children, there would still be 71 milkless days. While it is not to be inferred that increased consumption of milk would be a cure for all the malnourishment in the state, I am of the opinion that with an increased consumption of milk together with a bit of iron, many a malnourished child would be transferred into a normal one, many a mother would retain her teeth, and many a student have better eyes.

Annual Report of Secretary of International Association of Milk Sanitarians

An amendment to the Constitution, reducing membership dues, was adopted at the last meeting of the Association. This was done with the thought that many persons, interested and active in milk sanitation, would find it possible and desirable to enter the Association and participate in its activities. This belief was well founded. Five hundred and twenty new members have joined since our last meeting. At present we have members from 41 states, the District of Columbia, Alaska, Canada, British Columbia, Cuba, Mexico, British West Indies, South America, England, Ireland, and India. Impressive as these figures are, it is the interest that is being shown by all members which is significant. There are 285 Active Members and 622 Associate Members, making a total of 907 persons.

The *Journal of Milk Technology* has continued its progress in interest, value, and circulation. It is now well established, and there is every indication that the Association will be able to recompense the editor, the managing editor, and the necessary clerical assistants to a greater degree than has been possible heretofore. During the past year, the California Association of Dairy and Milk Inspectors, the Pacific Northwest Association of Dairy and Milk Inspectors and the Pennsylvania Association of Dairy Sanitarians have designated the *Journal* as their official publication.

There are three projects which are now under consideration and study by the Association:

1. Awards for meritorious and outstanding service in the field of milk sanitation.

2. Student training in relation to the Dairy Industries Exposition, and
3. Affiliations of local milk sanitarian organizations with this association.

Each of these projects has interesting and valuable possibilities, and merits serious study and consideration. Various individuals and committees have been working on these proposals and much has been accomplished. In the matter of affiliations—both local and International association organizations—constitutional and financial obligations must be considered. Studies so far indicate that a plan for affiliation can be devised and successfully operated.

However, it is believed that further studies should be given to these three proposals. As soon as this has been done, definite plans will be laid before the Association. It appears as if this could be done during the year and that definite action could be taken at our next meeting.

To the many individuals, committees, and organizations which have cooperated with the Association in its work, to the President for his mature counsel and advice, to the other members of the Executive Committee, and to the Managing Editor and Editor of the *Journal* for their interest and work, your secretary is deeply indebted. Any success which has been gained has been the result of splendid cooperation. This same cooperation will carry through successfully the problems and projects which are before us, with benefit not only to the Association but to the entire dairy industry.

Respectfully submitted,

C. SIDNEY LEETE, *Secretary*.

Oct. 15, 1940.

New Books and Other Publications

Veterinary Bacteriology, by I. A. Merchant. The Iowa State College Press, Ames, Iowa. 1940. 628 pages, \$7.00.

This book was written as a textbook to serve as an introduction of bacteriology to students in veterinary medicine. It emphasizes the general morphological and physiological characteristics of bacteria, and is concerned primarily with the species of bacteria, yeasts, molds, and filterable viruses which are pathogenic to animals. It acquaints the student with bacteria which are pathogenic to animals, those which are pathogenic to both man and animals, and with certain of those which are pathogenic to man only. The book contains no section on the pathogenic protozoa nor on laboratory methods of milk analysis.

The first 128 pages deal with the general biology of microorganisms. The second section of 82 pages deals with infection, resistance, and immunity. The third section of 318 pages deals with the classification and characteristics of pathogenic bacteria. The fourth section of 65 pages covers filterable virus diseases.

At the conclusion of each chapter, the author appends a list of references for further study.

Numerous illustrations and tables clarify the text. The index is adequate and well arranged. The printing is clear and well organized.

This book will be valuable to dairy farm inspectors. It will refresh the minds of veterinarians with much of what they have learned, and will bring to their attention some of the newer developments in the field. The long experience of the author in the application of veterinary practice to milk sanitation gives the book authoritativeness and usefulness in a field of practical importance.

J. H. S.

Milk Distribution as a Public Utility, by W. P. Mortenson. University of Chicago Press, Chicago, Illinois. 1940. 221 pages, \$2.50.

The author has compiled authoritative information on milk distribution. He develops the subject under the following headings: Part 1—Historical and introductory background (in which he compares the characteristics of public utilities with the fluid milk business); Part 2—Cost and profits of distributing milk and savings through unification (in which he analyzes operating costs and the possible savings through unification); Part 3—Legal considerations control; Part 4—Methods and difficulties of regulation; and Part 5—Economic effects of regulation.

