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Journal of

MILK and FOOD TECHNOLOGY

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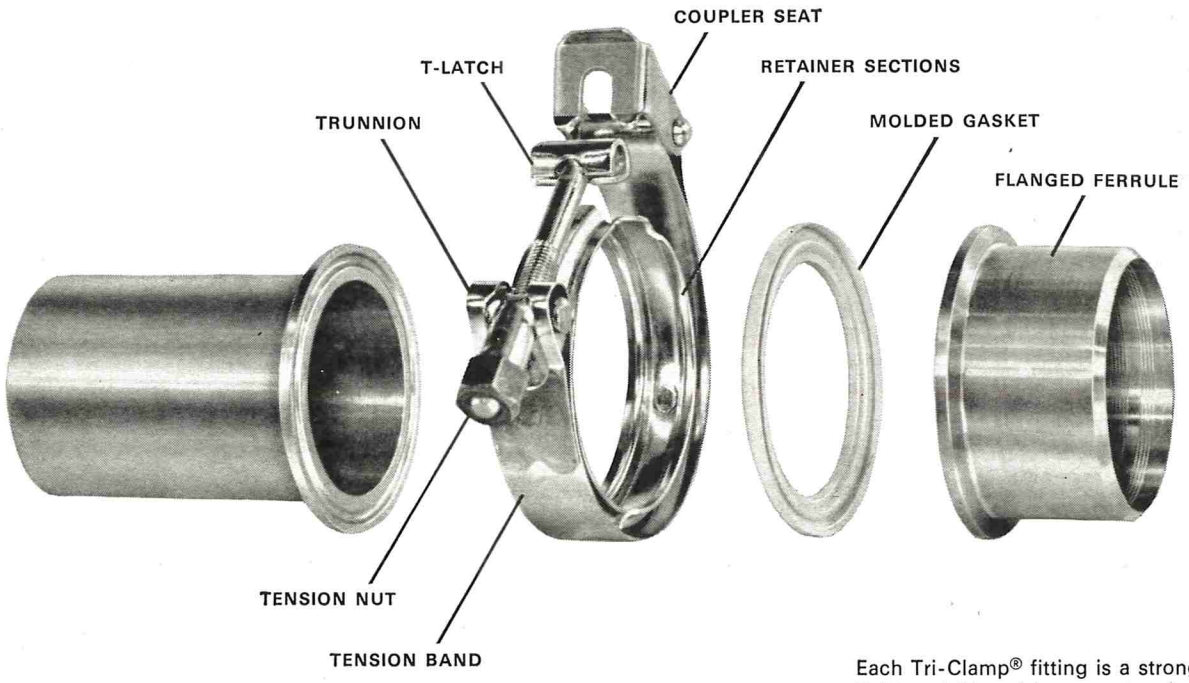

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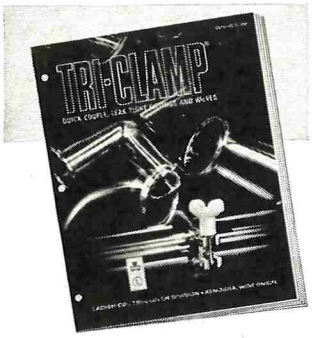

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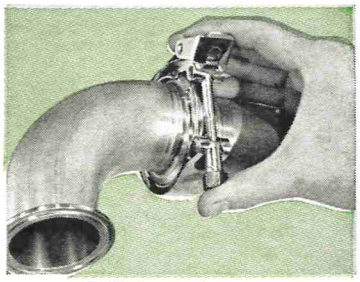

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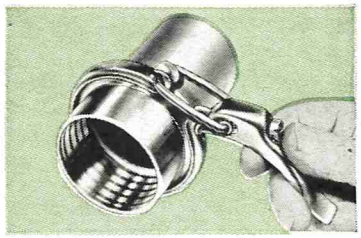

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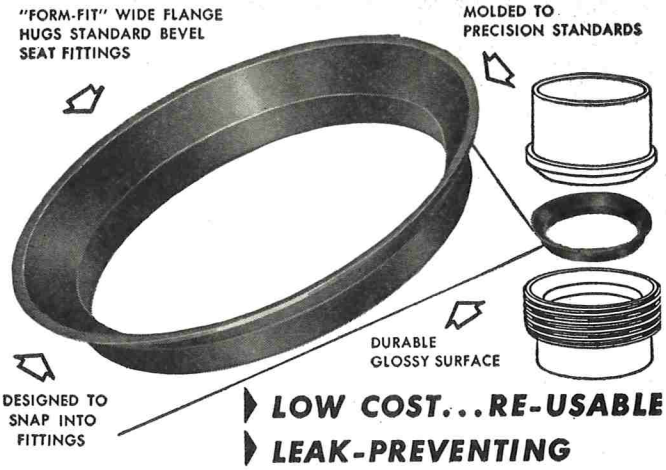

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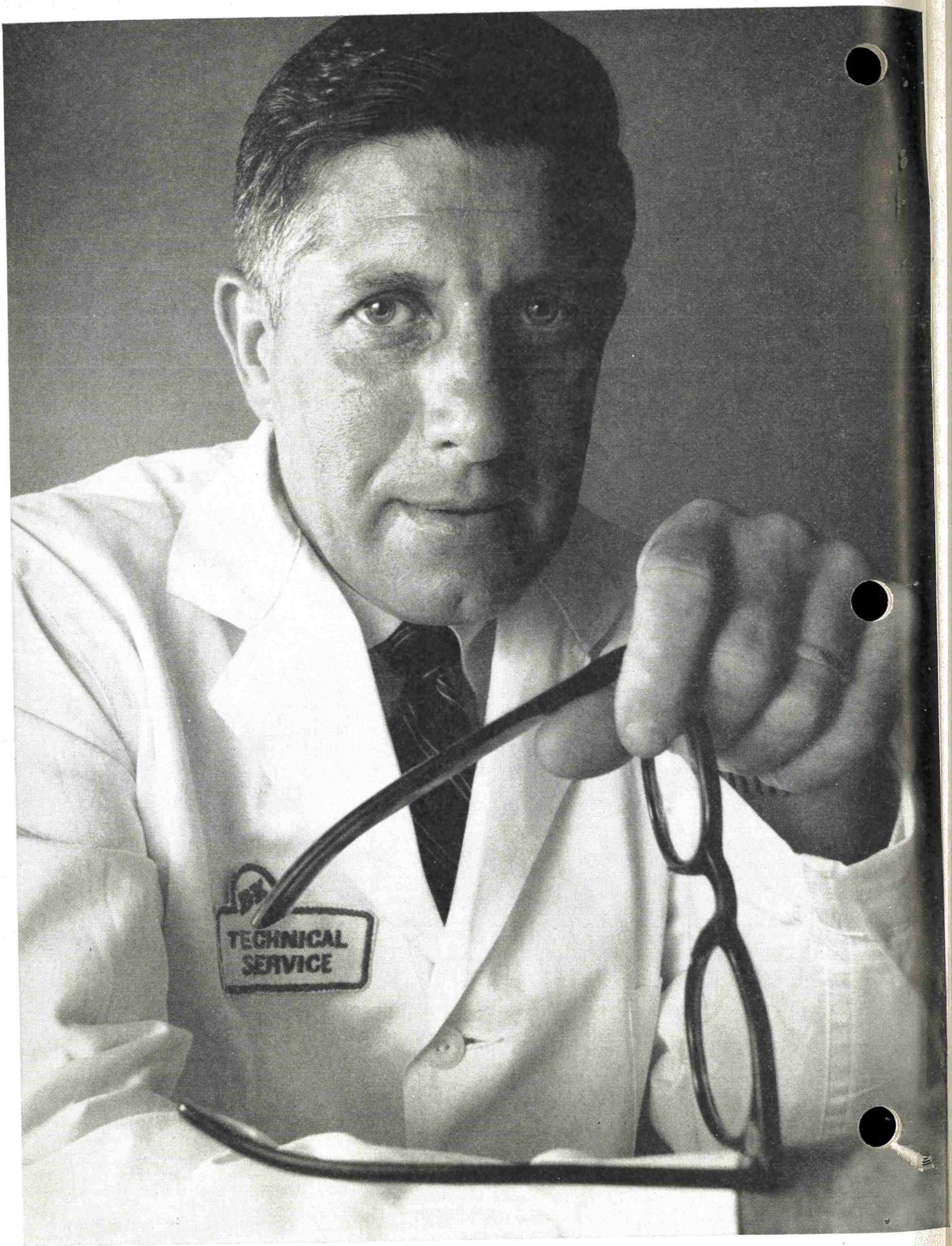
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THE EFFECT OF COPPER ON DISTILLED WATER QUALITY FOR USE IN MILK AND WATER LABORATORIES

GENE W. RONALD AND R. L. MORRIS

State Hygienic Laboratory
University of Iowa, Iowa City 52240

(Received for publication April 26, 1967)

SUMMARY

Levels of copper toxicity have been established in distilled water using the distilled water suitability test. It is shown that levels of copper, toxic by the distilled water suitability test, are not toxic to the test organism, *Aerobacter aerogenes*, in sterile milk or to the normal bacterial flora of a raw milk sample.

It is the contention of this paper that the distilled water suitability test is an unrealistically severe yardstick of distilled water quality for use in routine milk and water laboratories.

Standard Methods for the Examination of Water and Wastewater *(2)* states, "only distilled water and demineralized water which has been tested and found free from traces of dissolved metals and bactericidal and inhibitory compounds may be used for the preparation of culture media and reagents."

A statement of similar nature, though somewhat less restrictive, appears in Standard Methods for the Examination of Dairy Products *(1)*.

Each of the above publications then suggests the procedure of Geldreich and Clark *(3)* as the method of testing distilled water supplies for bactericidal compounds to determine their conformity to Standard Methods specifications.

In an attempt to satisfy this requirement, a statewide distilled water quality control program was initiated in Iowa. The program involves thirty approved milk and water laboratories and consists of analysis of their distilled water samples for pH, conductivity, copper and bactericidal agents.

Because so many distilled waters contain measurable concentrations of copper, it was decided to evaluate the direct toxicity effect of copper on the distilled water suitability test as well as its effect in actual distilled water use in routine milk and water laboratory procedures.

MATERIALS AND METHODS

Four, presterilized, 6-oz, "Whirl-pak" plastic bags were sent to each laboratory for the submission of their distilled water samples. The "Whirl-pak" bags are the same type as those used in many milk sheds around the nation for collecting raw milk samples.

