JULY, 1964
Vol. 27 P. 191-226 No. 7

Journal of

MILK and FOOD TECHNOLOGY

51st ANNUAL MEETING
AUGUST 18, 19, 20, 21, 1964
Portland Hilton Hotel — Portland, Oregon

Official Publication

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Babson, Bros., Co. ——Back Cover
Britex Corp. ——223
Difco Laboratories ——Inside Back Cover
Fiske Associates, Inc. ——225
IAMFES, Inc. ——I
Perfection Division, Sta-Rite Products, Inc. ——Inside Front Cover
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The Journal of Milk and Food Technology is issued monthly beginning with the January number. Each volume comprises 12 numbers. Published by the International Association of Milk, Food and Environmental Sanitarians, Inc. with executive offices of the Association, Blue Ridge Rd., P. O. Box 437, Shelbyville, Ind.

Entered as second class matter at the Post Office at Shelbyville, Ind., March 1952, under the Act on March 3, 1879.

EDITORIAL OFFICES: J. C. Olson, Jr., Associate Editor, Dept. Dairy Industries, University of Minn., St. Paul, Minn.; H. L. Thomasson, Managing Editor, P. O. Box 437, Shelbyville, Ind.

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Journal of

MILK and FOOD TECHNOLOGY

INCLUDING MILK AND FOOD SANITATION
Official Publication
International Association of Milk, Food and Environmental Sanitarians, Inc.
REG. U. S. PAT. OFF.

Volume 27 July, 1964 Number 7

Contents

Editorial:
The Changing Picture of Milk Control ---------- 191
Analysis of Bacteriological Utensils
Swab Counts and Dishwashing
Procedures From Field Reports
F. J. Post, J. W. Entz and J. J. Palmersheim ---------- 192
Growth Rate of Coliform Organisms
In Cottage Cheese and Reconstituted Nonfat Dry Milk
W. R. Shelton and L. G. Harmon ---------- 197
Analysis of Animal Food Products
for Chlorinated Insecticide Residues
1. Column Clean-up of Samples for
Electron Captures Gas-Chromatographic Analysis
B. E. Langlois, A. R. Stemp, and B. J. Liska ---------- 202
Comparison of Media for the Isolation
of Clostridium Perfringens from Food
Jill M. L. Southworth and Dorothy Hesseman Strong ---- 205
Ammendment to 3-A Sanitary Standards
for Storage Tanks for Milk and
Milk Products, Serial #0102 ---------- 215
Some Factors Which Govern Plaque Formation
by Lactic Streptococcal Bacteriophage
1. Seed Layer Requirements
E. M. Mikolajcik ---------- 210
In-Line Control of Bacteria Build-up in Processing
Amihud Kramer ---------- 216
News and Events ---------- 219
Index to Advertisers ---------- II

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Public and Educational Institution Libraries, 1 yr.	$6.00
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Orders for Reprints: All orders for reprints should be sent to the executive office of the Association, P. O. Box 437, Shelbyville, Ind

Membership Dues: Membership in the International Association of Milk, Food and Environmental Sanitarians, Inc., is $7.00 per year, which includes annual subscription to the **Journal of Milk and Food Technology.** All Correspondence regarding membership, remittances for dues, failure to receive copies of the **Journal,** changes in address, and other such matters should be addressed to the Executive Secretary of the Association, H. L. Thomasson, Box 437, Shelbyville, Indiana.

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THE CHANGING PICTURE OF MILK CONTROL

In the early days of milk sanitation literally millions of words were spoken to members of city councils, county commissions, and similar bodies in an effort to convince them that minimum standards for the production, processing, and distribution of milk and milk products should be adopted to assure the local milk consuming public a safe product. At that time, of course, the dairy industry was generally local in nature, and a high degree of control could be exercised over the milk supply by a strict enforcement of these minimum standards by local enforcement personnel. Since programs of this nature are of little or no value without a means of evaluation, the U. S. Public Health Service developed milk sanitation rating procedures. Activities conducted in accordance with these procedures made it possible to rather accurately present information to the local health authority and other administrative officials as to the efficiency and effectiveness of their program, its strong and weak points, and recommendations which if followed would assist in strengthening their overall milk sanitation activities.

We now find ourselves in the midst of an era of change in the dairy industry and, therefore necessarily, in milk sanitation control. Neither is strictly local in nature any more. This period of change has become most evident since the end of World War II and has progressed with increasing momentum since that time. It will continue to do so until we eventually have only a minimum number of fluid milk plants strategically located throughout the country being supplied by relatively few widely scattered dairy farms of extremely large production capacity. All of this, of course, has been brought about by the economics of the dairy industry, and has been made possible by the development of mass production automated equipment and the improved quality of both the raw supply and finished products. Taking full advantage of new distribution equipment and patterns has made it possible to increase the distribution radius of plants from a mere few miles to literaly hundreds of miles in some cases.

These changes have brought about an entirely new concept relative to the purpose and value of milk sanitation compliance ratings. While they were initially designed for the evaluation of local milk sanitation control activities to provide information to the local community officials they have now also become recognized as the "life-blood" of the dairy industry. Without them the Interstate Milk Shipper Program could not exist and milk could not move freely across jurisdictional boundaries on the basis of reciprocity of inspections. Because receiving areas in many cases do not ever see the conditions of production or processing it becomes all the more necessary that milk sanitation compliance ratings accurately reflect these conditions, not only as they exist at the time the rating is established, but on a day-to-day basis. To accomplish this goal, which is of such vital interest to the dairy industry, it must maintain constant vigilance over its own supply.

All of this throws an entirely different light on milk sanitation control programs as many of us have traditionally known them in the past. While many have always considered this to be an extremely expensive type of program to administer it is even becoming more of an economic burden to some communities because of the ever decreasing number of dairy farms. It is not unusual for procurement areas to be as much as 150 miles or more in radius under the supervision of one local health agency.

Direct supervision by an official agency has become more difficult. Where it was once possible to direct inspectional and supervisory activities toward a permanently located establishment such as a dairy farm, receiving station, or pasteurization plant we are now faced with the proposition of supervising bulk milk haulers who must be considered an integral part of any milk sanitation program. This becomes most difficult when their activities, which are numerous and varied, are conducted as many as hundreds of miles from the headquarters of the supervising agency.

Such factors as these, coupled with the increasing cost of supervision, have led many communities to ask the question "Why should we pay for the supervision of milk destined for sale in other areas?" Some communities have attempted to remedy this situation by the levying of permit fees or per hundredweight check-off fees. Such systems, however, are not always the most equitable or realistic considering the amount of service rendered. They also do not solve the problem of the inefficiencies created by the expansion of procurement areas and the subsequent overlapping of milk sheds.

All of this brings us to the question of who is responsible for each facet of a milk sanitation program, and what exactly should be the role of the responsible official milk sanitation agency, whether it be city, county, or state. It becomes increasingly evident that because of the lack of sufficient funds, availability of trained personnel, the large volumes of raw and finished products entering intra and interstate commerce, and the importance being placed on milk sanitation compliance ratings, the dairy industry must continue to gradually assume more and more responsibility for the development and maintenance of the quality of its supply. Such an approach would, therefore, leave the official agency in its rightful position of evaluating the conditions of production, processing, and distribution as they exist.

While each milk sanitation control agency undoubtedly feels that it is conducting a good program, none have reached the point where no further improvement is possible. In light of the past and possible future changes in the dairy industry, it would behoove all official agencies to periodicaly review and study their position to determine the weaker areas of activity and make the necessary modification to correct these, thereby enabling them to more completely discharge their milk sanitation control responsibilities.

H. H. Vaux, Director
Division of Dairy Products
Indiana State Board of Health

ANALYSIS OF BACTERIOLOGICAL UTENSIL SWAB COUNTS AND DISHWASHING PROCEDURES FROM FIELD REPORTS

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(Received for publication December 13, 1963)

Summary

Swab records of the Los Angeles County Health Department totaling 13,447 for the years 1959, 1960, and part of 1961 were analysed using the IBM 7090 computer of the Western Data Processing Center. Results indicated that the total number of illegal counts in the county for this period averaged 12.67%, far below comparable reports from other sections of the country. Analysis of the data indicated that bars had more illegal counts than restaurants and used hand washing far more than machine washing. About 38% of restaurants used machines. Type of sanitizer, time of exposure, utensil, and distribution of bacterial counts are also reported. Quaternary ammonium compounds appeared to be used less successfully than hot water and chlorine. Hot water was the most reliable sanitizer on the whole but chlorine was more frequently found at legal concentrations. It was concluded that supervision of operation was more important than the swab test itself.

The swab test for estimating bacterial contamination of surfaces has been in use for many years. The accuracy and validity of this method of sampling has been the center of considerable dispute *(2)*. The most recent edition of the Public Health Service eating and drinking establishment regulations *(7)* does not include the technique, whereas an earlier edition *(6)* did. Standard Methods for the Examination of Dairy Products (1960) has explicit directions for use with dairy equipment.

The continuing question of the usefulness of this technique prompted the following study to determine if valuable or useful information could be gained when all swab reports of a local health department were statistically evaluated. Inherent in the study was the question whether routine performance of this test is justified and whether information could be gained directed to this end.

Procedure

Swab reports were obtained from the Los Angeles County Health Department records for the years 1959, 1960 and part of 1961. The total number of records examined and coded was 13,447. A change in record form at the start of 1961 provided considerably more useful information than the earlier years and permitted more rigorous analysis of sanitizer, time of exposure, etc. Data was coded on IBM cards and a program developed for the IBM 7090 computer[2]. The 1959-60 data included 21 variables and the 1961 data 35 variables. The computer was instructed to generate as many one- and two-way and multivariant tables as the data seemed to warrant, determine percentages, correlation coefficients, and significance figures at the 95% level *(3)*. The output was collated, tables combined in the most advantageous ways and the most important ones selected for use in this study.

Missing values (i.e. blanks on the report forms) presented considerable difficulty in the 1959-60 data. An improvement was noted in the 1961 data along with the change in form. Also the "controls" presented some problems. In these the swab was removed from the vial, exposed to the air and replaced as a supposed check on technique. Judging from the results some were apparently deliberately contaminated as a "check on the laboratory". Missing values and controls were treated as indicated in the separate tables. In most instances they were removed from the calculations.

The technique of swabbing was that of the U. S. Public Health Service *(6)*. Four utensils were swabbed with a swab wetted in 4 ml of buffer. One ml portions were plated and reported as count per utensil. A count of 100 bacteria or more per utensil was considered illegal.

Results and Discussion

The Los Angeles County Health Department serves the larger part of Los Angeles County, an area of about 4 million population. This area is divided into districts and includes 71 cities (excluding the cities of Los Angeles, Long Beach, and Pasadena) as well as rural, mountainous, and desert areas. By far the largest number of swab reports came from districts with larger populations and only a few from the less populated areas.

The data presented here in tabular form were chosen primarily if some significance was associated and secondly if the distribution seemed interesting although not significant. Data not of sufficient import to include in tabular form included those in-

[1]Current address: Los Angeles City Health Department, Los Angeles, California.

[2]Western Data Processing Center, University of California, Los Angeles.

dicating no significant differences between urban and rural areas or high and low income areas, and no differences between the 14 districts studied. Icing of the samples had no influence nor did scraping of the dishes prior to washing. Wash temperature also showed no correlation with the number of illegals reported.

Table 1 shows the distribution of utensils sampled during 1961. The report form for 1959-60 did not include this particular breakdown, however, it is not unreasonable to assume that the same relative proportion of utensils was sampled in this period. During 1961 the most commonly sampled utensil was the glass. Although there was a higher percentage of illegal counts in this group, significance testing indicated that glasses were not anymore likely to have illegal counts than the other utensils.

TABLE 1. BACTERIAL COUNT AND UTENSIL (1961 DATA ONLY)

Utensil	No.[a]	% illegal[b]
Glasses	1089	18.92
Cups, Mugs	771	16.60
Dishes	19	15.79
Forks, spoons	12	0
Total	1891[a]	17.82

[a]The total number of utensils sampled is 4 x this figure.

[b]259 additional reports on which the utensil sampled was missing were not included in calculation.
No significant difference between the utensils by the t test at the 95% level.

TABLE 2. BACTERIAL COUNT DISTRIBUTION[a]

Count range	1959-60 No.	1959-60 %	1961 No.	1961 %	Combined No.	Combined %
9000	239	2.17	57	2.41	296	2.21
1000-8999	249	2.26	80	3.39	329	2.46
500-999	158	1.42	75	3.18	233	1.74
100-499	568	5.15	158	6.70	726	5.42
Total illegal	1214	11.72	370	17.22	1584	12.67
50-99	357	3.24	70	2.95	427	3.19
1-49	5845	52.99	1097	46.44	6942	51.84
0	3614	32.77	825	34.93	4439	33.15
Total legal	9816		1992		11,808	
Total	11,030		2362		13,392	

[a]Missing and control values not included in calculations.

One interesting aspect of the data presented in Table 1 is the extremely low percentages of illegals. Most other studies indicate that it is rather difficult to find 50% of the utensils below 100 organisms per utensil *(2, 8)*. Shook *(9)* found median counts in cities in Connecticut to be well above this level. Kleinfield and Buchbinder *(4)* found 15% of cups and glasses less than 100. In Los Angeles County 83% were less than 100 bacteria per utensil. In table 2 will be found the distribution of bacterial counts. It should be noted that of the counts above 100, the largest percentage were less than 500. Armbruster *(2)* reported that it is quite common to find 25% of utensils in excess of thousands. In this study this group amounted to 5% of the samples. There was a significant increase in percent illegals during 1961 in restaurants and bars but not fountains. The reason for this is not apparent from the data. The most likely reason for these low counts is good operation of dishwashing and sanitizing procedures. The possibility that new equipment and buildings in a growing metropolitan area may have some influence cannot be overlooked.

The type of establishment is given in Table 3. Restaurants and bars were most commonly sampled. Bars had a significantly higher percentage illegal as did fountains. Bars very likely have something inherent, possibly in the sanitizing practice, resulting in a significantly larger number of illegals than restaurants. This is not unexpected and corresponds to the practical experience of many sanitarians. The higher percent illegal may be partially due to the much greater use of hand washing in this type of establishment. Fountains seem to fall between bars and restaurants in this respect. Table 4 indicates the amount of hand and machine washing found in the various establishments. A slight but statistically insignificant rise in machine usage was noted in all establishments in 1961. In restaurants 36-38.5% used machine washing while in bars the amount of machine usage was negligible. In 1961, of the 9 bars using machine washing, 6 used quats and 2 hot water as disinfectants and for one this information was missing. The rinse temperature of the one missing was greater than 180 F and presumably was a hot water sanitizer; also it was the only one with an illegal count. Such breakdown was not possible with the 1959-60 data. Table 5 indicates that hand washing was more often associated with illegal counts than machine washing and this difference was statistically significant.

Data on sanitizer use is given in Table 6. Most establishments used chlorine as the sanitizing agent, quats were next and the least used was hot water. Hot water was significantly less often associated with illegal counts than was chlorine or the quats. This would appear to indicate that hot water is the most

TABLE 3. TYPE OF ESTABLISHMENT AND PERCENT ILLEGAL

	1959-60			1961		
	Number	% total	% illegal	Number	% total	% illegal
Restaurants	6089	54.93	10.35[a]	1194	50.55	15.24
Bars	3661	33.03	14.25[b]	813	34.42	20.66
Fountains	294	2.65	10.20	57	2.41	12.28
Camps	5	.05	0.0	3	.13)	Not
Others	41	.37	4.90	77	3.26)	determined
Controls	698	6.30	-[c]	213	9.02	-[d]
Missing	297	2.68	-	5	.21	-
Total	11,085			2362		
1959-61 Total	13,447					

[a]Based on 6056 restaurants, 43 lacked data on legality.

[b]Based on 3642 bars, 19 lacked data on legality.

[c]3 controls were illegal i.e. >100.

[d]all were legal counts i.e.<100.

At the 95% level differences between bars and fountains and restaurants with each year were significant. The increase in % illegals between years was also significant.

reliable as a sanitizer in field use. A further breakdown in usage of hot water as a sanitizer is given in Table 7. Although the total sample is not very large, more than half of the operations, whether hand or machine, were performing below the California requirement of 180 F. The differences, however, were not significant. Since many consider 170 F sufficient for adequate hot water sanitizing *(5)*, the number of operations above 170 F is also included. It is interesting to note that all of the hand operations below 180 F were actually below 170 F. The number of machines operating above 180 F was 39 and the total operating above 170 F was 64, an increase of 25. Although the number of hand operations is small it would appear that machines are somewhat easier to keep or at least more often found above 170 F.