He concludes that a unified system of milk distribution could bring about a reduction of milk distribution costs by amounts varying from about 1½¢ to even 2¼¢ per quart of milk handled. However, he emphasizes that there is no proof or certainty that they actually would be carried out, because of the difficulty of securing either an efficient management or freedom from political interference. The author raises many very practical and pertinent questions which are often ignored when the uninformed public endeavors to dictate how the milk business should be run. In view of the fact that the subject of the distribution of milk is such a recurring problem in so many communities, this book will be useful to those milk control officials and industrialists who want to be kept informed on this important question. The book is well-printed, easy to read, well-organized, thoroughly documented, and adequately indexed.

J. H. S.

The Streptococci, by W. D. Frost and M. A. Englebright. Reproductions of 120 photomicrographs, planographed by John S. Swift Co., Inc., Chicago, Ill. Willdof Book Co., Madison, Wis. 172 pages.

This book embodies the results of the authors' study of the isolations of streptococci during the past ten years of over 25,000 samples of milk drawn directly

from the cow's udders and plated on blood agate. The book describes in detail the methods used and the results found. Among the interesting data presented is information concerning their discovery of a new species which they call *Streptococcus and zoepidemicus*. They also cultured the throats of about 3,000 dairy plant employees working on five different farms. Ten species of hemolytic streptococci were identified. The most common ones were *pyogenes*, which was to be expected, *mastitidis*, which has not been reported before and was not expected, *angonosus*, which was expected, and *infrequens*, which has been reported as occurring infrequently. The most significant findings were the two species *zoepidemicus* and *epidemicus*, for which differentiation is given in detail. The authors append a bibliography of about 622 references.

J. H. S.

Frozen Desserts Ordinance and Code Recommended by the United States Public Health Service, May 1940 Edition. Federal Security Agency, Washington, 1940.

The combined ordinance and code for the control of frozen desserts has been issued by the United States Public Health Service in mimeographed form, accompanied by the "Frozen Desserts Plant Inspection Form". The general arrangement, form, and scope follow closely those now in use in the well-known milk ordinance and code. The long study that has been devoted to the drawing of this ordinance and code, preceded by the experience in the application of the milk ordinance and code, would seem to give this document an immediate and wide applicability.

The subject comprehends not only the commonly accepted frozen desserts but

also the partially frozen ones which are similar to these, including frozen custard, ice milk, and ices. Both the ordinance and code are arranged conveniently for adoption of either the grading or the non-grading type by merely deleting certain phrases.

Pasteurization is prescribed at a minimum heat treatment of 155° F. for 30 minutes (or a correspondingly effective method). Both the new tryptone-glucose-extract-milk agar and the old beef extract-peptone agar are allowed "where the new medium is found to yield considerably higher counts". Grade A bacterial standards are 50,000, Grade B 100,000, and Grade C no limit, per gram. The requirements are reasonable and practicable. Some parts, such as the medical check-up, might be made more specific for the guidance of the enforcement officer as well as the dealer.

J. H. S.

Dairy Bacteriology, by B. W. Hammer. Second Edition. John Wiley and Sons, Inc., New York, 1938, 482 pages. \$5.00.

In the ten years that have elapsed since the appearance of the first edition of this book, the field of dairy bacteriology has developed extensively. This new edition brings these fields into agreement with the present day concepts. Much new material has been added, and yet the size of the book has been kept very close to that of the first edition by the judicious elimination of some good text that was informative but not strictly necessary in a book of this type. The sections on spread of diseases through milk and the bacteriology of butter have particularly gained in amount of treatment. The index has been enlarged and improved. The format of the earlier edition has been maintained with its wealth of useful information.

J. H. S.

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Association News

California Association of Dairy and Milk Inspectors

Legislation favored by the Association will be handled differently in the future from what it has been in the past. In order to eliminate confusion, the members have appointed a legislative committee with Mr. H. E. Eriksen of Santa Barbara as chairman. With the aid of suggestions from the committee, Mr. Eriksen will clarify resolutions and represent the Association in matters of legislation.

LEONARD E. NISSON,
Secretary-Treasurer.

Central States Milk Sanitarians

The meeting of the Central States Milk Sanitarians, held on November 4, was attended by about one hundred members. Mr. L. E. Bober gave an interesting and enlightening address on "Mastitis". Dr. W. H. Haskel spoke at length, and among other things stated that the Chicago milk supply is one of the finest in quality of any in the country. The feeling was strongly apparent that it is most beneficial for men interested in the quality production of milk to be united in an association, such as this, and to attend the meetings where subjects in this field are discussed with such profit.