This plastic bag was decided upon as a sample container because of its inert effect upon the water sample and because no preparation of the bag is necessary prior to sample collection. Another important point is that a sample container such as the "Whirl-pak" bag precludes detergent residues that could possibly be left on other types of containers through routine washing procedures.

Copper was determined on each sample using the "cuperthol" method in Standard Methods for the Examination of Water and Wastewater *(2)*.

A stock solution of copper prepared according to the specifications given in the "cuperthol" method was used to prepare varying concentrations of copper for toxicity tests. pH was determined on each sample using a Corning, Model 12, expandomatic pH meter and the conductivity of the samples were determined on a conductivity-bridge equipped with a K-0.100 cell.

The procedure of Geldreich and Clark *(3)* was used to evaluate the distilled water samples for bactericidal activity. This procedure will hereinafter be referred to as the distilled water suitability test (DWST).

The control water used throughout the DWST was prepared by passing a first-distillation water through a mixed bed resin deionizer and a carbon filter after which it was collected and redistilled in an all glass still.

The pH of this control water varies from 5.8 to 6.2 with a conductivity range of 800,000 to 1.1 million ohms and the water is copper free.

TABLE 1. SCHEDULE OF FLASK PREPARATION TO DETERMINE TOXIC LEVELS OF COPPER

Flask No.	Ml of 1000 ppb[a] copper sol'n added per flask	Ml of minimal nutrient media added per flask[b]	Ml of control water added per flask
Control	0	9	21
1	21	9	0
2	15	9	6
3	10	9	11
4	5	9	16
5	1	9	20

[a]ppb = microgram/liter.
[b]Total of the constituent reagents per specifications of Geldreich and Clark *(3)*.

RESULTS AND DISCUSSION

Prepared levels of copper were utilized to determine what concentrations were toxic to the test organism *Aerobacter aerogenes (3)*.

Six 125-ml erlenmeyer flasks were prepared according to the schedule of Table 1. Each flask was

TABLE 2. TOXIC LEVELS OF COPPER AS DETERMINED BY THE DISTILLED WATER SUITABILITY TEST[a]

Flask No.	Concentration Cu^{++} ppb	Initial SPC	SPC (000 omitted)	Ratio SPC flask B / SPC flask A
Control-1	0	58	4600	
1	700	26	0.32	0.00007
2	500	59	1.4	0.0003
3	300	56	8.7	0.002
4	170	55	160	0.03
5	30	75	2300	0.5
Control-2	0	67	4100	
6	70	57	730	0.2
7	50	62	1500	0.4
8	30	56	1900	0.5
9	17	54	3000	0.7
10	3	72	3800	0.9

[a]Control-2 through flask 10 run in duplicate.

inoculated with a predetermined volume of a 24-hour subculture of the test organism and incubated 24 hours at 32 C. The results are shown in Table 2.

It should be pointed out here that the results in Table 2 are two individual determinations. Control-1 through flask 5 represents the first half of the experiment and Control-2 through flask 10 the final phase of the copper toxicity experiment. The schedule of Table 1 applies to the second half of Table 2 as well, except a 10^{-1} dilution of the initial copper solution was utilized in the preparation of these copper levels.

Geldreich and Clark *(3)* state that DWST ratios of 0.8 to 1.2 inclusive, indicate no bactericidal properties in a distilled water sample. Values below 0.8 are positive indication of biological toxicity while ratios exceeding 1.2 are indicative of growth stimulating substances. They concede however, that because of the sensitivity of the test, ratios up to 3.0 can be tolerated.

In Table 2 a copper concentration of 17 ppb results in a DWST ratio of 0.7 (toxic) while a copper level of 3 ppb gives a ratio of 0.9 (non-toxic). By extrapolation, a copper concentration of 10 ppb should then yield a DWST ratio of 0.8 (non-toxic). Ac-

TABLE 3. ANALYTICAL RESULTS OF DISTILLED WATER QUALITY CONTROL PROGRAM

Sample No.	Initial SPC Flask A	Initial SPC Flask B	SPC/flask A (000 omitted)	SPC/flask B (000 omitted)	Ratio SPC flask B / SPC flask A	Copper ppb	pH	Conductivity ohms (000)
1	39	34	3300	3000	0.9	0	6.2	450
2	38	35	2700	2800	1.0	0	5.8	660
3	31	29	3400	4200	1.2	10	6.7	83
4	32	34	3200	2600	0.8	0	6.4	590
5	28	29	2700	2200	0.8	<10	6.4	230
6	35	36	2700	12000	4.4	10	6.3	300
7	30	26	2400	930	0.4	0	6.6	170
8	27	31	3700	3000	0.8	<10	6.4	400
9	26	31	4500	2700	0.6	20	6.2	360
10	30	28	3200	2200	0.7	0	5.7	410
11	30	31	840	850	1.0	30	6.5	450
12	39	27	940	3100	3.3	<10	5.6	580
13	34	32	890	3100	3.4	0	6.3	420
14	46	39	1100	3000	2.7	0	6.3	590
15	42	37	970	670	0.7	30	6.4	210
16	31	31	770	780	1.0	100	5.8	750
17	19	31	1100	1400	1.3	<10	6.1	830
18	29	40	1100	360	0.3	10	6.4	140
19	34	36	1200	2200	1.8	0	6.5	200
20	41	40	1200	1300	1.0	10	6.3	530
21	79	80	5400	10000	1.9	<10	6.4	150
22	81	76	4300	4500	1.0	0	6.2	630
23	76	72	5100	3400	0.7	<10	6.1	510
24	70	49	4800	3700	0.8	0	6.1	620
25	70	70	5900	1300	0.2	0	6.8	200
26	105	82	3900	4100	1.0	<10	5.9	600
27	83	89	5300	15000	2.8	20	5.8	560
28	75	86	5200	3000	0.6	0	6.6	310
29	66	65	4600	6600	1.4	0	7.1	79
30	59	64	4800	950	0.2	10	6.1	530

cording to this data copper concentrations 17 ppb and above should be expected to yield DWST ratios indicative of biological toxicity.

Table 3 shows the analytical results of the distilled water quality control program on the first samples submitted by the local laboratories.

Nine distilled water samples had DWST ratios below 0.8 while three samples had ratios in excess of 3.0.

Levels of copper thought to be toxic exist in two samples (9 and 15) of the nine having a DWST ratio below 0.8. Not readily apparent is why three additional samples (11, 16 and 27) containing allegedly toxic amounts of copper had DWST ratios in the non-toxic range (0.8-3.0). Especially sample 16 since the copper concentration of this sample is almost six times greater than the level of copper found by us to cause toxicity (see Table 2).

At the risk of oversimplifying a highly complex situation it is conceivable that the above phenomenon might be explained if one considers that while toxic levels of copper are present in a given distilled water sample they may be overridden or masked by the presence of a more concentrated system of growth enhancement in the same distilled water sample.

The specific resistances of this group of samples ranged from a low of 79,000 ohms to a high of 830,000 ohms and averaged about 420,000. This average is considerably less than the 578,000 ohms recommended for "good quality" water *(3)*.

The results of samples submitted approximately two months later are similar in many respects to those found previously and are shown in Table 4.

Nine samples were toxic by the DWST procedure, and three were indicative of growth promoting substances.

Toxic levels of copper existed in three of the nine samples having a DWST ratio of less than 0.8, while two samples (28 and 29) had levels of copper thought to be toxic, but the DWST ratio for these samples indicated a suitable distilled water.

As explained previously however, this could possibly be the result of the interaction of systems of toxicity and growth enhancement.

TABLE 4. ANALYTICAL RESULTS OF DISTILLED WATER QUALITY CONTROL PROGRAM

Sample No.	Initial SPC		SPC/flask		Ratio SPC flask B / SPC flask A	Copper ppb	pH	Conductivity ohms (000)
	Flask A	Flask B	A	B				
			(000 omitted)					
1	53	35	7200	4600	0.6	30	6.0	690
2	52	54	1200	630	0.5	0	5.2	270
3	48	44	LA	4800	LA	10	6.0	340
4	39	55	7200	9300	1.3	0	6.6	36
5	52	52	4100	2300	0.6	20	5.9	370
6	59	59	2600	3200	1.2	0	5.8	750
7	34	52	3000	8200	2.7		No Sample	
8	49	58	3000	4300	1.4	Trace	6.5	150
9	53	42	4800	2800	0.6	0	5.8	620
10	61	51	2400	43000	18.0[a]	10	7.1	53
11	48	51	5800	730	0.1	0	5.8	550
12	66	38			LA	0	6.0	570
13	44	37	2400	1700	0.7	30	6.1	680
14	48	50	3700	2000	0.5	0	5.9	760
15	45	47	2800	2800	1.0	10	6.5	230
16	52	44	4700	9300	2.0	0	5.4	450
17	46	45	3100	4200	1.4	0	5.9	660
18	47	40	4100	10000	2.4	0	6.1	210
19	51	71	3500	1600	4.6	0	6.1	450
20	41	46	2000	3200	1.6	10	5.7	650
21	64	61	6200	4300	0.7	0	5.8	730
22	36	45	2300	8800	3.8	0	5.7	620
23	44	63	4700	5900	1.3	10	5.6	570
24	43	33	2800	170[b]	0.06	Trace	6.9	210
25	36	44	2400	2300	1.0	0	6.0	690
26	39	37	2700	7500	2.8	10	6.0	650
27	43	46	2300	2500	1.1	10	6.5	430
28	49	29	4800	12000	2.5	40	5.8	590
29	54	44	2200	5300	2.4	30	5.8	400
30	35	45	2300	3400	1.5	10	5.5	400

[a]Intense apple-like odor in this sample.
[b]172 colonies on 10^{-3} plate.