California regulations for sanitizing are; hot water 180 F or above, chlorine 100 ppm or above, or quats 200 ppm or above and for all sanitizers an exposure of 30 sec or longer. A breakdown of the 1961 data on the basis of concentration or temperature is given in Table 8. Although there was no significant difference, more units operated at less than the legal temperature or concentration with hot water and quats respectively, than operated legally. While there were more illegal counts when quats were below 200 ppm, there was no significant difference in the number of illegals when the concentration was above 200 ppm. Hot water also showed no significant difference in illegal counts between the two temperatures. Chlorine, however, did show a significant difference in illegal counts when below 100 ppm. Although it is rather difficult to interpret such retrospective data, one thing appears clear, chlorine was far more commonly (94.9%) found above the legally required minimum than either hot water or quats. Apparently it is easier to maintain chlorine above the legal threshold. In comparing total percent illegal counts for the three sanitizers, chlorine and hot water statistically gave the same result and quats resulted in significantly more illegal counts than either hot water or chlorine. When illegal

TABLE 4. DISTRIBUTION OF METHOD OF WASH BY ESTABLISHMENT[a]

	1959-60				1961			
Establishment	Machine	Hand	Total	% Machine	Machine	Hand	Total	% Machine
Restaurant	2123	3795	5918	35.87	460	734	1194	38.53
Bar	15	3524	3539	0.42	9	804	813	1.11
Fountain	48	236	284	16.90	14	43	57	24.56
Camp	0	5	5	0	0	3	3	0
Other	3	36	39	7.69	17	60	77	22.07
Total	2189	7596	9785	22.37	500	1644	2144	23.32

[a]Does not include controls or missing values.

There was no significance at the 95% level in machine usage in restaurants between years.

TABLE 5. BACTERIAL COUNT AND METHOD OF WASHING

	1959-60			1961		
Method	No.	% total	% illegal[a]	No.	% total	% illegal[a]
Hand	7827	70.61	12.22[b]	1596	67.57	18.17
Machine	2281	20.58	9.78[c]	501	21.21	14.57
Control	698	6.30	-	213	9.02	-
Missing	279	2.52	-	52	2.20	-
Total	11,085	-	-	2362	-	-

[a]Does not include control or missing values in calculation.

[b]Based on 7787 counts, 40 were without counts reported.

[c]Based on 2270 counts, 11 were without counts reported.

t test at the 95% level indicated a significant difference between machine and hand method in both years.

TABLE 6. BACTERIAL COUNT AND SANITIZER (1961 DATA)

Sanitizer	No.	% totals[a]	% illegal[a]
Hot Water	140	7.93	11.43
Quats	644	36.47	19.10
Chlorine	982	55.61	17.11
Total	1766	-	15.66

[a]212 controls and 384 missing values not included in calculations. t test at the 95% level indicates a significant difference between hot water and the other two methods but no difference between quats and chlorine.

counts of the proper operating conditions are compared, there is no significant difference between the sanitizers at the 95% level. This is probably due to the small number of samples. If one chooses the 90% level there is a difference between hot water and quats but not between hot water and chlorine or chlorine and quats. These results would seem to indicate that hot water is the better field sanitizer and that it has a wide latitude for temperature variation, i.e. the temperature can be considerably below the legal requirement and still produce good results. Chlorine on the other hand is much easier to keep at proper concentration although there is a slightly higher association with illegal counts even so. Quats appear to be far more often associated with illegal counts.

The second part of the operating requirement is sanitizer exposure time, Table 9. Again more operated at exposure times less than that required. Differences in percent illegals between times and between sanitizers were not significant.

Before final conclusions can be drawn it is important to comment on sources of inherent error. With retrospective studies using field records such as these, it is rather difficult to know whether the sample takers followed a set procedure in taking swabs. In this case there is no way of knowing whether the swabs were taken as the utensil emerged from the sanitizing process or after the utensil had been sitting on the shelf for a time. Presumably they were all taken as checks of sanitizing effectiveness but this probably is not entirely true. The fact that some dish machines operating over 180 F for more than 30 sec still produced some illegal results indicates that not all swabs were taken immediately after washing. It seems imperative that swab report forms have some provision for distinguishing between washing operation checks and "educational" uses, i.e. swabs from utensils standing on the shelf for a time.

CONCLUSIONS

The study presented here has several interesting aspects. The extremely low number of illegal counts in this rather large health jurisdiction seems outstanding. The reason for this is rather obscure but

TABLE 7. HOT WATER SANITIZER AND METHOD OF WASH

Temp. °F	Hand	%[a] at temp.	Machine	%[a] at temp.	Total	%[a] at temp.
>180	6	42.86	39	41.94	45	42.06
<180	8	57.14	54	58.06	62	57.94
Missing	1	-	32	-	33	-
Total	15	10.71	125	89.29	140	-
>170[b]	8	57.14	64	68.81	72	67.29

[a]Except for total percentages, missing values not included in calculations.

[b]Includes all those above 180 F and part of those below 180 F. t test for significance at 95% level indicates no difference between temperatures or methods.

is probably closely related to the excellent supervision of dishwashing operations by the health department and perhaps that the whole area is growing and the proportion of new buildings and especially new equipment may also play a role. Even so, bars, as is the experience of many sanitarians, showed significantly more illegal counts than restaurants with fountains in between. Handwashing is possibly a factor here since most bars used this method and hand washing is significantly more often associated with illegal counts than is machine washing. Machine washing is far more common in restaurants but is

TABLE 8. BACTERIAL COUNTS AND SANITIZER (1961 DATA)

Sanitizer		No. legal	No. illegal	Total	% illegal	% at temp. or ppm
Hot Water	>180 F	42	5	47	10.64	41.23
	<180 F	58	9	67	13.43	58.77
Total		100	14	114	12.28	-
Chlorine	>100 ppm	339	51	390	15.04	94.40
	<100 ppm	16	7	23	43.75	5.60
Total		355	58	413	16.34	-
Quat	>200 ppm	90	19	109	21.11	38.65
	<200 ppm	137	36	173	26.28	61.35
Total		227	55	282	24.23	-

t test for significance at 95% level with the % illegal indicates no difference between temperatures with hot water, or concentration with quats but does indicate a significant difference between chlorine concentrations. t test also shows no significance between hot water and chlorine total percent illegal but shows a significant difference between quats and each of the other sanitizers.

still only found in 35-38.5% of the establishments. Regardless of sanitizing agent machine dishwashing is significantly associated with fewer illegal counts. Hot water was the least used sanitizing agent but was significantly less associated with illegal counts than chlorine, possibly indicating greater reliability. More operations provided less than the required exposure time to the sanitizers and the precent illegals seemed more closely associated with concentration. The small number of samples limits interpretation of concentration or temperature results but hot water appears more reliable and chlorine appears to be easier for some reason to maintain at a legal concentration. Quats appear far more frequently associated with illegal counts even when operating at the required concentration.

If swabs are taken from utensils which have been out of the sanitizing process for a time, the swab count reflects airborne dust or other contamination and does not accurately reflect the sanitizing process. Therefore taking swabs of such utensils has no value in checking the operation of the sanitizing step and only has educational value to the personnel. Consideration must be given to whether this purpose warrants the time and expense of such test routinely. If the swab is used on the utensil as it leaves the sanitizing step as a check on the process, then it would not provide anymore information than correct operation of the process itself, i.e. concentration or temperature and time of exposure. This of course presumes that proper operation reduces counts below 100 per utensil in the field as it does in the laboratory. This point perhaps needs further corroboration.[3] In the opinion of the authors, based on the data presented here, close scrutiny and control of the washing and sanitizing procedures is the most important factor in keeping swab counts low.

TABLE 9. ILLEGAL COUNT AND TIME IN THE SANITIZER (1961 DATA)

	<30 sec.			>30 sec.		
Sanitizer	Total	No. illegal	% illegal	Total	No. illegal	% illegal
Chlorine	329	63	19.15	260	38	14.62
Quats	273	54	19.78	149	32	21.48
Hot Water	24	4	16.67	21	4	19.05
Total	626	121	19.32	430	74	17.21
% at Time	59.28			40.72		

t test indicates no significant difference between sanitizers at the 95% confidence level at either time interval nor between times for each sanitizer.

[3]After the preliminary report of these data, the Los Angeles County Health Department further studied this relationship under controlled conditions and substantiated this conclusion more scientifically. They have since abandoned routine swabbing in favor of more rigorous operations control, but will use the swab technique as a check on specific problems as the need arises.

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GROWTH RATE OF COLIFORM ORGANISMS IN COTTAGE CHEESE AND RECONSTITUTED NONFAT DRY MILK[1]

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Summary

The objective of this study was to determine the population trends of representative strains of *Escherichia coli* and *Aerobacter aerogenes* in cottage cheese and reconstituted nonfat dry milk (skim milk). Inoculated milk samples were stored at 32, 39, 50 and 90 F for a maximum of 130, 76, 119, and 11 days, respectively. The inoculated cheese samples were stored at 39, 50 and 55 F for 80 days. In milk at 50 F the generation time of *E. coli* during the logarithmic growth phase varied from 516 to 642 minutes and at 90 F the generation time varied from 33 to 39 minutes, respectively. The corresponding generation times for *A. aerogenes* were 540 to 648 minutes and 29 to 33 minutes. Both coliform species decreased in population at 32 and 39 F and the decrease was greater at 39 F.

In cottage cheese the population of both coliform species increased at 55 F and decreased at 39 F, but at 50 F the numbers of *E. coli* increased while those of *A. aerogenes* decreased. During the first five days at 55 F, the generation times of *E. coli* averaged 31.4 hours while *A. aerogenes* averaged 32.8 hours. At 50 F the generation time of *E. coli* averaged 48.2 hours.

Sanitation and keeping quality are important factors in consumer acceptance of food commodities. Between 1950 and 1960 the per capita consumption of cottage cheese increased from 3.1 to 4.8 lb *(13)* in the United States. The per capita sales of skim milk increased from 14.7 to 23.1 lb *(12)*.

Initial coliform populations of < 1 per ml or g in processed fluid milk and cottage cheese have shown significant increases during storage and movement through sales outlets. The normal growth range of the coliforms is 50 to 113 F, but they have been known to survive or even increase in milk products stored at 35 to 45 F *(2, 4, 6, 7, 15, 16, 17)*. The purpose of this study was to determine the population trends and generation times of typical strains of *Escherichia coli* and *Aerobacter aerogenes* in cottage cheese and reconstituted nonfat dry milk (skim milk) at various refrigeration temperatures. The growth pattern and generation time of the organisms were also determined at the optimum temperature of 90 F. The presence of coliform bacteria in cottage cheese has been reported by many workers, but the rate of increase of these organisms in cheese has not been investigated thoroughly. Overcast and Britton *(14)* manufactured cottage cheese which contained coliform counts of 10 or less per g after storage for 11 days at 40 F.

The generation time of coliform organisms grown in broth media has been determined by several workers. Barber *(3)*, using a single cell isolation technique, found the maximum rate of growth of *Bacillus (Escherichia) coli* occurred at 99.5 F with a minimum generation time of 17.2 min. Additional generation times were 25 min at 90 F and 12.5 hr. at 48 to 50 F. Jennison *(10)* determined that the generation times were 25 min at 90 F and 12.5 hr at 26 min and that of one *A. aerogenes* strain was 21 min when grown in nutrient broth at 90 F. Ingraham *(9)* reported that the generation times of *E. coli* (strain K-12) grown in a broth medium were 21 min at 99 to 111 F, 30 min at 90 F and 20 hr at 50 F.

Experimental Procedure

Preparation of Milk.

Low heat non-fat dry milk was reconstituted to 9% serum solids and steamed for one hour at 212 F on each of three successive days. Between heatings, the milk was stored at room temperature. After the last heat treatment, the samples were adjusted to pH 6.6 to 6.7 with a 10% solution of sterile trisodium phosphate and stored at 39 F until used. Sample bottles containing 100 ml of the sterile reconstituted non-fat milk were tempered at 32, 39, 50 and 90 F for 24 hr before the initial coliform inoculum was added.

Manufacture of Cottage Cheese.

The cottage cheese was manufactured by the short set method, cooked to 135 F and held in the whey at 135 F for 20 min to reduce the number of psychrophiles in the curd to non-detectable levels. The curd was washed three times with water containing

[1]Mich. Agric. Expt. Sta. Jour. Article No. 3269.

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15 ppm of a hypochlorite solution. During and after the manufacturing process the cheese curd was handled carefully to minimize contamination.

A creaming mixture containing 10.5% milk fat was steamed at 212 F for one hour. Sodium chloride equal to 3.5% by weight was then added. A sufficient quantity of the creaming mixture was blended with the cheese curd to give a concentration of 3% milk fat in the cottage cheese.

Isolation and Identification of Coliform Organisms.

Coliform bacteria were isolated from raw bulk tank milk initially on Violet Red Bile (VRB) agar plates incubated at 90 F. The isolates were purified and classified according to the characteristics described by Breed et al. *(5)*.

Propagation of Coliform Organisms.

Four representative strains of *E. coli* and three representative strains of *A. aerogenes* were selected and maintained on Tryptone Glucose Yeast (TGY) agar slants. Cultures were activated in sterile reconstituted non-fat milk, and transferred daily. An 18-hr culture was diluted with buffered sterile water to give the desired initial population and added to the reconstituted non-fat milk or creaming mixture. Initial coliform populations of 10 or less per ml were added to the milk stored at 50 and 90 F; whereas, 1500 to 2000 organisms per ml were added to the milk samples stored at 32 and 39 F. Fifteen hundred coliforms per ml added to the creaming mixture gave recoverable initial populations in the cottage cheese of about 250 per g. The reduction was attributed to the dilution of the creaming mixture with the cheese curd and to change in environment.

Preparation of Cottage Cheese Samples.

After the creaming mixture was mixed with the curd, four lots of creamed cottage cheese were prepared and inoculated with coliform organisms. Each trial included three groups of cheese samples: *(a)* control, *(b)* inoculated with *E. coli* and *(c)* inoculated with *A. aerogenes*. The creamed cottage cheese was measured into sterilized bottles and incubated at 39, 50 and 55 F.

Bacteriological Analyses of Samples.

Equal volumes of cottage cheese and 2% sodium citrate solution were weighed into a sterilized Waring blender jar and mixed for three minutes at slow speed. Appropriate dilutions of cheese or milk were plated on VRB agar and incubated at 95 and 90 F, respectively, for 24 hr. The higher incubation temperature was used to minimize psychrophilic growth which may have developed in the cottage cheese. Procedures outlined in Standard Methods *(1)* were used in determining all bacterial counts. The coliform count of control and inoculated samples of the reconstituted non-fat milk and cottage cheese was determined at appropriate intervals, selected according to the storage temperature of the samples.

Calculation of Generation Times.

The generation times were calculated according to the following formula:

$$\text{generation time} = \frac{(T_2 - T_1) \times \log 2}{\log b - \log B}$$

$(T_2 - T_1)$ = interval of time (minutes or hours)

b = bacterial population at time (T_2)

B = bacterial population at time (T_1)

pH Determinations.

All pH determinations were made with a Beckman H-2 pH meter equipped with glass electrodes.

RESULTS

The generation times of four strains of *E. coli* and three strains of *A. aerogenes* grown in reconstituted non-fat milk containing 9% serum solids and incubated at 50 and 90 F are shown in Table 1. The logarithmic growth phase usually occurred between the second and ninth days in milk incubated at 50 F and within the first 12 hr in milk incubated at 90 F. The data in this table also include the generation times computed during the interval between inoculation

TABLE 1. GENERATION TIMES OF COLIFORM ORGANISMS IN RECONSTITUTED NON-FAT MILK CONTAINING 9% SERUM SOLIDS

	Incubation temperature			
	50 F		90 F	
	Generation time during:			
	Logarithmic growth phase	Interval from inoculation to maximum population	Logarithmic growth phase	Interval from inoculaton to maximum population
	(min)	*(min)*	*(min)*	*(min)*
E. coli				
strain 1	516	942	34	52
strain 2	546	744	34	54
strain 3	522	774	33	53
strain 4	642	1038	39	56
A. aerogenes				
strain 1	540	870	33	53
strain 2	648	852	34	50
strain 3	636	1014	29	48

and attainment of maximum population. The latter interval extended for 24 hr for all coliforms incubated in milk at 90 F, and for 13 to 21 days for samples held at 50 F.

The curves showing the trends in population of a representative strain of *E. coli* in reconstituted nonfat dry milk containing 9% serum solids and incubated at 32, 39, 50 and 90 F are shown in Figure 1. The population curves of this organism were representative of the seven strains of coliforms used in this

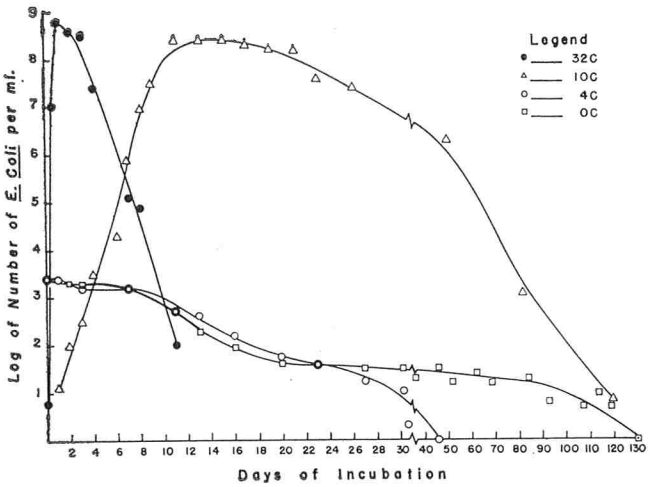

Figure 1. Population curves for *Escherichia coli* in reconstituted nonfat milk containing 9% serum solids and incubated at 32, 39, 50 and 90 F.

study. During incubation at 90 F an initial coliform inoculum of 10 or less per ml reached a maximum number of approximately 10^9 cells per ml in one day. The population decreased only slightly through the third day and abruptly thereafter.

