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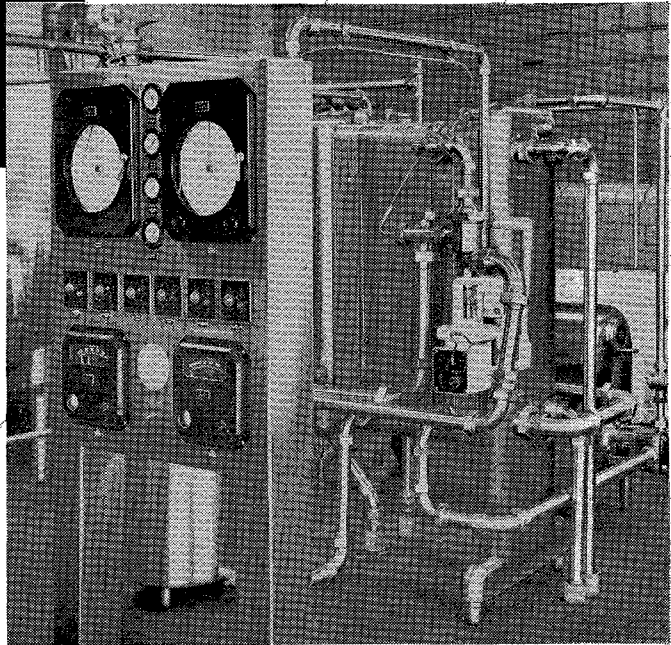
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(including MILK AND FOOD SANITATION)

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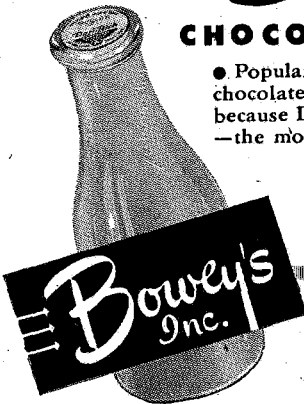
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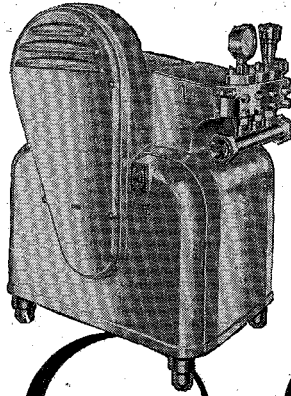
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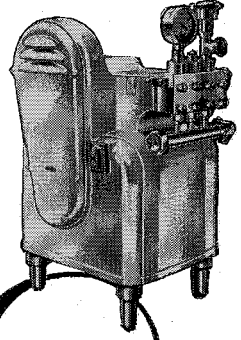
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Volume 11

September-October

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Editorials

*The opinions and ideas expressed in papers and editorials are those of the respective authors.
The expressions of the Association are completely recorded in the transactions.*

Thirty-Fifth Annual Meeting at Philadelphia

ON October 21 to 23, the INTERNATIONAL ASSOCIATION OF MILK AND FOOD SANITARIANS, INC., will hold its thirty-fifth annual convention. The headquarters hotel will be the Bellevue-Stratford. As soon as the convention is over, the great Dairy Show (as it is called) opens at Atlantic City and runs for the whole week. This arrangement makes it possible to attend both of these great events—come to Philadelphia beginning October 21st, remain there or go to Atlantic City over the weekend, and then attend the Dairy Industries Exposition on Monday and as many days thereafter as you can.

Attendance at such a combination of these meetings is a rare treat.

First, there is the opportunity to learn about the newer developments in control procedure and in equipment design and operation—and these are moving fast these days.

Second, there are the opportunities to meet old friends, make new ones, and look up the persons who can tell and show you what you have been wanting to know.

Third, there is the occasion when the assembly of many persons of similar outlook and with kindred problems engenders an enthusiasm for the work that serves as a great tonic against the discouragements that every one meets sooner or later in some part of his work.

Fourth, such occasions cement and foster professional solidarity—they make a fellow feel that he is not alone but is part of a great body of like-minded men with similar problems, aims, and ambitions.

Fifth, they advertise to the public the importance of milk and food sanitary supervision in the public health program.

Sixth, they constitute an excellent occasion to draw a fellow away from the daily grind, so that he gets a chance to view his own work in perspective, and then return home refreshed and inspired.

Attendance and participation in such assemblies pays dividends. Don't miss yours.

J. H. S.

A New Committee Proposed

THE relatively recent revival or emergence of interest in the sanitation of places in which food and beverages are served has been paralleled by an increase of activity (advertising and sales) by the detergent and bactericide industries which serve the food-dispensing and dairy industries. Numbers of new products have been developed, and their application in some instances involves new treatment procedures.

Most of the between-usage treatment procedures, especially for milk-handling equipment, had, after long experience, become rather standardized as the result of trial and error. Adoption of such standardized procedure does not imply that results are in all instances so satisfactory that nothing is to be gained by improvements in detergents and bactericides, or by logical modification of procedure. Accordingly, it has become accepted practice in the treatment of milk-handling equipment to: (a) rinse away all milk residue with cold or tempered water, (b) brush all milk-contact surfaces with detergent solution, (c) rinse surfaces free of detergent, (d) store portable metal equipment and parts dry, and store rubber or synthetic parts in caustic solution, and (e) apply bactericidal treatment to all milk-contact surfaces just prior to use. This treatment procedure includes five distinct phases, each of which has been made to appear essential in the voluminous literature on this subject.

Since V-J Day the developments in the field of detergents and bactericides have been numerous and have come in rapid sequence—so much so as to be somewhat confusing to many milk and food sanitarians as well as to the users of these products. It may be stated that, in some respects, the whole subject of between-usage treatment of utensils and equipment is in a confused and chaotic state. New synthetic detergents and chemical bactericides—and combinations thereof—have been introduced, and treatment procedures devoid of one or more of the heretofore-considered essential phases have been advocated—in some instances by individuals whose professional status is such as to land an aura of authority to their pronouncements.

In most instances milk control ordinances, statutes, or regulations restrict the use of chemical bactericides to one general type, and require amendment if the use of the newer bactericides, or of combinations of a detergent and a bactericide, is to be authorized. Sanitarians in administrative positions are frequently in a quandary regarding the action to be taken with respect to new products of other types. Should the milk control legislation be amended to permit their use? Should their use be tolerated on a provisional basis? The United States Public Health Service Milk and Food Section has not officially approved either type of the new products for milk-handling equipment, nor has the National Sanitation Foundation released a report of the conclusions

(Continued on page 317)

Thirty-fifth Annual Meeting
PHILADELPHIA, PA., Oct. 21-23, 1948
Hotel Bellevue-Stratford

A Combination of the Resazurin Test and the Direct Microscopic Count for the Bacteriological Examination of Milk*

A. L. BORTREE AND R. D. SPENCER †

Michigan Agricultural Experiment Station, East Lansing, Michigan

THE direct microscopic count, standard plate count, and reduction methods for determining milk quality are based upon staining characteristics of organisms, production of visible colonies, and dye reduction respectively, therefore the accuracy of these individual tests can not be determined by comparing one with another. Nevertheless the fundamental purpose of each of these tests is to determine whether a milk supply is satisfactory or unsatisfactory. A comparison of the results obtained by applying several tests to the same sample indicates the conditions under which one test may replace another without significant changes in the results.

Comparisons of the results obtained when samples are tested by both the methylene blue and resazurin dyes have been reported many times. Johns and Hawson (7) observed that resazurin was reduced to pink in three-fourths the time required for methylene blue to be reduced. Little (8) found that samples high in leucocytes and streptococci reduce resazurin to purple pink rapidly, require a long time to reach the pink color and reduce methylene blue slowly. Chilson and Collins (3) and Collins *et al* (4) reported that samples requiring five to six hours to reduce to the pink stage also show low standard plate counts. Golding and Jorgensen (6) likewise reported good agreement

between the plate count and resazurin test. Other workers (1, 4, 8) have observed that milk samples which reduce resazurin slowly, also have low microscopic counts. Schacht and Nichols (10) compared the results of various laboratory tests with the conditions observed on farms and concluded that the resazurin rennet test gave the best indication of the conditions under which the milk was produced.

Ramsdell, Johnston, and Evans (9) made an extensive study of the resazurin test and outlined a procedure for the interpretation of the results obtained on the basis of the rate of dye reduction at various intervals during incubation. Frayer (5) advised that the limitations of the test be kept in mind when interpreting the results and that doubtful results be checked microscopically.

Barrett, Rutan, and Keenan (1) have suggested a use of both the resazurin test and the microscopic count in a way which combines advantages of both tests. All samples were tested by the dye reduction method and those which were found to be satisfactory were not checked further. Those which were unsatisfactory were examined microscopically to determine the probable cause of low quality.

The findings of these workers suggest a combination of the resazurin test with the direct microscopic examination as an efficient method for the routine bacteriological examination of milk samples.

† Heatherwood Farms Company, Lansing, Michigan.

* Journal article No. 934 (n.s.) from the Michigan Agricultural Experiment Station.

EXPERIMENTAL

A procedure similar to that described by Barrett was used in this study. A total of 1020 producer samples were examined by both the resazurin test and the direct microscopic count. The resazurin test was carried out by adding 0.1 ml. of a 0.05 percent resazurin solution to 10 ml. of milk, mixing, and incubating at 37° C. Readings were made after 60 minutes of incubation in every case, 30 minutes and 90 minute readings were made on the majority of the samples. The samples were placed in one of four classes on the basis of the resazurin readings. The colors and the Munsell Designations for each class are shown in table 1. It will be

examined by the methylene blue reductase test, standard plate count, and direct microscopic count.

RESULTS

A total of 1020 producer samples were examined by the resazurin test. Thirty-minute readings were made on 916 of the samples while 60 minute readings and direct microscopic counts were made on all the samples. Data for the 30 minute and the 60 minute readings are shown in table 1. Results of the 90 minute readings are not presented.

On the basis of the 30 minute resazurin reading 537 samples were placed in class 1, 297 in class 2, 63 in class 3, and 19 in class 4. On direct micro-

TABLE 1

Class	Munsell Designation	DISTRIBUTION OF BACTERIA COUNTS OF MILK SAMPLES IN DIFFERENT RESAZURIN CLASSES				
		Up to 200,000	200,000 to 400,000	400,000 to 800,000	800,000 to 1,600,000	Over 1,600,000
<i>Direct Microscopic Clump Counts on 916 Samples</i>						
(30 min.)						
1 PB 7/4		500	18	12	6	1
2 PBP 7/5.5 & P 7/4		165	45	31	41	15
3 PRP 7/8		4	3	9	20	27
4 (colorless)		0	0	0	4	15
<i>Direct Microscopic Clump Counts on 1020 Samples</i>						
(60 min.)						
1 PB 7/4 & PBP 7/5.5		632	28	8	4	0
2 P 7/4		110	30	25	11	4
3 PRP 7/8		29	14	20	33	18
4 (colorless)		0	0	4	16	34
<i>Standard Plate Counts on 450 Samples</i>						
(60 min.)						
1 PB 7/4 & PBP 7/5.5		216	20	23	9	5
2 P 7/4		35	16	18	19	6
3 PRP 7/8		2	8	4	10	26
4 (colorless)		0	0	0	1	32

noted that PBP 7/5.5 and P 7/4 were combined for class 2 in the cases of the 30 minute readings while the PBP 7/5.5 was grouped with PB 7/4 in class 1 in the case of the 60 minute readings.

The direct microscopic count was made according to the technique described by Bryan *et al.* (2) Leucocyte counts of 1,000,000 or more were recorded.

The standard plate count and the thermoduric count were made on a portion of the samples examined. Another group of 379 samples was

examined. In the direct microscopic examination it was found that 93 percent of the samples in resazurin class 1 showed counts of 200,000 or less, and an additional 3.5 percent were in the range of 200,000 to 400,000. The majority of the microscopic counts on the class 2 samples was in the range up to 200,000, but there was a rather wide distribution of the counts on these samples. The class 3 samples also showed a wide distribution of counts with the high counts predominating. All counts on class 4 samples were high.

The data for the 60 minute readings are also shown in table 1. The distribution of the microscopic counts in the various classes is similar to that shown for the 30 minute readings. Likewise 94 percent of the samples were in the direct microscopic count range of 200,000 or less¹ and an additional 4 percent were in the 200,000 to 400,000 range. Again the class 4 samples were high in count and the classes 2 and 3 showed a wide variation of counts.

In table 1 the results of the 60 minute resazurin test are compared with the standard plate counts on 450 samples. Although there is fairly good agreement between the two tests, the results do not compare as well as the direct microscopic-resazurin results.

The results of the standard methylene blue test were compared with the direct microscopic counts and the standard plate counts for 379 samples. The data presented in table 2 indicate that in general the class 1 samples are of low count and the class 4 samples are of high count while those in classes 2 and 3 cover a wide range of counts.

The thermoduric count was determined for 209 samples and the results are presented in table 3. The limited amount of data shown here indicates that the thermoduric problem is not directly related to total count, therefore a procedure for the thermoduric count should be used together with a procedure for the total count in determining milk quality.

DISCUSSION

A study was made of the conditions under which the one hour resazurin test could be substituted for other laboratory tests without significantly changing the results obtained. The differences in rates of reduction of various groups of organisms, the effect of leucocytes on the reduction time and many other factors influencing the results of the resazurin test are recognized. Such factors as these undoubtedly account for many of the variations found in the samples in classes 2 and 3. However, no attempt was made to evaluate the influence of these factors other than to note that

TABLE 2

DISTRIBUTION OF BACTERIA COUNTS OF MILK SAMPLES IN FOUR METHYLENE BLUE CLASSES

Class	Munsell Designation	Up to 200,000	200,000	400,000	800,000	Over 1,600,000
			to 400,000	to 800,000	to 1,600,000	1,600,000
<i>Direct Microscopic Clump Counts on 379 Samples</i>						
1	126	5	8	0	0
2	43	10	6	4	4
3	32	17	22	23	14
4	2	3	3	7	50
<i>Standard Plate Counts on 379 Samples</i>						
1	123	5	6	5	2
2	40	9	6	5	6
3	31	14	23	24	16
4	3	2	3	6	50

TABLE 3

DISTRIBUTION OF THERMODURIC COUNTS OF MILK SAMPLES IN FOUR RESAZURIN CLASSES.

Resazurin Class (60 min.)	Munsell Designation	Up to 5,000	5,000 to 15,000	15,000 to 25,000	25,000 to 50,000	Above 50,000
		Thermoduric Counts on 209 Samples				
1	104	23	2	6	13
2	26	8	2	0	8
3	6	2	0	1	1
4	3	0	0	1	3

62 of the 139 low microscopic count samples which were in resazurin classes 2 and 3 on the basis of the 60 minute reading showed evidence of udder infection or a high leucocyte count or both.

Different procedures may be used for the combined resazurin microscopic test. When the submitted samples are in excess of 10 ml., the required 10 ml. may be transferred to another tube for the resazurin test and the remaining portion properly refrigerated. After the resazurin test has been completed, smears may be made of selected samples. When 10 ml. samples are submitted to the laboratory, smears may be made for each sample before incubation and an examination made of only those smears representing poor quality milk. A third method which may be followed is to make smears of the low quality samples after the one hour incubation. At this time the count will be increased and the proportion of the various organisms may have changed somewhat but the findings should be useful in aiding the fieldman in locating the source of trouble.

Although the proposed procedure involves only the resazurin test and the direct microscopic count, the plate counts are presented as additional information on the samples.

The data presented indicate that the methylene blue reduction test may be used with the direct microscopic count in the manner proposed for the resazurin test. However in comparing the results presented in tables 1 and 2 a more favorable relationship is found between the resazurin test and counting procedures than is found between the methylene blue test and counting procedures.

The data presented in table 3 indicate that the resazurin test like other tests which are based in part or entirely on the total count fails to detect many samples which are high in thermotolerant organisms.

Time spent in attempts to determine minor differences in bacteria counts of acceptable milks, contributes little to the improvement of milk supplies. Detection of unsatisfactory milk supplies and the determination of the cause of low quality should be the primary purpose of the bacteriological examination of milk. The direct microscopic examination is well adapted to this purpose except for the fact that a great deal of tedious work by a skilled individual is required if every sample is to be properly examined. When a sample of milk has been determined to be satisfactory, no additional information is needed for that sample. By the use of the relatively simple resazurin test the laboratory worker may eliminate from further examination the satisfactory samples. The data shown above indicate that in a high percentage of the cases milk samples found to be satisfactory by the resazurin test or the methylene blue test are also satisfactory by the more time consuming tests.

Since laboratory examination of milk samples for the purpose of improving milk supplies is of value only as it is used as a basis for doing field work, its extent should be determined by the amount of field work that is being done. The resazurin test appears to be very efficient in detecting the higher quality milk and the very poor quality milk. When field work is to be done with only the poorest producers, the samples in resazurin class 4 may be examined microscopically to determine the probable cause of low quality. Likewise, if field work is to be done with all patrons except those producing high quality milk, then a direct microscopic examination of those samples not in class 1 may be made to determine the probable cause of low quality.

SUMMARY

The data obtained indicate that milk samples placed in class 1 by the reduc-

(Continued on page 268)

Surveying Sediment Controls*

C. B. A. BRYANT

Johnson & Johnson, Chicago, Illinois

IT perhaps is most fitting at this time when our nation and the world are so concerned about our food supply, that we, as sanitarians, turn our attention to ways in which we may be of the greatest help. Milk and milk products are outstanding among the basic foods for supplying the world's nutritional needs. Much valuable work by our many college and experiment station research personnel has been done to bring information to our vast diversified farm population, to inform and show them how to produce more milk each day. They have been given practical and scientific data on breeding, feeding, better pasture and hay development, better milk methods, and personal care of cows. These factors have specifically contributed to volume milk production without adding more animals. This has been a direct contribution to our and the world's food needs.

We are all well acquainted with the fact that our present convenient yardstick for measuring cleanliness from a quality standpoint of commercial milk, is the sediment test—taken from a pint of milk, drawn off the bottom of a milk can at the milk plant's receiving dock. This is the standard method of the United States Food and Drug Administration, and by many state, county, city health, agriculture, and sanitary officials. So commonplace now is this method that most organizations have united on a standard set for judging. They have the same card for identifying sediment grades.

With our present advancement in this valuable milk industry, we, as sanitarians, are today faced with an opportunity to help our and the world's food needs by seeing that the production of milk is conserved and reaches market clean and that our finished milk products are wholesome and clean for human consumption.

Wendell Vincent, Chief of the Denver Station, United States Food and Drug Administration, in his address at the Utah Agricultural College, Logan, Utah, on March 12, 1947, said, "I believe that ultimately there will be just two kinds of milk offered to dairy plants. One is going to be good, fit for any use, and the other will be good for nothing other than animal feed. It will be rejected." Our service to this cause is equally as valuable as that of those first referred to, who have so excellently assisted in pointing the way to greater and better production. Therefore, it is timely that we "survey sediment controls" and through such a survey find methods for conserving all our milk supply so that it may be used for human consumption.

LOSSES FROM DIRTY MILK

In America, vast amounts of milk, daily produced and already hauled to market, are lost by sediment rejection. It does not measure up to cleanliness standards, as judged by the sediment test. Indicating this extensive loss, a recent public announcement in Indiana, under the heading "Quality Program", reads, "Indiana Milk Quality Improvement Program has shown fine progress in securing industry cooperation in producing high quality dairy

* Presented before Thirty-fourth Annual Meeting, International Association of Milk and Food Sanitarians, Inc., Milwaukee, Wisconsin, October 16-18, 1947.

products," according to a survey made by Purdue University, which supervises the program. For eleven months the program has been operating in Northwestern and East Central Indiana and five months in 1946, covering southern and northwestern parts of the state. An estimated 2,508,011 pounds of milk were rejected by companies participating in the program—a loss of about \$100,000, at reasonable price per hundred pounds. It is estimated that more than 4,500,000 pounds of milk will be rejected in 1947 because of inferior quality."

Again on this subject, from a paper given September 5, 1947, by Dr. J. C. Marquardt, New York State Department of Agriculture and Markets at the Dairy Industry Conference, Cornell University, Ithaca, N. Y., "During 1946 more than 8 percent of the milk was rejected as unclean on days that tests were made by the Department. At several plants rejections ranged as high as 15 to 30 percent. At the better plants, the rejections were as low as 3 percent."

I continue to quote from this paper, "We made several tests at this plant during May, and 33 percent of the milk was satisfactory. On federal standards, a small percentage of this milk would have been rated satisfactory." Another paragraph reads, "During May, a fluid plant was checked with the L and W. This milk was filthy. Less than 10 percent was satisfactory on our standards. Fifty percent of the milk was exceedingly dirty. On federal standards, practically none of this milk would have been rated satisfactory."

I personally stood at a receiving dock of one of our well-known national milk company's branch milk plants when sediment tests were being taken in the presence of a national regulatory authority. Of the milk received that day, I saw 42 percent of it returned to the farmers because of undesirable

sediment found in some of the cans of milk. It was significant to me to note that rarely did any one farmer receive back all of his cans of milk. Some farmers had 3 to 10 cans of milk in the daily shipment and received back only one or two because of bad sediment. The other cans of milk on that delivery were quite acceptable from a cleanliness standpoint. This immediately indicated to me, as an observer, that, by and large, our farmers who produced milk did want to keep it clean, or they would not have had any acceptable cans of milk in their daily shipments. Following through on the sediment study by visiting these farms at milking time and observing the milking practices, valuable observations were made.

In the past four years I have attended over 350 farms at milking time in all sections of the United States. All of these farms had milk returned because of undesirable sediment. To my way of thinking, and from this practical experience in field work, all farmers should be encouraged to save and mount the used filter disk and make a "Farm Sediment Check-Up."

This reveals many important factors on quality control to them. It first teaches them that a filter disk should never be used to remove dirt, carelessly permitted to get into the milk bucket, in order to make the can of milk acceptable for market. The only use of a filter disk should be to obstruct the stream of milk when it leaves a milk bucket, immediately after it has been drawn from a clean healthy cow, as it is poured into a clean milk can to show there was no dirt to be removed when mounted after use. When the used filter disk is clean, the producer may point to it with pride as his "Badge of Merit." Should unavoidable accidents occur, the filter disk is there to be the safeguard.

HOW TO KEEP EXTRANEOUS MATERIAL
OUT

Prof. G. Malcolm Trout, Michigan State College, in his recent articles appearing in the August and September, 1947, issues of dairy publications, has so well named the basic factors for quality milk production. I quote, "Many steps advocated today in sanitary milk production are antiquated. They belong to the 'horse and buggy' days. The quality of milk seldom improves after it leaves the udder of a healthy cow. All the milk producer can hope to do is to protect the milk from contamination and maintain, through cooling, the quality which already exists. This, in the light of present scientific developments, is not so difficult as it seems."

I should like to add here: The "Farm Sediment Check-Up" could well be the measuring yardstick of this accomplishment if we take time to teach and encourage its use.

Professor Trout further states, "Definition of milk quality is not simple. . . . The term 'quality' as applied to market milk often connotes entirely different concepts to various groups of people as previously suggested. In general, the specific concepts of individual groups are as follows." Dr. Trout goes on to classify these groups as 5, the fifth being, "The milk sanitarian or health officer; his concepts are: safety, low bacteria count, cleanliness."