TABLE 5. BACTERIAL COUNTS OF A STERILIZED MILK SAMPLE INOCULATED WITH *Aerobacter aerogenes* AND PLATED IN DUPLICATE WITH DILUTION WATER AND MEDIA CONTAINING VARIOUS AMOUNTS OF COPPER

Copper (ppb)	0	10	50	100	500	1000
SPC	49000	58000	58000	54000	67000	62000
Ratio (SPC containing copper) / (SPC zero copper)		1.2	1.2	1.1	1.4	1.3

TABLE 6. BACTERIAL COUNTS OF RAW MILK SAMPLE PLATED IN DUPLICATE WITH DILUTION WATER AND MEDIA CONTAINING VARIOUS AMOUNTS OF COPPER

Copper (ppb)	0	10	50	100	500	1000
SPC	41000	39000	39000	41000	39000	43000
Ratio (SPC containing copper) / (SPC zero copper)		.95	.95	1.0	.95	1.04

Specific resistances on these samples ranged from 36,000 ohms to 760,000 ohms with an average of 490,000. This average is slightly above that of the previous group of samples but is still well below the recommended level.

If the data shown in Table 3 and 4 is interpreted in terms of current recommendations for "good quality" distilled water, only 30% of the milk and water laboratories participating in this study would comply.

This appears to indicate that producing distilled water of the quality specified by Geldreich and Clark *(3)* is accomplished by only a small fraction of routine installations, and meeting these requirements would require major improvements in production mechanics and operation.

The DWST is a very sophisticated procedure both in design and operation. As a result, a high degree of technical skill is demanded of the user. Because of these points the procedure does not lend itself readily to the routine of milk or water laboratories. A quality control procedure is only effective if it can be applied at a frequency statistically significant insofar as the occurrence of the particular probem it measures is concerned. To utilize this procedure in milk or water laboratories only twice a year for the purpose of distilled water quality-control hardly seems significant.

Basically, the DWST procedure is utilized to determine the biological toxicity in a given system, and this it does very well. However, is the sensitivity at which the DWST determines biological toxicity a good measure of distilled water suitability for use in milk and water laboratories? The results of Tables 5 and 6 are offered with this question in mind.

Table 5 shows the results of a sample of sterile milk that was inoculated with a 24 hour subculture of *Aerobacter aerogenes* and plated in duplicate with phosphate buffer dilution water and standard plate count agar both containing various concentrations of copper. The counts obtained after incubation at 32 C for 48 hours were compared to counts obtained on control plates containing no copper in the dilution water and media. The results indicate no toxicity when both media and phosphate dilution water contain copper concentrations as high as 1000 ppb. However, as little as 17 ppb copper is toxic to the same organisms when tested by the DWST.

Table 6 shows the results of a raw milk sample when plated using the same conditions as in Table 5 in relation to the media and dilution water. This sample was not inoculated with *Aerobacter aerogenes* however, since it was desired to test the effect of the same levels of copper on the normal flora in the raw

TABLE 7. GAS PRODUCTION BY *Aerobacter aerogenes in* LACTOSE-BROTH MADE WITH COPPER FREE CONTROL WATER AND IN LACTOSE BROTH PREPARED WITH CONTROL WATER CONTAINING 100 PPB COPPER

	Lactose Broth (35 C)			
	Control		100 ppb Copper	
Tube No.	24 hr	48 hr	24 hr	48 hr
1	−	+	−	+
2	−	+	−	+
3	−	+	−	+
4	−	+	−	+
5	−	+	−	+
6	−	+	−	+
7	−	+	−	+
8	−	+	−	+
9	−	+	−	+
10	−	+	−	+

milk. The ratios calculated for the results obtained in Table 6 support the data of Table 5. Again, no reduction in bacterial population was evident even in the situation where both media and dilution water contained 1000 ppb copper.

The results of Table 7 indicate when lactose broth is prepared to contain 100 ppb copper and inoculated with a loopfull of a 24 hour subculture suspension of *Aerobacter aerogenes* no toxicity is evidenced. Gas is present in the tubes at 48 hours incubation at 35 C as is the case with similarly inoculated control tubes of lactose broth prepared with copper-free distilled water. In the DWST, however, 100 ppb copper is nearly six times greater than the copper level (17 ppb) that causes toxicity (see Table 2).

Copper toxic effects which are evident in the lower nutrient levels of the DWST apparently are inhibited by the higher nutrient levels in the actual media used for milk and water analyses. Therefore, it is felt the DWST is an unrealistically severe yardstick of distilled water quality for milk or water laboratories. In view of the infrequency of copper concentrations at the 100 ppb level, it appears that an acceptable quality distilled water could be evidenced by conductivities above 400,000 ohms specific resistance and copper concentrations below 100 ppb. In essence, this is a measure of good mechanical condition and operation of a typical laboratory still. The demand for a more sophisticated quality distilled water (particularly zero tolerance of copper), for use in milk or water laboratories, is not indicated by the facts presented herein.

ACKNOWLEDGMENT

The authors extend their thanks and appreciation to Miss Josephine Cerny, Associate Chemist, for her assistance and interest in portions of this paper.

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FOOD PROCESSING—RESEARCH AND DEVELOPMENT REPORTS

The components of flexible packaging containers, when exposed to the conditions of food processing or sterilization, are subject to migration or leaching into the food. A study has been completed of four different foil-laminated flexible-film materials potentially suitable for the packaging and heat processing of foods. The specific objectives were to determine the amount of film extractives which migrate into food-simulating solvents, the nature and source of these extractives, and the amount of volatile extractives that may be lost during the analysis of solvent extractives. The study was designed to provide information for developing methods with which to determine film extractives in fats or oils after high-temperature extractions of the film by fat and to establish a correlation between the amounts of solvent extractives obtained at 150 degrees F to the amounts of fat extractives obtained at the highest temperatures compatible with the films. Extraction methods and results are presented for n-heptane and water-film extractions performed under varying conditions. The report also includes evaluations of microscopic alterations in film structure following solvent extractions, as well as several procedures for determining film extractives in fat . . . Order AD-640 522—Migration of flexible packaging components into food . . . R. L. Ferm, The Pillsbury Co., Minneapolis, Mnn, for the Army, June 66, 61p.

PROTEIN "SOLUBILIZATION" AND CALCIUM AND MAGNESIUM PRECIPITATION BY "CHLORINATED CLEANERS"[1]

E. O. Wright, W. S. LaGrange, Connie Dennis and E. W. Bird[2]

Department of Dairy and Food Industry

and

D. H. Hotchkiss

Department of Statistics
Iowa State University, Ames 50010

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Summary

It has been stated that chlorinated cleansers increase the "protein solubilization" properties of cleanser solutions to which they are added *(7)*. Chlorinated cleansers, however, are alkaline materials which increase the active alkalinity of the solutions to which they are added; this seems to have been overlooked. The study reported here was undertaken to determine whether the added available chlorine or the increase in active alkalinity was responsible for the increase in protein solubilization.

Two chlorine sources were employed: Sodium hypochlorite (Chlorox) and chlorinated TSP. The approximate amounts by which these chlorine sources increased the active alkalinity (as % NaOH) at the 100 ppm and 200 ppm levels were: Chlorox, 0.0091% and 0.0199%, and chlorinated TSP, 0.0425% and 0.0800%.

When the active alkalinity was the same in two solutions, there was no significant difference in the amounts of protein they would dissolve, even though one of the solutions contained available chlorine and the other did not. However, the pairs of solutions containing the same percentage of active alkalinity as the solution containing 200 ppm available chlorine solubilized significantly more protein ($p < 0.06$) than did the pairs which contained the same active alkalinity as the solution containing 100 ppm; this is attributed to the higher active alkalinity of the former. These results were the same whether Chlorox or chlorinated TSP was the chlorine source, whether the direct or indirect method of determining protein solubilization was employed, and in the direct method, whether skim or homogenized milk was the soiling agent. On the basis of these data, the increase in active alkalinity, not the added available chlorine appears responsible for the increase in protein solubilization when a chlorine source is added to an alkaline cleanser.

No calcium remained in any of the spent cleansers from the direct method of evaluating protein solubilization. In general, the amount of magnesium remaining decreased as the active alkalinity of the solution increased. There was no significant difference in magnesium content between the chlorine-containing and chlorine-free solutions having the same active alkalinity. Chlorine sources apparently minimize precipitation of hard water cations onto equipment surfaces.

Recently there has been considerable use of "chlorinated cleansers" by the Dairy Industry, presumedly because of their ability to "dissolve" more protein than can non-chlorinated cleansers *(7)*. In a general study of cleansers, Harding and Trebler *(5)* indicate that the rate of protein peptization was roughly a function of the pH of a cleanser solution and that the total weight of protein dispersed was roughly a function of its active alkalinity (titration from the original pH of the solution to the phenolphthalein endpoint, usually expressed as % NaOH).

Because addition of available-chlorine sources to a basic cleanser solution will increase its active alkalinity, it was considered that the increased protein "solubilization" caused by the addition of the chlorine source, probably resulted from an increase in active alkalinity, not from the available chlorine added.

This hypothesis was tested by preparing several series of solutions within each of which there was *(a)* a basic 0.5% anhydrous tri-sodium phosphate (TSP) solution *(b)* this solution plus chlorinating agent to yield 100 ppm available chlorine and 200 ppm chlorine and *(c)* TSP solutions containing, respectively, the active alkalinities found in the two chlorinated TSP solutions in *(b)* but containing no chlorine. These solutions were employed in the indirect method of Merrill et al. *(7)*, and in a direct method described below.

Materials and Methods

The soiling material employed for the microscope slides was a clarifier-slime preparation, and that for the centrifuge tubes was either commercial skim milk (about 0.54% fat) or commercial homogenized milk (about 3.5% fat) from the Iowa State University Dairy.

Satisfactory soiling films were not obtained with the "suspension of 60% clarifier slime" recommended by Merrill et al. *(7)*. It was necessary to pasteurize the milk that was to be clarified; otherwise, enzymatic activity quickly rendered the soiling medium useless under working conditions. After considerable experimentation, the soft portion of the clarifier slime, which lay just beneath the skim milk in the bowl, was

[1]Journal Paper No. J-5666 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa, Project 1298.
[2]To whom inquiries should be sent.