At 50 F initial numbers of 10 or less *E. coli* per ml attained a maximum population of 1.7 to 5.0 x 10^8 after 13 to 21 days. The *E. coli* strain represented by the data in Figure 1 attained a population of 2.7 x 10^8 per ml in 13 days. The *A. aerogenes* strains attained a maximum count of 1.5 to 2.6 x 10^8 per ml in 15 to 17 days. In general at 50 F the most rapid growth of the coliforms occurred between the second and ninth days, followed by a stationary phase and then a gradual decline in population.

An initial inoculum of 2500 *E. coli* cells per ml was added to reconstituted non-fat milk which was stored at 32 and 39 F (Figure 1). For the first 23 days of incubation the gradual decreases in population at the two temperatures were similar. In subsequent incubation the rate of decrease was greater at 39 than at 32 F. At both 32 and 39 F minor fluctuations occurred in the population curves of the seven coliform strains. The data in Figure 1 are typical of the results obtained.

The data in Figures 2 and 3 show the population trends of representative strains of *E. coli* and *A. aerogenes*, respectively, when inoculated into cottage cheese and incubated at 39, 50 and 55 F. The population of all strains of both organisms increased at 55 F and decreased at 39 F but at 50 F the numbers of *E. coli* increased while those of *A. aerogenes* decreased.

Non-inoculated control samples of milk and cheese incubated at the same temperatures and subjected to the same analyses as the inoculated samples consistently contained < 1 coliform per g.

The generation times of *E. coli* and *A. aerogenes* grown in cottage cheese at 50 and 55 F are shown in Table 2. The generation times of the *A. aerogenes* were slightly longer than those of *E. coli.*

A comparison of the generation times of *E. coli* grown in cottage cheese and reconstituted non-fat milk incubated at 50 F is shown in Table 3. These data demonstrate the superiority of the reconstituted nonfat milk over cottage cheese as a growth medium for coliforms. The most rapid growth of *E. coli* occurred within the first five days in the cottage cheese, but between the 5th and 10th days in the reconstituted nonfat milk.

TABLE 2. GENERATION TIMES OF COLIFORM ORGANISMS IN COTTAGE CHEESE

Interval in which generation time was obtained	Incubation temperature			
	55 F		50 F	
	E. coli	*A. aerogenes*	*E. coli*	*A. aerogenes*
	(generation time)			
(days)	*(hr)*	*(hr)*	*(hr)*	
0-5	31.4	32.8	48.2	decrease in population
0-10	35.2	38.0	68.8	decrease in population
0-15	38.7	43.3	98.5	decrease in population
0-20	42.5	47.3	120.4	decrease in population

TABLE 3. COMPARISON OF THE GENERATION TIMES OF ONE STRAIN OF *E. coli* IN COTTAGE CHEESE AND IN RECONSTITUTED NON-FAT MILK INCUBATED AT 50 F

Interval for which generation time was obtained	Cottage cheese	Reconstituted nonfat milk
	(generation time)	
(days)	*(hr)*	*(hr)*
0-5	48.2	12.0
0-10	68.8	10.9
0-15	98.5	14.8
0-20	120.4	population in declining phase

DISCUSSION

Four strains of *E. coli* and three strains of *A. aerogenes* isolated from raw bulk tank milk and subsequently inoculated into sterile reconstituted nonfat milk showed decreases in population at 39 F and increases at 50 F. Kereluk et al *(11)* obtained similar results with *E. coli* isolated from frozen meat pies.

The differences between results reported herein and those of other workers *(2, 15, 16, 17)* who reported growth of coliforms in raw and commercially pasteurized milk incubated at 37 to 39 F may be attributed to *(a)* differences in the strains of coliforms and *(b)* the symbiotic effect of mixed populations found naturally in non-sterilized milk. Perhaps a more extensive study of raw milk supplies would yield psychrophilic coliforms capable of growing in milk at 39 F or lower.

The greater decrease in numbers of coliform organisms at 39 than at 32 F may be attributed to the fact that permease enzymes are inactivated at both 32 and 39 F, but since some metabolic activity occurs at 39 F, toxic products may accumulate which are detrimental to cellular metabolism. Also, the fact that water has maximum density at 39 F may be a contributing factor to the greater death rate at this temperature.

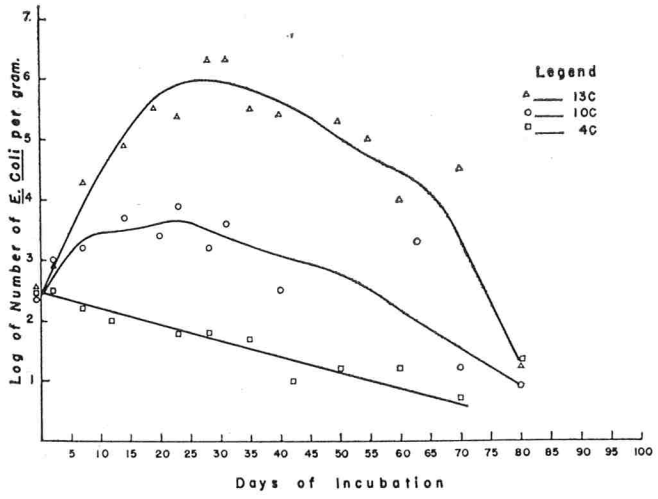

Figure 2. Population curves for *Escherichia coli* in cottage cheese incubated at 39, 50 and 55 F.

At 50 F the generation time for strains of *E. coli* from time of inoculation to time of maximum count was similar to the time reported by Barber *(3)*, but shorter than the time observed by Ingraham *(9)*. However, at 90 F the generation times of *E. coli* during the logarithmic growth phase were comparable to those obtained in broth and reported by other workers *(3, 9, 10)*. The generation time of *A. aerogenes* as reported by Jennison *(10)* was 8 to 12 min longer than found in this study. Age of inoculum and variation in medium used for propagation may account for this difference.

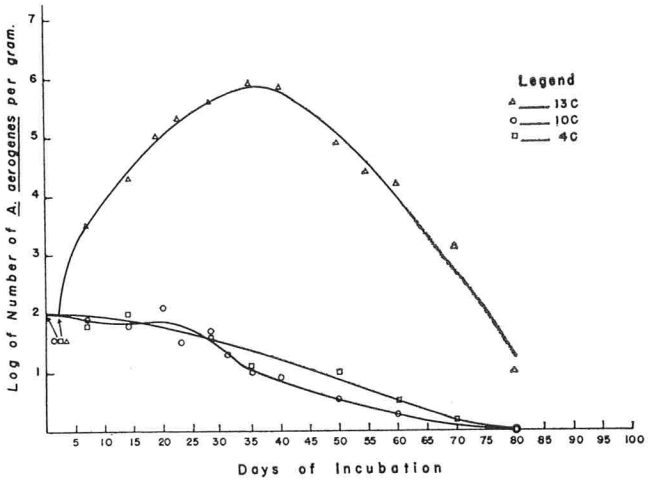

Figure 3. Population curves of *Aerobacter aerogenes* in cottage cheese incubated at 39, 50 and 55 F.

When incubated in cottage cheese *E. coli* tolerated lower temperatures than *A. aerogenes*. *E. coli* grew at 50 and 55 F; whereas *A. aerogenes* grew at 55 but not at 50 F. A decrease in cell numbers occurred at 39 F with both of the coliform organisms and the *A. aerogenes* population declined at 50 F. However, the diminution in cell population was negligible throughout the normal shelf-life of cottage cheese and coliforms persisted after the cheese had been stored for 15 to 20 days at 39 F. Harmon and Smith *(8)* reported that the shelf-life of cottage cheese stored at 42 F averaged 51% longer than corresponding samples held at 50 F.

In the reconstituted nonfat dry milk a coliform population of 1.7 to 5.0 x 10^8 per ml was reached within 13 to 21 days at 50 F., however, in the cottage cheese the maximum count of approximately 10^6 per g occurred between the 20th and 35th days in samples stored at 55 F. The differences in maximum popu-

lation between the two products were attributed to the lower pH and the presence of competitive organisms in cottage cheese. The coliforms are sensitive to pH below 5.0. Harmon and Smith *(8)* found that the coliform population declined in cottage cheese samples held at 42 F which had an initial and terminal pH of 4.8 or less. When the initial and terminal pH was above 5.1 continuous increases in numbers occurred.

Several pH determinations were made on cottage cheese samples during incubation. In one trial the initial pH was 5.1 and no change in pH occurred during the first 14 days at 39, 50 or 55 F. This may explain the rapid growth and shorter generation times during the first 10 days of growth. In another trial the initial pH of the cheese was 5.4 and at 60 days the pH readings were 5.3, 4.8 and 4.4 at 39, 50 and 55 F, respectively. This indicates that the decline in cell numbers at 39 F was primarily due to the effect of temperature rather than pH.

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ANALYSIS OF ANIMAL FOOD PRODUCTS FOR CHLORINATED INSECTICIDE RESIDUES

I. COLUMN CLEAN-UP OF SAMPLES FOR ELECTRON CAPTURE GAS CHROMATOGRAPHIC ANALYSIS.[1, 2, 3]

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(Received for publication December 23, 1963)

Summary

A one step method for sample clean up prior to analysis for chlorinated insecticides by electron capture gas chromatography is outlined. The method has been applied to a variety of animal product samples with excellent success. Sufficient sensitivity is attained so the procedure can be used as a screening procedure or a quantitative research tool. Using the procedure a technician can analyze 25 to 35 samples in an 8 hour day and detect nanogram quantities of chlorinated insecticides in a variety of samples.

Several methods have been used for the analysis of insecticide residues in animal products. Two methods used extensively for analysis of insecticide residues in animal products are the Mills paper chromatagraphic procedure and the Schechter-Haller colorimetric method for DDT *(5, 7)*. These methods require time consuming sample preparations for accurate, sensitive results.

Recently, two special detection systems for gas chromatography, the microcoulometric cell *(1)*, and the electron capture detector *(3)*, have turned the analysts' attention to instrumental analysis of insecticide residues.

Watts and Klein *(8)* reported on the use of electron capture gas chromatography for detection of residues in food products. These authors found it necessary to use a modified Mills clean up procedure to prevent contamination of the detector system.

Moats *(4)* reported the use of a single column clean up procedure for butterfat which was later adapted by Langlois, et al. *(2)* for clean up of all dairy products. Since the procedure was a simple, rapid means of sample preparation for dairy products, attempts to expand its use for clean up of other fat containing animal products were made. This paper presents results with the method on a variety of animal food products and tissue samples.

Methods

Reagent grade methylene chloride and technical grade petroleum ether, B.P. 30-60 C, were redistilled before use. The Entomological Society of America insecticides reference standards used were obtained from Nutritional Biochemical Corporation. The standards and unkown samples were prepared in hexane, B.P. 65-67 C, and stored at 2-5 C. Florisil, 60/100 mesh activated by the supplier at 650 C, was obtained from the Floridin Company, Tallahassee, Florida. The florisil was re-activated by heating at 140 C for 12-14 hr. Five per cent water was uniformly mixed with the florisil and the mixture was held in an air tight container for 48 hr before use. This time was necessary for the water-florisil mixture to reach equilibrium. The eluant was a mixture of 20% methylene chloride in petroleum ether.

Chromatographic columns were 20 mm OD x 600 mm pyrex tubes plugged at one end with glass wool. Analytical columns were ⅛-inch OD x 4 ft pyrex glass, packed with 2.5 or 5.0% Dow 11 Silicone on 60/80 mesh hexamethyldisilazane (HMDS) treated Chromosorb W. The Chromosorb W was obtained in the HMDS treated form from the supplier. The analytical instrument was either a Wilkens Aerograph Hi-Fi Model 600 gas chromatograph with an electron capture detector containing a 250 mc tritium ionization source, or the Wilkens Pestilyzer Model 680 with the same size tritium source. Both instruments were operated with a 90V potential across the detector. The recorder was a 1 mv Leeds and Northrup Model H with a disc integrator unit.

Fluid samples were weighed directly. Solid samples were cut as fine as possible prior to weighing. Weighed product samples were ground with 25 to 30 g of florisil in a glass mortar to form a free flowing powder. Twenty-five g of florisil were poured into a chromatographic column to form the bottom layer. This was prewashed with 50 ml of an equal mixture of methylene chloride and petroleum ether to remove some impurities from the florisil. The

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[1]Published with the approval of the Director of the Purdue Agricultural Experiment Station as Journal Series Paper Number 2256.

[2]Presented at the Fifth International Pesticides Congress, London, England, July 17-23, 1963.

[3]Supported in part by Research Grant No. EF-00-49-02 from the Division of Environmental Engineering and Food Protection, Public Health Service.

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washings were discarded. Next the sample-florisil mixture was poured into the chromatographic column to form the top layer. From 150 to 650 ml of the eluant mixture was used, depending on which insecticide residues were being eluted. The eluant was evaporated to dryness from a beaker in a water bath at 50-60 C. The residues were transferred from the beaker to a calibrated test tube and made up to a 5 or 10 ml standard volume with hexane.

The analytical column was operated at temperatures of 185 to 195 C with a nitrogen (high purity) carrier gas flow of 60 to 80 ml per minute. Standard insecticide solutions were analyzed before and after each series of unknown samples. Depending upon the quantity of insecticide present, from 5 to 25 μl of unknown samples in hexane were used for analysis.

RESULTS AND DISCUSSION

The sample size is limited by its fat content and the size of florisil clean up column used. Overloading the florisil column results in elution of fat and other impurities. If traces of fat are injected into the analytical instrument, they will cause poor stability of the analytical column and loss of instrument sensitivity. Typical sample sizes used for various products are presented in Table 1. By adhering to these sample sizes, proper clean up is achieved as indicated by the use of one analytical column for 400 to 500 analyses before replacement becomes necessary.

This one step extraction and clean up technique has been used for the detection of selected chlorinated insecticides in numerous types of samples as listed in Table 1. Recovery data for added insecticides to samples are presented in Table 2. This is a summary for all types of samples which explains somewhat the standard deviation of 3%. Duplicate samples of milk, for example, agree more closely.

Table 3 contains information on the amounts of eluant needed to eluate the various insecticides studied. Use of less eluant results in a reduction in per cent recovery of residues.

The sensitivity of the combination clean up procedures and electron capture gas chromatographic analysis for five chlorinated insecticides is presented in Table 4. A range is given for each insecticide to allow for various sample sizes as listed in Table 1. The effectiveness of the clean up procedure on a particular sample also effects sensitivity of the analytical procedure. Other factors which enter into the analysis will be discussed in a later paper.

Using analytical column conditions as outlined, an analysis on the instrument for the five insecticides was completed in less than 10 min. DDE and dieldrin were difficult to separate on this particular analytical column. Heptachlor and heptachlor epoxide were

TABLE 1. SAMPLE SIZE USED FOR ANALYSIS

Sample	g
Whole milk	10.0
Cream	2.0 to 5.0
Butter	1.0
Cheese	2.0
Dried whole milk	2.0
Evaporated milk	5.0
Egg yolk	2.0
Chicken fat	1.0
Chicken tissue	1.0
Lard	1.0
Blood	2.0
Soil	1.0
Ground animal tissue	1.0 to 2.0

TABLE 2. RECOVERY OF INSECTICIDES ADDED TO VARIOUS SAMPLES

	Percent recovery[a]	
Insecticide	0.1 ppm	1.0 ppm
DDT	94.0 ± 3%	95.0 ± 3%
Lindane	91.0 ± 3%	91.5 ± 3%
Heptachlor	91.5 ± 3%	92.0 ± 3%
Dieldrin	92.0 ± 3%	92.5 ± 3%
Endrin	88.0 ± 3%	88.5 ± 3%

[a]Average of 6 samples.

TABLE 3. AMOUNT OF ELUANT REQUIRED TO ELUTE SELECTED INSECTICIDES FROM FLORISIL

Insecticide	Ml of eluant
DDT	200
DDE	200
Lindane	200
Heptachlor	300[a]
Heptachlor epoxide	300[a]
Dieldrin	600[a]
Endrin	700[a]

[a]Eluted only from partially deactivated florisil.