Another outstanding authority on milk and milk products, Dr. J. C. Marquardt, states, "The consumer judges milk mainly by its flavor, appearance, and price. Unclean flavors are very objectionable. During 1944 the author demonstrated that when more than 25 percent of the milk received contained excessive amounts of sediment, an unclean flavor was developed in a mixed tank of milk. This according to numerous observations took place in less than 6 hours even when the milk

was cooled and held at 40° F. or below. . . . The flavor of milk is improved as the percentage of clean milk is increased." In this same article, Dr. Marquardt advocates, "The use of mounted and dried single service cotton discs by the producer proved to be most efficient as a sediment check-up. It affords a farm check-up on the progress in keeping milk physically clean."

This subject has so concerned national and state authorities who are taking steps for action that I herewith quote from a few. First, from Wendell Vincent, U. S. Food and Drug Administration: "Poor utensils and inadequate facilities for cleaning contribute to unfit milk, both from the bacterial and physical standpoint. . . . unclean animals make contributions of similar contaminants. . . . the placing of milk in a dirty can or one that is inadequately dried contributes to pollution of his product."

Second, Milton H. Button, Director, Wisconsin Department of Agriculture, Madison, in April of this year says: "The problem of sediment control, it seems to me, can be divided into two phases: First, how does the extraneous matter which causes poor sediment tests get into the milk? Second, how can we keep it out? These, it seems to me, summarize the problems which are facing the dairy industry. I like to emphasize the second point: How can we keep extraneous matter from getting into milk? As a dairy industry we are interested in CLEAN MILK. Our problem, is to KEEP the dirt OUT."

Harvey J. Weavers, Chief of the dairy division of the Wisconsin Department of Agriculture under Mr. Button, has this to add in instructions for quality farm milk production: "Keep Milk Covered While Cooling." "Milk cools through the sides and bottom of the can. For that reason it is not necessary to have the covers loose. The principle involved is

exactly the same as that used in heating water, where the heat is applied to the bottom of the kettle, and not directly to the water itself. The heat or cold is readily conducted through the metal wall of the container."

Following through to teach the producer "The Farm Sediment Check-Up" by mounting his used disk means work on the part of the leadership in our industry. It, however, assists in retaining at our markets all milk for human consumption, which, in turn, assists in maintaining a greater food supply.

CLEAN MILK DEMANDED

Quality milk is such a ringing note over the nation that during the past six months, all leading dairy trade journals had editorials with such headings as "F and D Isn't Bluffing", "Where Fighting Is Foolish", "Either Clean or Dirty", "Institute Organized to Improve Quality of Milk and Cream." Headlines appear on the front pages of leading city daily papers, such as, "U. S. Raids Cream Shipped into City: Few Cans Filthy" and another, "Dairy Man Gets \$2,000 Fine in Cheese Case."

Dairy cooperative house organs and independent dairy operations recently sent this information to their producers, "IMPORTANT" "The New York City Department of Health recently advised the milk industry it was necessary to take immediate steps to improve the quality of the milk going into our country plants and then shipped to the city pasteurizing plants." *Again I quote: "Don't Let Your Neighbor Down", "Produce Clean Milk." "We feel that our members believe in producing a quality milk and maintenance of Department of Health Regulations, particularly when they know the reasons for it, and have the cooperation of management and employees in obtaining the results."

I quote from another cooperative

* Noting this important counsel by the largest populated city in the world, it may be expected that all our cities are similarly reviewing their milk supply with respect to maintaining their high quality requirements.

milk organization, *The New England Dairymen*, August, 1947 issue, "With supplies of milk more readily available, our buyers are becoming quality conscious, and if at any time this office can be of service to you in attempting to straighten out your quality problems, feel free to call upon us."

A large independent dairy in their May-June, 1947, house organ to their producers, wrote: "Federal Food Inspectors Plan Careful Check for Sediment. There's trouble ahead for some milk producers."

Many states have given special attention to their dairy laws, in several cases revising and revamping them. Notably, Minnesota, Michigan, Ohio, Wisconsin, and Texas. Oklahoma, Iowa, and others, have established joint quality field service plans. A trade journal's news notes recently stated: "100 dairy plants in Iowa and an estimate of 75 in Wisconsin have adapted 'co-operative field service'".

A September, 1947, bulletin from C. O. Jacobson, Oklahoma Butter Institute, says in announcing 5 meetings during the week of September 27, 1947: "The State Department of Agriculture, Dairy Division, will furnish an inspector to supervise Sediment Testing and the Grading of All Producers' Cream at all of the above locations on Saturday, September 27, 1947."

Here is another from the August 27, 1947, Texas Dairy Products Association, Inc., trade letter by J. C. Davidson, Jr., who says, "Sediment Testing and Grading Days. The Oklahoma plan of sediment testing and grading all cream received at certain towns and certain days was adapted."

Following the work of national organizations on sediment control, we find such leaders as Dr. E. H. Parfitt of Quality Standards, Evaporated Milk Association, and his able force of associates have set and maintained leadership of high quality for farm production. The American Butter Institute, under the energetic leader-

ship of Russell Fifer, and his active associate, Ray Alberts, are way out in front on quality leadership. Up with this group is The National Cheese Association with quality work excellently directed by E. W. Gaumnitz and E. H. Richert. All of these are vigorously working toward one end: quality milk production from "Cow to Milk Plant" and on to the finished products. We can all join them by encouraging all who contact producers to encourage the use of "The Farm Sediment Check-Up."

USE OF FILTER DISK

I have visited the famous milk parlor with the "Rotolactor". Here the cows are given splendid preparation before milking. To my knowledge, no place in the world surpasses their sanitary milking methods. Here they take the added sensible precaution to obstruct the stream of milk from the cows going to the container vats by using a cotton filter.

My associates and I have visited over 3500 farms in these United States, who have had milk returned because of sediment. Two thousand visits were at milking time. These farmers mounted the used filter disk after the milking. By our observation, 50 percent were ashamed of their workmanship because of the amount of dirt which was removed from the milk. They immediately took steps to keep dirt from getting into the milk at future milkings. Twenty-five percent did not like the results as indicated on the mounted disk, but were principally encouraged to be more careful in their methods as they felt they were being policed. The remaining 25 percent were quite indifferent to the fact that the disk contained dirt.

Many of these were large producers—5 to 15 cans per daily delivery. Over one-half of these producers could be and were changed by this method of having them frequently mount and dry the used filter and send it to the milk plant by the milk hauler, to become

more careful and cleaner handlers of their milk. Continually mounting a used filter disk having excessive sediment, shamed them into taking reasonable precautions. One-half of these, however, could never be cured. Their personal conception of sanitation, that is rightfully needed for producing milk, is lacking. It is difficult to teach sanitation from the barn to the house. These producers in their homes are not as sanitary in their daily living habits as we find to be necessary for keeping milk clean. The sooner this small proportion are eliminated from producing milk and return to other farming activities, the better it will be for our industry.

This mounting of used filter disks is the "Farmer's Own Sediment Test." When the disk is clean as it should be, it is his "Badge of Merit." He can point with pride to his workmanship. He can show that from each healthy cow which gave clean milk, "He Kept It Clean." The industry has no better tool for quick, impressive results than this little piece of cotton, called a filter disk, to teach this valuable educational lesson. Just as sediment tests must be taken at the plant, this also must be done at the farm continually to assure protection from sediment. It is work. It takes effort on the part of the fieldman of milk companies. Those who have consistently followed it with their producers have received telling results.

When a producer is checking on one necessary feature of his production methods, he is apt to check on all requirements. Dirt is precious, but when it gets into milk it is filth. Encourage and instruct the farmer to SAVE his used filter disk. Let him periodically follow "The Farm Sediment Check Up." He then will come to these same conclusions regarding dirt. We must realize that in most things a farmer does in following his occupation, he works with dirt. Therefore, dirt to him, consciously or unconsciously, becomes glorified. He thinks

little about dirt when associated with food products in their raw producing stages. "The Farm Sediment Check-Up" gives him a visual picture and a different impression of dirt when it contaminates milk. We as sanitarians have an important, valuable, and very useful mission to render our nation and fellow man. Here is a worthy opportunity.

We can save much of this already produced daily milk supply now being returned to farms because its quality is below clean standards. This service can be rendered simply through the means of encouraging proper milk plant authorities to use the "Farm Sediment Check Up" as a basic feature in their quality program.

DUST

We, ourselves, need to be versed with the facts so we can impressively pass them on. Up to date I have viewed some 15,000 used filter disks that farmers have mounted and saved after filtering milk. The type and kind of sediment on these disks is not as prevalent as a manure base as years ago. Much is sand and dust. Farmers are generally taking better care of barn yards, bedding, and surroundings, providing better personal comfort to their herds.

However, they are not giving the attention to and protection against dust and wind blown earth; this control is also needed in quality milk production. We sanitarians need to be well versed with these dust contaminating features. Care of the milk cans and all milking utensils is most important. Sometimes the type of sediment on the lintine sediment test disk at the milk plant is different from the type of sediment on the used filter disk which filtered that same milk into the can at the farm. The "Farm Sediment Check Up" is the source of the study. Wind blown dust has been the offending element. The milk cans had not received proper care and protection, either before milk them.

A milk can must at all times be as ply. Following through to determine reasons why undesirable sediments was put into them, or after milk was in well cared for and clean as the receptacle that careful farmers use to bring into the house their personal milk sup- were experienced at the milk plants from cans of milk which were of good sediment test after milking at the farm, we discovered this bothersome element—wind blown dust. Milk cans must be properly washed at the milk plant. Frequently, the shoulders of milk cans harbor accumulations of dirt, not removed by the can washer. This needs elbow grease and brush applied either by milk plant management or by the farmer. A soapless cleaning solution must be used. It has been found that such milk cans, when full of clean milk, on the haul from the farm, did not measurably affect the sediment test taken at the milk plant. However, such cans partially filled with clean milk, on the haul from the farm, did affect the sediment test taken at the milk plant dock. Evidently, the dashing of milk in a partially filled can washed away some of this accumulated residue from the shoulder of the milk can.

Another important influence upon sediment from wind blown dust, with milk cans, is dust deposited on the pouring lip of our present standard type milk can. When a milk can is not properly protected at all times, this pouring lip is a perfect receptacle for fine wind blown dust.

Professor A. W. Rudnick, of Ames, Iowa State College, has done some outstanding work in this field and has given the industry most valuable data.* On the basis of follow up by

* Work by Dr. K. G. Weckel, University of Wisconsin on "Milk Cans Often Hide Sources of Bad Flavor" was reported in an article Nov. 1, 1946 in *Dairy News*. Dr. Weckel appropriately states: "Milk cans are strange devices. They are designed to convey milk. But what is a clean can, acceptable for conveying milk, as examined from the inside? What are the defects of cans as they are returned to farms? What can these defects do to quality of milk and products made from it? There are quite a number of things that should be worried about it."

"The Farm Sediment Check Up", we, in cooperation with others, have done some extensive follow through on this subject. In collaboration with Professor V. C. Manhart, Department of Dairy Husbandry, Purdue University, and again with R. H. Cronshey of California's Challenge Cream and Butter Association, special studies were conducted in field experiments studying the sediment problem of wind blown dust on milk cans.

This work was published in the *Southern Dairy Products Journal* and *The California Dairyman*, under the title, "A Study of Causes of Milk Rejection": "Influence of Wind Blown Dust on Sediment Content of Milk", and "Attention to Milk Can Design." Further work was done this past June in cooperating at a "Sediment School" with The Golden State Milk Company, Gridley, California. This was entitled "Following Through on Sediments" and was published in *The California Dairyman* and *The Pacific Dairy Review*.

OTHER FACTORS

It would seem reasonable that for future needs those in this industry could build a more serviceable milk can. This milk can should have built into it features to help correct these problems. Two national manufacturers are working on it and have placed out to the trade some of their early results. One is a stainless steel milk can; another, an aluminum milk can. Standard milk can manufacturers are alert to the needs and are intelligently working on the matter.

At some places the problem has been of sufficient trouble to cause the use of a cellophane cap to go over the milk can top. In Idaho one company is using a heavy duck canvas milk can top cap and are paying haulers extra to place them on all cans as they take them from the can washer at the plant and return them to the farms. Farm-

ers replace the cap as soon as the milk is placed in the can.

One of the contributing factors to sediment in milk when milk cans are not rinsed at the farm is the air intake for drying cans on the can washer at the milk plant. Here dust and dirt collects on the wire frame grill and breaks off in clusters and sometimes contaminates a washed can. A special filter cotton is obtainable to place over this air intake to catch this dust.

Some can washer manufacturers are giving special attention to this protective feature in present design and construction of their can washers.

We, as sanitarians, can carry the message and encouragement to both the milk industry and the producer to use this simple "Farm Sediment Check Up" as a basic tool for saving this valuable milk supply already produced and ready for market. It is as important as pointing the way to efficiently produce more milk.

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Quality Control in the Evaporated Milk Industry*

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NEARLY ten years ago manufacturers of evaporated milk formed a committee of technicians and sanitarians to develop a Sanitary Standards Code. The Evaporated Milk Industry was interested in establishing on a voluntary basis standards for measuring the quality of incoming milk, standards for equipment, and standards for sanitary operation of plants. At the present time ninety-nine percent of the industry, by volume, is supporting this program. To assure progress a staff of specialists in the field of dairy sanitation were employed to assist the industry in securing the objectives of the Code.

OBJECTIVES

The objectives of the Evaporated Milk Industry's Sanitary Standards Code are as follows:

1. To provide (for an industry that operates in 28 states) a uniform approach to the problems of sanitation as related to the plant, to processing, and to milk quality.
2. To provide definite goals and minimum sanitary standards for the entire Evaporated Milk Industry.
3. To provide a trained staff for the purpose of measuring Code compliance of each station and plant within the industry.
4. To assist companies in the problems arising as a result of the Sanitary Standards Code.

The organization work of the program is as follows:

Within each plant and station a definite procedure is established which

deals with sanitation of equipment, platform tests on incoming milk, inspection of milk cans returned to the farmer, and farm inspection work done by the fieldman. The results of the above work are tabulated in a manner that will permit its easy review¹ by members of the sanitary standards staff or public health officials.

ROUTINE OF WORK

At periodic intervals members of the sanitary standards staff visit each station or plant and make a spot check on all phases of the program. The time required to make a spot check depends upon the volume of milk handled; usually it requires from two to four days. The work at the plant consists of the following:

Detailed inspection of the plant for housekeeping, and the plant surroundings for appearance and sanitation.

Detailed inspection of the plant equipment for repair and cleanliness. This inspection is made just prior to use of the equipment.

Sediment testing of milk from 60 percent or more of the producers and bacteriological testing of milk from 60 percent or more of the producers supplying the plant.

The results obtained by sediment and bacterial testing are compared with the records maintained by the plant. All plants have records which show the grades and classes found at each testing period. This procedure shows the degree that the plant or station is complying to the industry program.

Inspection is made of the platform equipment used, to determine if it is adequate and in good working order. The methods used are those given in the A.P.H.A. *Standard Methods for*

* Presented at the Thirty-fourth Annual Meeting of the International Association of Milk and Food Sanitarians, Milwaukee, Wisconsin, October 17, 1947.

the Examination of Dairy Products.

The program of the Evaporated Milk Industry sets up minimum standards for milk production methods on farms. To determine the accuracy of fieldmen's reports the staff representative visits with the fieldman randomly selected farms located on a number of different routes. The conditions observed on these farms are summarized and this summary is compared with previous farm inspections made by the fieldmen. This procedure causes uniformity of work and significantly strengthens the quality of sanitation work done by fieldmen.

This procedure results in the staff representatives working with the fieldmen in the capacity of auditor and instructor.

The staff representative, before leaving a plant, meets with the superintendent, fieldman, and others, and reviews in detail his report. The completed report, after passing through the central office, is forwarded with comments to the executive officer of the company operating the plant.

The reports are tabulated and comparisons are made of several years work. Statistical reports are prepared so that the industry's position in relation to the Code can be determined on any item.

ACCEPTANCE OF THE PROGRAM

The Sanitary Standard Code of the Evaporated Milk Industry has formed a basis for the development of similar quality programs in other branches of the dairy industry. The fact that standards existed in the Evaporated Milk Industry has exerted significant influence on standards and methods used by regulatory officials whose activities have been confined to bottled milk. The program was adopted at the beginning of the war by the Veterinary Corps of the U. S. Army. In addition, the standards and methods advocated in the Code have been adopted by a number of regulatory officials.

SEDIMENT TESTING

The sediment test has been employed as a major yardstick to determine milk quality. In cooperation with the Federal Food and Drug Administration standards were formulated based upon known amounts of sediment. These standards have been accepted as state standards in eighteen states, one province in Canada, and two large cities.

The results of the sediment test are readily understood by producers whether it be in New York, Wisconsin, or Arizona. The establishment of standards that call for rejection of milk in excess of 3.0 milligrams per off-bottom pint is the most direct approach that can be taken to cause producer education in the production of an acceptable quality of milk. Sediment pads make a direct approach to the producer that can be readily understood, whether it be in New York, Wisconsin, or Arizona.

The ability to accept or reject a given can or shipment of milk from a producer has resulted in major improvements in milking methods and care of milk. This, in turn, has had its effect on quality as measured bacteriologically. The value of the sediment test as a means of measuring and improving milk quality is frequently overlooked by many milk sanitarians and this lack of interest retards industry progress.

WORK OF FIELDMEN

The working with the individual producer is considered an important part of the sanitary standards program. There are over 500 fieldmen in the Evaporated Milk Industry. Many are college-trained men; many were formerly county agents; vocational teachers, and state dairy specialists. There is need, however, for men trained in this work. A former fieldman in the Evaporated Milk Industry has joined the staff of one of our leading universities and is now instigating a four-

year curriculum for the training of fieldmen (sanitarians) for the dairy industry.

WORK WITH THE INTERNATIONAL ASSOCIATION OF MILK SANITARIANS

The dairy industry only recently has approached the problem of sanitary standards from an all-industry point of view. The interest in the past has been with each separate branch. In the past two years an all-dairy-industry committee, working with sanitarians, has functioned and secured definite results in the development of sanitary standards for equipment of specific interest to the entire industry. One of the initiating forces of this work, on which the chairman of the Committee on Sanitary Procedure, Mr. C. A. Abele, has reported, was the sanitary

standards program of the Evaporated Milk Industry.

REACTION TO THE PROGRAM

More fully to acquaint milk sanitarians, at all levels, with the quality control program in the Evaporated Milk Industry, there was published in the March-April, 1945 issue of the *Journal of Milk Technology* a paper entitled, "Sanitary Standards Program of the Evaporated Milk Industry." The favorable comments received from sanitarians on this publication, and the favorable reaction from regulatory officials who have had an opportunity to review the program first-hand, have done a great deal to assure the success of this first national sanitary standards program in the dairy industry.

RESAZURIN TEST AND DIRECT COUNT

(Continued from page 258)

tion tests are, in a very high percentage of the cases, of low count. Samples placed in class 4 are definitely of inferior quality, and microscopic examination should be made to determine the cause of low quality.

No attempt is made to explain the wide range in counts for samples in classes 2 and 3. Differences in reduction time by various microorganisms, failure of certain organisms to grow under the conditions provided or to stain by the methods used, and the use of a much larger sample for reduction tests (1,000 × or more) than for counting methods are recognized as factors which make impossible the direct comparison of these tests.

It is suggested that the resazurin test be used as a screening test to eliminate high quality samples from further examination and as a means of detecting the definitely low quality milk as a guide for further examination and field work.

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A Review of the Biological Aspects of the Quaternary Ammonium Compounds

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Although possessing numerous physiological activities, the germicidal properties of compounds of nitrogen were long overlooked, possibly because of unfortunate choices of compounds by early workers. Probably the earliest reference to the quaternary ammonium compounds is that of Jacobs *et al.* (1916 a, b, c) who reported on the bactericidal properties of quaternary salts of hexamethylenetetramine ($C_6H_{12}N_4$) which they found directly attributable to the presence of the $C_6H_{12}N_4$ nucleus of their compounds. Indications of specificity toward different organisms, and of different susceptibilities to influence by the presence of proteins were found, and were attributed to the molecule attached to the $C_6H_{12}N_4$ group. Again twelve years later Hartmann and Kägi (1928), on the basis of observations by Doerr, reported that several of their newly synthesized cation active substances possessed germicidal properties.

However, the real possibilities of this type of compound were overlooked until Demagk (1935) reported on the long-chain quaternary ammonium salts in which at least one radical is a long-chain aliphatic group (C_8H_{17} to $C_{18}H_{37}$) whether plain or substituted. The other three radicals, he found, could be one, two, or three lower alkyl- or benzyl-bound, or one aliphatically bound, phenyl radical. Two or even three of them would be arranged to form a cyclic compound as in salts of hexamethylenetetramine, pyridine, etc. The anion could be halide, sulfate, acetate, or another similar group. Since Demagk's report, a number of workers

have interested themselves in this field of studies and a considerable literature is being built up. General discussions of these compounds in their variety of applications have been presented by a number of workers including DuBois (1944), Marshall (1944), Botwright (1946), Lehn and Vignolo (1946), and Vignolo (1946 a, b).

GERMICIDAL EFFICIENCY AND CHEMICAL STRUCTURE

Leffler and Volwiler (1939) investigated a series of N-dodecyl heterocyclic amines and concluded that these, in general, were less germicidal than dodecyl - dimethyl - benzyl - ammonium chloride. For amines of the type dodecyl - $\begin{matrix} \text{R} \\ \diagdown \\ \text{N} \\ \diagup \\ \text{R} \end{matrix}$ the germicidal activity

against *Staphylococcus aureus* was greater when R was alkyl than when hydrogen. Valko and DuBois (1945) found this not to be the case in most of the tertiary dimethyl amines they investigated.

Shelton and coworkers (1939, 1940) studied over 100 quaternary ammonium compounds derived from aliphatic and heterocyclic amines and found optimum bactericidal activity in compounds possessing one cetyl ($C_{16}H_{33}$) group. The other three radicals, they found, could be very simple (e.g., three methyl groups) or they could be a part of a cyclic configuration as in the pyridinium salts. The alkyl pyridinium salts offered the most interest, and in that series optimum germicidal activity was found with cetyl-pyridinium chloride. These reports indicated that a maxi-

mum germicidal activity might be found for the $C_{16}H_{33}$ -substituted members of a homologous series of quaternary ammonium compounds. Confirmation of this observation for the series derived from tetramethylammonium bromide was reported by Hoogerheide (1945), who found that bactericidal properties became evident when one methyl group was replaced by a nonyl group, and increased with increased length of the substituted chain up to a definite maximum for cetyl-trimethyl-ammonium bromide.