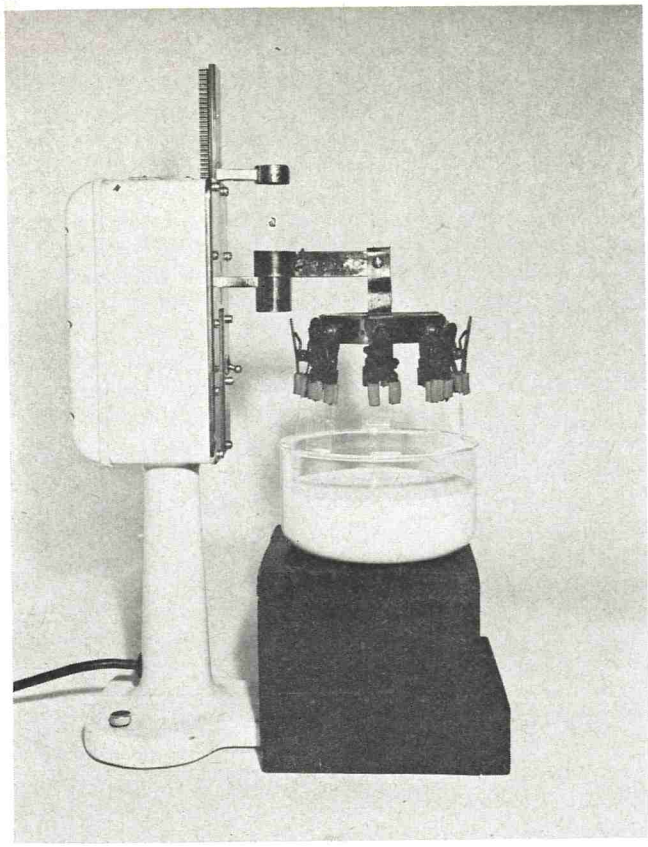

Figure 1. Apparatus for dipping glass slides into soiling medium or cleanser solutions; indirect determination of protein solubilization.

removed by hand. The diluting medium was the "skim milk" in the bowl. The slime (average total solids, 24%) was mixed with the skim milk (average total solids 10%) to yield a soiling mixture containing 14% total solids.

The slides were soiled by the method described by Merrill et al. (7) except that the slides were held 10 sec in the soiling medium during each dip. The apparatus shown in Figure 1 was employed for dipping the slides into the diluted separator slime, and the "cleanser solution." A switch was placed in th cord supplying current to the modified, Type 728, Submarine Signal Co. (Boston, Mass.), curd-tension meter. When the direction of travel switch at the low point of the path was tripped, the current could be shut off during the time for either the dipping or cleanser treatment. In actual operation, the wooden platform shown, was replaced by a Lab-Jack to facilitate setting the depth of immersion at a desired level. A second difference from the Merrill et al. (7) procedure was the method of predrying the slides. Forced, hot air from a hair dryer (Tropic Aire, model 38102, McGraw-Edison Co., Bersted Mfg. Div., Booneville, Mo.) was employed (Figure 2). A section of copper tubing, the same diameter as the outlet of the dryer, was expanded on one end to fit over the dryer outlet, closed on the other with a copper plate, and drilled (1/8-inch holes) along the top and sides to yield an even distribution of hot air. The drying time was approximately 10 min. After the final oven-drying period (13 hr., 80 C) the slides were held in a Boekel cabinet desiccator (Boekel, Philadelphia, Pa.) without desiccant, until used.

Skim or homogenized milk was dried onto the inside walls of 50-ml centrifuge tubes (Figure 3). The adapter was made from a 24/40 outer standard taper, sealed to a 35-mm length of 6-mm id. glass tubing. The air-intake tube at the inner-seal end consists of a 15-mm length of 6.0-mm i.d. tubing sealed at a right angle to a 12.0-cm length of 3.0-mm, i.d. tubing. Two such rotary evaporators (Rae Motor Corp., McHenry, Ill.) were attached to a single water aspirator operated at full water flow. The hot plate was set at medium heat. Two-ml samples of skim or homogenized milk were employed. Homogenized-milk samples were diluted with 1 ml distilled water to obtain relatively even drying. Skim milk samples were used without dilution at times. At other times samples were diluted with 0.5 ml distilled water. The skim milk film was more evenly distributed with the diluted samples but no difference in results was obtained between the procedures. The pre-dried tubes were dried in a constant-temperature over at 80 C for 1 hr and then held in the cabinet desiccator, until used.

The method of selecting slides for the nitrogen determination (in the indirect method) is important if this selection is not to invalidate statistical evaluation of the results. In each "run" there were five "cleanser" solutions and a tap-water "blank." Because of deterioration at room temperature, however, it was impossible to employ a single soiling mixture for six sets of slides. The procedure evolved was as follows: The soiling mixture was brought from a 4.4 C (40 F) refrigerator, two series of slides were dipped and the mixture discarded. Hence, three lots of mixture were employd for six sets of slides. Eight slides were soiled per set to insure a minimum of six in case of breakage. To eliminate differences that could be introduced from one set of slides to the next, through dipping, one slide was taken from each of the six soiled sets to constitute a group for "washing" with a single solution. The positions on the dipping holder were marked and kept

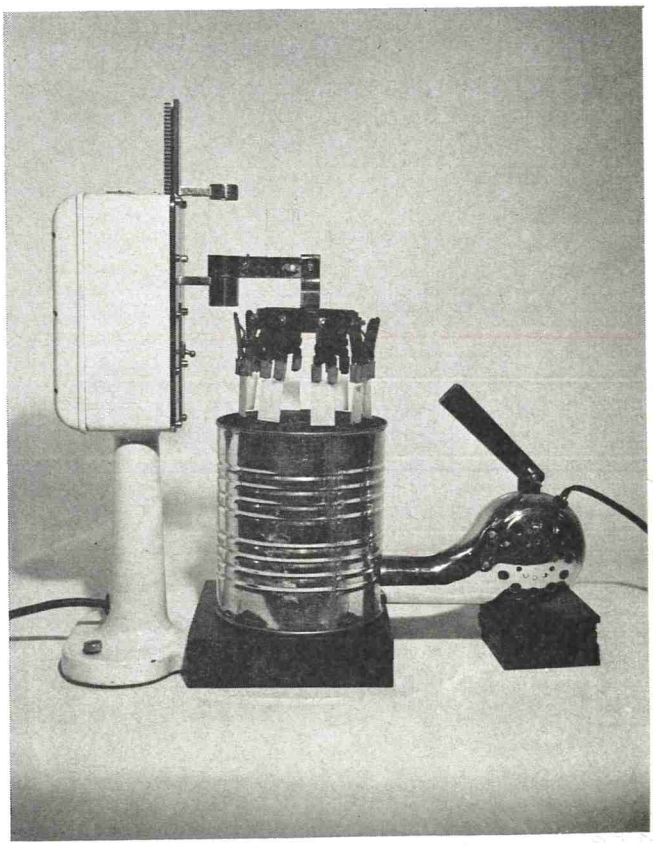

Figure 2. Apparatus for drying soiled and cleansed slides.

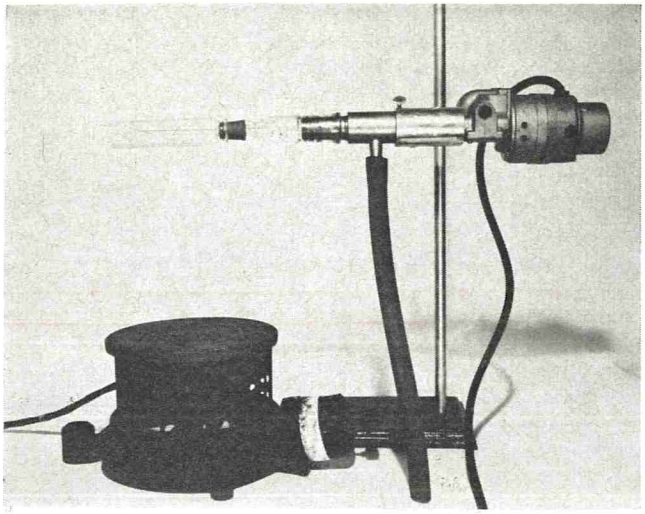

Figure 3. Apparatus for soiling centrifuge bottles employed in direct determination of protein solubilization.

in the same position for all dippings. To minimize effect of position of the slides in the holder, if the first slide of a set in run 1 started with slide 1, in set 2, it was in position 2, in set 3, in position 3, etc.

The cleansers for the 50-ml centrifuge tubes were heated to 65 C in a constant temperature water bath (Precision Scientific Co., Chicago, Ill.) in rubber-stoppered erlenmeyer flasks. Fifty ml of a solution were measured into a soiled tube with a pre-warmed graduated cylinder. The tubes were rubber-stoppered and held 10 min., during which time pairs were balanced in their shields for centrifuging. At the end of the 10 min hold, the stoppered tubes were centrifuged for 30 min at 2000 rpm (International size-2, centrifuge, 9.75-in diameter head to the bottom of the shields, International Equipment Co., Boston, Mass.), after which the solutions were filtered through No. 42, 11-cm, Whatman folded filter papers. The filtered solutions were held stoppered until analyzed.

The nitrogen determinations were made by the semi-micro total nitrogen procedure of Rowland *(8)* except that mercuric oxide was used as the catalyst; the ammonia was caught in 2.5% boric acid according to the method of Menefee and Overman, *(6)* and titrated with accurately standardized, approximately 0.04 N HCl. In the indirect method, 3 slides were used per determination, allowing duplicate analyses per solution. With the direct method, two soiled centrifuge tubes were used with each cleanser solution; 20 ml of each filtrate were used as a single determination, thus yielding duplicates from each tube. In both methods, 2 g of mercurous oxide-sodium sulfate catalyst, 8 ml of conc. H_2SO_4, 150 ml of dilution water, 24 ml of 50% (w/v) NaOH containing $Na_2S_2O_3$ and 35 ml H_3BO_3 were employed. To insure even boiling, a boiling rod was placed in each 300-ml Kjeldahl flask just before the addition of the sodium hydroxide solution. The boiling tubes were made from 12-cm lengths of 4-mm glass rod to the submerged ends of which 10-mm lengths of 3 mm i.d. glass tubing were sealed *(2)*.

The "cleanser" solutions were prepared from three stock solutions: *I*, TSP, 250 g $Na_3PO_4 \cdot 12\ H_2O$ were dissolved in distilled water and made to 2 liters with distilled water, *II*, sodium hypochloride solution, containing approximately 4500 ppm available chlorine (80 ml Chlorox made to 1 liter with distilled water) and *III*, chlorinated TSP containing approximately 3700 ppm available chlorine (105 g chlorinated TSP made to 1 liter with distilled water).