TABLE 4. MINIMUM SENSITIVITY OF THIS METHOD FOR SELECTED INSECTICIDES IN VARIOUS SAMPLES

Insecticide	Minimum levels detectable (ppm)
DDT	0.05 to 0.20
Lindane	0.01 to 0.05
Heptachlor or heptachlor epoxide	0.01 to 0.05
DDE	0.01 to 0.05
Dieldrin	0.01 to 0.05
Endrin	0.05 to 0.20

easily separated. Endrin was eluted as described by Phillips, et al. *(6)*.

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COMPARISON OF MEDIA FOR THE ISOLATION OF *CLOSTRIDIUM PERFRINGENS* FROM FOOD.[1, 2, 3]

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(Received for publication January 13, 1964)

SUMMARY

The purpose of the study was to determine how SPS agar of Angelotti et al. (2) compared with certain other solid media proposed for the isolation or identification of clostridia. An attempt was made, therefore, to recover viable cells of three strains of *Clostridium perfringens* from various suspending media using five plating media. The results were analyzed statistically.

The results may be summarized by stating that in every case SPS agar proved to be better than the other media examined for the recovery of the organisms; and that neither the food constituents tested nor mixed cultures of organisms affected the quantitative recovery of *C. perfringens*. The presence of sulfadiazine and polymyxin-B-sulfate in the medium did not affect the recovery of *C. perfringens* in a statistically significant manner.

During the last ten years, considerable interest has been aroused by accumulating evidence that *Clostridium perfringens* is the etiological agent of a mild form of food poisoning. Reports from public health laboratories in many countries, including those in the United States, indicate a substantial number of cases of food poisoning apparently caused by this organism. Symptoms usually consist of diarrhea and abdominal cramps and appear suddenly, 8-12 hr after ingestion of an infected food, although quoted figures vary somewhat on this point.

It has been suggested further that the number of cases of food poisoning recorded as caused by *C. perfringens* should in fact be considerably higher. Kemp et al. (8) indicated that some outbreaks reported to be of unknown etiology may have been caused by *C. perfringens* and that poor anaerobic techniques had prevented the bacterium being recovered.

Under these circumstances, the importance of a reliable medium for quantitatively isolating *C. perfringens* from food and fecal specimens in epidemiological work is obvious. Hobbs et al. (7) used direct plating on horse-blood agar for the isolation of *C. perfringens*. Other laboratories have preferred modifications of the sulfite-iron-glucose agar first proposed by Wilson and Blair (17). The use of the latter medium, and those developed from it, depends upon the ability of clostridia to reduce sulfite to sulfide with the resulting precipitation, in the presence of iron, of black iron sulfide.

Media suitable for the quantitative recovery of sulfite-reducing clostridia were proposed by Mossel et al. (11) and Mossel (10). The first medium suggested was a modification of the Wilson and Blair medium. In the latter paper the incorporation of 10 ppm of polymyxin-B-sulfate into the medium was suggested since the addition of this antibiotic suppressed the growth of many strains of Enterobacteriaceae. Mossel's medium was later modified by Angelotti et al. (2), by the addition of sodium sulfadiazine to suppress the growth of *Proteus, Pseudomonas* and coliforms.

The purpose of this study was to determine how the sulfite-polymyxin-sulfadiazine agar (SPS agar) of Angelotti et al. (2) compared with certain other solid media proposed for the isolation or identification of clostridia. An attempt was made, therefore, to recover viable cells of three strains of *C. perfringens* from three suspending media using five plating media selected for the study, and the results were analyzed statistically.

MATERIALS AND METHODS

The five plating media[4] selected for camparison are listed below:

1. Sulfite-polymyxin-sulfadiazine agar (SPS agar), prepared as outlined by Angelotti et al. (2) except that the final pH of the medium was 7.1-7.2.

2. Sulfite-polymyxin-sulfadiazine agar (SPS agar w/o) in which the sulfadiazine and polymyxin-B-sulfate solutions were omitted.

3. Sulfite-iron-agar (17). Bacto-Nutrient agar was used for preparing the medium. Since the original medium was developed for the examination of water, 20 ml of medium to be mixed with 20 ml of water under test, the quantities of nutrients and agar were halved for use in the present study.

4. Yeast-extract-blood agar (15). Seitz-filtered Bacto-Yeast Extract was used as a substitute for the yeast extract sug-

[1]Published with the permission of the Director of the Wisconsin Agricultural Experiment Station, Madison.

[2]This investigation was supported in part by Public Health Research Grant EF-131 (C1.) from the Division of Environmental Engineering and Food Protection.

[3]These data are a part of a thesis submitted to the Graduate School of the University of Wisconsin in fulfillment of the requirements for the Master of Science degree by the senior author.

[4]Media were prepared using products of Difco Laboratories, Detroit, Michigan.

gested in the original paper.

5. Lactose-egg-yolk-milk-agar *(16)*. The meat infusion broth used as a basis for the medium was prepared according to the method outlined in the *Manual of Microbiological Methods, (12)*. Bacto-Agar was substituted for New Zealand agar, the quantity being adjusted to give a final concentration of 1.5%.

The strains of *C. perfringens* used for the study included:

1. *C. perfringens* N.C.T.C. 8799 F 1546/52 of Hobbs serological type 10[5]. This strain was isolated from a 1952 food poisoning outbreak in Great Britain.

2. A strain (214d) isolated from a fecal specimen obtained from a patient who was afflicted in a food poisoning outbreak which occurred in Milwaukee, Wisconsin in March, 1962[6].

3. A classical strain of *C. perfringens* type A, Strain 26 #3624, obtained from the American Type Culture Collection.

Vegetative cells of the three strains employed were recovered from each of three suspending media using the five plating media outlined above. The suspending media were as follows: *(a)* thioglycollate medium without dextrose or indicator; *(b)* a commercially canned cream of chicken soup, diluted 1:1 with tap water to give the serving consistency recommended on the label of the product; and *(c)* a mixed culture in a food, the latter being cream of chicken soup described under *(b)*. Micro-organisms added to give a mixed culture were *Streptococcus faecalis* ATCC 9790, *Staphylococcus aureus* 196, *Escherichia coli* gratia. In preparing the broth cultures for inoculation of the soup, S. *faecalis* and S. *aureus* were grown in brain heart infusion and *E. coli* in nutrient broth.

Actively growing, 4-hr cultures of *C. perfringens* in thioglycollate broth w/o dextrose or indicator, adjusted to a predetermined optical density using a simple turbidimeter[7] were used for all inoculations. One in ten dilutions were made of standardized culture in thioglycollate medium or food, with or without the added mixed culture. For the mixed culture, 16-hr broth cultures of each of the "contaminating organisms" listed above were added to the diluted soup. The thioglycollate culture of *C. perfringens* was then added giving a final ratio of 1 part *C. perfringens* culture to 3 parts mixed culture to 6 parts soup.

Serial dilutions of the inoculated suspending media were made in phosphate buffered dilution water *(1)*. Triplicate plates were made immediately using 0.1 ml amounts of the 10^{-5} dilution as an inoculum for each plate for the five media being studied. Double layers of medium were poured to increase anaerobiosis in all cases; however, for YEB and LEYM agar both layers were thin because of the opacity of these media.

The plates were incubated at 37 C in an anaerobic jar[8]. After evacuating the jar with a water aspirator pump to 25 cm Hg negative pressure, the jar was refilled with a gas mixture of 90% N_2 and 10% CO_2. The procedure was repeated three times in all to insure an anaerobic atmosphere. Plate counts were made after 24 hr incubation, and checked again after an additional 24 hr.

The statistical design for these experiments was the split plot. For reasons to be explained, however, it was necessary to analyze the data obtained as a randomized complete block. In addition to an analysis of variance, Duncan's New Multiple Range Test *(13)* was applied.

RESULTS

The results of the study are presented in Table 1. The SPS agar and the SPS agar without polymyxin-B-sulfate or sulfadiazine appeared to permit the recovery of the greatest number of cells and did not differ significantly from each other. Under conditions of these experiments no growth for any of the three strains was attained on the SI or LEYM agar. The YEB agar permitted recovery of cells but at a significantly lower level than the SPS agars.

Also, on YEB agar, in testing the recovery of the *C. perfringens* from mixed culture, it was not possible to differentiate between the food poisoning strains of *C. perfringens* and the other organisms present; the classical strain of *C. perfringens* gave a typical zone of β-hemolysis, which permitted its differentiation from the other micro-organisms.

The original experimental design for the study was that of the split plot. Since no growth was obtained on the SI or the LEYM agar, these results necessarily had to be omitted from the statistical analyses. Likewise, because of the inability to distinguish between the food poisoning strain of *C. perfringens* and the "contaminating organisms" on YEB agar, certain results had to be excluded from the statistical calculations. For these reasons all data were analyzed as a randomized complete block.

Since the difference between treatments was significant, treatment means were compared using Duncan's New Multiple Range Test *(13)*. Comparisons were not made between all treatment means since only certain ones were relevant. Means compared were those in which cells had been recovered either from the same suspending medium, or using the same recovery medium. For all three strains, significant differences were found to exist between

[5]This culture was obtained through the courtesy of Dr. Betty C. Hobbs.

[6]This culture was obtained through the courtesy of Dr. E. R. Krumbiegel.

[7]Lumetron Photoelectric Colorimeter, Model 400-A, Photovolt Corporation, New York, New York.

[8]Anaero-jar, Case Laboratories, Inc., Chicago, Illinois.

TABLE 1. RECOVERY ON FIVE PLATING MEDIA OF THREE STRAINS OF *Clostridium perfringens* FROM THIOGLYCOLLATE MEDIUM, AND FROM CHICKEN SOUP, WITH AND WITHOUT AN ADDED MIXED CULTURE.

Strain	Suspending medium	SPS agar[a]	SPS agar[b] w/o	SI agar[c]	YEB agar[d]	LEYM agar[e]
			(Colonies per plate[6])			
8799 F	Fluid thioglycollate	151[a]	153[a]	0	120[b]	0
1546/52	Chicken soup	153[a]	161[a]	0	125[b]	0
	Soup + mixed culture	161[a]+*	161[a]+*	*	**	*
214d	Fluid thioglycollate	103[a]	106[a]	0	79[b]	0
	Chicken soup	110[a]	108[a]	0	77[b]	0
	Soup + mixed culture	109[a]+*	113[a]+*	*	**	*
ATCC type A	Fluid thioglycollate	40[a]	39[a]	0	27[b]	0
26#3624	Chicken soup	49[a]	47[a]	0	29[b]	0
	Soup + mixed culture	49[a]+*	49[a]+*	*	34[b]+*	*

[1]Sulfite-polymyxin-sulfadiazine agar *(2)*.

[2]Sulfite-polymyxin-sulfadiazine agar without polymyxin-B-sulfate or sulfadiazine.

[3]Sulfite-iron agar *(16)*.

[4]Yeast-extract-blood agar *(15)*.

[5]Lactose-egg-yolk-milk agar *(16)*.

[6]Each value represents the mean of three replicates, each replicate having been plated in triplicate. The inoculum for each plate was 0.1 ml of a 10^{-5} dilution of standardized suspension.

*+ indicates that an uncounted number of colonies of "contaminating organisms" were present.

**Indicates growth which may have included *C. perfringens* as well as "contaminating organisms".

[a, b]Where exponent letters differ within a strain, recoveries differ significantly.

SPS agar and YEB agar and SPS agar without antibiotics or sulfadiazine and YEB agar when cells were recovered from thioglycollate medium or the chicken soup. No other differences were significant for the two food poisoning strains. In the case of the classical strain, significant differences also existed between YEB agar and SPS agar, and YEB agar and SPS agar w/o antibiotics or sulfadiazine when *C. perfringens* was recovered from a mixed culture.

DISCUSSION

The objective of the study was to evaluate the effectiveness of SPS agar in quantitatively recovering *C. perfringens* from various suspending media. For purposes of comparison, four other media were used. The SI agar of Wilson and Blair *(17)* was chosen for its historical interest. This medium was proposed at an early date, in Britain, for the identification of sulfite-reducing clostridia in water supplies. In the present study, no growth was obtained on this medium from any of the strains of *C. perfringens* examined. It seemed possible that the peptone used in Bacto-Nutrient Agar differed in composition from that used by British workers.

To further elucidate this point, therefore, additional experiments were carried out in which an attempt was made to recover the three strains of *C. perfringens* used in the main study on Wilson and Blair medium in which Bacto-Peptone was replaced by Bacto-Tryptone or Proteose Peptone No. 3, in the preparation of the nutrient agar base. In addition, the medium of Thompson *(14)* and that recommended by the Ministry of Health *(9)* for the examination of water were also used. The latter two media were similar in composition to the original Wilson and Blair medium.

SPS agar was used as the control to indicate the numbers of cells used to inoculate plates for the Wilson and Blair media prepared using the various nitrogen sources. In every case, no recovery was effected on any of the sulfite-iron media so modified when the inoculum was of such concentration that 300 cells or less were present per plate as indicated by SPS agar. When the number of cells used to inoculate each plate was increased to approximately 3×10^6, some growth resulted, ranging from one large colony per plate to an uncountable number of colonies accompanied by complete blackening of the medium. Some strain difference was noted — the Milwaukee strain and ATCC Type A were recovered in greater numbers than Hobbs Type 10.

It thus appeared that a possible explanation for the failure to recover organisms on the Wilson and Blair medium in the original experiments lay, in

part at least, in the size of the inoculum used. This possibility is not supported by the observations of the British workers, however, who were able to detect relatively small number of cells of *C. perfringens* by use of this medium. Other possible explanations might be the use of the phosphate buffered water as a diluent (although cells did not remain in the dilution blanks for more than 15 min), the use of thioglycollate broth as the initial growth medium or a weakening of strains similar to that described by Gibbs and Hirsch *(4)*.

Hobbs et al *(7)* and Hobbs *(6)* recommended a blood agar for the initial isolation of *C. perfringens* from stool and food samples, hence the inclusion of a medium of this type in the present study. Wetzler's yeast-extract-blood agar was selected since its composition made it appear particularly suited to the recovery of clostridia. However, although *C. perfringens* grew on this medium and, in the case of the classical strain, ATTC Type A, could be identified by the production of the zones of β-hemolysis, for every strain examined recovery was inferior to that obtained on SPS agar.

Willis and Hobbs *(16)* recommended lactose-egg-yolk-milk agar for the preliminary examination of pure cultures of clostridia since egg yolk, milk and lactose plus neutral red act as indicators of lecithinase production, proteolysis and fermentation reactions respectively. This medium was included in the study since it constituents were very different from the sulfite-containing media and blood agar, making it useful for purposes of comparison. The only discernible difference in the preparation of the medium was in the source of agar and in the preparation of the meat broth. The negative results obtained here suggest that small modifications in the preparation and use of a medium for growing fastidious organisms may render that medium useless. The medium was chosen as one of the media of choice in the studies of Collee, Knowlden and Hobbs *(3)*. These workers were successful in obtaining growth on the medium.

Media of great interest in the study were the SPS agar and SPS agar w/o sulfadiazine and polymyxin-B-sulfate. Hac and Eilert *(5)* showed that sulfadiazine was highly effective in increasing the survival rate of mice experimentally infected with *C. perfringens*, therefore, it seemed possible that the amount of this substance recommended for use in SPS agar might be sufficient to prevent quantitative recovery of the organisms. Statistical analyses of results, however, showed no significant difference in the effectiveness of SPS agar with and without sulfadiazine and polymyxin-B-sulfate in recovering the strains of *C. perfringens* used.

In no case was there a significant difference between the numbers of cells recovered on SPS agar from thioglycollate broth and chicken soup with and without an added mixed culture. These data indicate that food constituents as represented here did not interfere with black colony formation, nor did they affect the ability of the medium to yield quantitative recovery of *C. perfringens*. These findings agree with those reported earlier by Angelotti, et al. *(2)*. It should be recognized the possibility exists that bacterial cells grown in a food product such as the chicken soup might conceivably react differently from cells grown on a different medium and later added to the soup.

The SPS medium was not designed to differentiate between members of the sulfite-reducing clostridia, although with experience, the colony form of *C. perfringens* can frequently be recognized. Growth of other organisms, however, is usually not significant except on prolonged incubation.

ACKNOWLEDGEMENT

The authors acknowledge, with appreciation, the cooperation of Dr. James H. Torries for assistance with the statistical design of the experiment.

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SOME FACTORS WHICH GOVERN PLAQUE FORMATION BY LACTIC STREPTOCOCCAL BACTERIOPHAGE.