Kuhn and coworkers have also studied a number of compounds of different types. In a series of dialkyl-methylbenzyl-ammonium chlorides (Kuhn, Jerchel, and Westphal, 1940), they found both surface tension and bactericidal action on *Streptobacterium plantarum* reached a maximum with the didodecyl compound, but toward staphylococci, diphtheria bacilli, *Bacterium coli*, and *Bacterium friedlanderi*, the dicetyl derivative was much more effective. For *B. paratyphosum* the maximum was intermediate. Kuhn and Jerchel (1940) also studied *o*-, *m*-, and *p*-dodecyl-oxyphenol-trimethyl-ammonium methosulfates and Kuhn and Westphal (1940a) studied the methosulfates of 3-, 6-, and 8-hydroxyquinoline dodecyl ethers. No positive isomerism effect of the dodecyl group, as regards bactericidal properties, was noted, and the order of effectiveness was different for different bacteria. Kuhn and Westphal (1940b) found that 1,3-diethyl-, dibutyl-, and dibenzylbenzotriazolium bromides have practically no action on lactic acid bacteria. The effects of octyl, dodecyl-, and cetyl-groups were found to be approximately in the ratio of 4:2:1. The most effective disinfectants against pernicious bacteria were those with two different alkyl groups such as 1-dodecyl, 3-ethylbenzotriazolium bromide. Very high results for the ethobromide of *n*-dodecyl-benzotriazole (1:615,000 against "Staph.") were reported, but more recently Westphal and Jerchel (1942)

of Kuhn's laboratory, reported that these results could not be reproduced. Neither could they be confirmed by Rawlins *et al.* (1943) or by Valko and Dubois (1945).

Eldred and Niederl (1941) reported that three factors determined the effectiveness of a germicidal compound: (a) general molecular structure, (b) type of N-alkyl radicals, and (c) length of the paraffin chain.

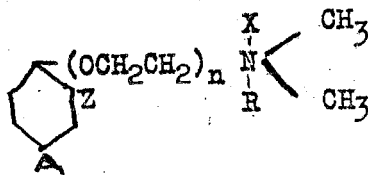
Baker, Harrison, and Miller (1941b) found two compounds (Zephiran and Phemerol) containing a benzyl group as well as long alkyl chains on the quaternary nitrogen were superior to compounds lacking such a benzyl group.

Kolloff *et al.* (1942) reported on preliminary studies on a group of 27 different alkylpyridinium and alkylpicolinium halides. Tests made by the Food and Drug Administration (F. D. A.) method (Ruehle and Brewer, 1931) showed that although each of the compounds possessed a definite bactericidal activity against *Staphylococcus aureus*, in general the introduction of a methyl group was not accompanied by any significant increase in such activity. The C_{16} derivative was found more effective against *Staphylococcus aureus* than the C_{12} or C_{14} members in the alkylpyridinium and alkylpicolinium bromide series.

Woodruff, Aspergreen, and Mantele (1942) with the series $RCOOCH_2CH_2-N-(C_2H_5)_2R_1$ found a maximum

of activity at $R = C_{16}$ when RX was methyl iodide, whereas it was at C_{12} when RX was allyl iodide.

Rawlins *et al.* (1943) studied a series of quaternary ammonium compounds of the general type



in which A is alkyl, aryl, aralkyl, or cycloalkyl; Z is halogen, alkyl, aryl,

or cycloalkyl; R is hydrogen, methyl, or substituted methyl, X is chloride or other anion, and n is 2 or 3. The Shippen modification of the F.D.A. technique was used in tests against *Staphylococcus aureus* and *Eberthella typhosa* to find the maximum dilution which killed at 20° C. in five minutes. The method of McCrea (1931) was used to test fungicidal activity. The general configuration of the quaternary salt was concluded to be as important as the exact chemical nature of its constituents, and the cation was found to contain preferably one long alkyl or alkylarpoly (oxyalkyl) or possibly alkylaro-alkyl chain, one short aralkyl, and two lower alkyl groups. The best chain length for the long chain was found to be 12 to 16 atoms, benzene rings being counted as equivalent to 4 atoms, and it was noted that any appreciable increase or decrease in this number interfered seriously with germicidal activity. These workers found that closed ring substituents on the aromatic nucleus were definitely inferior to alkyl groups in enhancing germicidal activity, and halogen substitution in the aryl groups sometimes even decreased such activity. It was found that any simple mineral or organic acid might serve as a source of the anion. One compound studied, *p*-*tert*-octyl-phenoxy-ethoxy-dimethyl-benzyl-ammonium chloride appeared superior to the others, and was selected for further study (see below: Joslyn *et al.*, 1943).

Epstein, Harris, and Katman (1943) made a comparison of the bactericidal activities of a number of fatty acid esters of ethanolaminoformylmethyl quaternary ammonium or pyridinium chlorides, and found the myriatic esters to have the maximum potency.

Valko and DuBois (1945) studied the germicidal activity against *Staphylococcus aureus* and *Eberthella typhosa* of halides of primary higher aliphatic amines; their tertiary dimethyl derivatives; quaternary methyl, ethyl, allyl, and benzyl derivatives of these ter-

tiary amines; and a few closely related ammonium halides. In the alkyl-dimethyl-benzyl-ammonium chloride series, maximum potency was obtained with the dodecyl (lauryl) and tetradecyl (myristyl) compounds. The decrease in efficacy was steeper toward the shorter chains than toward the longer ones. Surprisingly, unsaturation had a great effect, and the *n*-9-octadecenyl compound was more effective than either the dodecyl or tetradecyl homologs. In the alkyl-dimethyl-ethyl-ammonium bromide series, the maximum potency was obtained with the longer hexadecyl (cetyl) chain. Again the oleyl radical had a noticeably greater efficacy. In the series of alkyl-dimethyl-allyl-ammonium bromides and in the alkyl-trimethyl-ammonium bromides the maximum potency was obtained with a chain length of C_{14} (myristyl) to C_{16} (cetyl). Di-*n*-octyl derivatives were found not to be superior to the hexadecyl or dodecyl homologs. It becomes apparent that the maximum bactericidal potency is not reached with the same chain length of the high molecular aliphatic radical in various homologous series. The results confirm in a general way the conclusions of Eldred and Niederl (1941) and also show that the effect of the chain length of this radical and of the nature of the lower alkyl substituent on the germicidal activity are interdependent.

STUDIES ON THE GERMICIDAL PROPERTIES OF INDIVIDUAL QUATERNARY AMMONIUM COMPOUNDS

Alkyl-dimethyl-benzyl-ammonium chlorides. Dunn (1936) investigated the germicidal properties of a mixture of alkyl-dimethyl-benzyl-ammonium chlorides in which the alkyl radicals, derived from the fatty acids of coconut oil ranged from C_8H_{17} to $C_{16}H_{33}$. He reported that a number of both gram-positive and gram-negative pathogenic organisms were destroyed in ten minutes at 37° C., by the mixture in dilutions ranging from 1:35,000 to

1:90,000. The efficiency of the mixture was reported not to be affected by the presence of large concentrations of organic matter, nor by freezing or by heating at more than 50° C., for eighteen days.

The same worker (Dunn, 1937) later reported a more detailed study of the same germicide. The mixture was found to be readily soluble in water and to produce a low surface tension when so dissolved. Using the F.D.A. method, a high germicidal efficacy was found both in the absence and presence of horse serum. Specificity of activity was not demonstrated, but Dunn found greatest activity toward *Bacillus subtilis* at alkaline pH values. Definite bacteriostatic action was shown toward *B. subtilis* (gram-positive) but was of a low order toward *Escherichia coli* (gram-negative). The mixture proved superior to several market antiseptics, and was found not to be destroyed by storage for eight months at freezing or room temperatures.

Dunn (1938a) also made some fungicidal tests with alkyl-dimethyl-benzyl-ammonium chloride mixtures and found a 1:1,000 alcohol-acetone solution effective in destroying several pathogenic fungi as revealed by a modified F.D.A. technique. Blood serum was used in several tests with indications of good results.

Heineman (1937) has also studied the antiseptic properties of alkyl-dimethyl-benzyl-ammonium chlorides, using the regular phenol coefficient (F.D.A.) tests. High phenol coefficient values (154-293) were obtained using *Eberthella typhosa*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Escherichia coli*. A reduction of coefficient values at lower temperatures and in the presence of organic matter was noted, and was stated to be in accord with similar results obtained with other antiseptics. Even the lower figures, however, represent a high germicidal value. Spores of *Bacillus subtilis* and of several fungi were found to be destroyed in five minutes

by a 1:500 aqueous solution of the mixture.

Fanslau (1939) has likewise discussed the mixture of alkyl-(C₈H₁₇ to C₁₈H₃₇) dimethyl-benzyl-ammonium chlorides. He points out the free solubility in water and the resultant almost odorless, slightly alkaline solution. He states that an aqueous solution of 1:1,000 can be diluted 35 times and still destroy *Staphylococcus aureus* in less than ten minutes. Several years of storage are reported to have resulted in no change, and the mixture is stated to have no irritating effect on the body. A solution of 1:1,000 can be given orally or injected intraperitoneally daily for several months without causing any untoward reaction. Baker, Harrison, and Miller (1941b) tested this disinfectant against a number of organisms. It appears that the compound is somewhat less effective against the gram-negative than against the gram-positive organisms studied. Alkyldimethyl-benzyl-ammonium chloride has also been studied by a number of other workers (Hornung, 1935; Schneider, 1935; Maier and Müller, 1936; Thompson *et al.*, 1937; Leusden and Doring, 1938; White *et al.*, 1938; Wright and Wilkinson, 1939; Freeland, 1940).

Cetyl-pyridinium chloride. Blubaugh *et al.* (1939, a, b) reported on a series of investigations on a new compound, cetyl-pyridinium chloride. They found (1939b) that F.D.A. *in vitro* tests showed both tincture and aqueous solutions of this compound, in the absence or presence of organic material, to be highly bactericidal for virulent organisms. The activity of the compound against several pathogenic and non-pathogenic bacteria and fungi was determined, and the effect of changes in pH on the germicidal activity was noted. In further studies on this compound Blubaugh *et al.* (1940) used the F.D.A. method with provision for differentiation between bacteriostatic and germicidal activity both by subinoculation and by the use

of sodium thioglycollate broth. They found no bacteriostatic activity in the case of vegetative cells of a number of organisms. They concluded that for periods in excess of 24 hours the action is bactericidal rather than inhibitive. A dilution of 1:4,000 was found to be bactericidal in less than 15 seconds when tested against vegetative cells.

Green and Birkeland (1941) have tested the germicidal activity of methylpyridinium chloride and reported results indicating that the compound is an effective and practical germicide against spores of *Clostridia* and *Bacilli*.

Clarke (1942) showed the compound to compare favorably with other disinfectants and found no skin reaction following its use.

Ordal and Borg (1942) found cetylpyridinium chloride to inhibit lactic dehydrogenase of *Staphylococcus aureus* in 1.7×10^{-5} H ion concentrations. A concentration of 1.1×10^{-3} H ion was required to inhibit this enzyme in *Escherichia coli*.

Kolloff *et al.* (1942) found a 1:50,000 dilution would kill *Staphylococcus aureus* in ten but not five minutes when tested by the F.D.A. method.

Reed *et al.* (1943) have studied the effect of temperature on the germicidal activity of this compound and a review of the literature of cetylpyridinium chloride has been prepared by Huyck (1944). Recently Quisno and Foter (1946) have undertaken a new study of this compound.

Cetyl-dimethyl-benzyl-ammonium chloride. Gershenfeld and Milanick (1941) have studied the action of a number of surface tension depressants. Among those studied is a quaternary ammonium compound, cetyl-dimethyl-benzyl-ammonium chloride. Data are presented which show a rather high germicidal efficacy against *Staphylococcus aureus* at alkaline pH values. A slightly increased efficiency against *Eberthella typhosa* was revealed by these tests made according to the F.D.A. technique. It appears that a

1:10,000 dilution will kill *Eberthella typhosa* in 5 minutes at 37° C. at pH 8.2 while *Staphylococcus aureus* required 1:9,000.

Cetyl-trimethyl-ammonium bromide. Barnes (1942) found that solutions of cetyl-trimethyl-ammonium bromide would, when applied to the skin, greatly reduce the normal skin flora, and that they were effective in sterilizing surgical equipment. Clinical experience was reported to have shown the compound to be painless and harmless when applied to the skin and to be a very effective skin disinfectant.

Hoogerheide (1945) has made a study of this compound using a modified F.D.A. technique. Cetyl-trimethyl-ammonium bromide is soluble in alcohol and chloroform but insoluble in a number of other organic solvents. In water it is sparingly soluble at low temperatures but its solubility increases remarkably between 20° and 30° C. Like the other quaternary ammonium salts, it is a surface tension depressant. Three ug/ml. were found to prevent the growth of *Staphylococcus aureus*, and it was noted that the potency increased with increasing pH. Gram-negative and gram-positive species were found to be equally affected, and a considerable part of the germicidal potency was lost in the presence of serum. Comparison with a series of commonly used disinfectants again revealed that the compound was one of the outstanding bactericidal and bacteriostatic agents.

p-tert-octyl-phenoxy-ethoxy-ethyl-dimethyl-benzyl-ammonium chloride. Rawlins *et al.* (1943) picked this compound from a series of similar compounds as the one offering the most promise as a good disinfectant. Joslyn and coworkers (1943) subjected this compound to a more detailed study. Water solutions had a pH of 5 to 6, and possessed a somewhat bitter taste. Such solutions were not affected by dilute acid or alkali, but precipitated upon the addition of concentrated mineral acids. Aqueous and alcoholic

solutions were stable toward light, air, and temperatures up to 100° C. The compound was incompatible with soaps and iodides. It did not affect rubber, plastics, fabrics, or metals with the exception of copper and brass. A 1:1,000 dilution gave a surface tension of 36 dynes/cm. at 25° C. The compound was tested against ten diverse organisms, and dilutions ranging from 1:3,500 to 1:180,000 were found to be effective. A dilution of 1:1,000 was ineffective against *Pseudomonas aeruginosa* in five minutes at 20° C., but at 37° C., a 1:3,500 dilution was effective in five minutes. The compound was also found effective as a fungicide in *in vitro* tests against seven fungi and appeared to show promise as a general fungicide. The germicidal action of this compound was reported not to be reduced when incorporated as the active ingredient in either an aqueous or tincture formula. Baker, Harrison, and Miller (1941b) found this compound more effective against gram-positive than gram-negative organisms.

9-octadecenyl-dimethyl-ethyl-ammonium bromide. The general properties of this compound have been discussed by Botwright (1946). This worker found that 10 p.p.m. of the compound would kill *Streptococcus pyogenes* in ten minutes. One percent concentration produced little or no skin reaction when applied to the forearms of 28 persons for 24 hours. The compound proved effective for the germicidal treatment of drinking glasses, and a test kit for the rapid determination of the compound is available.

Stearyl-trimethyl-ammonium bromide. Baker, Harrison, and Miller (1941b) found this compound effective against four gram-positive and two gram-negative organisms in ten minutes at 1:3,000, but not at 1:30,000 in all cases. A 1:1,000 dilution would not kill the gram-negative *Proteus vulgaris* in ten minutes, but 1:3,000 was sufficient for a ninety-minute exposure. This compound appeared to be less effective against the gram-negative species studied.

Other compounds studied by Baker, Harrison, and Miller (1941b) were the following:

N (lauric acid ester of colamine formyl-methyl) pyridinium chloride, lauryl pyridinium iodide, and NNN'-tetramethyl-NN'-didodecyl-betahydroxy-propylene diammonium bromide.

Alkyl-dimethyl-3,4-dichlorobenzyl-ammonium chlorides. This relatively new mixture in which alkyl represents radicals derived from the fatty acids of coconut oil (C_8H_{17} to $C_{18}H_{37}$) appears not to have received much mention in the literature. Hucker *et al.* (1947) found the compound superior to the non-chlorinated alkyl-dimethylbenzyl-ammonium chloride mixture against most of a variety of organisms tested. These workers used a method different from the F.D.A. phenol coefficient method and found that a 1:22,000 dilution would kill twenty-four hour culture of *Aerobacter aerogenes* in ten minutes. For the same effect on *Escherichia coli* (a heat resistant strain) a dilution of 1:14,000 was required. The mixture was less effective against spores.

A recent communication by DuBois and Dibblee (1946a) corroborates the lesser effectiveness against spores (*Bacillus metiens* was used) and points out that the killing of 60 to 75 percent of the spores almost immediately is not consistent with a logarithmic order of death. This same observation had been noted both with this compound and with oleyl-dimethyl-ethyl-ammonium bromide by Hucker *et al.* (1947), for vegetative cells, and is a commonly observed phenomenon.

FACTORS INFLUENCING THE GERMICIDAL ACTIVITY OF QUATERNARY AMMONIUM COMPOUNDS

Organic matter generally reduces the bactericidal efficiency of disinfectants, and it appears that the quaternary ammonium compounds are not an exception. The fact that surface-active ions combine with or are adsorbed by

protein materials has been definitely established (Valko, 1946), and fat droplets and other types of organic matter seem to react similarly.

Although Botwright (1946) has stated that these compounds are not as greatly affected by the presence of oxidizable organic matter as the chlorine compounds, Klarmann and Wright (1944) have found that certain of the less effective germicides of the coal tar, cresylic, or pine oil variety, or the less active synthetic phenol derivatives may be impaired to a lesser extent than some of the more efficient quaternary ammonium compounds.

Domagk (1935) reported that alkyl (C_8H_{17} to $C_{18}H_{37}$)-dimethyl-benzyl ammonium chlorides possessed strong inhibitory properties even in the presence of 10 percent blood serum, and Dunn (1936) reported a similar observation. The following year Dunn (1937) found that 50 percent of normal horse serum would markedly reduce the activity of alkyl-dimethyl-benzyl-ammonium chlorides toward a number of different organisms. Tested against *Staphylococcus aureus* at 20° C. the phenol coefficient of the germicide was reduced from 279 to 54.2 by the presence of the 50 percent serum. It was his observation, however (Dunn, 1938b), that in the presence of this amount of serum, the quaternary ammonium compounds showed greater efficiency at 20° C. toward *Staphylococcus aureus* than any of several other disinfecting agents with which it was compared. At 37° C. it ranked with the top three germicides. Heineman (1937) also noted a reduction in the presence of blood serum. Tested against *Staphylococcus aureus* at 37° C. the phenol coefficient was reduced from 275 to 154 by the presence of 10 percent serum.

Kramer and Sedwitz (1944) found cetyl-pyridinium-chloride to be effective in the presence of 10 percent blood serum in slightly increased concentrations, and Fair *et al.* (1945) showed that it was necessary to increase the concentration of cetyl-pyridinium bro-

mide in order to effect killing of *Entamoeba histolytica* cysts in the presence of 50 to 200 p.p.m. of finely ground agar agar, egg albumin, or lecithin. The increased dosages necessary were not large, but were significantly different from the amounts that were effective in the absence of these foreign substances.

Neter (1942a) has shown that blood serum also interferes with the inactivation of tetanus toxin by alkyl-dimethyl-benzyl-ammonium chloride. Similar reductions in germicidal efficiency have been noted in the presence of other types of organic matter. Thus Domagk (1935) noticed that soaps interfere with the action of the quaternary ammonium salts, and Baker *et al.* (1941c) and Brewer (1943) have shown that phospholipids inactivate the quaternary salts. Neutralization by lecithin has recently been reported by Quisno *et al.* (1946a) and interference by agar has been demonstrated by Sherwood (1942) and Quisno *et al.* (1946b).

Many workers have also noticed an increased activity of these compounds in alkaline pH ranges, or, conversely stated, a reduced activity in the more acid pH ranges (Dunn, 1937; Blubaugh *et al.*, 1939b; Gershenfeld and Milanick, 1941; Hoogerheide, 1945; and others). This effect is readily apparent in the data presented on Zephiran (alkyl-dimethyl-benzyl-ammonium chlorides) by Gershenfeld and Perlstein (1941). Here a dilution of 1:70,000 killed *Staphylococcus aureus* in less than five minutes at pH 7.0 as revealed by the F.D.A. technique. At pH 6.0 a dilution of 1:10,000 was required, and at pH 5.0 a dilution of 1:5,000 was not sufficient. At pH values below 5.0 the germicide evidently becomes quite worthless. It is especially interesting to note that Rahn (1945) and Quisno and Foter (1946) find Ceepryn (cetyl-pyridinium chloride) almost equally effective in acid and alkaline solutions.

Reed *et al.* (1943) have studied the effect of temperature on the germi-

cidal activity of cetyl-pyridinium chloride, and noted that while activity increased with increased temperature, considerable variation in temperature effects occurred when tests were made with different organisms.

It is sometimes possible to increase the germicidal activity of a disinfectant by the addition of a surface active agent. In spite of the fact that quaternary ammonium salts are themselves surface active, experiments of this sort have been tried with them. Usually the surface active agent is of the anion active type, and these react with the cation active quaternary compound to form a biologically inactive salt in most instances. Gershenfeld and Perlstein (1941) found this to be the case when Aerosol OT (di-octyl) sodium sulfosuccinate was added to solutions of Zephiran (alkyl-dimethyl-benzyl-ammonium chlorides). Petroff and Schain (1940) obtained apparently similar results when Aerosol OT, Tergitol 4T (triethanolamine tetradecyl sulfate), and Tergitol 4T (sodium tetradecyl sulfate) were added to Zephiran in tests run against *Bacterium pyocyaneus*. Their data seem to indicate the opposite effect of Tergitol 4T in a test against *Staphylococcus pyogenes aureus*, and it is difficult to account for these results.

BEHAVIOR OF QUATERNARY AMMONIUM SALTS IN THE PRESENCE OF VARIOUS TYPES OF SURFACES

Tice and Pressman (1945) made a study of the behavior of quaternary ammonium salts in the presence of positively and negatively charged gelatins. They presented evidence which indicates that negatively charged gelatins are capable of producing coacervates with quaternary salts in certain ratios. No such phenomenon was observed with positively charged gelatins in any ratio. Tice and Pressman reported that the bactericidal efficiency of Phemerol (*p*-tert-octyl-phenoxy-ethoxy-ethyl-dimethyl-benzyl-ammonium chloride) was not reduced in the presence

of either type of gelatin even when coacervation existed. They found that there was even a possibility that gelatin augmented the bactericidal efficiency of this compound.

In this connection it is interesting to note that Rahn (1946) found no protective action of gelatin upon bacteria dried upon a gelatin surface when the dry bacteria were exposed to Zephiran (alkyl-dimethyl-benzyl-ammonium chloride) or Ceepryn (cetyl-pyridinium chloride). Rahn's explanation is that the hydrophilic (germicidal) ends of the molecules were directed outward. The formation on the hands of a similar film, but with the organophilic ends toward the oily skin and the germicidal-NOH groups directed outward, explains the phenomenon reported by Miller *et al.* (1943) that hands dipped in 1 percent Zephiran remain apparently sterile for about two hours through an imperceptible film, but that bacteria under this film are still alive when the film is later broken. Rahn found that no protection was offered by glass or sand surfaces, but that fat surfaces would protect his dry bacteria for as long as twenty hours.