The basic cleanser solution, A, was prepared by making 185.5 ml of solution *I* to 2 liters with tap water. When soiled centrifuge tubes were employed, a volume of 50 ml was sufficient. For each 4-liter batch of solution *I* prepared, three other solutions were prepared containing 210.0 ml (A_1), 250 ml (A_2) and 285 ml (A_3) of solution *I* in 2 liters of final tap-water solution. The active alkalinity of these four solutions (A, A_1, A_2 and A_3) was determined. The active alkalinities of solutions A, A_1, A_2 and A_3 were plotted against the ml of solution *I* required to prepare 2 liters (or 500 ml) of solution. The ml of solution *I* needed to prepare a solution with the same active alkalinity as a chlorine-containing solution, were read from this curve.

Solutions containing 100 ppm, B, and 200 ppm, D, available chlorine were prepared by making 185.5 ml of *I*, plus the calculated amount of *II* or *III* to 2 liters (or proportionate quantities to 500 ml) with tap water. The stock solutions were measured with 100-ml or 50-ml burettes, depending on the volume required.

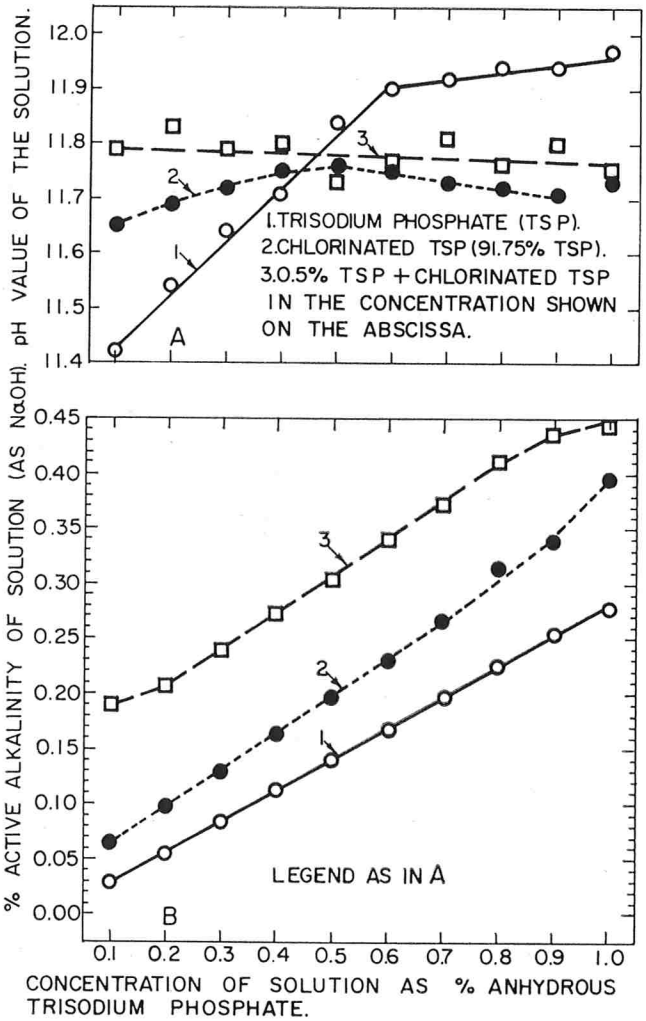

Figure 4. pH values (A) and per cent active alkalinity (B) *vs.* concentration of solutions of trisodium phosphate, chlorinated trisodium phosphate and 0.5% trisodium phosphate plus chlorinated trisodium phosphate.

Solutions C (equivalent to B) and E (equivalent to D) were prepared by interpolating the active alkalinities of solutions B and D on the curve described earlier, reading the volume of *I* needed for the volume of solution desired, and preparing these solutions with tap water.

Available chlorine determinations were not affected by the flocculant precipitates of calcium phosphates in the tap-water solutions. These phosphates reacted and increased the measured active alkalinity when determined by the method to be described. For this reason, 250-ml aliquots of each cleanser solution were placed in 250-ml, rubber-stoppered, centrifuge bottles and centrifuged in an International size 2 centrifuge at 2000 rpm for 30 min. The precipitated calcium phosphates packed tightly; the supernatant was poured off and used for the chlorine and active alkalinity determinations.

Available chlorine was determined in the usual manner: 50 ml of the solution were pipetted into a 125-ml erlenmeyer flask, 2 g of solid KI were added and rotated until the KI was completely dissolved. 10 ml of 1 N H_2SO_4 were added and the solution was titrated immediately to a pale straw color with 0.0141 N $Na_2S_2O_3$. 2 ml of 1% starch solution were added and titrated to the disappearance of blue color. The ml titration x 10 = ppm available chlorine in the solution titrated. For the analysis of solutions *II* and *III*, 50 ml of the stock solution were diluted to 1 liter with distilled water. 50 ml of the dilute solution were titrated with 0.0141 N $Na_2S_2O_3$ solution. The ppm chlorine were obtained by multiplying the ml of $Na_2S_2O_3$ solution by 20.

Active alkalinity cannot be determined by a direct titration of chlorine-containing solutions because of the effect of the chlorine on the end-point of phenolphthalein. The following indirect procedure was employed: 50 ml of the cleanser solution were pipetted into a 300-ml erlenmeyer flask, 50 ml of 0.1 N H_2SO_4 were added by pipette, a "boiling rod" was placed in the flask to ensure even boiling and the solution was boiled on a hot plate (set at medium) for 5 min. The solution was cooled in a tray of water at room temperature. The boiling rod was rinsed and the solution was shaken from the open end, three times, and the boiling rod was removed from the flask. Two g solid KI were added, the flask was rotated to dissolve the KI, and, if iodine was released, the solution was titrated to a pale straw color with 0.0141 N $Na_2S_2O_3$. Two ml of 1% starch solution were added and the solution was titrated just to disappearance of the blue color. Thirty drops of 1% alcoholic phenolphthalein were added and the excess sulfuric acid was titrated with 0.1 N NaOH solution until 1 drop of the alkali yielded a faint but definite red-violet color that persisted for 30 sec. The solutions were sufficiently buffered that an attempt to use an increment smaller than one drop introduced a greater error than the use of one drop because of the inability of the eye to discern the smaller color change. If no iodine was liberated on the addition of potassium iodide, the 2 ml of starch solution were added. No thiosulfate solution was added, and the titration was carried out as described. Blanks containing 50 ml distilled water and 50 ml of 0.1 N H_2SO_4 were subjected to the same treatment as were the samples which did not contain chlorine. The active alkalinity of the solution was equivalent to the ml of NaOH for the blank minus the ml of NaOH for the sample. The active alkalinity, as sodium hydroxide was calculated as:

% Active Alk. = (ml titration, blank-ml titration, samp) x N NaOH x m.eq. NaOH x (100/ml cleanser soln)

Calcium and magnesium were determined by a modification of the method of Bird et al. *(1)*. Determinations were made on the filtrates equivalent to those from the direct protein determination. To obtain sufficient filtrate for these determination, 8 ml of skim milk, plus 2 ml of redistilled water[3], or 8 ml of homogenized milk, plus 4 ml of redistilled water, were dried in 250-ml centrifuge bottles, using the apparatus employed with the 50-ml centrifuge tubes. The amount of cleanser solution was 250 ml; the method of cleansing was the same as that for the 50-ml tubes.

Because of the high phosphate content of the cleanser solutions, the following procedure for calcium and magnesium was necessary: 100 ml of the centrifuged, filtered, spent cleanser solution were pipetted into a 250-ml, g.s., graduated cylinder; 115 ml of potassium metastannate and 3 ml of conc. HNO_3 were added, the mixture was made to 220 ml with redistilled water, thoroughly mixed, and allowed to stand for 5 min. The mixture was transferred to a 250-ml, rubber-stoppered centrifuge bottle and centrifuged for 5 min at 2000 rpm. Approximately 140 ml of clear supernatant were obtained. Because several preliminary analyses of 100-ml aliquots yielded no calcium, 100 ml of the supernatant were employed for the magnesium determination, and 40 ml for the calcium determination.

pH values were determined with a model G, Beckman glass electrode unit; the values reported were not corrected for alkali-ion errors.

RESULTS AND DISCUSSION

The concentration of TSP in cleanser solutions and their pH and active alkalinities. Figure 4A shows that the pH of TSP solutions increases as the concentration increases to between 0.5% and 0.6% anhydrous TSP, after which the rate of increase in pH with rise in concentration is not great. This is in agreement with the observations of Merrill et al. *(7)*. The rise in pH with increase in concentration is much less for chlorinated TSP solutions in the concentration range 0.1% to 0.5% anhydrous TSP equivalent than for TSP solutions; at higher concentrations, the pH actually is depressed slightly. When 1% chlorinated TSP was added to a 0.5% anhydrous TSP solution, although the pH of the resulting solution is greater than that of a 0.1% chlorinated TSP solution, it is slightly less than that of a 0.5% TSP solution; as the concentration of chlorinated TSP is increased above 0.1%, the pH of the solutions decrease slightly in essentially linear fashion.

If the active alkalinity of these same solutions is considered (Figure 4B), the active alkalinities of all three types of solutions increase as the concentration of the alkaline ingredients increase. These data confirm the hypothesis that the active alkalinities of the cleanser solutions increase as a result of the addition of a chlorinated cleanser.

Data obtained but not presented, show that, whether a chlorinated cleanser is used alone or is added to an alkaline cleanser solution, the concentration of

[3]Prepared as described by Handwerk and Bird *(4)*.

TABLE 1. INCREASE IN ACTIVE ALKALINITY CAUSED BY ADDITIONS OF CHLORINE SOURCES TO ALKALINE CLEANSER SOLUTIONS TO YIELD 100 AND 200 PPM AVAILABLE CHLORINE

ppm Available chlorine in the solution	100	200
Soiling agents: Clarifier slime – skim milk[a]; skim milk[b] (7 values)[c]		
Chlorine source: Chlorox		
Increase in % active alkalinity by chlorine source	0.0091	0.0199
Increase as % of 0.03% active alkalinity[d]	30.3	66.3
Chlorine source: Chlorinated TSP		
Increase in % active alkalinity by chlorine source	0.0424	0.0800
Increase as % of 0.05% active alkalinity[d]	84.8	160.0
Soiling agent: Homogenized milk (3 values)		
Chlorine source: Chlorox		
Increase in % active alkalinity by chlorine source	–0.0007[e]	0.0161
Increase as % of 0.03% active alkalinity[d]		53.7
Chlorine source: Chlorinated TSP		
Increase in % active alkalinity by chlorine source	0.0379	0.0758
Increase as % of 0.05% active alkalinity[d]	75.8	151.6

[a]Indirect method of protein determination.
[b]Direct method of protein determination.
[c]Mean weighted for number of samples in each group.
[d]Harding and Trebler (5) recommended 0.03% and 0.05% active alkalinities (as NaOH) in cleanser solutions for general purpose cleaning.
[e]No explanation can be given for this single anomalous value.

available chlorine in the solution is dependent solely on the amount of chlorinated cleanser added to the solution.