I. SEED LAYER REQUIREMENTS[1]

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(Received for publication December 20, 1963)

SUMMARY

A study was conducted of factors which may influence the accuracy of the plaque count obtained by the double-agar layer method as applied to lactic streptococcal bacteriophage. Host cell cultural media were skimmilk or hydrolyzed milk protein broth (Sobol's). *Streptococcus lactis* strains ML3, C10, C2 and *Streptococcus cremoris* strains C3, KH, R1, US3 and the homologous bacteriophages were used. Plaque formation on Trypticase Soy Agar was generally enhanced by: *(a)* modification of the cultural media or the seed layer agar by the addition of $CaCl_2$, phenylalanine, proline, riboflavin, niacin, calcium pantothenate, and biotin; *(b)* using a six-hr culture of host cells rather than cells incubated for 18 or 24 hr; and *(c)* selection of the proper serial transfer system for reactivating the host cells from a frozen or lyophilized state. Sobol's broth yielded lower plaque counts than skimmilk irrespective of the modifications used. Responses to plaque enhancement factors were influenced by species and strain differences of S. *lactis* and S. *cremoris*. In general, strains of S. *cremoris* had a more exacting requirement for calcium, media enrichment, and length of incubation of host cells than S. *lactis* strains.

Several procedures for determining bacteriophage (phage) titer are in current use: namely, the double agar layer technique, dilution end-point, changes in turbidity, and failure to produce acid. Of these methods, Adams *(1)* concluded that the agar layer technique yields the most accurate information.

It was observed in our laboratories that the agar layer technique gave varying and inconsistent results when applied to certain lactic streptococcal phage. As a consequence, plaque counts obtained did not reflect accurately the level of phage infection.

This study was undertaken to ascertain the nature of these inconsistencies and to develop a standardized procedure for the enumeration of lactic streptococcal phage.

PROCEDURE

The following Lancefield Group N streptococcal strains and the homologous phages were used: *Streptococcus lactis* C10, ML3, C2, and *Streptococcus cremoris* C3, KH, US3, R1.

Plaque counts, with certain modifications, followed the agar layer technique described by Adams *(1)*. Trypticase Soy Agar (TSA), obtained from Baltimore Biological Laboratory, Inc., was used as the basic agar.

The foundation agar layer was prepared by pouring 12-15 ml of melted TSA agar onto an 100 mm heavy bottom petri dish. The poured plates were dried overnight in a 30 C incubator.

The seed (cover) layer consisted of 4 ml of melted TSA agar, tempered to 46 C, and diluted with 4 ml of a host cell suspension.

Routinely, 6-hr growth of host cells in Sobol's broth *(4)* or reconstituted 11% T.S. skimmilk (Matrix Mother Culture Media, Galloway-West Co., Fond du Lac, Wisc.) was used for the preparation of the seed layer cell suspension. The skimmilk was sterilized by autoclaving at 15 lbs for 10 min. Host cells carried in skimmilk were diluted with sterile distilled water to give a 10% concentration. This was done because preliminary studies had shown that cells grown in skimmilk and used directly produced plaque plates which were opaque and covered with clotted milk, and thus did not lend themselves to ideal observation of lysed areas. Furthermore, use of skimmilk instead of distilled water as the diluting vehicle induced excessive foaming which interfered with accurate sample measurement and produced air bubbles in the solidified seed layer. Cells carried in broth were usually added to the melted seed layer without further dilution.

Additives were used as follows: *(a) amino acids* — 100 mg L-proline and 200 mg DL-phenylalanine were added directly to 1 liter of agar or liquid media prior to sterilization; *(b) vitamins* — prepared as Seitz sterilized stock solutions and stored at 4 C, were added directly to the melted seed layer or cultural broth just prior to inoculation with host cells to yield a final concentration per liter of 1 mg niacin, 1 mg riboflavin, 1 mg Ca pantathenate, and 10 μg biotin; *(c)* $CaCl_2$ — was used as a sterile stock solution, with an 0.1 ml portion added just prior to use, yielding a final concentration of 0.33% in the media, as recommended by Deane and Nelson *(3)*.

[1]Article Number 20:63. Department of Dairy Technology, Ohio Sate University. Supported by a grant from the U. S. Public Health Service (National Institutes of Health).

TABLE 1. INFLUENCE OF ADDED $CaCl_2$ UPON THE PLAQUE-FORMING ABILITY OF LACTIC STREPTOCOCCAL PHAGE UTILIZING HOST CELLS CARRIED IN SKIMMILK OR BROTH

Organism	No. trials	Host cell cultural media: Skimmilk, Control	Skimmilk, With $CaCl_2$[a]	Sobol's broth, Control	Sobol's broth, With $CaCl_2$[a]
		(*PFU/ml*)			
S. cremoris C3	6	75	234 (212%)[b]	<1	71 (700%)[b]
S. cremoris KH	6	193	261 (35%)	133	209 (57%)
S. cremoris R1	7	113	226 (100%)	100	183 (83%)
S. cremoris US3	7	175	206 (18%)	121	153 (26%)
S. lactis ML3	6	238	306 (22%)	164	236 (44%)
S. lactis C10	4	260	264 (<1%)	228	227 (<1%)
S. lactis C2	4	124	126 (<1%)	163	174 (<1%)

[a]$CaCl_2$ was added to the seed layer at a final concentration of 0.33%.

[b]Percentage increase in PFU upon the addition of $CaCl_2$.

Fresh phage stock solutions in Sobol's broth were held at 4 C for periods not exceeding 14 days. The phage titer did not decline significantly during this period. For the preparation of plaque plates, phage stock solutions were serially diluted in buffered water (2) and added directly in 0.1 ml quantities onto the surface of the dried foundation layer plates. Dilutions were adjusted to result in a multiplicity of infection of approximately 0.1.

The melted seed layer with the suspension of host cells, which comprised a total volume of 8 ml, was mixed gently and equal quantities were added to duplicate foundation layer plates containing added phage. The plates were rotated in a circular fashion to insure maximum phage-host cell contact and proper distribution of plaque areas. The plates were incubated in an up-right position at 30 C. Incubation was continued until a thick mat of host-cell growth was observed with clearly discernible plaques. This condition was usually attained within 6-8 hr. The clear, lysed zones were counted in a Quebec bacteria colony counter (2) and reported as plaque-forming units per ml (PFU/ml).

RESULTS AND DISCUSSION

Results obtained for the effect of calcium on plaque formation for 7 different lactic streptococcal phage races are presented (Table 1). For both the skimmilk and broth series, the addition of $CaCl_2$ to the seed layer produced significant increases in the number of plaques formed by five of the phages (c3, kh, us3 and ml3), but had no effect upon plaque formation of the other two phage races studied (c10 and c2).

The degree of response of the phages to the addition of calcium varied with individual phage races and with the type of cultural medium used to propagate the host cells. With c3 phage, calcium increased plaque formation from 75 to 234 PFU for host cells carried in skimmilk and from <1 to 71 PFU for host cells cultured in Sobol's broth. This would indicate that, with the double agar layer method, lysis of *S. cremoris* C3 cells by its homologous phage is depressed unless calcium is present, with this effect being particularly pronounced when host cells are grown in a partially defined broth.

TABLE 2. EFFECT OF ENRICHMENT OF THE SEED LAYER OR CULTURAL BROTH WITH SELECTIVE ADDITIVES UPON THE PLAQUE-FORMING ABILITY OF *S. cremoris* C3 WITH c3 PHAGE

Additive	Seed layer[a] PFU/ml	Seed layer[a] Change	Sobol's broth[a] PFU/ml	Sobol's broth[a] Change
		(%)		(%)
Control (no additives)	154	0	145	0
Riboflavin (1 mg/1)	187	+21	156	+ 8
L-Proline (100 mg/1)	172	+12	207	+43
DL-Phenylalanine (200 mg/1)	203	+32	231	+59
DL-Tryptophan (200 mg/1)	128	−17	123	−15
Combination of 4 above additives	240	+56	243	+68

[a]Average of 4 trials for the seed layer and 5 trials for Sobol's broth.

TABLE 3. PLAQUE-FORMING ABILITY OF LACTIC STREPTOCOCCAL PHAGE AS INFLUENCED BY FORTIFICATION OF THE HOST CELL CULTURAL MEDIA

Organism	No. trials	Host cell cultural media: Skimmilk Unfortified	Skimmilk Fortified[a]	Skimmilk % [b]	Sobol's broth Unfortified	Sobol's broth Fortified[a]	Sobol's broth % [b]
		(PFU/ml)			*(PFU/ml)*		
S. *cremoris* C3	6	234	294	+26	71	107	+51
S. *cremoris* KH	7	261	307	+18	209	256	+23
S. *cremoris* R1	7	226	240	+ 6	183	227	+24
S. *cremoris* US3	7	206	271	+32	153	222	+45
S. *lactis* ML3	6	306	329	+ 8	236	320	+36
S. *lactis* C10	4	264	231	−13	227	228	0
S. *lactis* C2	4	126	170	+35	174	218	+25

[a]Fortification was at the rate of 1 mg riboflovin, niacin, and Ca pantothenate/liter, 10 μg biotin/liter; 100 mg L-proline/liter; and 200 mg DL-phenylalanine/liter.

[b]Percentage change upon the fortification of the basic host cell cultural media.

The other phage races which were active against S. *cremoris* strains exhibited a varying and somewhat lower requirement for calcium than the c3 phage. For example, the addition of the calcium increased the plaque counts of phage r1 from 113 to 226 PFU in skimmilk and from 100 to 183 PFU in broth.

Of the three S. *lactis* phage races studied, only m13 showed a significant increase in plaque numbers upon addition of calcium, with the increase amounting to 22% with the skimmilk cultures and to 44% with the broth cultures.

It was evident that the calcium requirements for plaque formation by lactic streptococcal phage are strain dependent, and, in general, phage active against S. *cremoris* organisms have a more exacting requirement for calcium than phage lysing S. *lactis* strains. Consequently, the characteristic of a given host cell-phage combination must be known in respect to the calcium requirement for lysis in order to attain effective plaque formation on double agar layer plates.

As a result of these findings, $CaCl_2$, to yield a final concentration of 0.33%, was added routinely to all seed layer preparations used in the agar layer technique.

To investigate further the findings that host cells cultured in Sobol's broth tended to produce less PFU than did cells grown in skimmilk, an experiment was conducted in which the seed layer or cultural broth was fortified with riboflavin, proline, phenylalanine and tryptophan and the influence of the fortification upon plaque formation. S. *cremoris* C3 host cells with homologous phage were used in this phase of the study. The results reveal (Table 2) that the additives increased the plaque counts for all series with the exception of that for tryptophan which exhibited a depressing action. Of the single additives, phenylalanine was the most effective stimulant for plaque formation, increasing plaque counts by 32% when the seed layer was fortified and 59% when added to the cultural medium. However, the mixture of additives produced the greatest increase in PFU in all trials.

At about the time these studies were in progress, a paper appeared by Reiter and Oram *(6)* in which it was reported that niacin, pantathenate, and biotin were required by lactic streptococcal organisms. Consequently, in all further work, these compounds, in addition to riboflavin, proline and phenylalanine were included as part of the fortification mixture.

Results were obtained on the effect of fortification of host cell cultural media (skimmilk and broth) upon the plaque formation by 7 different phage races (Table 3). With the exception of phage c10, the enrichment of the cultural media had a stimulatory effect upon plaque formation by lactic streptococcal phage with increases ranging from 6 to 35% for the skimmilk medium and 23 to 51% for Sobol's broth. However, plaque counts of phage c10 were depressed 13% upon fortification of skimmilk and no response was noted with enrichment of Sobol's broth.

As a host cell cultural medium, fortified skimmilk yielded higher plaque counts for phage races c3, kh, r1, us3, m13 and c10 than fortified broth. Thus, despite the increased plaque formation observed upon fortification, Sobol's broth did not support maximum plaque formation, generally. Phage c2 was the only phage race studied which yielded higher plaque numbers for Sobol's broth than for skimmilk. However, fortification of the cultural medium increased c2 plaque formation by 35% and 25%

TABLE 4. RELATIVE EFFICIENCY OF PLATING AS INFLUENCED BY THE AGE OF THE HOST CELL CULTURED IN VARIOUS MEDIA

Organism	Age of cells	Cultural media: Skimmilk, Unfortified	Skimmilk, Fortified[a]	Sobol's broth, Unfortified	Sobol's broth, Fortified[a]
	(hr)	*EOP*[b]			
S. *cremoris* C3	6	1.00	1.00	1.00	1.00
	18	0.90	0.87	0.70	0.47
	24	0.84	0.63	0.58	0.35
S. *cremoris* KH	6	1.00	1.00	1.00	1.00
	18	0.94	1.02	0.63	0.61
	24	0.90	0.91	0.42	0.32
S. *cremoris* R1	6	1.00	1.00	1.00	1.00
	18	0.89	0.93	0.64	0.49
	24	0.76	0.75	0.31	0.19
S. *cremoris* US3	6	1.00	1.00	1.00	1.00
	18	0.81	0.64	1.02	0.57
	24	0.92	0.58	0.84	0.20
S. *lactis* ML3	6	1.00	1.00	1.00	1.00
	18	1.03	1.12	0.50	0.64
	24	0.78	0.84	0.24	0.68
S. *lactis* C10	6	1.00	1.00	1.00	1.00
	18	1.21	1.25	0.96	1.04
	24	1.22	1.42	0.82	0.86
S. *lactis* C2	6	1.00	1.00	1.00	1.00
	18	1.16	1.01	0.89	0.86
	24	1.10	1.01	0.80	0.52

[a]Fortification was at the rate shown in Table 3.

[b]Relative efficiency of plating was based upon the arbitrary value of 1.00 assigned to the 6 hr cells in each of the media.

with skimmilk and Sobol's broth, respectively.

Various investigators *(1, 5)* have suggested the use of overnight or 18-hr cultures for the preparation of the host cell suspension used in the seed layer. Observations revealed, however, that the phage absorption rate appeared to be depressed with aged lactic streptococcal host cells *(4)*. Consequently, a study was made of the influence upon plaque formation of the length of incubation (age) of the host cell used for the preparation of the seed layer. Fortified and unfortified skimmilk and broth were used to culture the host cells. Plaque counts were determined using host cells incubated at 30 C for 6, 18 and 24 hr. Relative efficiency of plating (EOP) was calculated arbitrarily assigning the value of 1.00 to plaque counts obtained with host cells incubated for 6 hr in each of the media.

The results reveal (Table 4) that regardless of the cultural media, phage races which were active against S. *cremoris* strains, generally, yielded maximum plaque formation when 6-hr host cells were used, with plaque counts decreasing progressively as the age of the host cell was increased to 24 hr. S. *cremoris* host cells carried in fortified broth for 18 or 24 hr had a lower EOP than similarly handled cells cultured in any of the other three media.

Similar results were obtained for S. *lactis* strains carried for 6 hrs in unfortified or fortified Sobol's broth. In contrast, when skimmilk (unfortified or fortified) was utilized for S. *lactis* host cells, plaque counts obtained with 18-hr cells were equal to or greater than counts obtained when 6-hr cells were used. The use of 24-hr S. *lactis* cells cultured in skimmilk yielded variable results for phage m13, phage c10, and phage c2 as compared to the series using younger cells.

Thus, considerable variability in response to age of host cells was noted between strains, with phage lysing S. *cremoris* organisms demonstrating a more exacting requirement for the use of 6-hr host cells in the preparation of the seed layer suspension than the phage which were active against S. *lactis* strains. This strain difference was observed earlier for the calcium additive requirement. As a result of these studies, it is recommended that young (6 hr) host cell be used routinely in the preparation of host cell suspensions for the double agar layer technique.

Normally, host cells used in these studies were either reactivated from frozen skimmilk cultures or from a lyophilized state on a regular biweekly basis. This was done as a precaution against possible contamination and in order to insure an actively growing culture. It was noted that where S. *cremoris* C3 host cells were used, which had been kept in an active state for an extended period of time, less plaques were formed than when the host cells were freshly activated from a lyophilized or frozen culture. In order to ascertain this effect more closely, an experiment was conducted in which one set of host cells had been kept in an active state by daily transfer of an 0.1 ml aliquot of the previous day's culture into fresh cultural media and by repeating this proc-

TABLE 5. INFLUENCE OF THE NUMBER OF SERIAL TRANSFERS OF S. *cremoris* C3 HOST CELLS UPON THEIR ABILITY TO FORM PLAQUES WITH c3 PHAGE

Host cell serial transfer media	No. of transfers of host cells: 4-10	30-36	Change
	(PFU/ml)		*(%)*
Skimmilk—unfortified	168	132	−21.4
—fortified[a]	205	145	−29.2
Sobol's broth—unfortified	91	135	+48.4
—fortified[a]	107	113	+ 5.6

[a]Fortification was at the rate shown in Table 3.

ess over a period of 30 days: the other set of host cells were freshly activated (4 serial transfers) from a lyophilized culture.

Results reveal (Table 5) that the host cells which were propagated for 30 days in unfortified or fortified skimmilk lost 21 to 29% of the plaque-forming ability when compared with plaque counts obtained with freshly reactivated host cells. In contrast, successive transfers of host cells in unfortified Sobol's broth improved the plaque-forming ability of the host cells by 48%. Only a slight (5.6%) effect was evident when fortified broth was used as the cultural medium.