STUDIES DEALING WITH THE MECHANISM OF THE INHIBITORY ACTION OF QUATERNARY AMMONIUM COMPOUNDS

A number of workers have discussed the mechanism of the antibacterial action of quaternary ammonium salts. Albert (1942) reviewed the methods by which antiseptics kill bacteria and considered that cationic germicidal wetting agents contain basic ions or cations which interact with the acid groups of bacterial protoplasm to form feebly ionized compounds. Miller and Baker (1940) reported that several of these compounds markedly inhibited the metabolism of six gram-positive and six gram-negative organisms. This statement was amplified by Baker, Harrison, and Miller (1941a). The rate of respiration or glycolysis was deter-

mined in the Warburg apparatus, and at a concentration of 1:3,000 all of the quaternary ammonium salts studied exerted a marked inhibitory action on all of the studied organisms. At 1:30,000 a similar effect was noted in most cases. Gram-positive and gram-negative organisms were inhibited to the same degree, and the exerted inhibitory effect was usually complete within fifteen minutes. For a number of compounds the inhibition appeared to be irreversible.

Later, Baker *et al.* (1941b) found that quaternary ammonium compounds were slightly less effective against the gram negative organisms tested, especially *proteus vulgaris*. They also found (1941c) that phospholipids such as lecithin, cephalin, and sphingomyelin prevented this inhibition of bacterial metabolism by cationic detergents. They felt (1941c) that the action of the detergents was two-fold: (a) a disorganization of the "cell membrane" by virtue of surface activity, and (b) denaturation of proteins essential to metabolism and growth.

Sevag and Ross (1944) studied the mechanism of the inhibitory effect of Zephiran (alkyl-dimethyl-benzyl-ammonium chlorides) on yeast cells. They found that Zephiran in concentrations of 1:1,000 or higher caused a fading of the absorption bands of cytochrome c, and in a manometric measurement found a 1:3,500 dilution reduced the "oxygen consumption of p-phenylenediamine." This would seem to indicate the inhibition of the cytochrome-cytochrome oxidase system by the disinfectant. One and eight-tenths percent serum was found to compete for the Zephiran, counteracting the inhibition.

Ordal and Borg (1942) have shown that cetyl-pyridinium chloride inhibits the oxidation of lactate by *Staphylococcus aureus* and *Escherichia coli*. Roberts and Rahn (1946) also noted enzyme inactivation by bactericidal concentrations of Ceepryn. They noted that inactivation was not complete at

bacteriostatic concentrations, and were in doubt as to whether enzyme inactivation was not a result of death rather than the cause.

Valko and DuBois (1942, 1945) found the bactericidal effect of a surface active cation such as the N-n-dodecyl-N'-ethyl-benzotriazolium ion greatly diminished in the presence of other less toxic surface active cations. This led to the conclusion that either on the surface of, or in the bacteria, there are certain "spaces" available for the surface active cations. If these are occupied by harmless cations, the bacteria are protected against toxic cations, provided the former are more firmly attached than the latter. They state that the initial process can satisfactorily be described as a reversible adsorption by the bacteria which function as exchangers.

Valko (1946) assumed that following this initial step the surface active ions penetrated into the cell and resulted in some further action, such as denaturation and precipitation of proteins, cleavage and inactivation of complexes, or lysis of cells, to produce the final lethal reaction.

On the other hand, Hotchkiss (1946) seems to feel that the surface active ions cannot penetrate into the cell while it remains alive, and he therefore assumes that the quaternaries first disrupt the cell membrane, making it completely permeable and resulting in reduction of enzyme activity and cessation of multiplication.

Rahn (1946) has shown that 1 gram of bakers' yeast in 100 p.p.m. Zephiran adsorbed nearly 20 p.p.m. of the germicide, and Valko and DuBois (1945) have found that killing of bacteria by alkyl-dimethyl-benzyl-ammonium chloride could be reversed by sodium lauryl sulfate.

METHODS OF EVALUATING THE GERMICIDAL ACTIVITY OF QUATERNARY AMMONIUM COMPOUNDS

In order to evaluate the germicidal activity of disinfectants and compare

the activity of different compounds, it is obvious that a test method must be used which is standardized with respect to the origin, cultivation, and resistance of the bacterial strain employed, and with respect to the conditions under which the tests are run. The standard F.D.A. method as developed by Ruehle and Brewer (1931) appears to meet these conditions, and has, of course, been used successfully for a number of years by the Food and Drug Administration in testing a variety of germicidal substances.

The F.D.A. method has been used almost exclusively in studies on the quaternary ammonium compounds (as cited above), chiefly because it is the only available, recognized, standard method. Some workers (Valko and DuBois, 1945) still seem to feel that the F.D.A. method is capable of giving reproducible results from which valid conclusions may be drawn, but recently there has been presented evidence which indicates that this method does not always lead to reproducible results when applied to highly effective germicides, and in particular to the quaternary ammonium salts.

Brewer (1943) found that when tested against *Eberthella typhosa*, the germicidal activity of disinfectants containing synthetic detergents was greatly altered by the phospholipid content of the culture medium (peptone). Brewer (1944) also mentions as factors leading to discrepancies the mutation of the test organism between the smooth and rough strains and the variation in the number of organisms transferred from the medication tube. The latter is dependent upon the quantity of liquid picked up by the transfer loop, and this in turn is affected by the surface tension of the liquid. In cases where the disinfectant tested is a surface tension depressant, serious error may be introduced.

Mallmann (1944) points out several difficulties in interpretation of results of F.D.A. tests, particularly when tests are made in the presence of organic

matter or when the effect of pH is introduced.

Tobie and Ayres (1944) found a distinct lack of correlation between cup-plate tests and phenol coefficient determinations on dodecyl-trimethyl-ammonium bromide and octadecyl-trimethyl-ammonium bromide. The dodecyl compound proved to be superior by the plate test, but with the phenol coefficient method the octadecyl compound appeared superior. However, the unreliability of agar cup-plate tests has been shown by the reports of Sherwood (1942) and Quisno *et al.* (1946) on the reduction of germicidal efficiency of quaternaries by agar.

It has now become a common experience that the quaternary ammonium compounds yield inconsistent results when tested by the F.D.A. phenol coefficient method, not only in the hands of different workers, but also in tests run by the same worker in the same laboratory over a period of time. Klarman and Wright (1946a) point out that there have been several instances where a higher concentration of the tested quaternary compound evidently permitted survival and subsequent proliferation of the test organisms while a lower concentration appeared to have a germicidal effect.

These workers (Klarman and Wright, 1946a, b) have tried various modifications of the F.D.A. technique and showed wide discrepancies between these and the usual technique. It appears that the minimum bactericidal concentration may be considerably higher than that indicated by the F.D.A. tests. The difficulty seems to depend upon the development of a condition in the medication tube whereby it is impossible to secure a representative transfer. Presumably there occurs an agglutination and precipitation of the bacteria upon the walls of the test tube which causes an irregular distribution.

It becomes increasingly apparent that a new method of evaluation must be devised for these compounds if rational

scientific and regulatory work is to be carried on. A number of workers are engaged in trying to develop such a test. Klarman and Wright (1946 a, b) have reported that a technique based on the transfer of standard pieces of filter paper from the medication tube is showing indications of success. Klarman and Wright (1946b) and Pressman and Rhodes (1946) have also reported promise of success with methods involving the transfer of larger samples from the treated cell suspension. Reddish (1946) considered the usual F.D.A. method completely unsatisfactory for quaternary ammonium compounds and suggested that some modification of the "use-dilution" method might be satisfactory. The use-dilution method has been briefly described by Mallmann (1945).

It appears that bacteria transferred from the medication tube have adsorbed on their surfaces sufficient quaternary cations to result in a bacteriostatic effect. To avoid this and obtain more accurate results, Quisno *et al.* (1946) recommend the use of a neutralizing medium containing lecithin and a dispersant, "Tween 80," both of which help to neutralize the quaternary ammonium compound.

Rahn (1947) has discussed the advisability of a number of these proposed procedures for the evaluation of these compounds.

USES OF THE QUATERNARY AMMONIUM COMPOUNDS

Sanitation of eating and drinking utensils. Krog and Marshall (1940) using a swab test technique, studied the sanitizing effect of alkyl-dimethylbenzyl-ammonium chloride (Zephiran) on glass tumblers artificially contaminated with suspensions of bacteria from human feces. The effects of fats (sour cream) and of the two types of lipstick on the efficiency of the compound under these conditions were determined, and a series of field tests was run in two restaurants and two taverns. These workers concluded that a 1:5,000

solution demonstrated marked bactericidal action against bacteria found on eating and drinking utensils; that detergents and soaps had little or no effect on the potency of the compound in two- or three-compartment washing procedures; the stability of the compound was favorable to the application studied; temperature did not affect the stability or bactericidal efficiency adversely above 70° F.; and a one minute exposure was apparently sufficient to reduce surviving bacteria to below 100 colonies per tumbler rim.

Walter (1941) and Walter and Hucker (1942) reported on a similar study of the use of alkyl-dimethylbenzyl-ammonium chloride in six taverns over a two-month period. They found that the substitution and proper use of a 1:5,000 solution for the plain water or chlorine being used resulted in a marked decrease in the number of organisms and the elimination of coliform organisms from the rims of the glasses tested. McPherson (1944) showed that a 1:5,000 dilution of the same compound was effective in reducing plate counts from swabs of various eating and drinking utensils in restaurants and hospitals, and also showed that the substitution of a 1:5,000 dilution for the cold water used for storing ice cream scoops would be desirable.

Recently Botwright (1946) has reported on studies with another quaternary, oleyl-dimethyl-ethyl-benzyl-ammonium bromide (Amerse) in which glasses were examined in several restaurants and taverns. Under actual use conditions, this compound and two other unnamed quaternaries were found superior to certain chlorine compounds in reducing the bacterial count from beverage glasses. Mallmann and co-workers (1946) have also recently reported that alkyl-dimethylbenzyl-ammonium chloride (Roccal and B. T. C.) and *p*-tert-octyl-phenoxyethoxy-ethyl-dimethyl-benzyl-ammonium chloride (Hyamine 1622) in 1:6,400 dilutions would sanitize 800

glasses effectively with an exposure of approximately 30 seconds. In this study the presence of neutralizing agents in the cleaners (wetting agents and polyphosphates) had no effect on the sanitizing value of the quaternaries when 800 glasses were sanitized using two- or three-compartment procedures.

From the above studies, it is evident that the reaction of the quaternary ammonium compounds with the various components of cleaning compounds (see Powney, 1943) will not lower their effectiveness when properly used in two- or three-compartment washing procedures. In addition, the quaternaries have an advantage in being relatively odorless and tasteless in the concentrations recommended for use. According to Botwright (1946) they have already been accepted as sanitizing rinses by more than 160 cities in 18 states throughout the country.

Recently, there has been considerable interest in the possibility of developing a dishwashing compound which would be germicidal as well as having good detergent properties. Quaternary ammonium salts have been suggested for this purpose because of their highly bactericidal nature, but unfortunately they react with the best anionic detergents, and both are precipitated out of solution. Some success along this line has recently been reported by Guiteras and Shapiro (1946) who used a formulation embodying trisodium phosphate, sodium bicarbonate, tetrasodium pyrophosphate, an alkylated aryl polyether alcohol (wetting agent), and a cetyl-dimethyl-ethyl-ammonium bromide preparation.

Sanitation on the dairy farm and in the dairy industries. Scales and Kemp (1941) reported that alkyl-dimethyl-benzyl-ammonium chloride (Zephiran) was excellent for sterilizing cows' udders prior to milking, and recommended wiping the udder with a cloth wet with a 1:5,000 dilution of the compound after first removing adhering soil with a cloth wet with water. They further suggested that a 1:5,000

solution would be useful for sterilizing the cups of milking machines, etc. Frayer (1944) also states that some experience with the use of alkyl-dimethyl-benzyl-ammonium chloride (Roccal) in wiping cows' udders prior to milking indicates that it has distinct merit for the purpose since, in addition to its germicidal property, it has detergent and some emollient values. Recently Hughes and Edwards (1946) have reported lesions developing on cows' teats, presumably as a result of the continued application of cetyl-trimethyl-ammonium bromide (see below under veterinary medicine).

Jamieson and Chen (1944) found that washed and supposedly clean milk cans had heavy loads of bacteria which could be reduced over 90 percent by *spraying* with alkyl-dimethyl-benzyl-ammonium chloride or N (acyl col-amino formyl-methyl) pyridinium chloride. They used one ounce of 10 percent solution to one gallon of water and reported no appreciable difference between the results obtained with these two compounds and those obtained with a hypochlorite and another undescribed disinfectant.

Krog and Marshall (1942) have made a study of the application of alkyl-dimethyl-benzyl-ammonium chloride (Roccal) in a dairy pasteurizing plant. They found that the use of 1:5,000 to 1:8,000 dilutions of the compounds following the usual cleaning treatment exerted a definite bactericidal action on the flora associated with milk handling equipment, resulting in 68 to 99 percent reduction in bacteria counts obtained from swabbings made at specified stations throughout the plant. The compound was reported to be definitely stable, not decreasing greatly in concentration when used to sanitize properly cleaned equipment. In addition, the quaternary was said to impart no taste or odor to milk products, to be safe to use because of its low toxicity, and to be no more corrosive to metal and rubber than ordinary water. The fact that it is

a wetting out agent increases its value in cleaning milk handling equipment.

Frayer (1944) states that a few trials have indicated that alkyl-dimethyl-benzyl-ammonium chloride (Roccal) is relatively inert toward aluminum, copper, tinned copper, and tinned iron, and points out that this is not surprising in view of the pH of about 7.0 in effective concentrations. Hucker *et al.* (1947) after testing a number of nickel, tin, aluminum, magnesium alloy, and stainless steel samples, concluded that alkyl-dimethyl-3,4-dichloro-benzyl-ammonium chloride (Tetrosan) and *p-tert-octyl-phenoxy-ethoxy-ethyl-dimethyl-benzyl-ammonium chloride* (Hyamine 1622) had little if any effect on those metals and alloys commonly used in the fabrication of dairy and food processing equipment. These workers also concluded "tentatively" that the same metals do not appreciably affect the germicidal activity of the cationic germicides.

Levowitz (1944) and Vignolo (1946a) have made suggestions as to how the quaternary ammonium compounds may possibly be applied in the dairy industry. An important point in connection with the use of these compounds was made by Rahn (1945), namely, that these compounds become less efficient in the presence of milk and hence can be used only to sterilize equipment which has been washed free from milk.

Krog (1942) considered that because of its lack of flavor or odor, alkyl-dimethyl-benzyl-ammonium chloride (Roccal) could be used in 1:5,000 aqueous solution to soak wiping cloths for the sterilization of novelty equipment in the ice cream industry.

Recently, DuBois and Dibblee (1946b) have reported that alkyl-dimethyl-benzyl-ammonium chloride (B. T. C.) did not influence the bacterial count of raw or pasteurized milk at concentrations ranging from 1:500 to 1:25,000, and hence could not be added to milk by unscrupulous dealers to cover up unsanitary practices.

According to Botwright (1946), about thirty cities in the United States have accepted quaternary ammonium salts for dairy uses.

Plant sanitation in the food industries. Vignolo (1946, 1946a) has suggested the use of quaternaries for sterilizing lug boxes used to bring fruits and vegetables to the plant and for sterilizing various pieces of food processing equipment by spraying or flushing treatments. This is not a technical article, however, and no data as to the effectiveness of such treatments are given.

Penniston and Hedrick (1944) showed that the usual count of three to six million microorganisms found on eggs after washing in water could be reduced to a few thousand or none by a three to five minute rinse in a mixture of nearly equal amounts of the lauric and myristic esters of colamino formyl-methyl pyridinium chloride (E607 Special). The mixture was found to be somewhat less effective on molds, but it did nevertheless kill a large percentage. The disinfectant was considered useful in preventing contamination from the shell of eggs intended for dehydration.

Sanitation in the brewing industry. Lehn and Vignolo (1946) have suggested that a quaternary compound in a dilution of one ounce of 10 percent solution to four gallons of water can be used to sterilize lauter tubs, brew kettles, hop strainers, fermenting tanks, and surface coolers by spray application, and to sterilize plate coolers, filtering equipment, beer and wort lines, fittings, and pumps by circulating the solution through the various pieces of equipment. Again, this is a general, non-technical paper, and no data as to the effectiveness of the quaternaries are cited.

Water supply disinfection. Fair *et al.* (1945) have studied the cysticidal action of 10 quaternary ammonium compounds against *Entamoeba histolytica* cysts. They found that for the best substances, 300 p.p.m. was

required for killing in a 10-minute contact period, and 10 p.p.m. for a 2-hour period. The necessary concentration increased with increasing density of cysts to be killed, but suspended organic matter and pH appeared to have only slight effects. On the basis of their observations they concluded that at least four of the ten compounds were effective cysticides: cetyl-pyridinium bromide (Fixanol), lauryl-dimethyl-benzyl-ammonium chloride (Nopco QCL), cetyl-pyridinium chloride (Cee-pryn), and an unnamed compound (Sapamine K_w). In addition, alkyl-dimethyl-benzyl-ammonium chloride (Zephiran) and cetyl-dimethyl-benzyl-ammonium chloride (Triton K₆₀) may be effective, but their data on these compounds are not complete.

The detergent and disinfecting properties of the compounds studied led the authors to suggest exploration as to their possible usefulness in water disinfection, sanitation of eating utensils, safeguarding of shellfish, cleansing and disinfection of contaminated vegetables and fruits that are to be consumed raw, cleansing and disinfection of water mains, cleansing of water filters, and control of *Psychoda* in trickling filters.

Sotier (1946) has reported that *p-tert-octyl-phenoxy-ethoxy-ethyl-dimethyl-benzyl-ammonium chloride* (Polymine D), when used in solid form combined with mild alkalis, proved effective for sterilizing two jute-packed water mains that had proved refractive to chlorine sterilization. For the longer main (3,000 ft.) one 7-day application of the germicidal solution was effective. The relatively rapid action was attributed to the high germicidal power and detergent and surface-active properties of the compound.

Disinfection in surgery. Domagk (1935) spoke of the sterilizing effect of alkyl-dimethyl-benzyl-ammonium chlorides (Zephiral) on the skin, and suggested their possible use in surgery. Walter (1938) made an investigation of this quaternary salt (Zephiran) to

determine its possible effectiveness as a skin disinfectant. He sterilized operative fields with 1:1,000 tincture of alkyl-dimethyl-benzyl-ammonium chloride and then removed full-thickness biopsies of skin from one edge of the wound immediately after incision. These were suspended in 1.5 liters of Ringer's solution to dilute the residual bactericide and prevent continued action. The biopsies were subsequently transferred to dextrose broth and were incubated at 37° F. (*sic*, but must mean ° C.) for 96 hours. Cultures yielding no growth of bacteria were then inoculated to determine any bacteriostatic condition in the tubes. Of 75 cases, 25 yielded positive cultures, and on the basis of these results the author concluded that the preparation was an efficient bactericide for operating room use and gave directions for disinfection of skin and hands. White *et al.* (1938) similarly removed full-thickness pieces of skin both before sterilization and after sterilizing with a 1:1,000 tincture of alkyl-dimethyl-benzyl-ammonium chloride (Zephiran). Their exact method of culturing the tissue sections is not given, but they reported that all of the untreated pieces of excised tissue gave positive results while only two sections from treated areas yielded growth in the cultures. They state that these results are sufficient to warrant further trial. Wright and Wilkinson (1939) stated that the use of alkyl-dimethyl-benzyl-ammonium chloride on skin in preparation for operation gave evidence of high germicidal potency. Their exact methods were not stated. Hauser and Cutter (1944) treated hands with Zephiran and then touched blood agar plates. Tests on skin (1:1,000) revealed no resultant dermatitis, and introduction of drops in the human eye gave a minimum of subjective symptoms. They stated that clinical experience had shown that it can be safely used as a pre-operative cleansing material for the surgeon's hands as well as on the operative field. Gardner and Seddon (1946) found that in 1½

minutes Zephiran was effective in disinfecting skin artificially contaminated with *Pseudomonas pyocyanea*, but cetyl-trimethyl ammonium bromide (CTAB) was not effective in 10 minutes on skin contaminated with *Staphylococcus epidermitis*.

Barnes (1942) reported studies on this latter compound, cetyl-trimethyl-ammonium bromide (CTAB). On the basis of plate counts from swabs before and after treatment with 1 percent CTAB, he concluded that such a treatment was capable of materially reducing the number of bacteria on normal skin. Williams *et al.* (1943) also considered CTAB highly effective in removing dirt and bacteria from the hands, and suggested that it would be an admirable substance for application to the hands in gloveless surgery. They emphasized the necessity for first removing organic matter, and noted certain skin reactions in a small percentage of cases. Cetyl-pyridinium bromide was said to be less likely to produce these reactions.

Kramer and Sedwitz (1944) found a tinted tincture of 1:200 cetyl-pyridinium chloride (Ceepryn) to be a highly effective non-irritating cutaneous disinfectant for securing a sterile operative field, and used Ceepryn successfully in 1:1,000 aqueous solution on all surgical patients requiring a germicidal irrigant or wet dressing. Helmsworth and Hoxworth (1945) studied the use of cetyl-pyridinium chloride as a skin antiseptic using both swab technique and the culturing of tissue fragments to determine the efficiency. Tincture of cetyl-pyridinium chloride applied to operative fields washed with soap and water, alcohol and ether was reported to have a definite germicidal effect (1:1,000 and 1:500 tincture). One to one hundred aqueous solution also produced a striking germicidal effect when used to scrub operative fields. Aqueous cetyl-pyridinium chloride was well tolerated by the skin of patients.

Brown *et al.* (1944) employed small culture plates placed in direct contact

with the abdomen as soon as the applied antiseptic had dried. Two sides of the abdomen were used for comparative data, and using this technique they reported that tincture of Phemerol (*p-tert* - octyl - phenoxy - ethoxy - ethyl-dimethyl-benzyl-ammonium chloride) in 1:500 dilutions was superior to the mercurials studied and to tincture of green soap and alcohol. It seemed about equal to tincture of iodine, and did not cause skin irritation or interfere with wound healing. It was considered suitable as a skin antiseptic for surgery.

Neufeld, Schiemann, and Schutz (1940) recognized that alkyl-dimethyl-benzyl-ammonium chloride (Zephiran) was strongly adsorbed on human skin and could kill pneumococci placed on a Zephiran-treated hand. They also stressed a point which appears to have been neglected by the writers previously mentioned, namely, the need of using a specific neutralizing agent in all bacterial studies with the cationic detergents to avoid misleading results due to bacteriostasis (see under "Methods of Evaluating Germicidal activity," page . . .).

Miller, Abrams, Huber, and Klein (1943) made another point which placed the whole question of skin disinfection with quaternaries on a questionable basis. Using chiefly Zephiran and Phemerol, they demonstrated that these cationic detergents deposit an invisible, non-perceptible film on the hands, and showed that this film retains bacteria underneath it and is very resistant to mechanical trauma. The inner surface of the film has a low bactericidal power whereas the outer surface exerts a strong germicidal action. The formation of such a film on the hands has been explained by Rahn (1946) as due to an oriented adsorption of the molecules of the detergent on an organophilic surface with the harmless organic end directed toward the skin and the germicidal-NOH group toward the outside. The outside of the film remains sterile for considerable periods of time, but beneath the film the bacteria on the skin remain alive.