D. V. FITZGERALD,
Secretary.

Chicago Dairy Technology Society

On November 19, 1940, the Chicago Dairy Technology Society met and heard Dr. N. E. Fabricius of Iowa State College speak on "New Developments in the Butter Industry, with Special Reference to the Vaccination of Cream for Improving the Quality of Butter, also for Improving the Quality of Ice Cream Mix." He described the Vaccinator, a device for pasteurizing and treating cream and other products under vacuum, and discussed experiments connected with its performance.

Mr. Bonewitz, of the P. W. Bonewitz Chemical Company, summarized the research on control of proteolytic organisms in milk cans by treating with steam acidulated with gluconic acid.

J. T. THORNE.

Massachusetts Milk Inspectors' Association

At the Massachusetts Milk Inspectors' Association meeting in Springfield, Massachusetts, on November 1, President Enright introduced Dr. Edwin M. Knight, who gave a paper on "The Milk Serum Test used in Detecting Bang's Disease."

Professor J. H. Frandsen of Massachusetts State College spoke on the new equipment for milk plants and ice cream manufacturing which he saw at the Dairy Show in Atlantic City. Dr. Carl Fellers, also of Massachusetts State College, gave a talk on "Food Poisoning."

Nomination of officers for the year 1941 was held at the business meeting, and election will take place at the meeting in Worcester, Massachusetts, on January 8 and 9. Dr. Wolman from Philadelphia will talk on "Homogenization," and Dr. Workman of Connecticut, on "High-Temperature-Short-Time Pasteurization."

The Association extends a cordial invitation to all to attend its meetings in connection with the Union Agricultural meeting and display of equipment at the Memorial Auditorium, Worcester, on January 8 and 9.

ROBERT E. BEMIS,
Secretary-Treasurer.

Michigan Association of Dairy and Milk Inspectors

In conjunction with the Michigan Allied Dairy Association Convention and Machinery show, the annual meeting of the Michigan Association of Dairy and Milk Inspectors will be held in Grand Rapids on March 12 and 13, 1941. The program will consist of reports and discussions on the progress and work of the Committees on standardization of requirements for dairy farms, dairy plants, ice cream, and butter plants; new tests for quality in the dairy field; can and bottle washing problems; and a question and answer period.

H. J. BARNUM,
Secretary-Treasurer.



Photograph of banquet session of joint meeting of New York State Association of Dairy and Milk Inspectors, with International Association of Milk Sanitarians, New York, October 18, 1940. Photo by Drucker-Hilbert Company.

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"Doctor Jones" Says—

"One of the military commentators, here awhile ago—he was discussing the proposal about turning over those destroyers to Great Britain and so on and he said it'd be unfortunate if the decision in a matter like that should become, as he expressed it, 'the subject of ill-informed and irresponsible agitation'. Then he went on to say that matters of policy were quite properly the subject of public debate but when it came to an executive decision in a war situation, that was something else.

"It struck me, as I was reading that: encouraging 'the voice of the people' to express itself and still get the things done that ought to be—it's one of the real problems in a democracy. If they don't have their say, within reason—if the people don't, it ain't democracy. But there's times when too much voice is a serious handicap. Like Louie, the tailor down here: over to the Firemen's Ball he came off the floor and somebody thought he was sick or something. 'No', Louie says, 'it was just that voman's voice: it was so strong it made me dizzy. Ven they eat onions', Louie says, 'they ought to stay home'. Of course, if he hadn't wanted to take a chance on onions he could've stayed home himself. That's democracy.

"But take it in our public health line: there's things we know'd benefit the public if they were done—like cleaning up tuberculosis and pasteurizing milk and so

on—us health officers, but it's pretty well agreed you can't move much ahead of public sentiment. And it's awful irritating, sometimes, when you're trying to get something done and you run up against what looks to you like some o' that 'ill-informed and irresponsible agitation': people that don't know what it's all about leading the opposition and others following 'em like a flock of sheep. I'm a great believer in the freedom of the press but I do know a good health officer: one of the newspapers in his town—I don't suppose they've missed an opportunity in twenty years to oppose or criticise what he did. On the other hand, we can look back and see some things we thought were awful necessary at the time, that it'd have been just as well if they hadn't been done—like building some of those small tuberculosis hospitals. I've been sure I was right, before now, when I wasn't.

"Ill-informed agitation against health measures—the best way to avoid it, the way it looks to me—there's two things: have a full-time health officer that's so competent everybody'll have confidence in him and make a business of keeping folks well-informed. Bad leadership and ignorance—they're termites you've got to look out for in the underpinning of a democracy." *

PAUL B. BROOKS, M.D.

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