It would appear that adaptation by host cells to growth in a partially defined broth increases their susceptibility to lysis by phage; for cells carried in skimmilk the reverse occurs. This finding may have practical commercial application as current starter culture practices recommend the use of freshly reactivated cultures on a regular basis which would, in turn, increase the susceptibility of such cells to phage action. Conversely, earlier cheese plant practices have involved reuse of starter cultures over long periods of time by carrying cultures in an active state by daily transfer or by using whey from the previous day's run. This practice may, in effect, have reduced the phage susceptibility of the cultures when considered in terms of the findings in this study.

ACKNOWLEDGMENTS

Appreciation is expressed to I. A. Gould and T. Kristoffersen for counseling in respect to the study and for assistance in preparation of the manuscript.

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AMENDMENT TO 3-A ACCEPTED PRACTICES FOR SUPPLYING AIR UNDER PRESSURE IN CONTACT WITH MILK, MILK PRODUCTS AND PRODUCT CONTACT SURFACES

Formulated by
International Association of Milk, Food and Environmental Sanitarians
United States Public Health Service
The Dairy Industry Committee

The "3-A Accepted Practices for Supplying Air Under Pressure in Contact with Milk, Milk Products and Product Contact Surfaces," published April 1964, effective date July 26, 1964, are hereby amended in the sections indicated below:

Substitute the following for subsection C.(3) of the April 1964 issue of these Practices:

3. PIPING: Air distribution piping, fittings, and gaskets between the downstream terminal filter and the processing equipment except where the compressing equipment is of the fan or blower type and except as provided in section H shall conform to 3-A "Sanitary Standards for Fittings Used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Milk and Milk Products," and Supplements thereto except that where air distribution piping, or fittings and gaskets do not actually contact the product or form a part of the product contact surfaces, transparent plastic tubing may be used.

Delete the words, "High pressure" from subsection D.(1)(c), and alter subsection to read:

(c) Water-lubricated or non-lubricated blowers.

Add a new Section H, to follow Section G, as follows:

H. SPECIAL REQUIREMENTS FOR MOVING CONTAINERS FROM ROTATING MANDRELS

(1) When air under pressure is used for moving containers from rotating mandrel assemblies with integral air passages, the parts forming the air passages shall be of non-toxic, relatively non-absorbent materials.

(2) A disposable media filter shall be located at the closest possible point upstream from the rotating mandrel assembly (See Figure #5).

Append a new drawing, designated, "Figure 5 Rotating Mandrel Assembly," to the series of figures.

This amendment shall become effective July 26, 1964.

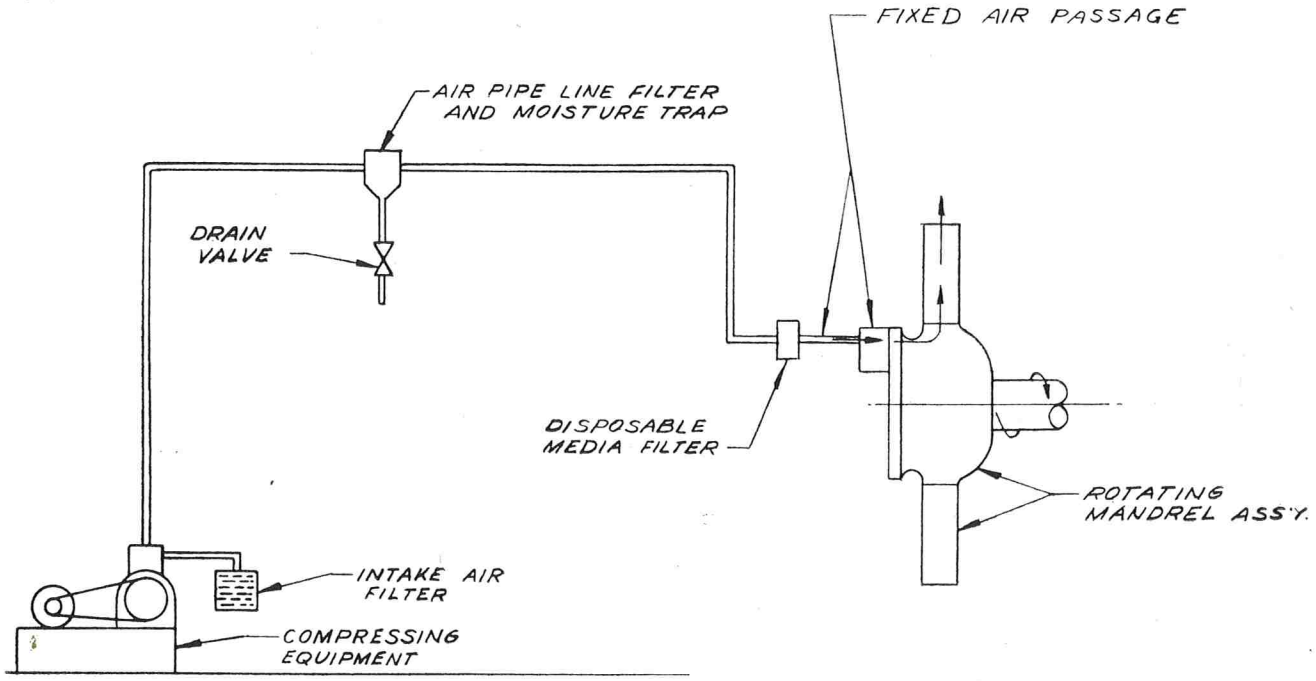

FIG. 5

ROTATING MANDREL ASSEMBLY

IN-LINE CONTROL OF BACTERIA BUILD-UP IN PROCESSING

AMIHUD KRAMER

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In setting up any control system, it is first necessary to define exactly what is to be controlled; find a precise, accurate, and rapid testing procedure; set-up control stations where the tests are to be taken and post results so that action can be taken promptly when needed.

Definition.

In this discussion our problem may be defined as the detection and prevention of bacterial build-up anywhere in the processing line that may result in the production of an unwholesome, spoiled product. Although bacterial spoilage may be eliminated by the application of excessive heat or acidification, such excessive treatments may play havoc with flavor, color, texture, and other quality attributes of the food. The ultimate goal of providing a good quality end product may, therefore, be accomplished only by the use of sanitary materials and by maintaining sanitary manufacturing conditions. These may be controlled by routine testing for bacterial build-up.

Testing Procedures.

The absolute test of the effectiveness of the process is the quality and performance of the finished product. If no cans of product have swelled, or found to be off-flavor, or otherwise adversely affected by unsanitary conditions, then it can be said that the process is under control; however, if some of the cans are lost as a result of swelling or other detrimental changes, the damage will have already been done irretrievably. Thus, the test must be one that will *predict* rather than measure the final quality and performance of the finished product.

In selecting an appropriate test procedure we must consider not only precision, that is, whether duplicates check out closely; but also accuracy, that is, whether the test does in fact predict the ultimate quality and performance of the finished product. The time required to complete the test is also of major importance. If a test can be performed within fifteen minutes of the time the sample is obtained and the results indicate that some difficulty may be encountered, there may yet be time to change the process to maintain the quality of the finished product. However, if a time lapse of days is required before test results are obtained, large quantities of product may have been manufactured during this incubation period, which may end up in a hazardous, perhaps unsalable condition.

When comparing the plate count method to the direct microscopic method in terms of these three criteria, we find that the plate count method is perhaps more precise, undoubtedly more accurate since it counts only live bacteria, but much slower; the results becoming available generally in no less than 24, usually 48, hours after sampling, whereas, there is no time required for incubation when the direct microscopic method is used.

It should be pointed out that all bacteriological tests, and other microanalytical tests such as mold or insect fragment counts, *appear* to suffer from lack of precision; that is, reproducibility, when in fact they may be quite precise, but duplicates may vary widely because of the inadequacy of the sample. It should be recognized that the occurrence of such things as bacterial spores or mold filaments in foods is in the nature of a rare accident. Under such conditions the characteristic frequency distribution is the Poisson. The standard deviation of the Poisson distribution is the square root of the mean occurrence. Thus, for example, a sample having a true average count of 4 would have a standard deviation of the square root of 4 or 2. This means that if one determination were made there would be about 2 chances out of 3 that the actual count would be 4 ± 2, and about 1 chance in 3 that the count would be less than 2 or more than 6. There would even be a remote chance (about 1/100) of the count exceeding 10 or being 0. This chance for error has nothing to do with the precision or the accuracy of the test method itself. It is simply due to the nature of the material which is being examined which no improvement in the method will alleviate. The only opportunity for reducing this error is by increasing the number and frequency of sampling. The more tests that are made, the more closely will the average value of such tests approach the true value for the lot.

In view of the urgency of obtaining results while the product is still in the production line, it is suggested that the plate count methods be reserved for survey types of investigations, such as those conducted by the National Canners Association in order to detect specific sources and locations of contamination. Such investigations may indicate the appropriate place where further routine quality control type testing is to be made, preferably by the direct microscopic method, for no other reason but that the results will be immediately available.

Control Station.

The tests should be made and the results posted as closely as possible to the location in the production line where the problem may exist, and where corrective action can be taken, rather than in a central laboratory which may be far removed from the line.

The complete survey should reveal whether the contamination is introduced with the raw material or other ingredients, whether bacteria grow on surfaces of equipment or are built-up as a result of accumulation of product or water. Surveys most frequently indicate the following equipment as sources of bacterical contamination: washers, pumps, flumes, pipes, tanks, dewatering screens, conveyor belts, picking tables, quality graders, blanchers, filler hoppers, filler spouts, mixing tanks, blending tanks, boning tables, dicers, closing machines, blanching or cooling water.

The location of the control station can then be established intelligently on the basis of the information provided by the survey. This may consist of one particular spot where the sample is taken, which the survey indicated is a particularly troublesome point in the line or several spots may be indicated, where samples may be removed or material collected in any other manner would be utilized for a direct microscopic count.

How many tests should be made and how frequently they should be made will depend entirely on the precision required, and the probability of a change in the situation. For example, if it is found that a count of less than 6 is satisfactory, and the typical count is ordinarily 0 or 1 (except when something goes out of control) there is no particular reason for making more than one test during a given time interval. If on the other hand typical counts are about 4, there would be a probability of getting a value greater than 6 too frequently by chance alone to rely on a single count. In such a situation it would be advisable to make perhaps four or five counts at one time and report average values. All the information available on the characteristics of the process should be utilized to make the counts where and when they will be most useful. For example, if it is known that counts are generally high at the beginning of an operation, or immediately following a meal break, testing should be concentrated at such times. If the use of a new lot of raw material may cause a shift in count levels, testing should be increased at such times.

Reporting.

Results obtained at the control stations should be posted immediately on the control charts so that action may be taken promptly whenever counts go out of control. For the greatest usefulness and likelihood of success, the results should not go into a laboratory notebook only, but should be posted prominently at or near the location where action if needed would be taken. Posting the data in the form of a control chart makes it possible to view the progress of the production operation on a continuous basis, indicating clearly and promptly when action should or should not be taken, and also indicating developing trends which may point to action even before the situation has gone out of control. In Figure 1 is an example of a P chart which would apply to such counts. We assume that the counts on the average are about 5 and in order to obtain the desired precision, we average 5 counts. We set our upper and lower control limits as follows:

$$3\sqrt{\frac{c}{n}}$$

where c is the average count, and n is the number of tests averaged. Thus in our example:

$$3\sqrt{\frac{5}{5}} = 3$$

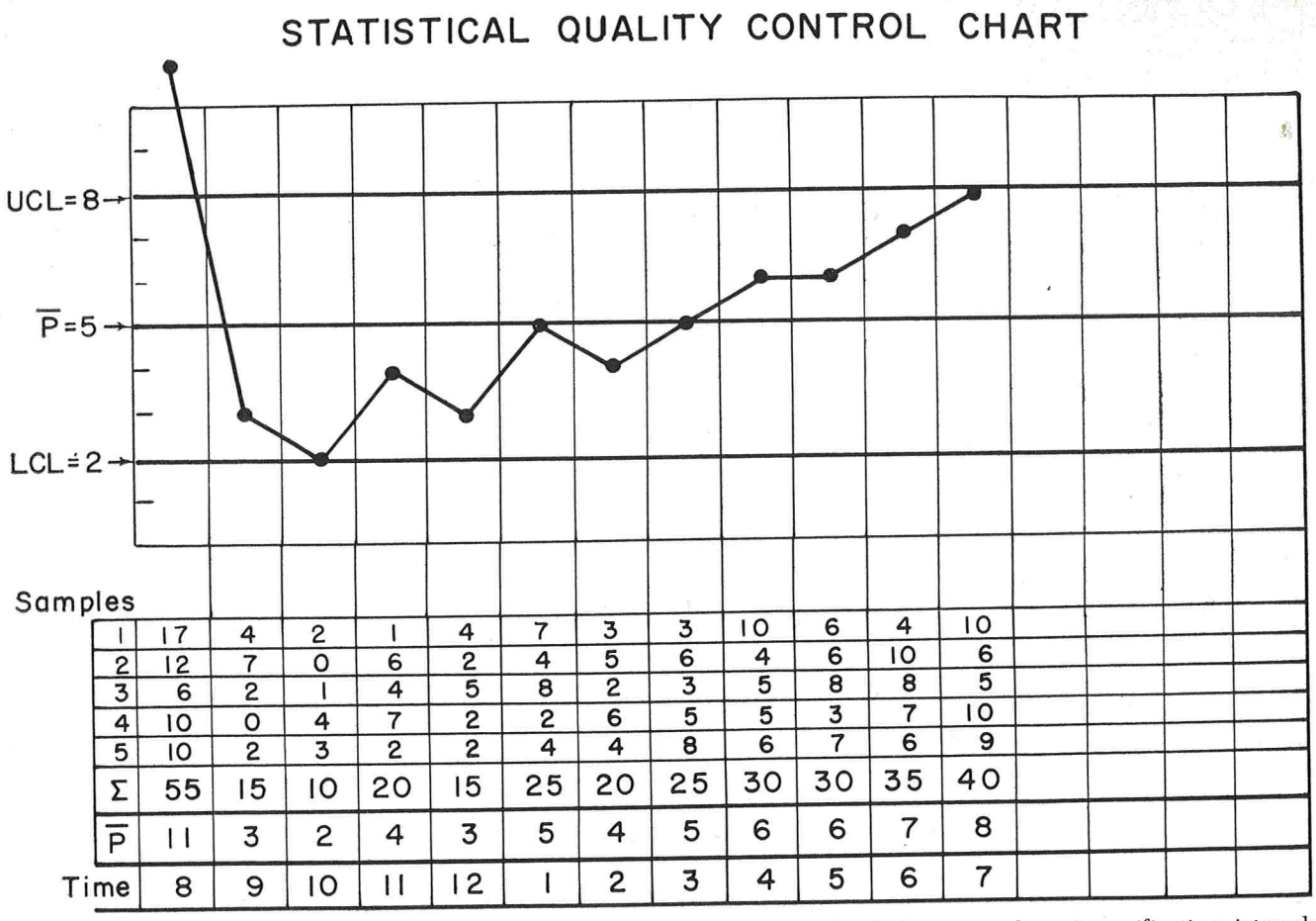
Thus, the upper control limit is $5 + 3 = 8$ and the lower control limit is $5 - 3 = 2$. We decide that these five tests should be done at hourly intervals, and come up with the results shown in Figure 1.

Thus in practice we decide to take five samples within an hour period, and post these individual results in the lower part of the Figure, opposite the spaces filled under "Samples", 1. . . 5, for "Time" 8. We then sum these five counts as shown in the Figure in the row starting with the symbol "Σ". Next the mean of this sum is obtained by dividing the sum (Σ) by the number of individual tests (5), and this is recorded in the next line starting with the symbol "$\bar{P}$". This mean value is then located on the control chart shown as the upper part of Figure 1, where the vertical axis is the scale of counts, and the horizontal axis is time in hours.

Note that the first set of five tests taken at 8 a.m. averaged 11, indicating an out of control situation which apparently was corrected by 9 a.m. Although the remaining averages shown did not exceed the upper control limit of 8, the trend is unmistakable, thus it would be well worthwhile to take further action by 2 or 3 p.m., without necessarily waiting for a given average to again exceed the count of 8.

Action.

The control chart will indicate when action should be taken. It may or may not point directly to the specific action to be taken. If, for example, the results are taken from a test of a conveyor belt which is continuously dipped in a sanitizing solution, it may be obvious that the solution should be changed or more material added. In other instances the cause of the build-up may not be obvious, so that something of an investigation may be required before the cause of the build-up is found and corrected. The following are some suggestions given by G. A. Vacha,



Samples												
1	17	4	2	1	4	7	3	3	10	6	4	10
2	12	7	0	6	2	4	5	6	4	6	10	6
3	6	2	1	4	5	8	2	3	5	8	8	5
4	10	0	4	7	2	2	6	5	5	3	7	10
5	10	2	3	2	2	4	4	8	6	7	6	9
Σ	55	15	10	20	15	25	20	25	30	30	35	40
P̄	11	3	2	4	3	5	4	5	6	6	7	8
Time	8	9	10	11	12	1	2	3	4	5	6	7

Figure 1. A statistical quality control chart for recording count levels. Individual counts taken at specific time intervals are recorded, summed, and averaged in the lower part of the Figure, and the mean count values posted on the chart. UCL is the upper control limit. P is the desired mean count, and LCL is the lower control limit.