It is easy to see why the swabbing techniques of Barnes (1942), Williams *et al.* (1943), Gardner and Seddon (1946), and the contact plate methods of Hauser and Cutter (1944) and Brown *et al.* (1944) give such good results. They are testing only the germicidal outer side of the adsorbed quaternary film. Much better are the skin-sectioning techniques (Walter, 1938; White, *et al.*, 1938; Helmsworth and Hoxworth, 1945), but these also are not entirely reliable for no antidote was used, and it appears that enough quaternary may be adsorbed on bacteria to cause a continuing bacteriostatic effect, even though the bacteriostatic condition does not exist in the medium itself. In surgery the quaternary ammonium compounds could not be expected to exert a bactericidal action in incisions where they would come in contact with organic matter, phospholipids, etc., which would act as antidotes.

It is possibly significant, however, that Hauser and Cutter (1944) have actually used quaternaries for surgeons' hands and on operative field with no infection resulting, and White *et al.* similarly had infection in only 1.3 percent of cases in a quaternary-treated series, but in 3.5 percent in tincture of iodine-treated series. Kramer and Sedwitz (1944) reported that Ceepryn tincture 1:200 had been used routinely in operative cases at the Youngstown (Ohio) hospital for three years (*ca.* 18,000 cases) with no observed irritation and no single instance of infection.

Besides hand and skin disinfection, there are other proposed surgical uses of the quaternary ammonium compound. Walter (1938) recommended a 1:5,000 aqueous solution of alkyl-dimethyl-benzyl-ammonium chloride (Zephiran) for use in glove basins during operations, and recommended immersion in a 1:1,000 solution for the sterilization of such instruments as will not stand heat treatment. He stated that metal instruments could not be stored in the solution. Williams *et al.*

(1943) were of the opinion that cetyl-trimethyl-ammonium bromide (CTAB) could be used to sterilize surgical instruments.

Barnes (1942) on the basis of swab tests before and after treatment reported that dirty bowls and baths could be sterilized by swabbing 2 percent cetyl-trimethyl-ammonium bromide (CTAB) on the surface, and Forman (1943) cleaned two wash basins and two baths with 2 percent CTAB and reported that subsequent cultures showed the surfaces to be sterile.

Human medicine. Quaternaries have also been used in attempts at combatting various infections. Wright and Wilkinson (1939) were convinced that alkyl-dimethyl-benzyl-ammonium chloride was effective in combatting infection in injuries and found that in proper dilution (1:1,000), it did not damage tissue and was not uncomfortable to the patient. They reported that its use on burns gave good results in combatting secondary infection. Barnes (1942), on the other hand, stated that although CTAB was efficient in cleaning burns, swab-tests revealed no sterilizing action. Forman (1943) reported Cetavlon to be effective in the removal of grease and ointments from skin, and for the removal of scabs and crusts. He further stated that the compound appeared effective in curing boils and dermatitis due to strapping within 10 days when a 1 percent solution was applied daily. He postulated the theory that the germicide reduced the staphylococcal population of the skin so that the natural bactericidal powers of the skin would be sufficient to cope with the infection. Forman also reported satisfactory progress of severe nummular eczema, chronic infective dermatitis, and impetigo of the face when treated daily with Cetavlon, although one case appeared to show sensitivity. Fischer (1944) also has reported success with CTAB in the prevention of impetigo neonatorum. He presented a comparative study of three methods of prophy-

laxis against impetigo neonatorum over a period of seven years in the same hospital. There was a material decrease in the incidence of the infection following the use of a new antiseptic baby lotion containing cetyltrimethyl-ammonium bromide 0.16 percent, boric acid, lanolin, and mineral oil. Sarber (1942) reported some success in preventing fatal *Streptococcus hemolyticus* peritonitis in white mice which had had the test organism massaged into abrasions of the abdomen. Cetyl-pyridinium chloride (1:200 tincture) proved somewhat superior to three other disinfectants tested.

Neter (1942a) showed that alkyl-dimethyl-benzyl-ammonium chloride (Zephiran chloride) 1:2,000 would almost completely inactivate tetanus toxin. This suggests its use for the prevention or treatment of infection by toxin-producing bacteria. He points out that addition of human serum to the solution interferes with the toxin-destroying properties, but that the addition of serum to Zephiran-inactivated toxin does not cause reactivation. Neter (1942b) also showed that Zephiran chloride in 1:10,000 dilution inhibits (and up to 1:1,000,000 delays) clotting of oxalated human plasma by staphylococcal cultures and also that Zephiran chloride up to 1:100,000 inhibits fibrinolysis by hemolytic streptococci.

Investigations have also been made of the possible use of quaternaries in the prevention of influenza and other virus diseases. However, the results have not been too satisfactory. Krueger *et al.* (1942) noted that Zephiran, while possessing strong bactericidal powers for the majority of pathogens, did not inactivate type A or type B influenza virus in concentrations as high as 1:10,000 acting for one hour at room temperature. Knight and Stanley (1944); however, reported that Phemerol and Roccal proved highly viricidal, causing immediate inactivation of an egg-adapted PR8 strain of influenza virus at 0.5 and 0.05 N

concentrations and inactivation after 2 weeks at 0.005 and 0.0005 N concentrations.

Klein and Stevens (1945) tested 8 quaternary ammonium compounds against a strain of influenza A virus. Three of these, cetyl-pyridinium chloride, Phemerol, and Zephiran were found to be active *in vitro* in high dilution against this strain of virus, but administered by intra-nasal spray, all of these compounds failed to protect mice against infection. Klein, Kalter, and Mudd (1945) later showed that these same three compounds were active against *Shigella paradysenteriae* phage, *Staphylococcus albus* phage, influenza A virus, and vaccinia virus, but completely ineffective against an *E. coli* phage. This latter observation led Kalter *et al.* (1946) to describe a method for isolating *Escherichia coli* phage from sewage. Emulsol 607, Zephiran, and cetyl-pyridinium chloride were recommended for the purpose. Maier (1939) had previously used Zephiran in a dilution of 1:50,000 for the successful preservation of staphylococcal bacteriophage, vaccines, and venom solutions. The personnel of U. S. Naval Research Unit 1 (1942) also outlined a technique whereby Zephiran 1:10,000 in normal salt solution could be added to an equal volume of virus suspension to free it of bacterial contamination. Knight and Stanley (1944) concluded from their results (above) that if these compounds are used as bactericidal agents in the presence of virus, they must be used only in very small concentrations such as those reported by the Personnel of U. S. Naval Research Unit 1 (1942).

Miller, Baker, and Harrison (1939), searching for a compound which could effectively inhibit the metabolism of microorganisms found in lesions of dental caries or in plaques associated with such lesions, studied the action of alkyl-dimethyl-benzyl-ammonium chloride (Zephiran) on those bacteria. A lactobacillus; *Micrococcus tetragenus*, *Staphylococcus albus*, an unidentified aerobic, acid-producing, gram-positive

diplococcus, and a mold of the genus *Monilia* were isolated, and it was found that the alkyl compound exerted a pronounced inhibitory effect on both respiration and glycolysis in concentrations estimated at 1:10,000 to 1:50,000 molar (effected 1×10^9 cells in 5 to 10 minutes). Cells so inhibited did not regain their metabolic activity when resuspended in fresh glucose-buffer. For the purpose the quaternary compound was found to be superior to the well-known metabolic inhibitors, fluoride and iodoacetate.

Miller, Muntz, and Bradell (1940) demonstrated that 1:3,000 alkyl-dimethyl-benzyl-ammonium chloride when incubated with dental plaque material in glucose solution would completely inhibit the metabolism of this material. They also showed that application of a 1:500 solution of the germicide *in situ* for 2 minutes prevented appreciable acid formation by the plaque material when it was subsequently removed after 10 minutes and incubated with glucose solution. While it seems likely that the metabolism of dental plaque material is intimately associated with the pathogenesis of dental caries, these authors are careful to point out that the efficiency of any agent proposed for the cure, prevention, or mitigation of dental caries can ultimately be determined only on the basis of results obtained from clinical studies.

Huyck (1945) examined saliva (collected by having the subjects chew a sweetened gum) before and 3 hours after a 2-minute treatment with an alcohol, glycerin, and water solution containing 0.025 percent (1:4,000) cetyl-pyridinium chloride. The acid produced in the saliva in a 4-hour incubation period before and after treatment, was compared, and the bactericidal effectiveness was calculated to be 90.64 percent (average of 21 cases). On this basis, the authors concluded that cetyl-pyridinium chloride in 1:4,000 concentration is bacteriostatic or bactericidal to bacteria found in the oral cavity.

Toxicity. In connection with the numerous medicinal purposes for which the quaternary ammonium compounds have been suggested, their toxicity becomes of great importance. Further data may be needed but preliminary studies seem to indicate that at least some of these compounds may be sufficiently non-toxic to permit rather widespread use (Thompson *et al.*, 1937; Heineman, 1937; Harshburger, 1942; Sarber, 1942; Warner *et al.*, 1942; Welch and Brewer, 1942; Huyck, 1944; Hauser and Cutler, 1944).

Veterinary medicine. Scales and Kemp (1941), after performing some laboratory tests of the efficiency against *Streptococcus agalactiae* of a mixture of alkyl-dimethyl-benzyl-ammonium chlorides (Zephiran), a dispersant (Triton No. 720) and 33 percent milk, suggested that the mixture would be effective as a treatment for chronic mastitis if injected into the udder at body temperature directly after milking. Bryan *et al.* (1944) have made a more extensive study using *p*-tert-octyl-phenoxy-ethoxy-ethyl-dimethyl-benzyl-ammonium chloride (Phemerol). Besides laboratory tests, experiments were performed in which 176 cows were treated by infusing each quarter with 75 ml. of 1:1,000 aqueous solution. Of 171 cows with chronic mastitis, but showing no marked induration of the udder, 147 (86 percent) were reported to have been freed of the infection. In most cases, a transitory edema of the mucous lining the teat resulted, and abnormal milk was produced for varying periods after treatment. Five other cows showing marked induration of the udder were similarly treated, but failed to recover. The greatest decrease in milk flow as a result of infusion in cows in the early stages of lactation, and it was suggested that cows should be treated near the end of lactation so that microscopic examination could show recovery or failure of treatment before the onset of the dry period. The bac-

terial count of milk produced before treatment and upon recovery after treatment yielded reductions or from 20 to 99 per cent.

That caution will have to be exercised in applying the quaternary ammonium compounds in the control of mastitis is indicated by the recent studies of Hughes and Edwards (1946) in England. Their *in vitro* experiments with cetyl-trimethyl-ammonium bromide (Cetavlon) showed this compound to be superior to chlorine or chloroxyleneol disinfectants when tested against *Streptococcus agalactiae*, and disinfectant action of 1 percent of the compound persisted on the surface of dry hands and cows' teats for a period of at least 3 hours, although subsequent moistening of the hands with milk reduced the efficiency of the disinfectant. The inclusion of the compound in a lanette wax-oil base did not appear to reduce the efficiency, and application of this mixture to the hands of milkers and the teats of cows twice daily at milking time appeared to prevent the spread of *S. agalactiae* infection. However, after three months of application, lesions of the teats developed, accompanied by a rapid increase in *S. agalactiae* infection of the teats and milk. One percent cetyl-trimethyl-ammonium bromide cream failed to control this infection, and it was suggested that the increase in the number of cows with teat lesions was due to the use of this compound.

Bryan and Young (1945) have described another application of a quaternary ammonium compound in the field of veterinary medicine. They found that the application twice weekly of 1:1,000 aqueous *p-tert*-octyl-phenoxy-ethoxy - ethyl - dimethyl - benzyl - ammonium chloride (Phemerol) solution relieved ringworm infection in calves. The solution was applied by means of a cotton swab to the scabs until they appeared to have "taken up" all of the solution possible. Within one month, ringworm lesions disappeared from the twenty-two calves treated.

Miscellaneous other uses. Wallace (1940) tested benzyl phenol, sodium-*o*-phenyl phenate, and alkyl-dimethyl-benzyl-ammonium chloride to determine their germicidal activities and their effects upon bathing suit materials. One and one-quarter inch squares of the material were saturated with suspensions of *E. coli*, *S. aureus*, and *T. interdigitalis*, dried 24 hours, and then placed in the various disinfectants for intervals of 1, 4, 6, and 10 minutes. They were then removed, rinsed, and cultured. The alkyl-dimethyl-benzyl-ammonium chloride was found effective in a dilution of 1:1,000, and in this dilution had no effect on the color or elasticity of the bathing suit material, while the other two compounds had a slight effect on the elasticity in effective dilutions.

The use of quaternary ammonium compounds in the textile industry has been discussed by Katz (1939) and Borghetty (1945).

METHODS FOR THE ESTIMATION OF QUATERNARY AMMONIUM COMPOUNDS

With the ever-widening range of applications in diversified fields to which the quaternary ammonium salts are being found suitable, methods for their estimation are becoming important. Dubois (1945) has given a number of methods which may be used. One of these is based on the adsorption of iodine from solution by the quaternary ammonium compounds. The method necessitates selection of a different end-point for each compound titrated. Another is that of Hartley and Runnicles (1938) who developed a method reportedly accurate within 1 percent at concentration of N/1000. The paraffin-chain cation is titrated directly against a paraffin-chain anion, in a slightly alkaline solution. As titration proceeds, bromphenol blue changes from purple to pure blue. A third method is that described by Flowtow (1942) which is based on precipitation of the quaternary salt

with an excess of potassium dichromate and iodometric determination of the free dichromate. As an alternative, the solvent extractable permanganate of the high molecular cations could be estimated.

Still another method given is that of Auerbach (1943, 1944). This is a colorimetric determination based upon the extraction of the solvent-soluble salt formed in alkaline solution between the anion of bromphenol blue and the cation of high molecular quaternary ammonium salts. This method is similar to one proposed by Krog and Marshall (1940) but the solvent-soluble dye-high molecular cation complex is determined by the photoelectric colorimeter.

Dubois also lists a number of methods for the estimation of quaternary ammonium salts on fabrics and paper applicable in various phases of the textile industries and the like, and has also (1945b) described an argentimetric method.

For field use, where a minimum of equipment is a requisite, a rapid test has been developed by Brooks and Hucker (1947). The method consists of adding, dropwise, 0.04 percent bromphenol blue buffered at pH 4.5-5.0 to 1 ml. of the quaternary salt solution and 1 ml. of ethylene dichloride in a test tube. At the endpoint, a yellow-green color in the ethylene-dichloride layer contrasts with a blue-violet color in the aqueous layer. The test is rapid, simple to use, and could be operated in taverns and the like by any technician or health officer.

Dubois (1946) has recently published a review covering the method of Brooks and Hucker and several other methods currently in use. There are also available methods for the estimation of quaternary ammonium compounds in foods (Wilson, 1946) and in fruit juices (Harris, 1946).

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MILK and FOOD SANITATION

The Chemistry and Biology of Milk Waste Disposal*

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MILK, a mixture of proteins, fats, sugars, and salts containing calcium, phosphorus, iron, copper, and magnesium, all of which are essential to maintenance of life processes, is often termed as a nearly perfect food for humans. When milk is processed a certain percentage is lost to sewers and is discharged to streams or treatment plants where it is an excellent food for bacteria that break down complex organic materials into simpler, more stable, and fairly innocuous substances.

Modern methods for disposal of milk wastes are a combination of devices for confining in a small space all the physical, chemical, and biological processes that otherwise might be spread over a long stretch of receiving streams. Microorganisms play an important part in intermittent sand filters, high and low rate trickling filters, the activated sludge process, and in sludge digestion.

These microorganisms, regardless of size, all have common requirements that must be met in order for them to remain alive. Food must be of a suitable type and of adequate supply, there must be sufficient oxygen, proper temperature range, and a suitable medium in which to live. All of these conditions are normally provided by the milk wastes in a properly functioning treatment plant. The organic waste constituents include the three classes of food: carbohydrates, proteins, and fats, plus their decomposition products.

The inorganic compounds include mineral salts ordinarily found in water plus ammonium salts, nitrates, and nitrites. Some waste matters are carried in suspension, others in solution, and still others in a finely divided colloidal state. The presence of these foods and minerals qualify milk wastes as a suitable culture medium capable of supporting multitudes of various microorganisms. Of these, bacteria are the most predominant both in numbers and in extent of activity.

Milk wastes contain acid forming, alkali forming, and proteolytic bacteria all of which are active in the stabilization of the wastes.

The efficiency of bacteria in reducing organic matter lies in their ability to produce a variety of enzymes, the complicated protein compounds that bring about digestion without being used up in the process. These are specific in action. Carbohydrases act only on carbohydrates, proteases only on proteins, and lipases only on fats. The result of digestion here is the same as in the digestive apparatus of man. Compounds are reduced to soluble form or made chemically more simple. Bacterial enzymes may be external or internal. External ones are liberated from the bacterial cells and diffuse into the wastes, transforming organic substances so that they may pass through bacterial cell membranes. See Figure 1. This is important since it is the only method by which saprophytes may obtain food. Internal enzymes are retained by the bacterial cell and are effective in the release of energy from the digested food. Energy is required in such vital processes as

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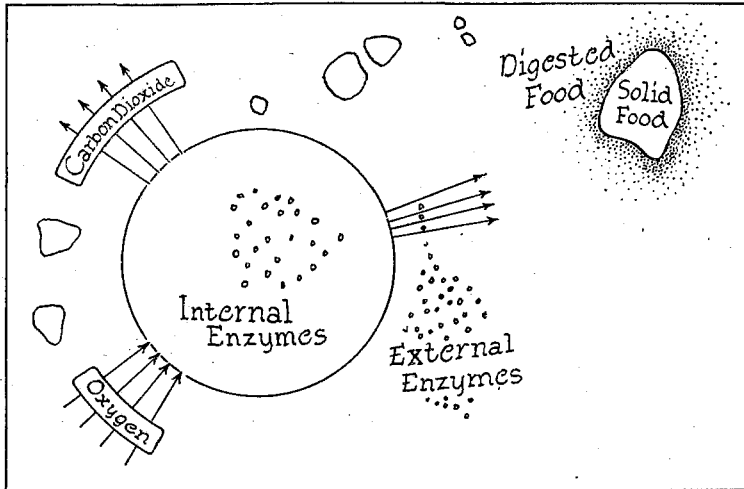


FIGURE 1

growth, reproduction, and movement. Energy release is brought about by action of oxygen upon the food contained in the wastes. It is the oxidation reaction called respiration. Most organisms require molecular oxygen for this activity with bacteria extracting it from the wastes in which they are immersed. Biochemical oxygen demand (B.O.D.) is a measure of the degree to which they are able to extract oxygen dissolved in the medium for action upon the available food supply. Organisms functioning in the presence of free oxygen are spoken of as aerobes.

Certain other bacteria are able to function where the free oxygen concentration is zero provided suitable compounds containing oxygen are available. These compounds are reduced by the bacteria in order to obtain oxygen to apply to food. Such bacteria are called anaerobes and function primarily in sludge digestion.

TRICKLING FILTER

The trickling filter is a device for providing and maintaining desirable living conditions for a complicated society of organisms. The efficiency of the filter is not readily explainable on the basis of its simple structure. It is

merely a bed of crushed stone or other medium over which the wastes are caused to flow in small streams. It is a method for tremendously increasing the surface area to which the wastes are exposed. The time of passage is about 15 or 20 minutes. Yet biochemical oxygen demand removal is in the neighborhood of 75 or 80 percent, equal to removal by storage in a bottle with excess oxygen for 7 days. This efficiency can be explained only on the basis of biological activity occurring in the filter. The mechanism is not truly that of a filter and yet a definite separation of materials from sewage has resulted from passage through it. The material removed has somehow been collected upon the filter medium.

Whenever water carried organic wastes are allowed to touch, trickle, or flow over a suitable contact surface, slimy films of growth are formed. These gelatinous or zooglear films make up the normal biological growth on trickling filters and may vary in color from gray to green or red. The growth is widespread over and at different depths and may be variable in nature at different points. It consists of a jellylike matrix in which millions of bacteria are embedded and on which a variety of other organisms find a suitable place to live.

Some of these organisms are in motion swimming in the surface film of moisture, gliding over the filter growth, on the surface of the rock, and in the interspaces. Their life is a continuous scramble to obtain food, to grow, and to propagate. Their inter-actions in carrying out these require-ments results in waste purification.

Certain factors are essential to the maintenance of trickling filter efficiency.

Food

The microorganisms have their supply of food pumped to them at all times. It is only necessary for them to take it as it goes by. The quantity, therefore, is adequate. As far as type is concerned, there are carbohydrates, proteins, and some fats plus inter-mediate decomposition products. They may be in the form of suspended particles, colloidal, or in solution. The living mass has ability to absorb or-ganic matter from solution, coagulate the colloidal matter, and flocculate the finely divided suspended matter. These substances, then, are permanently re-moved from the fluid or their passage is effectively delayed.

There is a direct relationship be-tween total mass of bacteria produced and the supply of food available. The greater the supply of food, the higher will be the population value. Starting with a small bacterial population, the rate of bacterial increase will follow a sigmoid curve. The leveling off point in the curve will be determined by total food supply. There is, then, equilibrium between bacterial mass and concentration of food substance. If the population ceiling is reached, bacteria are practically inactive in waste puri-fication. Only rapidly multiplying bac-teria are efficient in this process. It would appear, then, to be an advantage to keep bacteria always at the maxi-mum growth rate. This can be done only by supplying unlimited food or by preventing the population from reaching the ceiling value. Any method of killing off some, but not all,

of the bacteria would do the job. It will be shown, however, that there are natural forces that bring about this result.

Bacterial Population

While some trickling filter efficiency can be obtained by the presence of only a single strain of bacteria, a filter con-tains under natural conditions a wide variety of kinds. The number of kinds will vary with each filter, its con-dition, the type of waste applied, and the season of the year. It has been found that any increase in the kinds of bacteria present results in an increase in the percent removal of biochemical oxygen demand. This is significant when considered in the light of the kinds and location of bacteria actually found in normally operating filters. The kinds located at different levels have different habitat preferences, uti-lize different kinds of foods, and pro-duce different end products. The products formed by those in the upper layer make up the food requirements of those in the next layer and so on to the bottom of the filter. There is thus a series of reactions starting with food applied at the top of the filter and end-ing with the mineral products that pass out in the effluent.

As the wastes pass over the zoogleal mass in the rock filter, soluble sub-stances pass into the matrix, enzymes are added to the fluid, and colloidal and suspended particles adhere to the film and are made soluble. Foods digested by the external enzymes cannot be uti-lized until they are taken into the bac-terial cell where they may serve one of two functions:

1. They may serve as raw material in the repair and growth of the filter life. Usually digestive products of proteins are used for this purpose and are transformed to living matter, there being no immediate products.

2. Food also serves as a source of energy. This is mainly true of carbo-hydrates and fats. They will be used up in this order of preference (carbo-

hydrates, fats, proteins). The greatest amount of energy is required by bacteria while at the maximum growth rate. Since the release of energy is an oxidation process, biochemical oxygen demand reduction is greatest at this stage also. In the presence of internal enzymes of the proper kind, oxygen combines with the absorbed foods, energy is released, and acids, carbon dioxide, and water, or other products are formed. These pass into the matrix, to the wastes, and into the effluent.