Increases in the active alkalinity of alkaline cleanser solutions caused by addition of chlorine sources to yield 100 and 200 ppm available chlorine are presented in Table 1. The increases range from 0.0091% to 0.0199% active alkalinity when Chlorox was the chlorine source and from 0.0379% to 0.0800% active alkalinity when chlorinated TSP was employed. These increases appear small at first glance. However, Harding and Trebler (5) recommended only 0.03% and 0.05% active alkalinities (as NaOH) for alkaline solutions for general purpose cleaning. With these percentages as reference values, the increases caused by addition of Chlorox represent from 30.3% to 66.3% of the lower recommended level, and those resulting from addition of chlorinated TSP are equivalent to from 75.8% to 160.0% of the higher recommended level. Such increases in active alkalinity would lead one to expect considerable increases in protein solubilization as a result of the addition of chlorinated cleansers in amounts sufficient to furnish 100 to 200 ppm available chlorine.

The accuracy with which it is possible to attain desired active alkalinities is shown in Table 2. The mean values presented for the pairs of solutions, B:C and D:E, are representative of the agreement between individual pairs of solutions. In both the direct and indirect methods of evaluating the cleansers, there was no significant difference ($p < 0.05$) between the active alkalinities of the matched pairs of solutions. The available chlorine concentrations were attained within $\pm$ 3 ppm of the desired values.

Protein solubilization by chlorine-containing solutions and those containing no chlorine are summarized in Table 2. The means of the data indicate that chlorine-containing solutions, at either 100 ppm or 200 ppm available chlorine, have no greater protein-solubilizing powers than do solutions containing the same concentrations of active alkalinity but no chlorine. A least squares treatment of the data showed no significant differences between the chlorine-containing and non-chlorine solutions. If the pairs of solutions at the 100 and 200 ppm available chlorine levels are compared with the control (0.5% anhydrous TSP), there is significantly more protein ($p < 0.05$) solubilized by the control solution than by the experimental pair solutions. When the pairs of solutions at the 200 ppm available chlorine level are compared with those at the 100 ppm available chlorine level, the difference in protein solubilization approaches significance ($p = 0.05$) in favor of the pairs with the higher active alkalinity. Similarly, when the Chlorox-containing solutions are compared with those containing chlorinated TSP at the same available chlorine levels, the protein-solubilizing powers of the latter are highly significantly greater ($p < 0.01$) than the former. This difference is attributed to the higher active alkalinities of the solutions containing chlorinated TSP (Table 1).

None of the comparisons indicate a greater protein-solubilizing power of solutions containing available chlorine when compared with solutions containing no chlorine but the same active alkalinity. It is considered that the increase in active alkalinity, when "chlorinated cleansers" are added to alkaline cleanser solutions, results from the increase in active alkalinity, not from the available chlorine added.

The effect of available chlorine on the precipitation of calcium and magnesium was investigated (Table 3). The active alkalinities of all the cleanser solutions were high enough to cause complete precipitation of the calcium in the hard water (approximately 260 ppm calcium and 125 ppm magnesium hardness). As regards the residual magnesium, there was no significant difference between the chlorine-containing and the non-chlorine solutions if the active alkalinities of the two solutions were the same. The Chlorox-containing solutions precipitated sig-

TABLE 2. PROTEIN DISSOLVED BY CHLORINATED AND NON-CHLORINATED CLEANSERS CONTAINING EQUIVALENT ACTIVE ALKALINITIES[a]

	Solution designation[b]					
	Tap H_2O	A	B	C	D	E
Indirect Method of Study[c]						
Soiling agent: Soft clarifier slime + bowl skim milk (T.S. 14.0%)						
Chlorine source: Chlorex (3 runs)						
Av. ppm avail. Cl	0.0	0.0	101.5	0.0	201.4	0.0
Av. % active alk.	0.0	0.1680	0.1770	0.1779	0.1855	0.1860
Av. resid. N value[d]	80.8	28.4	27.0	29.3	26.5	27.8
Chlorine source: Chlorinated TSP (4 runs)						
Av. ppm avail. Cl	0.0	0.0	101.4	0.0	200.7	0.0
Av. % active alk.	0.0	0.1670	0.2069	0.2065	0.2449	0.2434
Av. resid. N value[d]	79.3	34.4	38.3	36.3	30.0	33.2
Direct method of study[e]						
Soiling agent: Skim milk						
Chlorine source: Chlorox (4 runs)						
Av. ppm avail. Cl		0.0	100.0	0.0	199.4	0.0
Av. % active alk.		0.1665	0.1757	0.1763	0.1882	0.1890
Av. soluble N value[d]		19.36	16.81	16.97	17.62	18.62
Chlorine source: Chlorinated TSP (4 runs)						
Av. ppm avail. Cl		0.0	99.7	0.0	199.2	0.0
Av. % active alk.		0.1665	0.2107	0.2114	0.2480	0.2481
Av. soluble N value[d]		19.36	17.65	19.91	19.68	21.54
Soiling agent: Homogenized milk						
Chlorine source: Chlorox (3 runs)						
Av. ppm avail. Cl		0.0	100.8	0.0	200.5	0.0
Av. % active alk.		0.1651	0.1614	0.1609	0.1812	0.1804
Av. soluble N value[d]		23.36	18.67	17.88	19.28	20.18
Chlorine source: Chlorinated TSP (3 runs)						
Av. ppm avail. Cl		0.0	100.4	0.0	200.5	0.0
Av. % active alk.		0.1651	0.2030	0.2027	0.2409	0.2390
Av. soluble N value[d]		23.36	21.98	22.08	21.05	22.70

[a]Expressed as % NaOH.
[b]Solutions: A. 0.5% anhyd. TSP; B. A + 100 ppm avail. Cl.; C. TSP soln. with same active alk. as B, no Cl; D. A. + 200 ppm avail. Cl; E. TSP soln. with same active alk. as D, no Cl.
[c]Slides soiled by alternately dipping in soiling medium and alkaline chlorine-calcium solution, several times, were dried, immersed in a cleanser solution (10 min; 65 C) and the nitrogen remaining was determined.
[d]Expressed as mcg. N per mg. original soil.
[e]Definite amounts of skim or homogenized milk were evenly dried onto the inner surface of 50-ml centrifuge tubes; 50 ml of a cleanser solution (65 C) were added and held 10 min, centrifuged, the supernatant filtered and analyzed for nitrogen.

nificantly less magnesium than did the chlorinated TSP solutions ($p < 0.01$). This is attributed to the higher active alkalinity and higher phosphate contents of the chlorinated TSP solutions.

The type of soiling material had no effect on either protein or magnesium solubilization in the experiments in which protein solubilization was evaluated by the direct method. It is concluded that, in these experiments, the difference in fat content in the soiling material was without effect in protein and magnesium solubilization.

The experimental results presented indicate that the presence of available chlorine in an alkaline cleanser is not responsible for increasing protein solubilization or for decreasing the precipitation of hard-water cations such as calcium and magnesium. Such improvement in the solubilization of proteins of alkaline cleanser solutions as results from the addition of chlorinated cleansers is effected by the increase in the active alkalinity of the solutions.

The active alkalinities of the cleanser solutions employed in this study were selected to be in accord with those employed by Merril et al. *(7);* in both studies, these active alkalinities are about five to six times higher than the lower concentration recommended by Harding and Trebler *(5)* for general purpose cleaning and between three and four times greater than the higher levels proposed by them.

TABLE 3. CALCIUM AND MAGNESIUM CONTENT OF SPENT ALKALINE SOLUTION PAIRS CONTAINING THE SAME PERCENTAGE OF ACTIVE ALKALINITY[a]; ONE MEMBER CONTAINED A CHLORINE SOURCE, THE OTHER DID NOT

		Solution designation[b]					
		Tap H_2O	A	B	C	D	E
		Direct method of study[c]					
		Soiling agent: Skim milk					
		Chlorine source: Chlorox (2 runs)					
Av. ppm avail. Cl.		0.0	0.0	100.2	0.0	199.5	0.0
Av. % active alk.		0.0	0.1651	0.1756	0.1746	0.1862	0.1855
Av. mg in 100 ml spent soln	Calcium	33.8	0.0	0.0	0.0	0.0	0.0
	Magnesium	13.1	1.24	2.15	2.93	0.42	0.77
		Chlorine source: Chlorinated TSP (2 runs)					
Av. ppm avail. Cl.		0.0	0.0	100.0	0.0	199.2	0.0
Av. % active alk.		0.0	0.1651	0.2042	0.2032	0.2432	0.2429
Av. mg in 100 ml spent soln	Calcium	33.8	0.0	0.0	0.0	0.0	0.0
	Magnesium	13.1	1.24	0.34	0.29	0.34	0.45
		Soiling agent: Homogenized milk					
		Chlorine source: Chlorox (2 runs)					
Av. ppm avail. Cl.		0.0	0.0	100.8	0.0	200.3	0.0
Av. % active alk.		0.0	0.1651	0.1738	0.1731	0.1913	0.1919
Av. mg in 100 ml spent soln	Calcium	26.3	0.0	0.0	0.0	0.0	0.0
	Magnesium	8.9	1.14	0.54	0.60	0.73	0.79
		Chlorine source: Chlorinated TSP (2 runs)					
Av. ppm avail. Cl.		0.0	0.0	100.5	0.0	199.5	0.0
Av. % active alk.		0.0	0.1651	0.2099	0.2102	0.2461	0.2465
Av. mg in 100 ml spent soln	Calcium	26.3	0.0	0.0	0.0	0.0	0.0
	Magnesium	8.9	1.14	0.43	0.52	0.37	0.34

[a]Expressed as % NaOH.
[b]Solutions: A. 0.5% anhyd. TSP; B. A + 100 ppm avail. Cl; C. TSP soln. with same active alk. as B, no Cl; D. A + 200 ppm avail. Cl; E. TSP soln. with same active alk. as D, no Cl.
[c]Definite amounts of skim or homogenized milk were evenly dried onto the inner surface of 250-ml centrifuge bottles; 250 ml of a cleanser solution (65 C) were added and held 10 min, centrifuged, the supernatant filtered and analyzed for calcium and magnesium.