Minnesota Department of Agriculture for action which may minimize contamination:

1. Before cleaning dismantle or open the equipment as far as possible.

2. Disconnect lines where possible or open cut-outs to avoid washing debris from one piece of equipment to the next.

3. Remove as much waste as possible with brush, shovel, broom or other appropriate tool.

4. Rinse surfaces to be cleaned with water to remove food residues.

5. Clean surfaces with hot water with an added detergent specifically formulated for the removal of a particular type of soil. Use cleaning aids such as high pressure or brushes to remove tenacious deposits.

6. Complete the cleaning by thoroughly flushing with hot water to remove detergent residues, and finally rinse with cold water to cool equipment below 80 F.

7. Allow equipment to drain and air-dry.

8. Do not depend on high pressure steam to sterilize equipment; in many instances the steam spreads contamination by blowing it from crevices and cracks onto equipment which has been cleaned.

9. Before resuming operations, sanitize equipment by rinsing or spraying the equipment with a chlorine solution which contains 100 to 200 parts per million.

10. Sanitize in same manner water pipes used for recirculating wash water and for pumping peas, corn, etc., as well as brines and syrup.

11. Avoid contamination of equipment by spatter from floors or from contaminated equipment.

12. Keep hoses used for rinsing equipment off the floor.

13. Make sure that water used for brine, syrups and for cleaning is free from contamination. Water storage tanks must be frequently drained, cleaned and sanitized so as to eliminate bacterial build-up.

14. Thoroughly back-wash and sanitize regularly water filters and water softeners. Bacterial build-up by accumulation and actual growth is very common in such equipment.

15. Eliminate dead ends in water pipes, brine and syrup pipes and pipes used for transferring foods from one place or equipment to another.

16. Eliminate dead ends in flumes, sharp curves, bad solder and welded joints.

17. Provide in-plant chlorination and maintain a chlorine residual of 1 ppm in the plant water supply. Provide controls so the chlorine content can be increased to 25 ppm or higher during cleaning operations.

18. Eliminate scale from the surfaces of pipeline blanchers, water pipes and equipment surface. Such deposits may harbor thermophiles and other types of microorganisms.

19. Keep viners clean so as to reduce the contamination of peas and lima beans.

20. Pea boxes, bins, etc. must be in good repair and washed after each trip to the plant. Rinsing the boxes and bins with a chlorinated final rinse is recommended.

21. Clean daily and sanitize corn huskers and cutters.

22. Replace wooden husker and cutter bins with metal ones, and clean and sanitize daily.

23. Keep cooling tanks clean and chlorinate cooling tanks or canals. Satisfactory chlorine residual is 2 to 5 ppm.

24. During canning and freezing operations periodically rinse equipment, conveyor belts, picking tables with water to prevent accumulation of debris, thereby physically removing large numbers of microorganisms.

25. During a breakdown, rinse off equipment and cool it down below 90 F so as to arrest bacterial growth.

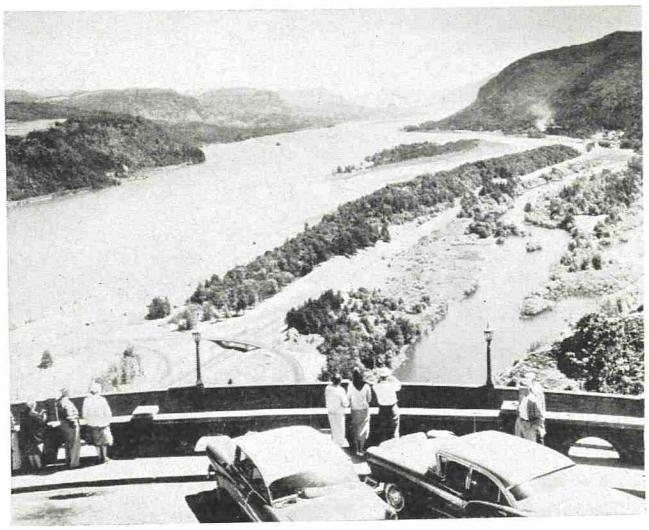
26. During short period shutdowns keep washers, dewatering screens, blanchers and similar equipment running, and cool down to below 90 F.

27. Use only sugar, starch, salt, spices which have been tested and approved by a reliable laboratory.

NEWS AND EVENTS

"AND THIS IS PORTLAND!" SITE OF IAMFES 51ST ANNUAL MEETING

Portland, Oregon, located near the head of the river navigation for deep-sea vessels, is the Columbia Empire's gateway to the seven seas of the world. It is a thriving industrial and seaport city of some 402,300 people, a transportation hub of rails, steamship and airplane routes. Majestic Mt. Hood, 62 miles distant, 11,245 feet high, snow covered the year round is clearly visible from the city.



The Columbia River has carved one of the world's most beautiful and majestic gorges through the heart of the Cascade Mountains. Here at Crown Point the Gorge stretches in magnificent panorama. Across the river loom the Washington shores. Crown Point, 24 miles east of Portland, Oregon, is 750 feet about the river.

A CITY is a horde of natives in an orderly jungle, its air heady with the pungent perfume of industry and raucous with the cry of the untamed taxicab.

It is a seat of government, a hub of commerce, a center of entertainment and learning, and a rainbow terminus where some find a pot of gold and some find a pot of free mission stew.

It is churches and theaters, parks and parking lots, freeways and alleys, hotels and suburban Edens. It is a potpourri, spiced by variety.

To the dweller in this concrete honeycomb it's a place to take a vacation from (but to return to inevitably with fascination), and to the country dweller the city is a mecca to visit as often as an excuse can be found.

All of these things are of a metropolitan atmosphere. But a city can have something else — undefinable but perhaps best called personality. It is this intangible attribute that inspires people to refer to a population center as "The City," with the capital letters apparent even in their speech.

Every state has at least one such metropolis. In

Oregon, it's Portland, a city that is many things to many persons.

"Why do Portlanders need vacations?" a recent newcomer asked, and he was more than half serious. "They live in the kind of place most people look for when they're vacationing."

A remark like this can jolt a native Portlander into a new awareness of his surroundings. Perhaps nowhere else are there so many people who take for granted a setting so spectacular.

There are few places in Portland from which there is no view of Mt. Hood, the craggy, snow-capped 11,245-foot peak that is the highest point in the state and a winter wonderland that is little more than an hour's drive from downtown Portland. On its slopes can be found skiing all year around — it's best from November to May — and activities to intrigue the hiker, mountain climber, camper, huckleberry picker and those who perfer the less physical pastimes like picking olives from martinis and staring pensively into the giant stone fireplace in Timberline Lodge.

Only a little farther in the opposite direction from Portland is Oregon's unspoiled coast, where only whim and pocketbook dictate whether a visitor throws himself into the informal hubbub of a resort town or walks in solitude on a beach of white sand where the only sounds are the sigh of surf and the reedy cries of gulls. The camera and the artist's easel are as pertinent as the clam shovel in a beachgoer's gear.

Portlanders and their visitors need not go that far for water fun. If a tugboat captain rams one of the eight bridges that span the Willamette River on its course through the city, he can blame the distraction of damsels sunning themselves on the decks of pleasure craft. These boats rub gunwales with sternwheelers, ocean-going freighters and tankers and the excursion boats that ply both the Willamette and the nearby Columbia River. A look at Portland's port is as easy as a walk to the foot of any east-west street.

No visitor can ever forget an excursion boat ride up the Columbia, for a mid-river vantage point perhaps offers the most breath-taking view of the towering, timber-topped cliffs and the white plumes of waterfalls that make the Columbia Gorge a scenic wonder. Rooster Rock State Park, a swimming and boating paradise only 23 miles east of Portland in the Gorge, is the second most frequently visited of Oregon's state parks, and it is an afterwork spa for many Portlanders who enjoy the relaxation of a swim and a beach barbecue supper, followed by the deep peace that comes with sunset on a beach dotted with the dying embers of campfires.

While stream and lake angling, deep sea fishing, hunting and other outdoor sports are readily available, many a guest of Portland does not have the time for or inclination toward such activities. For these, the city itself offers diversion in abundance.

The badge of the conventioneer is seen in very corner of the metropolitan area, be its wearer in search of night life, unusual cuisine, theater, symphony, art, golf, church services of his denomination, scenic drives or simply a look at the blooms that have made Portland known as the City of Roses.

Has he a taste for crawfish? He can find them, prepared to succulent perfection by an expert chef. Does his palate yearn for French, Mexican, Chinese, Italian, Japanese, Swedish, German, Polynesian or Western "chuckwagon" delicacies? No problem, for Portland's cosmopolitan cookery embraces everything from steaks to sauerbraten, from pizza to prime rib, from chicken cacciatore to chow mein and from buttermilk to Bavarian beer. He can dine in a sedate Victorian home, a South Sea garden of bamboo and waterfalls, a candle-lighted Early American inn or a contemparary lounge with a view.

These things are within a short distance of the major hotels. So are the night spots, the movie theaters, the Civic Theater with its stage plays, the Auditorium with its local and visiting symphony orchestras and solo artists, the Art Museum with its fine collection of Northwest Indian artifacts, the Oregon Historical Society's museum and library, churches of all denominations and the International Rose Test Gardens established in 1917 and one of only a handful of such gardens currently operating in this country.

The test gardens, located in Washington Park, consist of a series of display beds in which new varieties of roses from many parts of the United States and from countries abroad are carefully nursed by Curator Rudy Kalmbach, an expert on rose culture.

Portland is a city of greenery. It is emerald-studded with parks and golf courses. The tree-canopied Park Blocks, a verdant strip through the heart of the business district, serve as a campus for Portland State College students and as an oasis of grass, fountains and park benches for foot-weary shoppers, tourists and citizens who seek a quiet haven in which to enjoy a book or newspaper, feed pigeons or just plain loaf.

Not only in the Park Blocks but elsewhere in the city it would be difficult to throw an inkwell without hitting a college student. Portland's intellectual climate is warmed by Reed College, which has an international reputation as an incubator of Rhodes scholars; Lewis and Clark College, the University of Portland, Cascade, Multnomah and Warner Pacific Colleges, and the University of Oregon's Medical and Dental Schools.

Anyone who knows Portland tries to be on hand in June. Early in that month each year the entire population willingly drops city sophistication to par-

ticipate in a week of infectious fantasy. During the annual Rose Festival, the bemused mayor and city commissioners find themselves subjects of a teenage Queen of Rosaria, chosen and crowned in elaborate ritual. Parades, pageantry, stadium shows and more events than any one person could witness in seven days make the fete a carnival that yearly draws visitors from many states and Canada.

From farther away than that have come many of the residents of Portland's modern zoo, an artistically designed attraction that fascinates even those urbanites who are somewhat blase about being stared at by monkeys and bears. A former golf course, the new zoo shows off animals from all corners of the earth in natural, unbarred grottoes.

Prime attractions are the penguins, netted near the South Pole in a personal expedition by Portland's zookeeper, Jack Marks. Two miles of narrow gauge track winding through the zoo and linking it with Washington Park are kept shiny by constant passage of miniature trains, almost always hauling more adults than children. Moonlight rides carry passengers through scented firs in the cool of night, with a stop at a high point for a view of the light-jeweled city.

For the convention visitor and his family there is leisurely shopping in nearly every district. Newest of the giant marts is the Lloyd Center, complete with department stores, shops of every description, restaurants, markets, a cocktail lounge overlooking an ice skating arena, an ultra-modern hotel and a park.

Portland's restless vigor is evident in other new landmarks. An addition to the skyline is the $8,000,000 Memorial Coliseum, a striking architectural marvel of glass, concrete, steel and wood surrounded by vast parking facilities. It is designed to handle anything from a hockey game to a regional basketball tournament, and from a local meeting to a national convention session.

A new airport terminal is the first part of Portland seen by visitors who arrive by air, and an impressive introduction it is — with acres of lobby, escalators between all levels, coffee shop, restaurant, cocktail lounge, shops and observation deck. A persistent rumor is one that a newcomer spent a week living in one of the plushy furnished conference rooms, convinced he was in a fine Portland hotel — but this story is given no credence by airlines officials.

Anyone who visits this Oregon metropolis is likely to talk about it for some time. Many of the staunchest boosters among Portland's permanent residents are those who came on what they intended to be a visit. They came, they saw and they were conquered.

PETER L. MILLER ELECTED PRESIDENT OF NADEM

Elected as directors of the National Association of Dairy Equipment Manufacturers at its 19th Annual Meeting held at the Kenwood Golf and Country Club, Bethesda, Maryland, on May 19 to serve for a three year term were Marvel J. Heinsohn, Sales Manager, Portersville Stainless Equipment Corp., Portersville, Pennsylvania, and Walter Z. Meyer, Sales Manager, Food Processing Equipment Division of the Paul Mueller Company, Springfield, Missouri.

Peter L. Miller, Vice President of the Chester-Jensen Company, Chester, Pennsylvania, was elected President of the Association at the Board of Directors meeting immediately following the Annual Membership Meeting on May 19. Walter Z. Meyer was elected Vice President and Marvel J. Heinsohn was elected Treasurer.

Peter Miller succeeds Robert Walker who is retiring President of NADEM and President of Walker Stainless Equipment Company, New Lisbon, Wisconsin.

At the Board of Directors meeting on May 19, John Marshall was re-appointed as Executive Vice President and the firm of Fistere and Habberton was again retained as General Counsel for the ensuing year. Other members of the Board of Directors are James Brazee, Vice President for Sales & Marketing, CP Division, St. Regis, Chicago, Illinois, Emil Howe, Manager Market Development, Ladish Company, Tri-Clover Division, Kenosha, Wisconsin, and Robert Walker.

CONGRESSIONAL HEARINGS ON FEDERAL GOVERNMENT PAPERWORK

Congressman August E. Johansen of Michigan, Acting Chairman of the House Subcommittee on Census and Government Statistics of the Post Office and Civil Service Committee, presided at the Public Hearing held in the Cannon Building June 1, 1964, concerning the problem of the federal government "Paper Work Jungle."

He welcomed the first witness, John Marshall, Executive Vice President of the National Association of Dairy Equipment Manufacturers, Washington, D. C., who is also the Regional Vice Chairman of the Manufacturing Trade Association Group of the National Industrial Council and Chairman of its World Trade Committee, a group representing more than 75,000 plants engaged in the manufacture of machinery, equipment, etc.

Mr. Marshall stated that his association which rep-

resents 38 major manufacturers of industrial dairy plant processing and packaging machinery and equipment most of whom are considered as small businesses are greatly interested in cooperating with the Bureau of Census of the Department of Commerce and with the Department of Agriculture in completing necessary forms to secure relatively complete and reasonably accurate industry statistics. Such statistics are important to both industry and government since the dairy industry is not only one of the largest farm enterprises representing a return to dairy farmers of nearly five billion dollars of cash income each year or about 1/7 of the total income of all farms.

He stated that in 1962 the latest available Bureau of Census data showed that our members' customers, that is, milk processing plants, employed more than 275,000 persons whose total payroll exceeded 1 billion 415 million dollars and the value of shipments from these plants of all dairy products exceeded 11 billion 450 million dollars that year.

Mr. Marshall commended the Committee for its interest in the paper work problems of businessmen, and for providing a forum where their complaints could be heard.

Mr. Marshall's complaint to the Committee was that while for many years his members had been filing numerous government reports required of such manufacturers, that it was impossible to obtain from any government bureau either (1) the dollar value of industrial dairy plant processing and packaging machinery and equipment manufactured annually in the United States, or (2) the dollar value of imports of such equipment which we know amounts to three to five million dollars or more per year. He further stated that it was most annoying and beyond comprehension to find top government career officers charged with the responsibility of establishing policy to permit of tabulation of reasonably adequate and correct data, throw stumbling blocks in his way and seemingly use means to delay government action to compile such information from reports now being filed.

Congressman Johansen stated that the Subcommittee would look into these charges when it calls in the federal agencies later this month.

FOOD FORUM FOR TOP MANAGEMENT TO BE FEATURE OF DAIRY & FOOD EXPOSITION IN CHICAGO IN FALL

"Food and the Future: Concepts for Planning" will be the theme of a two-day industry-wide forum for top management of food processing operations, October 8-9, 1964, at McCormick Place in Chicago.

The forum will occur on the final two days of the Dairy and Food Industrial Exposition, October 4-9 at McCormick Place, and persons eligible to attend the Exposition will also be eligible to participate in the seminar.