Proteins also are finally used as a source of energy. This type of food is extremely complicated in structure and requires considerable preparation before it is ready for bacterial use. This preliminary process is a digestive one and takes place outside the bacterial cell. Protein is changed to progressively more simple compounds such as proteoses, then peptones, and finally amino acids. Most amino acids are readily soluble and may pass through bacterial membranes and into the cell. Here a fraction of the amino acid unit may be oxidized with the release of free ammonia, hydrogen sulfide, foul smelling mercaptans, and allied substances. They, in turn, may provide energy by being oxidized to sulphates, nitrites, and nitrates. When this stage has been reached, protein has been disposed of and placed on the mineral side of the ledger. Another increment of biochemical oxygen demand has been satisfied.

The organic food is continually changed in character as it passes downward through the filter. The environmental conditions also change continually. It would not be surprising, then, to find bacteria with particular food habits located at particular levels taking up positions where the altered materials reaching them are of the proper kind. It has been found that bacteria in the upper layer are ones capable of decomposing carbohydrates with the production of acids. There

are others here capable of digesting proteins and liberating hydrogen sulfide and ammonia from some of the amino acids. In the middle layer bacteria are present which can act upon these products and decompose them further. In the lower layer are forms capable of oxidizing ammonia and nitrites to nitrates, and sulfides to sulfates.

Oxygen

Biochemical activity in the trickling filter is primarily aerobic. Outside oxygen must, therefore, be supplied for each increment of biochemical oxygen demand satisfied. It is known that the oxygen reserve in the wastes is entirely insufficient. In properly designed filters, natural ventilation is induced by the difference in temperature between the wastes and air. During warm weather an air current moves downward through the filter; in cold weather the air current moves upward. Because of the large filter surface area exposed to the air, oxygen dissolves in the surface moisture, passes through the zooglyphic matrix and is available for absorption by the bacterial cells. Sometimes, and for various reasons, a portion of the filter may become clogged. Pooling then results. Oxygen supply will become inadequate in those areas and anaerobic conditions may become established. Filter efficiency will be impaired in proportion to the extent of pooling.

Temperature

Within limits, temperature has an effect upon the rate of biological activity. As a general rule it may be said that the rate of biological activity approximately doubles for each increase of 10° C. While this statement is generally true, temperature still has only a little effect upon trickling filter efficiency. Rate of activity here apparently is governed by the speed at which organic matter is transferred into the biological film and by seasonal changes in filter population.

Biological Association

So far filter function has been discussed only in the light of bacterial activity. Actually the living matter on the filter is a complex society of a variety of competing organisms. The relative components of the society are not static. Filter growth is a seasonal succession in which certain organisms appear, become prominent, and are in turn replaced by an increasing population of another type. A list of different kinds of organisms in the filter is long and impressive. How long it actually is, no one knows because all the forms have not been identified. But among them both plants and animals are represented. In the plant groups are such forms as bacteria, algae, and molds.

Algae are simple green plants that may occur as single cells or filaments. They depend for life upon their ability to synthesize sugar from carbon dioxide and water in the presence of light. For this reason they occur only in the upper illuminated zone. While they may utilize certain protein digestion products, their purifying role appears to be a minor one. Their primary contribution is probably the addition of oxygen to the wastes that flow by.

Molds resemble algae in general structure but lack green pigment. They are unable to manufacture sugar and therefore, must depend upon the food supply in the waste. See Figure 2. While they function in reducing biochemical oxygen demand and in concentrating dissolved matter, they may also function as binders in holding filter growth in place. They are most numerous during winter.

The animal constituents consist of protozoa and metazoa. Protozoa are the simplest of animals. Each consists of a single unit of mass and structure called a cell. It actually is only a blob of jelly-like material held in shape by an elastic membrane. It is capable of all activities necessary to life. Protozoa are classified according to the mechanism by which they move about.

1. Sarcodina — flowing
2. Flagellata — whip
3. Ciliata — cilia

Although flagellates may be present in tremendous numbers, they are small in size, absorb only soluble materials, and have little to do with purification. Many of the *Sarcodina* and *Ciliata* are bacterium eaters or predators. The *Sarcodina* take in bacteria or other

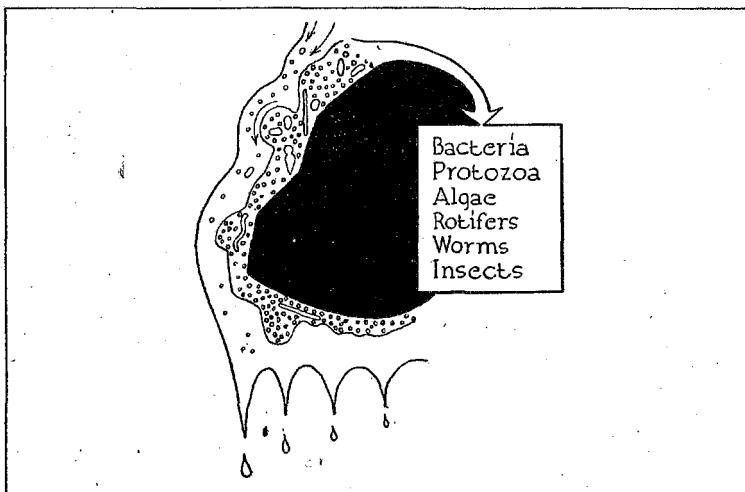


FIGURE 2

food by flowing over and engulfing it. The *Ciliata* are equipped with an opening or mouth lined with cilia. Currents of water are set up by the cilia so as to sweep bacteria and other particles into the mouth. These are then digested and oxidized for energy and for use as a building material. To this extent biochemical oxygen demand is satisfied and suspended matter is precipitated in the protozoan body.

The more important function of protozoa is their bacterial scavenging action. By attacking and devouring bacteria in the filter there eventually is established a balance between the number of bacterial predators, and a ratio between bacterial population and possible population ceiling. When bacterial population balance is maintained near the zone of maximum growth, biochemical oxygen demand reduction is greater than at the bacterial population ceiling value. The protozoa function then in continually decreasing the bacterial numbers so that bacteria are stimulated to increase their numbers.

Protozoa are concentrated in the upper layer of the filter with the highest number at the two-foot level. This is also about the level of bacterial concentration.

Metazoa are larger animals composed of a number of organized cells. In this group are such diverse forms as eel worms, sludge worms, rotifers, water fleas, insect larvae, and certain adult insects. These forms move about through the filter, feeding upon solid material and even on filter growth. Some feed upon the protozoa. Others may burrow through the filter, feeding on all types of organic matter and loosening masses of filter growth. This results in a certain amount of unloading at all times although intensive unloading occurs in fall and spring.

It is apparent, therefore, that the trickling filter is merely a mechanism for maintaining ideal biological conditions. See Figure 3. High rates of biochemical oxygen demand removal result through oxidation of organic

compounds and through flocculation and storage. Filter treatment is not completed until unloaded filter growth has been settled and disposed of.

ACTIVATED SLUDGE

The transformation of waste constituents in the activated sludge process is similar in many respects to that of the trickling filter. The biological working units or flocs are kept in suspension and are swept through the waste. They are thus kept continually in contact with their food supply. Large flocs are formed by biological populations and accumulation of waste substances. They function in absorbing materials from suspension and solution and in oxidizing these materials. The results, therefore, are clarification and biochemical oxygen demand reduction. An extreme acceleration of the oxidizing reaction is inherent in this process due to design and development of ideal conditions for growth of desirable biological forms.

It has been determined that the activated sludge floc may vary somewhat in character but always contains a predominance of zooglycal bacteria. Findings by all workers point to the primary importance of these bacteria as the basic functional units in the floc. They are short, rod-shaped with rounded ends, and grow in colonies that are spherical, evenly lobed or tree-like. The gelatinous matrix of the colony is composed of the capsules of the bacterial cells. They also may occur free in the medium and swim about by flagellar activity.

The bacteria of activated sludge resemble those of the trickling filter and can function interchangeably in either situation.

Since food material must pass through the bacterial cell wall before it can be used for energy or built into living material, the bacterial surface available determines the purifying efficiency. It has been calculated that each cubic foot in the aeration chamber

contains at least 250 square feet of bacterial surface. If free swimming bacteria and protozoa are added the area may reach 500 square feet.

The purification of the wastes by the activated sludge process takes place in two stages. The first of these stages is called clarification. In it carbonaceous and nitrogenous organic matter in all stages of dispersion is removed from the wastes by coagulation, ab-

are made soluble and may pass into bacterial cells where in the presence of internal enzymes and the absorbed oxygen supply they are oxidized and energy is released.

For high operating efficiency, it is important here as in the trickling filter that bacteria be maintained at the maximum growth rate. This may be accomplished in two ways; by removal or wastage of sludge and by predation

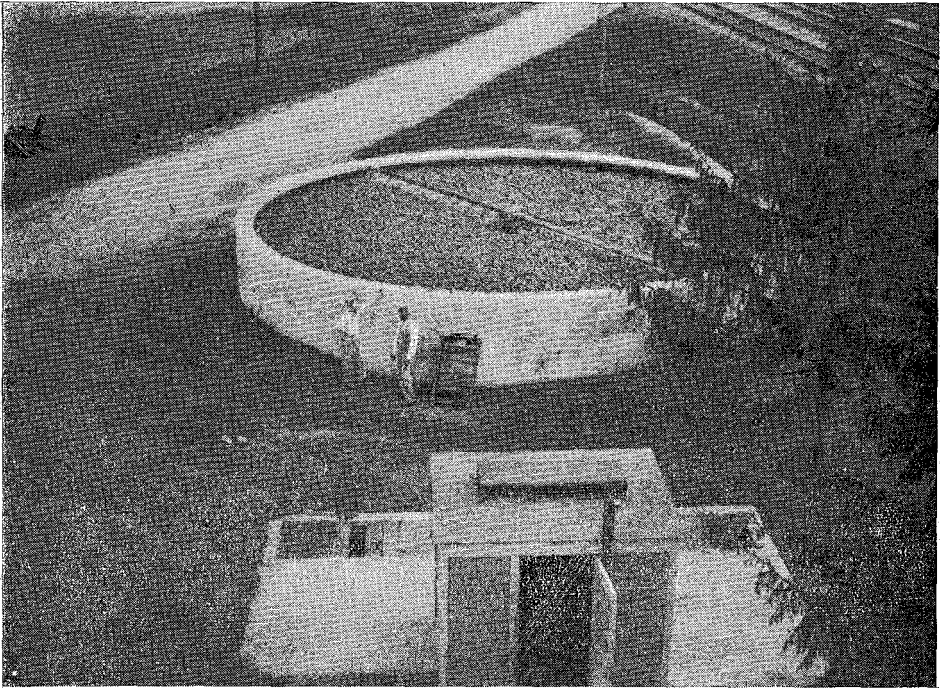


FIGURE 3. Trickling filter for treatment of milk plant wastes, Union Center, Wisconsin.

sorption, and other mechanisms and becomes a part of the floc. Clarification is a rapid stage reaching equilibrium in 30 to 40 minutes of passage.

This stage is followed by a reactivation stage during which the clarification powers are restored to the floc. While this is occurring, oxygen is supplied by aeration of the medium, and is used by bacteria to oxidize the food load of the floc. Enzymes of many kinds are effective in the floc matrix and in the waste itself. Complex foods

of protozoa and other bacteria feeding organisms. The presence of a large number of ciliates is an indication that activated sludge is in good operating condition.

The activated sludge process can be considered a biological type of filtering apparatus in which the active life rapidly strains out polluting materials and then slowly breaks them down to a non-polluting condition. Purification of milk wastes cannot be considered complete until settled sludge

has been drawn out of this system for disposal.

SLUDGE DIGESTION

Digestion is the term applied to the decomposition of settled sludge. The process is a combination of processes including true digestion by enzymes and reduction and oxidation of compounds by anaerobic organisms. Anaerobic organisms live in the absence of free oxygen and can obtain their supply from oxygen-containing compounds. Digestion is usually carried on in separate sludge digestion tanks. The object of digestion is the alteration of the collected settled solids in order to permit disposal without creating a nuisance. The activities of bacteria, molds, protozoa, and other forms are involved. A wide variety of extra-cellular enzymes are formed by the bacteria and are mixed into sludge. The first stage is characterized by active fermentation involving gas production and formation of organic acids.

The gases are mainly methane and carbon dioxide. In the second stage the acids are neutralized or decomposed by alkaline reaction products.

Except for molecular oxygen supply, organisms active in digestion are affected by the same conditions as aerobic organisms. Food must be supplied at frequent intervals by introduction of undigested sludge, temperatures must be maintained at the optimum and the maximum growth rate must be maintained.

These treatment processes embodying combinations of physical, chemical, and biological processes including aerobic and anaerobic methods of milk waste stabilization can prove effective in substantially reducing or eliminating unnecessary stream pollution.

Grateful acknowledgment is extended to Dr. A. F. Bartsch, Senior Biologist of the Wisconsin State Board of Health, for his assistance in the preparation of this paper.

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(Continued from page 292)

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* Seen only in abstract.

The Jamieson Kit—Its Construction and Use

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Most sanitarians will agree that the job of teaching better sanitation has been a difficult one. Too often, bacterial plate count reports and the most convincing explanation of 'how and why' fail to produce the desired improvements. The sanitarian's story often contained too much theory for the practical-minded people he was dealing with. What was needed was a means of presenting this story in a simple, tangible manner, and of providing an on-the-spot visible demonstration which would analyze the work being done.

In the past few years, food handlers' schools of various types have improved the sanitarians' teaching facilities. Most of the states, provinces and larger cities have instituted these courses with great success. However, even this improvement failed to provide the sanitarian with the instrument he required—a means of demonstrating to an operator and his food handling employees, in unmistakable terms, a fairly accurate picture of the sanitation being practised in their establishment.

During the summer of 1945, Jamieson *et al* (1) developed the "seeing is believing" approach to sanitary control. This procedure made use of a traveling laboratory for the swab-slant testing of equipment and utensils. Slants were inoculated and left to incubate on the premises where all employees might see for themselves how well they were doing their jobs. Here, at last, was a simple and practical method for bringing food-handling personnel to a realization of the need for better sanitation.

As the Manitoba Department of Health and Public Welfare was in a position to make first-hand observations of Jamieson's work, this Depart-

ment early realized that here was a teaching method that could be readily adapted for use in the supervisory work as carried on by a health department. With but very few alterations, a traveling laboratory was prepared for each health unit and immediately put to use. Arrangements were made with one of the Provincial Bacteriological Laboratories to keep the kits supplied. Results obtained have been so gratifying that weekly use of the kit has become routine for each sanitarian.

This article has been prepared to provide details of the kit's construction and contents. It serves, as well, to recognize the senior author of the original work by naming the traveling laboratory "The Jamieson Kit".

CONSTRUCTION OF THE JAMIESON KIT

Each kit (Figure 1) when equipped for use contains the following:

- 32-36 Jamieson Bottles
- 4 Bottle racks
- 1 Bottle stand
- 6-8 Bottles of sterile water
- 36 Sterile swabs
- 1 Clean towel
- 1 Record book

The Case

The traveling case is of pine board construction covered with leatherette and lined with white oilcloth. Its construction and dimensions are shown in the upper half of Figure 2. A swing handle makes for easy carrying of the handy case which weighs about 15 pounds when loaded ready for operation. A loaded kit contains enough equipment to analyze two restaurants.

Bottles

An inexpensive, durable, flat-sided type of bottle, with a Bakelite screw-

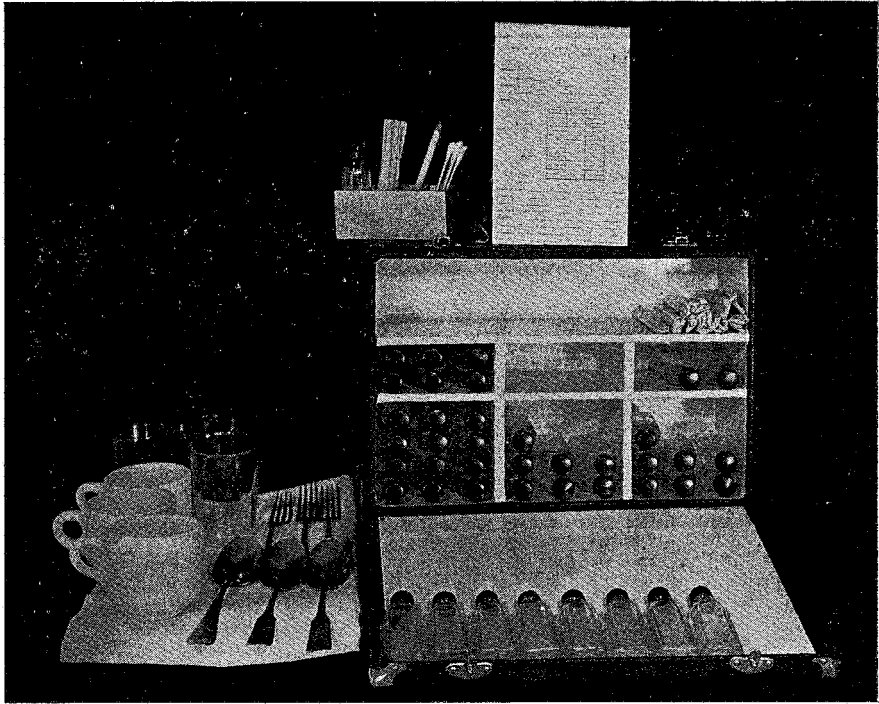


FIGURE 1. The traveling laboratory used by the personnel of the Manitoba Department of Health and Public Welfare, for the seeing is believing teaching of better sanitation in food handling establishments

cap is recommended. One ounce "Prince of Wales B" bottles have been very serviceable. (This is the trade name for a bottle made in Canada. We understand that a bottle known as "Royal Arch" is produced in the United States, and is very similar to the "Prince of Wales" bottle, although it lacks some of the advantages of the latter bottle.)

Using a mask, one-half of one side of each bottle was sand-blasted with a fine sand. The resultant roughened panel is used for labelling each bottle.

The above bottles each containing an agar slant are referred to as Jamieson Bottles. The slant is prepared by allowing 5 ml. of medium to solidify with the bottle lying on the side next to the sanded edge.

*Medium**

The preparation of large lots of the culture medium is quite simple. The medium found satisfactory for even some fastidious bacteria as well as yeasts and moulds is that used for milk analysis with a few simple additions. The formula per liter amount, using Difco constituents, is as follows:

Tryptone 3 gms.
 Dextrose 1 gm.
 Beef extract 5 gms.
 Yeast extract 4 gms.
 Agar 25 gms.

The heavy agar content provides the firm gel necessary to withstand considerable pressure from a swab rubbed

* Provided by M. C. Jamieson, October 10, 1947.

over the medium. After liquefaction by heating 10 ml. of good quality skim milk are added while stirring. This milk may be reconstituted by mixing 10 gm. of spray process skim milk powder with 100 ml. of water. The

medium is standardized to pH 7.0 and filtered through absorbent cotton. It is then dispensed into the bottles in 5 ml. amounts, sufficient to form at least $\frac{1}{8}$ inch layer complete over one inner side of the one ounce bottles, laid flat.

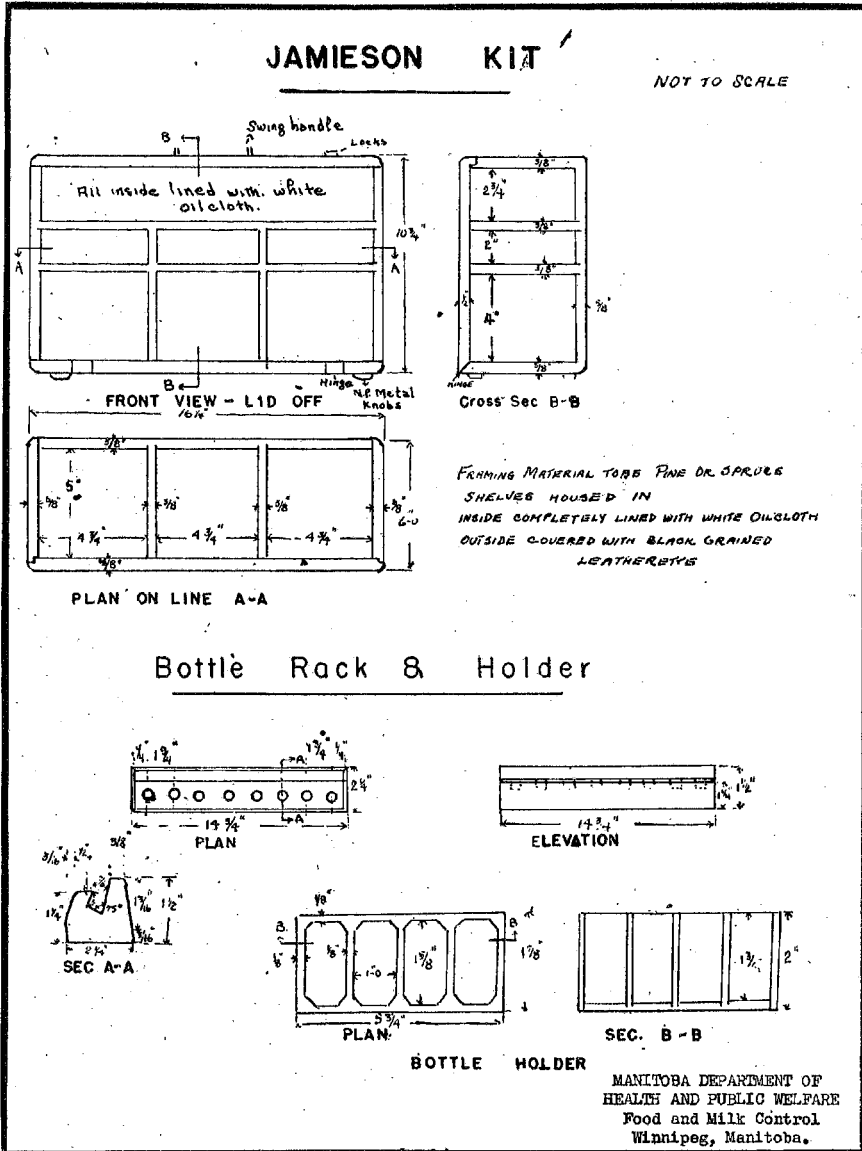


FIGURE 2. Inside view of Jamieson Kit

An automatic pipetting machine is a great convenience for delivering the medium, with uniformity and speed, into bottles arranged in suitable trays. The bottles are capped loosely. After sterilization at 15 pounds steam pressure for 30 minutes, the medium is slanted by laying the bottles flat on the side closest to the sanded beveled edge. Space may be economized by stacking the bottles in tiers or directly in the sterilizing trays if these are suitable for being laid flat on the proper side. To avoid considerable loss of the slants, that too frequently results from contamination apparently drawn into the bottles during cooling, it is advisable to cover the bottles tightly with towels soaked in a disinfectant solution. Treatment in alkyl-dimethyl-ammonium-chloride at a concentration of 1 ounce to 3 gallons of water has proven superior to dry sterile towels, probably due to the impregnation of the fabric by the cationic germicide. After solidification the slants need considerable conditioning to dry the surface of the medium. The dry surface grips the swab and serves to extract more material from it. Also, it reduces the tendency to develop spreader colonies. This may be accomplished in 3 or 4 days at 37° C. if the bottle caps are loose and the bottles are upright. Then, after tightening the caps the bottled medium may be stored almost indefinitely.