Likewise, these active alkalinities (0.1651% to 0.2465% as NaOH) are higher than those found satisfactory for in-place cleaning;, *viz.,* 0.1032% as NaOH (0.08% as Na_2O) *(3).*

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METHODS FOR THE QUALITATIVE AND QUANTITATIVE DETERMINATION OF AFLATOXINS[1]

E. H. MARTH

Department of Food Science and Industries
and
The Food Research Institute

University of Wisconsin, Madison

SUMMARY

Qualitative and quantitative measurements of aflatoxins are most often routinely made by means of thin-layer chromatography. The accuracy of the quantitative determination by this procedure can be improved by substituting a densitometric "reading" of the thin-layer plates for visual examination. Examination of small samples (down to 0.3 mg) can be accomplished by a modified thin-layer chromatography procedure which employs an acetone-water extraction technique. Confirmation of toxicity is most often done with the duckling bioassay although embryonated chicken eggs have been used successfully for this purpose. Other methods which, under certain circumstances, may find application in the measurement of aflatoxins include: paper chromatography, microbiological assay, spectrophotometry, and a tissue culture assay.

Interest in food-borne mycotoxins is of relatively recent origin. Aflatoxin, the mycotoxin which has received most research attention, was unknown seven years ago. It is true that nearly 25 years ago cereal grains which overwintered in the field had supported the growth of several toxigenic molds and later caused numerous deaths when products made from the grains were consumed *(12)*. Undoubtedly there were other instances in which humans or animals consumed moldy products and became ill, but these observations, if they were made, appear to have been largely disregarded until early in 1960 when large numbers of turkey poults and ducklings on English farms died after ingesting moldy peanut meal imported from Brazil *(12)*. Subsequently it was demonstrated that *Aspergillus flavus* formed the toxin as a consequence of its growth in the peanut meal *(12)*. These initial observations led to development of considerable interest in and experimentation with aflatoxin. The need for a technique to detect and quantify aflatoxin was soon apparent.

Since ducklings were initially found to be highly sensitive to the effects of the toxin, it is not surprising that early investigators utilized them in assay procedures. Use of animals for this purpose is expensive, time consuming, and slow in providing results. Consequently, thin-layer chromatography techniques were soon found applicable to detect and measure aflatoxin. This procedure, when properly conducted, also yields additional information on which of the four known aflatoxins are present in a test sample. Comparison of spots produced by test samples with those from standards is done visually and, hence, can be a source of substantial error when this method is used for quantitative purposes. To minimize this error, an appropriate densitometer has been used successfully to "read" the thin-layer chromatographic plates. These are the principal methods to be discussed in this paper. Several other approaches have been suggested for measurement of aflatoxin and these will also be briefly mentioned.

[1]Presented as part of a Round Table on Analytical Methods in Food Microbiology at the 67th Annual Meeting of the American Society for Microbiology, May 3, 1967, at New York, New York.

THE DUCKLING BIOASSAY

The duckling bioassay was developed by Sargeant and co-workers *(17)* in 1961 at the Tropical Products Institute in London and at the Central Veterinary Laboratory in Weybridge, England. The procedure used by these investigators is essentially as follows:

1. The sample of peanut meal is continuously extracted with methanol for 18 hr using a Soxhlet apparatus. The solvent is then evaporated and a black oily residue remains.

2. This black oily residue is dispersed in water and the resultant mixture is continuously extracted for 3 hr by means of chloroform. Removal of chloroform yields a black, molasses-like residue.

3. The residue just described is dissolved in a mixture (50:50) of light petroleum (hexane) and methanol and a dark brown solution is obtained. Water is added and the mixture is shaken vigorously. The lower layer (which contains the aflatoxin) is removed, washed with hexane, and evaporated to dryness. The resultant product is brown and solid.

4. The extract thus obtained is suspended in water and administered to ducklings (usually day-old) by dosing them into the lower part of the esophagus through a thin polyethylene tube attached to a hypodermic syringe. Generally, ducklings are treated

for five consecutive days and then are sacrificed three days later.

5. The criteria for toxicity are mortality and/or liver lesions. The LD_{50} for one-day-old ducklings is approximately 0.5 mg toxin per kg of body weight *(12)*. Ducklings which die during the dosing period display massive necrosis of the liver parenchymal cells and diffuse hemorrhages throughout the liver. Administration of less toxin is accompanied by the characteristic proliferation of bile duct epithelium.

The procedure just described was modified slightly by Armbrecht et al. *(3)* at the U. S. Food and Drug Administration laboratories. Their method employs an initial triple extraction with hot methanol, a 6-hr extraction with methanol in a Soxhlet apparatus, a 3-hr extraction with chloroform, washing with petroleum ether, evaporation of solvents, and dissolving the residue in propylene glycol before administering it to the ducklings by the tube method. They receive 0.5 ml of sample the first day and the dosage is increased by 0.1 ml each succeeding day for a total of seven days. Surviving birds are all sacrificed after an additional seven days without treatment and major organs and tissues are examined for gross pathological changes.

Although the duckling bioassay appears to yield accurate information about the toxicity of a sample, it is now employed primarily as a confirmatory test. Furthermore, it is difficult to quantitate aflatoxin in an unknown sample by this method.

THIN-LAYER CHROMATOGRAPHY

The initial method for detection of aflatoxin by thin-layer chromatography was reported by Broadbent et al. *(5)*. Although numerous modifications of the original method have been suggested, they all employ, essentially, four major steps. These are: *(a)* defatting if the product is high in fat, *(b)* extraction, *(c)* purification and *(d)* resolution.

Instead of reviewing all of the proposed methods, attention will be directed primarily to the procedure recommended in June, 1965 by the Aflatoxin Methodology Working Group *(1)*. This method is probably the most rapid and has been used extensively (although not without some modifications) by numerous investigators. The following steps are involved:

1. The sample (50g) is extracted with a mixture of 1 part hexane and 2.5 parts aqueous methanol (55:45 v/v methanol:water) in a Waring Blendor. After appropriate blending, the material is transferred to a centrifuge bottle and is centrifuged for 20 to 30 min. The aqueous methanol layer (middle layer) is removed and retained for partition column chromatography.

2. A portion (50 ml) of the aqueous methanol layer is mixed with Celite and the mixture is placed into a glass chromatographic column previously equipped with a cotton plug at the bottom. Initially the column is eluted with hexane only (500 ml) and this eluate is discarded. Then the column is eluted with a 50:50 mixture of chloroform and hexane until 600 ml is collected in the receiver. This fraction contains the aflatoxin.

3. The eluate is evaporated to near dryness and transferred, using chloroform, to a sample vial. Chloroform is then evaporated and the vial is sealed until the sample can be examined by thin-layer chromatography.

4. Thin-layer chromatographic plates are prepared using Silica Gel G-HR and the suspension of silica gel is applied to the plate at a thickness of 0.25 mm. After the suspension has gelled, plates are dryed at 80 C for at least 2 hr after which they are stored in a drying cabinet until used.

5. A developing tank is lined with blotter paper previously saturated with chloroform and the trough is filled with a mixture of 7% methanol in chloroform.

6. The sample (in the vial) is dissolved in chloroform and, together with appropriate standards, is spotted on a line 4 cm from the bottom edge of the plate. A line is drawn across the plate 2 to 3 cm from the top edge and 0.5 cm from each side edge. The plate is inserted into the tank and is removed when the solvent front has reached the top line. After the solvent has evaporated, the plate is examined in the dark with the aid of a long wave UV lamp. In order of decreasing R_f values, the spots will be aflatoxins B_1, B_2, G_1, and G_2. The B-toxins display a bluish fluorescence, whereas the G-toxins are slightly green in color.

7. It is possible to quantify the aflatoxins by controlling the size of the spot through the application of various amounts of sample and standard and then comparing the size of the spots produced by both materials. It is also possible to obtain quantitative values by dilution of sample and standard until neither produce visible spots.

Modifications of this method which have recently been suggested include the use of: *(a)* an unlined tank *(16)* and *(b)* solvent systems consisting of benzene (95%), ethyl alcohol, and water in the ratio of 46:35:19 (v/v); chloroform and acetone (85:15 v/v), and chloroform, acetone, and 2-propanol (825:150:25 v/v) *(16, 19)*.

It should also be mentioned that a micro procedure for the quantitative determination of aflatoxins was developed by Cuculla et al. *(9)* and reported early in 1966. This method employs aqueous acetone for extraction in a manner similar to that of Pons and

Goldblatt *(15)* who used this chemical to extract aflatoxin from cottonseed. The solvent is suitable for use with fatty products (such as peanuts) since it dissolves the aflatoxin without extracting appreciable quantities of oil. When the concentration of aflatoxin is sufficiently high, a sample as small as 0.3 mg can be accurately assayed. The procedure employs the following steps:

1. The sample, in a conical tipped centrifuge tube, is soaked for 30 min in 70% acetone and is stirred occasionally to insure maximum extraction.
2. A small amount of 20% lead acetate is added, the mixture is stirred and distilled water is added.
3. The sample is centrifuged for 10 min at 4 to 5 thousand rpm and the supernatant is decanted.
4. More 70% acetone is added to the residue and the mixture is allowed to stand for 30 min after which distilled water is added and the mixture is centrifuged a second time—this time for 5 min. The supernatant is removed and combined with that obtained initially.
5. The liquid is shaken twice with chloroform and the chloroform layer is filtered through anhydrous sodium sulfate.
6. The chloroform is removed and concentration of aflatoxins is determined using chromatostrips.

It has been indicated earlier in the present discussion that thin-layer chromatography can be employed to determine the concentration of aflatoxin in a sample. Until recently quantification was dependent on visual comparison of spots produced by the sample and standard. This subjective procedure, which depends on visual discrimination and acumen gives poor quantitative values unless plates are read by an experienced observer.

Ayres and Sinnhuber *(4)*, in an attempt to overcome the obvious disadvantages of the visual technique, proposed the use of fluorodensitometry to measure the intensity of spots on thin-layer chromatographic plates. This method has been evaluated by other works and their results suggest it to be a very useful tool in aflatoxin measurements *(16, 19)*.