Four major speakers have already been retained to address the seminar. These are:

Dr. James R. Bright, Professor of Business Administration, Harvard Graduate School of Business, who will discuss the impact of technological change on the food industry;

Dr. E. M. Foster, Professor of Bacteriology of the University of Wisconsin, who will discuss microbiology of the food supply and environmental control in the food industries;

Dr. A. T. McPherson, Physical Sciences Administrator, U. S. Department of Commerce, a recognized expert on synthetically produced nutrients, who will discuss possible chemical bases for the food supply of the next century; and

Dr. R. G. H. Siu, Chairman of the Army Research Council, Department of Defense, Washington, D. C., whose subject is yet to be announced.

The forum is sponsored by Dairy and Food Industries Supply Association, which is also the sponsor of the Dairy and Food Industrial Exposition. The Exposition, with more than 300 exhibits of supplies, equipment, and services for the dairy and food processing industries, will occupy the entire seven-acre exhibit level of McCormick Place.

The forum sessions will be held in the meeting rooms on the registration level of McCormick Place. Admission will be by badge only and advance reservations will be required for forum attendance.

Further program details and reservation procedures will be announced at a later date.

LITTER JAM

This year's highway litter, if laid end to end, would create the world's record traffic jam.

In fact, it would bring all traffic to a dead stop on the preferred 3,000-mile route between New York and San Francisco, reports Keep America Beautiful, Inc., the national anti-litter organization.

KAB says the rubbish that will be dumped on streets and highways in 1964 if concentrated would bury the transcontinental route a foot deep in discarded wrappers and cartons, paper cups, plates, tissues, cans, bottles, garbage and such assorted trash as old mattresses and automotive parts.

KAB based its graphic picture of the national "litter harvest" on an estimate from the U. S. Bureau of Public Roads, that up to 20 million cubic yards of litter will be dumped this year on the nation's streets and highways.

"It will cost taxpayers over a hundred million dollars to clean up the highway mess this year," said

Allen H. Seed, Jr., executive vice president of KAB. "And the cleanup won't be permanent! The only *real* solution is education.

"We can all give a hand," Seed continued. "Put a litterbag in your own automobile and use it. When the bag is full, empty it in a trash receptacle along the way, or take it back home with you and get rid of it there. It may seem like a very little thing to do. But today's highway litter jam is made up of 'little things' – millions and millions of them! If each person will just dispose of his own litter properly, there would be no highway litter problem."

THIRD DRAFT OF PHS MILK ORDINANCE TO BE CIRCULATED

Public Health Service representatives and 12 milk sanitation specialists met in Washington, D. C., June 1-5, to review the draft of the proposed *Pasteurized Milk Ordinance and Code – 1964 Recommendations of the Public Health Service.*

Seventeen hundred copies of the draft had been distributed in March to the States, selected communities, and all segments of the dairy industry for critical review and comment.

At the Washington meeting, personnel of the Milk and Food Sanitation Branch of the Service's Division of Environmental Engineering and Food Protection, and a panel representing the fields of milk sanitation administration, technology, enforcement, and education, evaluated the comments and recommendations received.

Following this review, the panel recommended for further study the preparation of another draft of the proposed revision, incorporating many of the suggestions.

The Service endorsed the panel's recommendation and anticipates completion of the draft rewrite by the latter part of August for distribution to milk regulatory agencies and the dairy industry for review.

If necessary, the panel will be reconvened during the Fall to assist the Service in completing the revision. The panel includes the following members:

Mr. Harold J. Barnum, Chief, Milk Sanitation Section, Environmental Health Services, Denver Department of Health and Hospitals, 659 Cherokee Street, Denver, Colorado 80204.

Mr. George Bauer, Director, Department of Public Health and Welfare, Springfield, Missouri.

Mr. Paul Corash, Executive Director, Metropolitan Dairy Institute, Inc., 60 East 42nd Street, New York 17, New York.

Mr. William A. Dean, Jr., Bowman Dairy Company, 140 West Ontario Street, Chicago 10, Illinois.

Mr. G. A. Houran, Vice President, Sales, Milk Plant Division, The DeLaval Separator Company, Poughkeepsie, New York.

Mr. Shelby Johnson, Director, Food and Drug Program, Division of Environmental Health, Department of Health, 275 East Main Street, Frankfort, Kentucky 40601.

Robert W. Metger, DVM, Director of Quality Control, Dairymen's League Co-Operative Association, Inc., 402 Park Street, Syracuse, New York.

Mr. Alfred E. Reynolds, Chief, Bureau of Dairy Service, State Department of Agriculture, 1220 N. Street, Sacramento, California 95814.

Mr. Elbrege D. Sullivan, Vice President, Central Oklahoma Milk Producers Association, Route No. 2, Lawton, Oklahoma 73501.

Mr. Clinton Van Devender, Supervisor, Milk Control Program, State Board of Health, Jackson 5, Mississippi.

Mr. H. H. Vaux, Director, Division of Dairy Products, Indiana State Board of Health, 1330 West Michigan Street, Indianapolis, Indiana 46207.

Dr. K. G. Weckel, Professor, Dairy and Food Industries, Babcock Hall, The University of Wisconsin, Madison, Wisconsin 53706.

KOSIKOWSKI DECORATED BY FRENCH GOVERNMENT

A Cornell University professor, Frank V. Kosikowski of the dairy and food science department, has been decorated by the French Government "for services rendered to the science of dairying, both in your homeland and in the international field."

Presentation took place during an international meeting on dairy education sponsored by the Food and Agriculture Organization of the United Nations in Paris, June 2-8. In attendance were some 100 scientists and teachers from more than 40 countries.

Kosikowski received the "Croix D'Officier Du Merite Agricole," a decoration established by Napoleon III. He is only the second American to have received this since its establishment.

In a speech delivered at the presentation, a representative of the Minister of Agriculture of the French Republic said, in part, of the Cornell professor:

"You have contributed to the advancement of knowledge by at least 130 scientific publications on dairying and food in general, including a book in collaboration with M. Mocquot, head of the Milk Research Centre at Jouy-en-Josas.

"You have also carried out numerous missions: In France and Europe in 1952 and 1955; in Ireland in 1959; in Puerto Rico in 1961; in Denmark in 1962; and in Italy in 1963.

"In your capacity of consultant at FAO, you have devoted a great deal during the past 12 months to

the preparation of this international meeting on professional teaching and training which shows every sign of being a great success.

"It is these outstanding merits and this devotion to the cause of teaching and research in dairying, to which such significance is attached by M. Pisani, the French Minister of Agriculture, and by the Director General of Agricultural Education, M. Soupault, that the French Government wishes to honour in conferring this distinction, seldom bestowed on foreigners."

LANGLOIS APPOINTED ASSISTANT PROFESSOR AT UNIVERSITY OF KENTUCKY

Dr. Bruce E. Langlois has been appointed assistant professor of Dairy Science at the University of Kentucky. Dr. D. M. Seath, chairman of the Department of Dairy Science at the University made the announcement recently. Dr. Langlois assumed his new duties the first part of June.

Dr. Langlois will work in the field of dairy bacteriology. He will be continuing a project on market qualities of fluid milk as well as initiating other projects both in the field of bacteriology and biochemistry. The appointment combines both research and teaching duties.

Born in New Hampshire, Dr. Langlois received the Bachelor of Science degree from the University of his native state in 1959. He majored in Dairy Technology. He then moved to Purdue University where he earned the Ph. D. degree in 1962. His dissertation was based on a study of free fatty acids in the swiss-type cheese produced at Purdue.

After receiving his advanced degree, Dr. Langlois was appointed an assistant professor at Purdue and worked on a number of projects involving insecticides in poultry and dairy products. He has published 9 papers in the past several years dealing with residues of the material in food and methods of identification of the pesticides.

Dr. Langlois is married and the father of one child. He is a member of the American Dairy Science Association and Sigma Xi. His appointment fills a vacancy in the Department of Dairy Science created by the untimely death of Dr. W. E. Glenn.

ISM ANNOUNCES ANNUAL CONFERENCE PROGRAM

The Institute of Sanitation Management has announced the complete program for its forthcoming Sanitation Maintenance Conference. The Conference will be held September 21-24, at the Hotel Commodore in New York City. The accompanying Sanitation Maintenance Show will be held September 20-22, also on the ballroom floor of the Commodore.

Registration fees will be $10 for members, $15 for non-members for the full Conference, with fees of $5 for members and $7 for non-members for any one day. There is no fee for visiting the Show exhibits.

A total of 8 sessions will be held during the Conference, but with many concurrent "Cracker Barrel" sessions, the actual number of individual meetings totals 26, with 32 different subjects up for discussion. The Cracker Barrel sessions are also designed as informal meetings, so that a far wider range of subjects will be taken up than appears on the program.

The Show will again open on Sunday, September 20, from 1:00 p.m. to 5:00 p.m., for those registrants who are unable to get away from their work during the weekday Show hours. On Monday, the exhibits will be open after the morning Conference sessions, from noon to 5:00 p.m. The final session of the Show will be held on Tuesday morning, from 9:00 a.m. until noon, before the Conference opens. ISM reported a brisk sale of exhibit space, promising to match its first Show in 1956 at the New York Coliseum.

The Conference program has been so designed by the Chairman, Richard Ehmann of the Port of New York Authority, that there will be no major meetings running concurrently, and that no meeting will conflict with Show hours. The emphasis will be on the intense, informal discussions that have developed over four years of the Cracker Barrel type of session.

FOOD EXECUTIVES ADDRESSED BY EXPERTS ON INTERNATIONAL LAW

Food law experts from Canada, Mexico and the United States were among the featured speakers at Foods On The Move '64, the fifth in the Food Update seminar series for food executives, held recently at the St. Moritz in New York City.

Keynote speaker at the session devoted to international food law developments was Franklin M. Depew, Esq., President of The Food Law Institute, Inc., whose organization is sponsor of the seminars. Mr. Depew discussed the work of groups and individuals in different parts of the world to evolve generally acceptable food standards and thus promote international trade. He cited the leadership of Dr. Carlos A. Grau of Argentina in helping to develop the Latin American Food Code as being of particular value. Mr. Depew noted that educational forums such as Food Update have helped to alert American businessmen to both the hazards and opportunities presented by developments in the field of international food law. He observed that the informed counsel of American industry is essential if the United States Government is to be truly effective insecuring the adoption of acceptable food standards by the Joint FAO-WHO Codex Alimentarius Commission (Food and Agriculture Organization-World Health Organization). While much progress has been made, Mr. Depew said, the Commission still faces difficult problems in attempting to develop safety and purity standards suitable for both technologically advanced and developing countries. There is also general agreement on the need to proceed promptly with work in harmonizing existing standards, he stated.

John L. Harvey, Esq., Deputy Commissioner, Food and Drug Administrations, USA, spoke on recent and current matters concerning food laws and regulations in this country. In discussing food additives, color additives, and pesticides, Mr. Harvey pointed out that in order to protect the public health and safety, the need for adequate governmental inspection and analytical control in these areas is approaching that now applicable to drugs. He described how the FDA had recently been reorganized to provide better, more efficient consumer protection. Mr. Harvey also discussed the FAO-WHO sponsored move toward international food standards from his vantage point as Chairman of the Codex Alimentarius Commission. The objectives of the Commission, he said, include the establishment of food standards which will serve as a sound basis for international trade and aid in eliminating trade barriers set up in the guise of "standards." He also noted that the Commission aims at serving as the point of guidance and coordination for many different organizations already engaged in elaborating international food standards in order to utilize most effectively the wealth of expertise in this field.

Dr. L. I. Pugsley, Associate Director, Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Canada, traced some of the principles of food legislation which have evolved over the years in Canada, and indicated how they have been consolidated in the present Canadian Food and Drugs Act. Dr. Pugsley also reported on the FAO regional seminar on food legislation held at Bangkok, Thailand, in 1962, which he attended as a consultant. The food regulatory legislation and methods of enforcement used by each country represented at the regional seminar differed widely, Dr. Pugsley stated. Many of the governments were taking active steps to up-date their food legislation and to provide more comprehensive standards for foods. While the countries are interested in laws to protect people against health hazards and deception, there is also much interest in the international marketing aspects of food legislation, especially in establishing grade standards to augment the reputation of their foods in world markets, Dr. Pugsley said. He also outlined a proposed model food law drawn up for the countries in Asia and the Far East.

Dr. Rafael Illescas Frisbie, Director of Food & Drug Control, Mexican Ministry of Health, spoke on the development of legislation covering foods, drugs, and cosmetics in his country.

Dr. Illescas noted that the Mexican food industry has expanded rapidly and includes a number of large American as well as national plants, but there is still a need for improvement capital and for modernization of production methods to keep pace with the growing population. He noted that imports to Mexico are handled through the Secretariad de Industria y Comercio, and all trade activities are guided and governed by international commercial treaties. In outlining some of the factors to consider in exporting foods to Mexico, Dr. Illescas suggested that particular care be given to the size and nature of prospective markets for relatively expensive ,imported foods and beverages. Dr. Illescas also cited the importance of considering local food customs and tastes, remarking that the fusion of colonial Spanish foods and indigenous Indian foods has given rise to a genuine Mexican Cuisine.

With reference to the IX Latin American Food Congress which is scheduled to take place in Puerto Rico in 1965, Dr. Illescas indicated that he has proposed that all Latin American countries prepare addendum of their typical national food products, vegetables, meats, and beverages, not presently covered by the existing Latin American Food Code (Codigo Latinamericano de Alimentos).

Dr. Kenneth Morgareidge, Vice President and As-

sistant Director, Food and Drug Research Laboratores, Inc., New York, discussed presently emerging ground rules covering food additive petitions in the United States. He noted that heretofore the major effort in the field of food additive regulations has been in "clearing" old materials having well recognized uses but which could not be "generally recognized as safe" as interprted under the legal definition of a food additive. It seems reasonable to assume that this phase has now been nearly completed, and that henceforth the major emphasis will shift to newer chemicals and those for which experience in common use is not available, he stated. This trend is already evident in the decreasing number of new petitions recorded in the Federal Regiter, he noted. Dr. Morgareidge also discussed classifications of food additives, the proper format for petitions, and some of the problems which manufacturers may encounter in filing and obtaining approval of particular additives. Looking ahead, he forsaw continuing, expanded use of additives along with a greater degree of governmental supervision and surveillance. He also anticipated the ultimate passage of further legislation which will greatly augment the enforcement powers of FDA in the food additive field, especially in the form of expanded factory inspection authority. In addition, the format of petition writing may change somewhat as greater reliance can be placed on stopping a questionable product from reaching interstate commerce rather than on apprehending violations after the fact.

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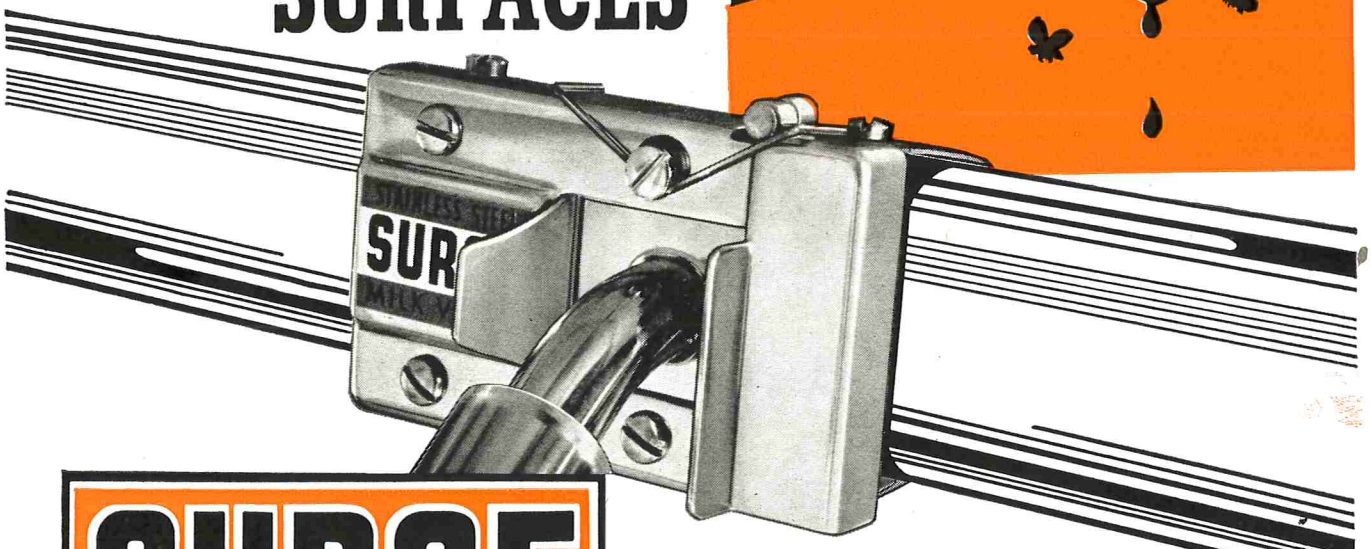

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