Bottle Racks

This rack measures $14\frac{3}{4} \times 2\frac{1}{4} \times 1\frac{1}{2}$ inches; it is constructed of hardwood and is finished with shellac and varnish. Each rack contains eight circular holes just large enough to hold the cap of a Jamieson Bottle and set at an angle of 75°. Details of construction are shown in the lower half of Figure 2.

Bottle Stand

This small four-compartment varnished wooden stand is used to hold

the various supplies during operation of the kit. Details are shown in the lower half of Figure 2.

Sterile Water Supply

A supply of sterile water is carried in "Prince of Wales B" bottles. These bottles of water are sterilized at 15 pounds steam pressure for 30 minutes. The sterile water is used to moisten the swabs.

Swabs

Four inch cotton-tipped applicators are prepared in the laboratory. These are placed in $4\frac{1}{2} \times 3$ inch glassine coin envelopes, four swabs to each envelope, folded and sterilized. Swabs placed in corked test-tubes were tried in place of the 'bagged' swabs but were found to be less satisfactory.

Record

A suitable form was developed to record the results of each use of the kit (Figure 3). These were printed on the handy 5×8 inch size of paper and bound in booklets of 50 each.

USE OF THE JAMIESON KIT

The technique employed in using the kit is simple and varies little, regardless of whether the establishment being analyzed is a restaurant, beverage room, dairy, cannery, etc. The variety of utensils and equipment examined will depend upon the point the sanitarian wishes to emphasize.

In the case of a restaurant, the usual routine is to examine cups, glasses, forks, spoons, and cream jugs. Three of each item are swabbed, using a separate sterile swab and bottle for each item. The swab, after being moistened in the bottle of sterile water, is wiped around the lip surface three times and then used to inoculate a Jamieson Bottle. After proper labeling, the bottle is placed cap down in the rack so that the side containing the medium is toward the front of the rack.

By using two racks for each estab-

Form F12.

MANITOBA DEPARTMENT OF HEALTH AND PUBLIC WELFARE
BUREAU OF FOOD AND MILK CONTROL

Jamieson Kit Report

RESTAURANT NO. _____ INNOCULATION DATE _____
 READING DATE _____
 NAME OF RESTAURANT _____
 ADDRESS _____ MANAGER _____
 TYPE OF RESTAURANT _____ CLASSIFICATION _____
 GENERAL RATING _____

	GOOD	FAIR	POOR
1. CUP			
"			
"			
2. GLASS			
"			
"			
3. FORK			
"			
"			
4. SPOON			
"			
"			
5.			
6. CONTROL			
TABULATION			

METHOD OF WASHING: _____
 DETERGENT _____
 SANITIZER _____
 PERCENTAGE OF CERTIFIED EMPLOYEES _____
 REMARKS _____

 INSPECTOR _____ SUPERVISING AGENCY _____

FIGURE 3. Record form for reporting results

lishment, three each of five items can be analyzed. The sixteenth bottle is a control, inoculated by a sterile swab dipped in the sterile water. The sanitarian is very careful to explain that this bottle will prove that all the equipment used for the kit is sterile. Experience has taught that a wide variation of items can be analyzed. Such items as dish-washing rinse water, a meat slicer, an employee's hands, and others can be used.

After all bottles have been inoculated, labeled, and placed in the racks, the racks are set in a prominent place in the kitchen or elsewhere in the establishment. Here the colonies grow at room temperature under the watchful eyes of all employees. The sanitarians point out that the fewer the spots (colonies), the better the sanitation. In order that operators and their staffs may have more time to study and

understand the technique being employed, an introductory letter is left which describes the method in simple, straightforward terms. To assure an even broader understanding, the same letter has been printed in both French and Chinese.

The reading and interpretation of the results of inoculated bottles can be made in 72 hours, for in most cases, maximum growth has been reached by that time. However, the program of most sanitarians makes it more convenient to read results, a week after the day of inoculation. The additional time allows the employees more time to examine the very evident results and to be impressed. With few exceptions, the operators are so convinced that they immediately seek advice as to the methods of attaining improvements.

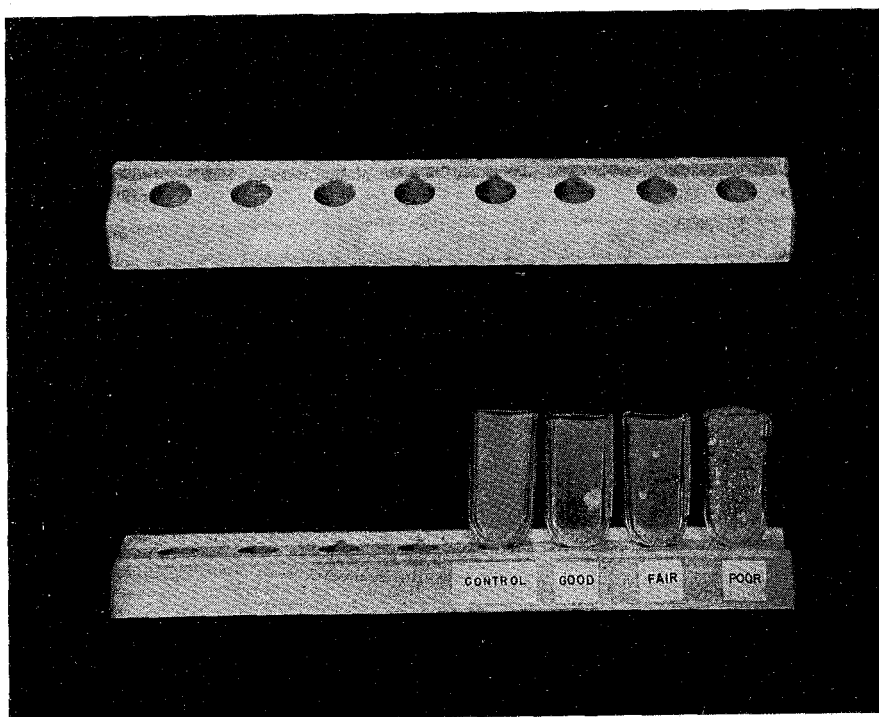


FIGURE 4. Illustration of grades

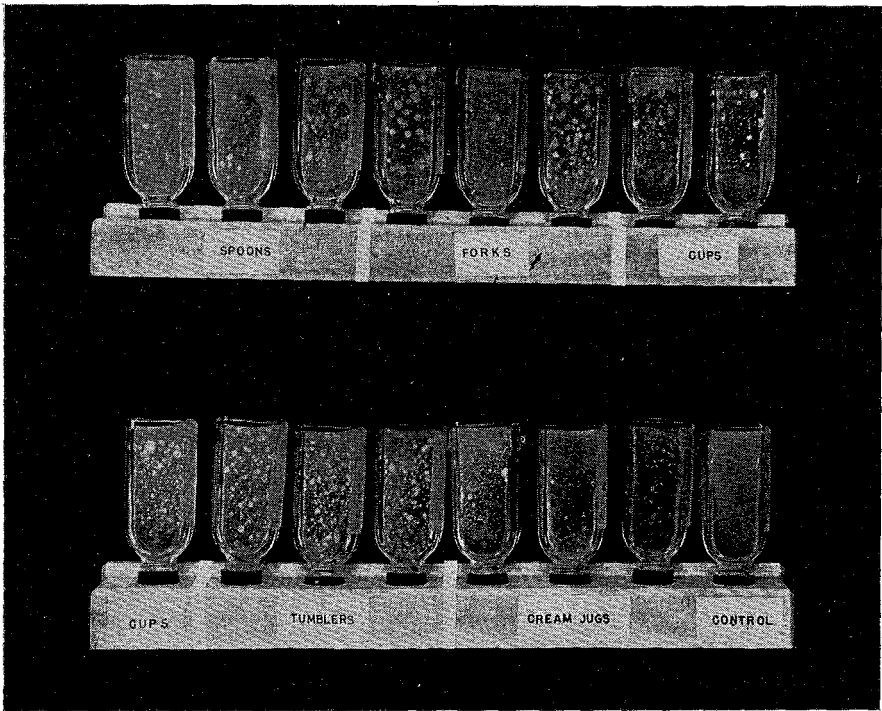


FIGURE 5. *Before*—The results observed at the first visit of a kit to an establishment

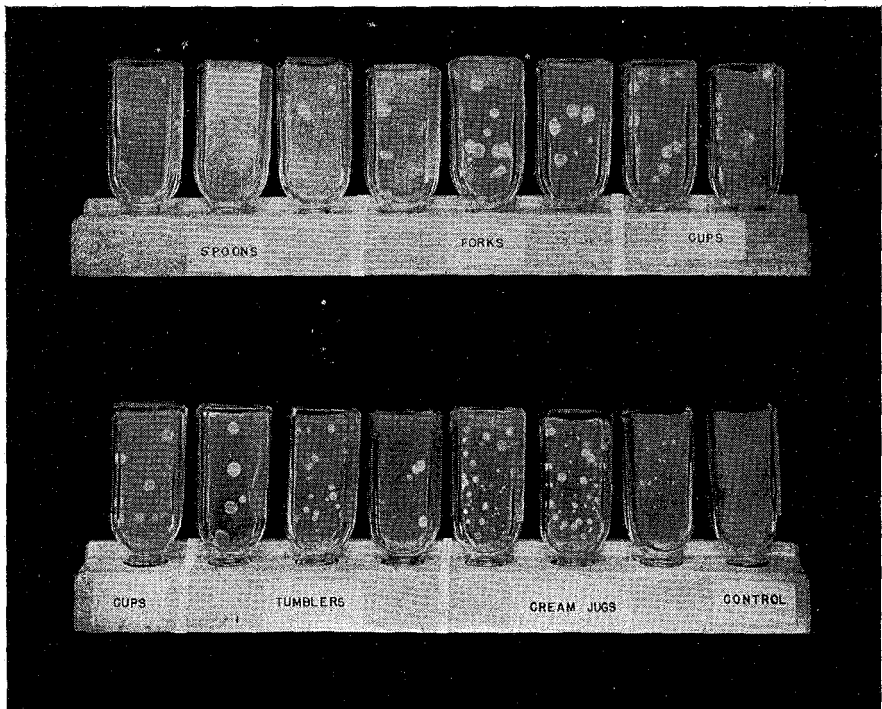


FIGURE 6. *After*—Results observed in the same establishment (as Figure 5) after cleansing and sanitization had been improved

The actual reading of results is made according to the following simple standard: Good, 0 - 10 colonies; fair, 11 - 50 colonies; poor, over 50 colonies, (Figure 4). Little experience is necessary to differentiate the grades with relative ease. At this point, it should be noted that this test was never developed as a substitute for the standard plate count, as established by the United States Public Health Service (2). Work already carried out (3) has shown that the grades used in conjunction with the Jamieson Kit compare favorably with the standard of the United States Public Health Service (2).

Much could be written about the improvements obtained through the

use of Jamieson Kits, and of the interest and enthusiasm shown by operators, employees, and public health personnel. Considerable material has been published about the "seeing is believing swab testing," (1, 4, 5, 6) and, in addition, many requests have been received for instructions regarding the assembling and use of kits. It is expected that the foregoing will answer these requests, and, in addition, will standardize the construction of kits, thereby permitting reasonable comparisons of results obtained in different areas.

Acknowledgments

The development of Jamieson Kits, now in use by the Manitoba Department of Health and Public Welfare

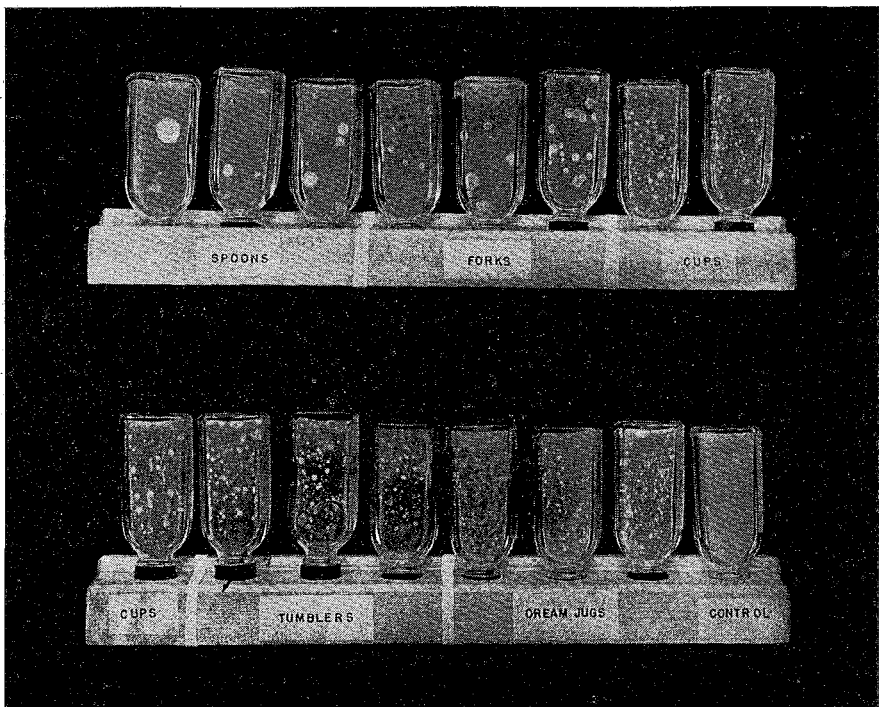


FIGURE 7. Results showing fairly good sanitation of cutlery but poor sanitation of dishes

has been made possible through the generous cooperation of Professor M. C. Jamieson, Department of Bacteriology and Animal Pathology, University of Manitoba, Winnipeg, Manitoba. Appreciation is also expressed to the Officer Commanding and photographic staff of the Manitoba Headquarters of the Royal Canadian Mounted Police for assistance in the preparation of illustrations:

LITERATURE REFERENCES

1. Jamieson, M. C., Chen, H. K., & Wiligan, D. A.; *Canadian J. Pub. Health*, March, 1946.
2. U.S.P.H.S. *Pub. Health Bull.* No. 280, 1943, Ordinance & Code Regulating Eating and Drinking Establishments.
3. Jamieson, M. C., University of Manitoba. Results as yet unpublished.
4. *J. Milk and Food Technol.* 10, 164 (1947).
5. *Can. Dairy & Ice Cream Journal*, 25:7, (1946).
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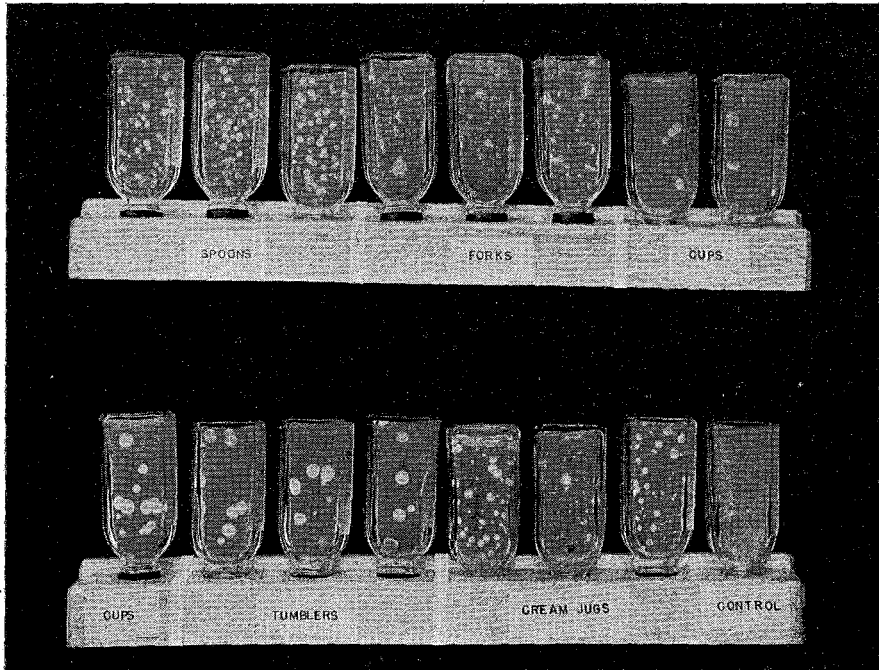


FIGURE 8. Results showing poor sanitation of spoons and forks but fair sanitation of dishes

Thirty-fifth Annual Meeting
 PHILADELPHIA, PA., Oct. 21-23, 1948
 Hotel Bellevue-Stratford

JOURNAL of MILK and FOOD TECHNOLOGY

(including MILK AND FOOD SANITATION)

Official Publication of the

International Association of Milk and Food Sanitarians

(Association Organized 1911)

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Association News

Chicago Dairy Technology Society

George Gelman, Technical Director of the Quartermaster Food and Container Institute, spoke at the last meeting on "The Army Food Research Program."

Mr. Gelman stated that they are carrying on the research of value to Army, Navy, and Air Forces. Their advancement has rendered World War II rations obsolete.

As for the dairy industry, Mr. Gelman stressed the need for a good powdered milk with a better flavor and more easily reliquified. At present, evaporated is the only milk used in operational rations. It would be desirable to have a product that would not require turning.

Canned bread is now available. It will hold up two years and a canned cake even improves with age.

Studies were conducted to determine the frequency at which foods are consumed. Plain milk may be consumed without affecting the appetite.

H. P. SMITH
Recording Secretary

Detroit Announces Examination for Veterinarians

The Detroit Civil Service Commission has recently announced examinations for the positions of Junior and Senior Veterinarian, with the written examinations scheduled for August 9, 1948, October 11, 1948, and December 13, 1948. These positions are with the Detroit Department of Health, and afford excellent opportunities for qualified registered veterinarians. Since residence qualifications have been waived, applications are being accepted from all eligible persons anywhere in the United States.

The salary rates for these positions are as follows: Junior Veterinarian, \$2,875 to \$3,275 per year; Senior Veterinarian, \$3,432 to \$3,909 per year. The official examination announcement giving further details as to examinations and qualifications may be obtained by writing to the Detroit Civil Service Commission, Water Board Building, Detroit 26, Mich.

Minnesota Dairy Fieldmen and Inspectors Association

The Minnesota Dairy Fieldmen and Inspectors Association will hold its annual meeting and banquet at the President Cafe, Minneapolis, Minnesota, at 6:30 P.M. on September 23, 1948. Mr. W. A. Gordon, Editor of the *Dairy Record*, will be the guest speaker.

During the day the Association membership will be in attendance at the Annual Fieldmen's Conference presented by the Dairy Division, University of Minnesota. The fieldmen's conference is included as a part of the Minnesota Dairy Products Institute which will take place at the Dairy Division, September 20-23. The program of the fieldmen's conference will be as follows:

- Quaternary Ammonium Compounds and Their Use as Sanitizing Agents
J. J. Jezeski
- Milk House Construction and Arrangements.....J. J. Handy
- A Discussion of Farm Water Supplies.....D. M. Ryan, H. M. Bosch
- The Inspector's View of the Minnesota Milk and Cream Grading Program.....C. M. Pesek
- The Fieldman's Viewpoint of the Minnesota Milk and Cream Grading Program.....C. H. Mattson
- Management's Viewpoint of the Minnesota Milk and Cream Grading Program.....C. J. Moulton
- Comparison of Methods for the Evaluation of Milk Quality.....J. C. Olson
- Keeping Milking Machine Clean
George H. Hopson

As a result of a meeting of the Board of Directors of the Association July 14, 1948, a number of items are to be brought before the membership at the annual meeting. It is important that attendance be as complete as possible.

It is suggested that all present members act as a committee of one to encourage non-member fieldmen and other interested persons to be present at the conference and to join the Association. An intensive membership drive will take place in the near future. Carl Mattson, Land O'Lakes Creameries, Minneapolis, Minnesota, is the membership chairman. Inquiries may

be sent to him. A special effort will be made to interest the Municipal Dairy Inspectors in becoming members. We have several in the Association at the present time; in fact, Dr. George S. Failing, Winona Dairy Inspector, is a member of our Board of Directors.

Remember, then, the Annual Meeting, September 23, 1948.

J. C. OLSON, JR., PH.D.
Secretary-Treasurer

Ellenberger Retires

Dr. H. B. Ellenberger retired July 1, 1948 as head of the Department of Animal and Dairy Husbandry, University of Vermont and State Agricultural College, Burlington, Vermont, after 31 years of service.

He came to the University of Vermont in 1917 and was made head of the department a year later. When he took over, it was a one-man department. During his regime the staff has increased to nine full-time teachers and research workers.

In 1931 he was president of the American Dairy Science Association.

In 1931 and 1932 he served as chairman of the New England Governors' Dairy Advisory Board and helped to set up New England Dairies, Inc., an organization designed to assist the dairy industry with problems arising in connection with the marketing of milk from Vermont and other New England States. Then he served for nearly a year as Administrator of the Greater Boston Milk Market Agreement.

Chemiatric Markets Stone-Marshall Test Paper

Charles G. Marshall, President of the Chemiatric Corporation of Sparta, New Jersey, announces the acquisition of all patent rights to the Stone-Marshall test paper, extensively utilized for testing quaternary ammonium compounds used in the food, dairy, and allied industries. A national promotional campaign is now under way. Samples are available.

PRELIMINARY PROGRAM FOR THE ANNUAL MEETING

Hotel Bellevue-Stratford, Philadelphia, Pa.

October 21, 22, 23, 1948

Thursday, October 21

Morning Session

Registration 8:30-10 A. M.

Address of Welcome—Dr. Rufus S. Reeves, Director Department of Public Health, Philadelphia, Pa.

A Reciprocal Milk Inspection Program—R. P. Farrell, State Department of Public Health, Nashville, Tenn.

Should Milk Be Strained on the Farm?—*Affirmative:* R. G. Ross, Oklahoma City, Okla. State Health Department.

Negative: Morle Baker, Iowa State College, Ames, Iowa.

Report of the Committee on Food Handling Equipment—C. W. Weber, State Department of Health, Albany, New York.

Afternoon Session

Special Field Inspection Trips—Under direction of W. S. Holmes, Dairy Council, Inc., Philadelphia, Pa.

1. Campbell Soup Company Plant, Camden, N. J.

2. General Baking Company Plant, Philadelphia, Pa. (Busses will leave promptly at 1:30 P. M.)

Evening Session

A Dairy Farm Management Program—G. H. Hopson, De Laval Separator Company, Poughkeepsie, N. Y.

The National Sanitation Clinic—A. W. Fuchs, United States Public Health Service, Washington, D. C.

Sanitation Problems in the Carbonated Beverage Field—H. E. Medbery, American Bottlers of Carbonated Beverages, Washington, D. C.

Report of the Committee on Sanitary Procedure—C. A. Abele, The Diversey Corporation, Chicago, Ill.

Friday, October 22

Morning Session

Program Sponsored by the Dairy Technology Societies—Dr. C. W. England, Chairman, C. Y. Stephens Dairy and Poultry Industries.

The Use of Cleaner Sterilizers on the Dairy Farm—Dr. Franklin Barber, National Dairy Research Laboratory, Baltimore, Md.

What A Dairy Technologist Can Do for His Employer—Dr. G. C. North, Beatrice Foods Company, Chicago, Ill.

Phosphatase Test—Dr. H. Sharer, Department of Health, New York City.

Experiences with High Bacteria Counts Due to Udder Infections—Dr. Kenneth Wilson, Sylvan Seal Milk, Inc.

Afternoon Session

What's Wrong with Milking Machines?—Emil Domingo, Health Department, New York City.

The Timing of High-Temperature, Short-Time Pasteurizers—Wm. Jordan and R. F. Holland, Cornell University, Ithaca, N. Y.

Report of the Committee on Communicable Diseases Affecting Man—N. O. Gunderson, Commissioner of Health, Rockford, Ill.

Business Session

Saturday, October 23

Morning Session

Report of the Committee on the Professional Status of Sanitarians: H. B. Robinson, U. S. Public Health Service, New York, N. Y.

Report of the Committee on Applied Laboratory Procedures—K. G. Weckel, Univ. of Wisconsin, Madison, Wis.

Testing Quaternary Ammonium Compounds in the Field—Luther Black and George R. Weber, United States Public Health Service, Cincinnati.

Report of the Committee on Dairy Farm Methods—M. P. Baker, Chairman Iowa State College, Ames, Iowa.

NEW YORK STATE ASSOCIATION OF MILK SANITARIANS

Twenty-Fifth Annual Conference

Buffalo, N. Y., Sept. 22-24, 1948

Hotel Statler

PROGRAM

Tuesday, September 21

7:00 P.M. to 9:00 P.M.

Registration.

Wednesday, September 22

9:00 A.M. to 10:00 A.M.

Registration.

Address of Welcome, B. F. Mattison, Erie County Commissioner of Health, Buffalo, N. Y.

Presidential Address, E. R. Albee, Bacto-B Laboratories, Buffalo, N. Y.

Studies of the Thermal Death Ranges of Certain Bacteria in Milk, F. W. Gilcreas and J. E. O'Brien, New York State Department of Health, Albany, N. Y.

The National Sanitation Foundation Clinic, W. D. Tiedeman, New York State Department of Health, Albany, N. Y.

The 3A Standards for Milk and Sanitary Standards for Food Handling Equipment, C. W. Weber, New York State Department of Health, Albany, N. Y.

The Professional Status of Milk Sanitarian, H. B. Robinson, U. S. Public Health Service, New York City.

2:00 P.M. to 5:30 P.M.

The Present Day Conception of Milk Sanitation, H. E. Hilleboe, Commissioner of Health, New York State Department of Health, Albany, N. Y.

A Proposed Plan for Five State Reciprocity for Milk Inspection

W. S. Anderson, Penn. State Department of Health, Harrisburg, Pa.

H. Clifford Goslee, Dept. of Farms & Markets, Hartford, Conn.

H. E. Bremer, Dept. of Agriculture, Montpelier, Vt.

Paul Corash, New York City Department of Health, New York City.

An industry representative (name to be announced later).

Milking Machine Contamination (with demonstration), Emil Domingo, New York City Department of Health, New York City.

Thursday, September 23

9:00 A.M. to 12:30 P.M.

Quality Improvement Milk Programs

J. Russell Fox, New York State Milk Distributors Assn., Albany, N. Y.

R. W. Metzger, Dairymen's League Coop. Assn., New York City.

Wilber Rothery, Department of Health, Auburn, N. Y.

State Plan for Dairy Barn Improvement and Management, G. H. Hopson, DeLaval Separator Co., New York City.

Discussion by a County Agricultural Agent.

Control of Mastitis by Injection, Amos Stuff, Hopewell, N. J.

Control of Traffic of Mastitis Cows, J. W. Fink and W. H. Grunge, New York City Department of Health, New York City.

2:00 P.M. to 5:00 P.M.

Importance of Maintaining Pasteurizing Plant Inspections, C. W. Weber, New York State Department of Health, Albany, N. Y.

Design, Construction and Operation of Can Washers, Floyd Carkuff, Crowley Milk Company, Binghamton, N. Y.

Evaluation of the Rinse Test for Determining Sterility of Milk Cans, N. A. Milone, New York State Department of Health, Albany, N. Y.

Business Meeting.

7:15 P.M.

Annual Banquet.

Friday, September 24

9:00 A.M. to 12:30 P.M.

The Rochester Restaurant Sanitation Program in Action, G. A. West, Department of Public Safety, Health Bureau, Rochester, N. Y.

A Method of Determining Sediment Removal Efficiency of Filter Discs, E. O. Anderson, University of Connecticut, Stowe, Conn.

Question and Answer (leader to be announced later).

New Members

ACTIVE

- Avis, Kenneth E., Manon City, Ill.
 Bosworth, Grover, 3125 Nicollet Ave., Minneapolis, Minn.
 Bowman, A. W., Cable Dairy Products, Inc., Box 140, Lexington, N. C.
 Cartwright, Dewey S., 8130 S. Maryland Avenue, Chicago 19, Ill.
 Chapplear, C. G., Caddo Co. Health Dept., Anadarko, Okla.
 Damisch, George A., Jr., 1663 N. Bilbert Street, Danville, Ill.
 Davis, Herbert, United Milk Prod. Co., Garden Prairie, Ill.
 Demaree, Chester C., Health Dept., City Hall, Asheville, N. C.
 England, Ross, St. Louis Dairy Co., 2001 Chestnut, St. Louis, Mo.
 Ford, Bruce R., 1229 South Clay Ave., Phelps Grove Park, Springfield 4, Miss.
 Frederick, Franklin B., Savanna, Ill.
 Fox, Earl C., 613 N. Broadway, Shelbyville, Ill.
 Gile, Robert M., 7 W. Geneva Street, Elkhorn, Wis.
 Hallett, R. C., Brunswick Gr. Meck. Health Dist., Boydton, Va.
 Hansen, George K., 506 N. Edwin, Champaign, Ill.
 Harms, Dwight E., 524 Second Avenue, Dixon, Ill.
 Henke, Herbert W., 744 Troy Road, Edwardsville, Ill.
 Hewes, Warren F., 3654 N. Monticello Avenue, Chicago 8, Ill.
 Johnson, Merdith R., 418 W. Tompkins, Galesburg, Ill.
 Katcheloffer, Elmer, 417 W. Jefferson St., Joliet, Ill.
 Kinate, Milton, Forrest Milk Prod., Forrest, Ill.
 Lamer, Beatrice, 3726 N. Pine Grove Avenue, Chicago 13, Ill.
 Mathews, R. Kay, Choctaw-McCurtain Co. Health Dept., Hugo, Okla.
 Mendizabal, Osvaldo Luis, Pedro Goyena 985, Buenos Aires, Argentina
 Orr, Garland E., St. Louis Dairy Co., 2001 Chestnut Street, St. Louis, Mo.
 Patrick, Clyde E., Box 137, Lovington, Ill.
 Schauf, Bernard, 490 Randolph Street, Burlington, Wis.
 Schytt, Bernard B., Pulaski-Wythe Health Dist., Pulaski, Va.
 Smith, Harold Winston, W. T. Boyd & Sons, Nashua, N. H.
 Towle, John E., Tri-County Dist. Health Dept., 4200 E. 9th Ave., Denver 7, Colo.
 Van Keuren, W. C., 2 Waldo Ct., Wellesley 81, Mass.
 Watermann, W. C., 709½ Palmyra Avenue, Dixon, Ill.
 Zichis, Dr. Joseph, 9246 Vincennes Avenue, Chicago 20, Ill.

CHANGES OF ADDRESS

- Allquist, John E., 621 Carl St., St. Paul, Minn., to 621 Case St., St. Paul, Minn.
 Bulmer, L. C., 230 Park Ave., New York, N. Y., to 670 63rd Ave. South, St. Petersburg 5, Fla.
 Cone, J. Frank, State College of Wash., Pullman, Wash., to Dept. of Bacteriology, Pennsylvania State College, State College, Pa.
 Culp, J. R., State Dept. of Health, Atlanta, Ga., to Ga. State Health Dept., Eng. Div., Atlanta, Ga.
 Davidson, Ralph M., 531 U. S. Customs Bldg., Denver 2, Colo., to 2335 Hudson St., Denver 7, Colo.
 Davies, Ben, Chippenham, Wiltshire, to United Dairies Limited, 34, Palace Court, Bayswater, London, W. 2.
 Doetsch, Raymond N., 1120 E. Capitol St., Washington 8, D. C., to Dept. of Bacteriology, University of Maryland, College Park, Md.
 Johnson, Lloyd, 255 Homestead Ave., Hartford 1, Conn., to United Farmers of New England, Morrisville, Vt.
 Krog, Andrew J., Paterson, N. J., to Lily-Tulip Cup Corp., 122 East 42nd St., New York 17, N. Y.
 Larson, R. A., 5 East Long St., Columbus, Ohio, to Indiana Dairy Prod. Assn., 623 Board of Trade Bldg., Indianapolis 4, Ind.
 Lawson, George W., 397 S. St. Marks Ave., Chattanooga, Tenn., to Consolidated Dairies, Inc., P. O. Box 3025, Avondale Sta., Birmingham, Ala.
 Miller, M. M., 4305 Chamberlayne St., Richmond 22, Va., to Univ. of Denver, University Park, Denver 10, Colo.
 Morrison, Donald E., 926 West Peachtree St. N.W., Atlanta, Ga., to Cobb County Health Center, 208 S. Waddell, Marietta, Ga.
 Myers, Dr. Robert P., 1403 Eutaw Place, Baltimore, Md., to c/o National Dairy Research Lab., Inc., Oakdale, L. I., N. Y.

Nusser, Dr. Wm. A., 824 Fidelity Bldg., Baltimore, Md., to 425 Camden Ave., Salisbury, Md.

Pegge, Capt. Chas. Elmer, Jr., Station Hospital, Tuskegee, Ala., to Station Hospital, LAAB, Columbus 17, Ohio.

Register, Joe Collins, State Hygienic Lab., Jackson, Miss., to 135 Delamore St., Jackson 22, Miss.

Richardson, Prof. G. A., Univ. of California, Davis, Calif., to Dept. of Dairy Husbandry, Oregon State College, Corvallis, Ore.

Sattell, Irving, 35-45 81st St., Jackson Heights, Queens, New York, N. Y., to 294 Webster Ave., Brooklyn 30, N. Y.

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A NEW COMMITTEE PROPOSED

(Continued from page 254)

reached at the National Sanitation Clinic, held late in June, 1948. The general confusion is aggravated by the fact that the new products are currently being employed in some milk sheds in which their use is not officially permitted.

In view of the widespread uncertainty and indecision which currently contribute to the confusion, it appears desirable that the Executive Committee of this Association consider the organization of a new standing committee, the function of which shall be to study and report on the effectiveness of new detergents and bactericides (or to review and act upon the findings of other non-commercial agencies), and to formulate standard treatment procedures for milk- and food-handling equipment. A committee has been active in the formulation of sanitary standards for the design and construction of milk-handling equipment, and one has been organized to develop similar standards for food-handling equipment. Effective sanitation involves more than design and construction details, however; and the time has come to act upon that knowledge.

Such a committee, when organized, may find it expedient to collaborate with other organizations, as has the Committee on Sanitary Procedure. It may have to provide for the collection and collation of data on the results of trials of treatment procedures in the field; and it may be necessary to establish a standard procedure for the assay of the effectiveness of proposed treatments. When such a committee is organized, the Executive Board may find it advisable to alter the title of the Committee on Sanitary Procedure, to avoid confusion of the functions of the two committees.

The publication of standard between-usage treatment procedures, in the JOURNAL OF MILK AND FOOD TECHNOLOGY, will serve the detergent and bactericide industries and sanitarians, by authenticating those which are effective. It is only in some such manner that the current confusion on this subject can be composed.

C. A. A.

Correction

The article in the July-August issue, page 206, entitled "Abstracts of the Literature of Milk and Food During 1947" under the authorship of C. K. Johns was compiled in large part by the Board of Associate Editors of this Journal. Dr. Johns condensed, edited, classified, and added to this material.

J. H. S.

POSITION OPEN

A college graduate to assist municipal milk and food control officer in his official duties. The incumbent must be capable of operating a laboratory for general bacteriological and chemical examination of milk and milk products, also the necessary tests in connection with restaurant sanitation control, and to assist in general administrative duties.

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JOURNAL OF MILK AND FOOD TECHNOLOGY

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“Doctor Jones” Says—*

PAUL B. BROOKS, M.D.

Let's see: how long's it been since we had a milk-borne epidemic of streptococcal sore throat in this state? (Scarlet fever or septic sore throat, that is). Well, it's two and a half years, anyway. And, for years, there wasn't one passed but what we had anywhere from one to three or four—bad ones, some of 'em. Not that it's surprising, their dropping off that way. With better'n 95 per cent of our milk pasteurized you'd expect it.

Of course (just to keep the record straight) the last one we had—sixty cases back in 1945—that *was charged* to pasteurized milk. The circumstances—pasteurized milk delivered to a summer camp: it was handled, when they got around to use it, by a cook or somebody with a sore throat. The same milk was used elsewhere around that section and no trouble from it. It was an epidemic, yes; but not the fault of the milk.

But I'm off on a sidetrack. What I was thinking about when I started was rheumatic heart disease, a form of rheumatic fever. You've heard plenty about rheumatic fever in the past few years. Its responsible for most of the deaths from heart disease in folks from five to twenty-four years old. After tuberculosis and syphilis it's the most

common chronic infection, so I was reading.

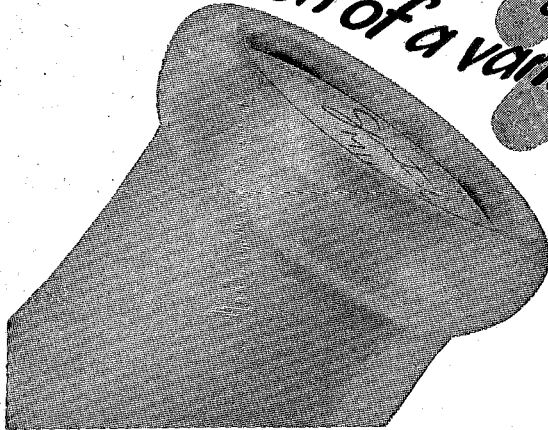
Attacks of rheumatic fever are usually—probably always—preceded by hemolytic streptococcal infections, most often sore throat. That's what seems to be responsible for the rheumatic fever.

A couple of other things I also read. Dr. Madsen, of Denmark, reported, a few years ago, that several epidemics of rheumatic fever had followed milk-borne epidemics of streptococcal sore throat. The other: deaths from rheumatic heart disease, in this country, had fallen off something like 25 per cent in 30 years. Over about that same period pasteurization of milk has been increasing, steadily, all over the country.

So I got to wondering if those might not be another two and two that'd add up to four and, maybe, the falling off in milk-borne streptococcal infection resulting from pasteurization might've had something to do with the decline in rheumatic heart disease. I haven't much confidence in my mathematics, so it's just a thought but—well, anyway, I'll swear that two and two make four.

* *Health News*, New York State Department of Health, Albany, N. Y., August 9, 1948.

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the touch of a vanished hand!"*



(with apologies to Tennyson)

If there is one place on a milk bottle which should never be promiscuously handled—that place is the pouring surface.

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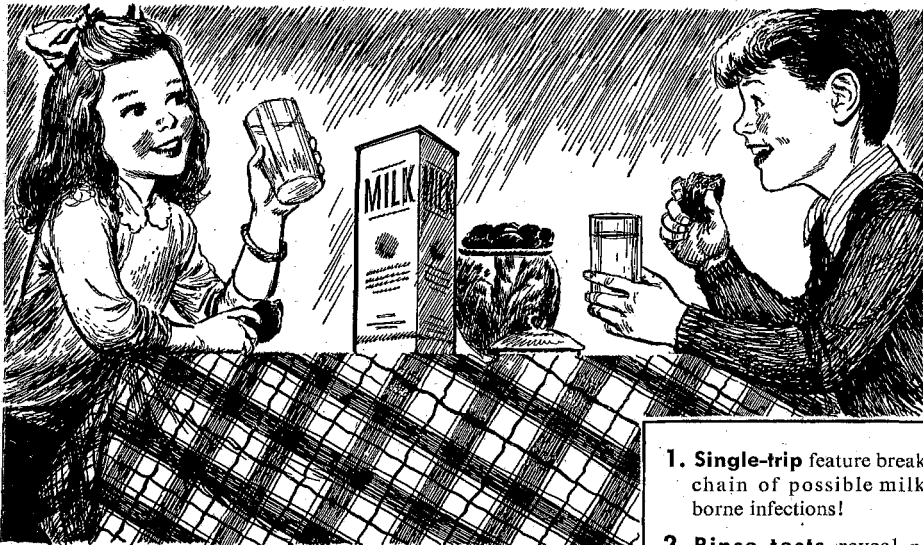
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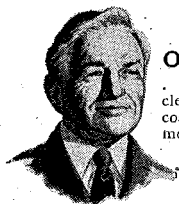


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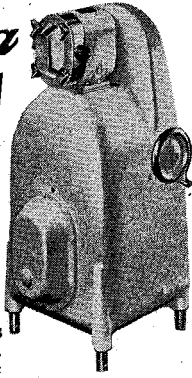
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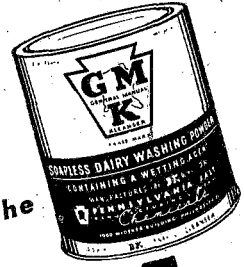
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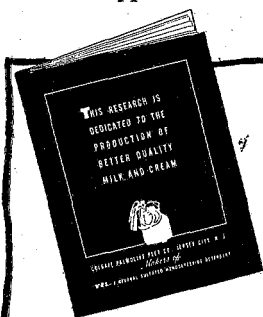
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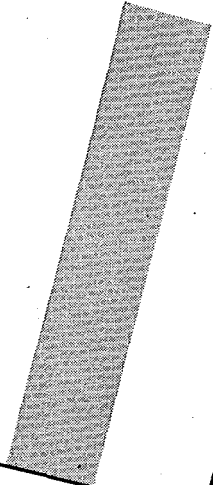
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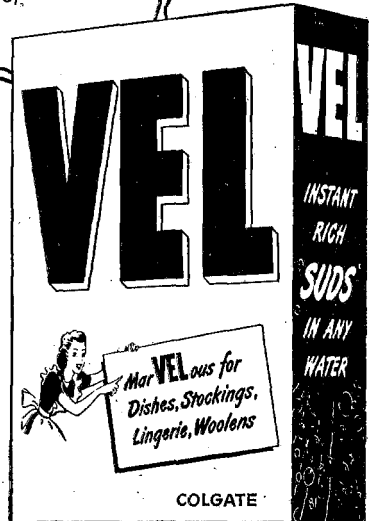
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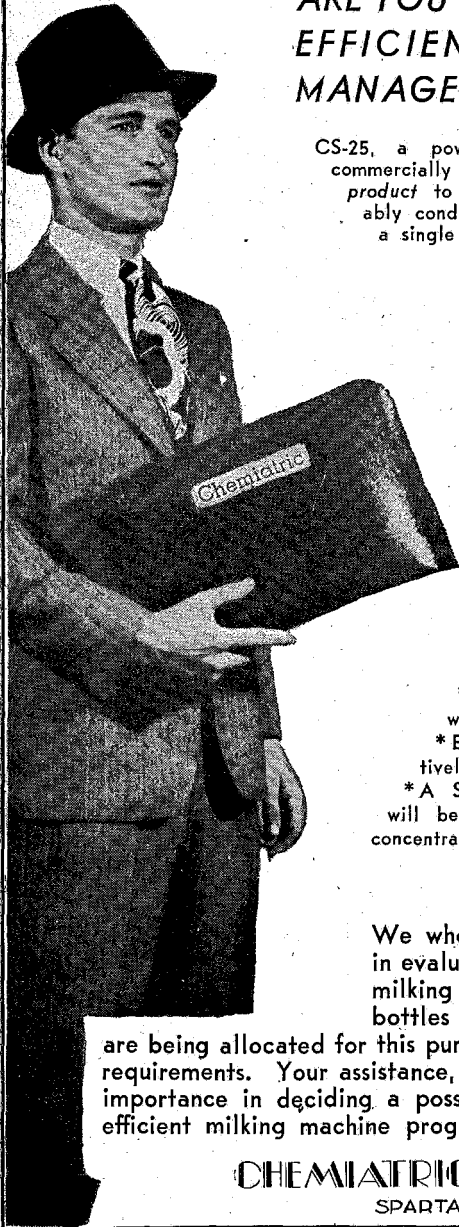
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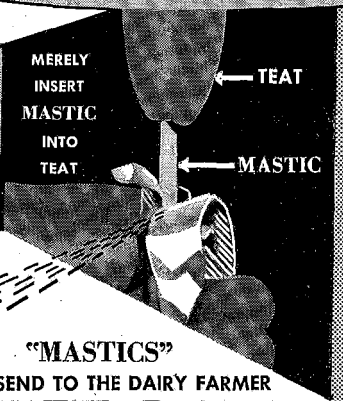
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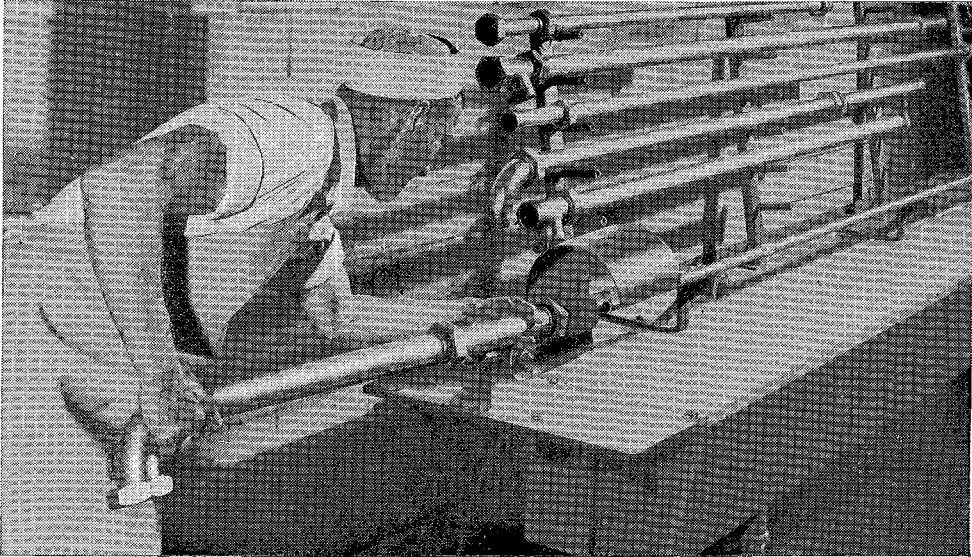


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