DENSITOMETRY

Densitometric determinations are made using thin-layer chromatographic plates prepared as described earlier in the present discussion. Visual examination of the plates is replaced by "reading" them with the aid of a TLC densitometer. This instrument is equipped with an automatic-scanning thin-layer plate stage composed of a search unit with an ultra-violet light source, primary and secondary filters, and a primary slit.

The densitometer, as just described, is coupled with a varicord recorder and an automatic integrator. The product of this instrument is a densitometric recording of the test samples which shows the four aflatoxin peaks and the integrated areas under each peak. According to results of one study, the average deviations, using the densitometer, were 2 to 3% for aflatoxins B_1 and G_1 and 5% for B_2 and G_2 *(19)*. These same authors suggested that the deviation using the visual approach may be 20% *(19)*. Furthermore, low concentrations (e.g. <2.0 μg/ml) of aflatoxin can be accurately measured by the densitometer. Usually all four aflatoxins can be determined from one diluted sample by means of the densitometer, whereas two or more dilutions may have to be made when thin-layer chromatographic plates are examined visually.

OTHER METHODS

Several other approaches have been employed for detection and measurement of aflatoxin. A few of these will be mentioned below.

Paper chromatography has been used by some investigators *(7, 8, 18)*. This technique permits one to distinguish between the B and G toxins but is less sensitive than thin-layer chromatography.

Burmeister and Hesseltine *(6)* have examined numerous microorganisms for their sensitivity to aflatoxin. They noted that growth of one strain each of *Bacillus brevis* and *Bacillus megaterium* was inhibited by the presence of approximately 3.7 and 1.8 μg aflatoxin per ml, respectively. More recently Arai et al. *(2)* observed that 7.5 μg aflatoxin B_1 per ml inhibited growth of *Streptomyces virginiae* and *Streptomyces netropsis*. Use of a microbiological procedure in the measurement of aflatoxin could replace thin-layer chromatography but would not eliminate the extraction (and concentration) procedures. Consequently, the time required for an aflatoxin determination would be extended by the incubation period required for growth of the test organism. There would be no appreciable gain in sensitivity.

The use of embryonating chicken eggs has been suggested for use in detection and measurement of aflatoxin *(10, 14, 20, 21)*. This method could conceivably, replace thin-layer chromatography but is probably quite unsatisfactory for this use since it is more complicated and, in all likelihood, more expensive. In fact, the chick embryo procedure has, in some instances, replaced the duckling bioassay as a confirmatory test. The method appears to be useful in this application.

A spectrophotometric assay procedure applied to aflatoxin determinations was reported in 1965 *(13)*. This technique seems to have generated little enthusiasm for only a few investigators have reported its use since then.

Finally, it should be mentioned that aflatoxin, in concentrations of 0.05 ppm or more, retards the growth of lung cells in tissue culture and exposure of the cells to 0.1 ppm aflatoxin results in giant cell formation *(11)*. This technique could also be applied for measurement of the toxin, but again is probably more complex and no more accurate than thin-layer chromatography coupled with densitometry. Use of the tissue culture technique might be suitable as a confirmatory test.

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ANNUAL VPI DAIRYMEN'S SHORT COURSE

The Annual VPI Dairymen's Short Course is scheduled for December 5-7, 1967 at Blacksburg, Virginia. It will be held in the pavilion at the Dairy Cattle Center.

The first day's program will deal with nutrition and feeding under 3 main topics: Facts for maximum forage utilization; Balance grain mixture to fit forage program; and Special topics, including problems in low milk fat, potential in dairy beef, urea utilization by calves and heifers, complete feeds, and nitrates.

Dairy farm management talks and discussions will include rapid adjustment changes as is being made on some farms; systems of leasing buildings and feed storage and contracting silage and grain; some projections into the types of dairying in the future; and the art and science of management.

Milking machine demonstrations dealing especially with vacuum fluctuations, its causes and the effect on udder health are scheduled.

The final half day is on genetics and reproduction. Some of the topics included are: rates of maturity and longevity; reproduction-facts and fantasies; days open-losses in production and remedial action; and sire and cow indexes.

For more information and a copy of the program, write Paul M. Reaves, Dairy Science Department, Virginia Polytechnic Institute, Blacksburg, Virginia.

AVOIDING COMMON DEFECTS IN CHEDDAR CHEESE[1, 2, 3]

GEORGE W. REINBOLD AND E. R. VEDAMUTHU

Department of Dairy and Food Industry
Iowa State University, Ames 50010

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SUMMARY

Most of the commonly occurring defects in Cheddar cheese are directly attributable to errors of judgment, observation, measurement, and technique. A general outline of the principles of Cheddar cheesemaking has been presented, calling attention, when possible, to the possible presence of these errors. Sufficient, continuing, and effective on-the-job training is suggested as the best means of avoiding the development of defects in Cheddar cheese.

Although consumer preference, in the final analysis, dictates the definition of what constitutes defects in Cheddar cheese, years of manufacturing and marketing experience have accorded certain abnormalities definite and permanent status in cheese judging and scoring. Since the late 1800s, when the first textbooks on Cheddar cheese manufacturing were written, the defects have not changed—they remain essentially problems of body, flavor, texture, color, and composition (including yield).

There are no statistics available to indicate the trends in the incidence of defects over the years. We can only guess that great improvement has been made in the curtailment of each defect. Certainly, our present usage of almost impermeable flexible films for wrapping blocks, cuts, and slices would indicate that some types of gas problems have been reduced. The increased sales of slices and precision cuts require that better body and less-open texture be developed. More people are now enjoying natural cheese; our tastes have become more sophisticated. Better flavors and different flavor levels are required. No longer is processed cheese used as a burial ground for undergrade cheese. Present-day manufacturers insist that uniformity of moisture, fat, salt, color, body, texture, and flavor be provided in the cheese they purchase for processing.

As better make procedures, starters, milk, equipment, and knowledge have evolved, the cheesemaker has been able to more carefully control the bacteriological, chemical, and physical properties of his product. Because of the variability of some of these factors, however, certain defects still commonly occur. Frequently, these defects may be attributed to errors of judgment, observation, measurement, and technique. At times, several or all these errors may be committed simultaneously. The cheesemaker may err in his judgment of the proper time to initiate cooking; he may incorrectly judge the amount of starter culture to use under changing milk conditions. Poor observation of body and texture changes during cheddaring frequently occur as do violations of proper measurement procedures of titratable acidity or pH. Inadequately trained personnel make errors in the techniques of curd cutting or salting.

We present in this article a general outline of the principles of cheesemaking, calling attention, wherever possible, to the possible sources of errors.

IMPORTANCE OF QUALITY AND UNIFORMITY

Cheesemaking, defined as briefly as possible, is the controlled expulsion of whey from coagulated milk. The cheesemaker who learns how to effect this change with *the greatest uniformity from vat to vat* will experience fewer occurrences of the common quality defects in his cheese.

The rate and degree of moisture loss (or whey expulsion) is dependent upon acidity (amount, rate, and time of development), temperature (time of exposure and elevation), and curd handling (the factors of cutting, such as time, fineness, and uniformity of cut, are most important initially—stirring, matting, and cheddaring also are involved). Through judicious manipulation of each of these factors, body, flavor, texture, color, and composition are controlled.

Moisture control, as mentioned, is the essence of successful cheesemaking. If moisture control is achieved, the finished curd will possess the necessary physical, chemical, and microbiological characteristics. The firmness of the curd is determined by the amount of moisture left in the curd. The elasticity or brittleness at times, even the texture of the curd, is dependent upon how the moisture content is established. Chemical changes that occur in cheese curd are irrevocably bound to the microbial

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[1]Editorial Note: Second of two papers dealing with two important aspects of cheesemaking technology having particular significance relative to the public health safety and consumer acceptance of cheese.

[2]Journal Paper No. J-5672 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project 1188.

[3]Presented at the Dairy Industry Conference, Iowa State University, Ames, Iowa, March 29, 1967.

population. Starter cultures, as well as adventitious types, grow and die at rates established by their environment. Starter growth, as influenced by the environment, has a predetermining initial effect upon flavor, body, and texture in the finished curd. Moisture control is not easily attained. Even though the usual steps in cheesemaking were first established through trial and error, they have fundamental scientific significance. Their relationship to moisture control is readily determined.

In the following outline of the usual pattern of Cheddar cheese manufacture, we attempt to point out only the more common errors of judgment, observation, measurement, and technique.

COLLECTION AND PREPARATION OF MILK

Since the actual condition and suitability of the milk in the vat for cheesemaking defies rapid determination, extensive milk-quality fieldwork is necessary. Reduction tests may be used as a quality-control test, but they reveal only a part of the true bacterial condition of milk. Cheese plants should seriously consider the use of the plate-loop count to provide fieldmen with a more accurate list of producers that may require help. Inhibitory tests are not used frequently enough; an extremely low level of antibiotic may perceptibly slow starter growth, but will have no effect on the growth of gram-negative, gas-producing microorganisms. Milk should be filtered or clarified. The manufacture of different types of Cheddar cheese may require dissimilar milk-heat treatment or pasteurization; however, extremes in either direction should be avoided. One of the most frequent errors in the use of fully pasteurized milk is the tendency to use too much starter. Accurate thermometers are an absolute necessity; flow diversion valves are rapidly becoming so.

STARTER SELECTION AND USAGE

Improper starter selection and use appears the most effective of all ways to achieve undergrade cheese. There is virtually no defect that cannot be traced back to starters in one way or another. Excessive moisture in cheese (from poor starter usage) will produce sour or acid flavors; weak, sticky, and pasty bodies; open or gassy texture; bleached or "acid-cut" color, and a poor finish. Too little moisture in cheese (again from improper starter usage) will cause too little flavor development, requiring extended curing. Bodies may be too hard or corky, and color, texture, and finish may suffer. Defective flavors such as acid, bitter, or fruity are directly attributable to improper starter or starter usage. The occurrence of fruity flavors requires separate comment. For years, most people believed that the sole cause of this defect could be attributed to the presence of coliforms, yeast, and other deleterious microorganisms in the milk. We now know that *Streptococcus diacetilactis*, a fairly common starter organism, may be involved.

There have been volumes written on starter transfer and maintenance, and most of them are reliable sources of information. The most frequent errors that occur, however, include: over-ripening and undercooling after ripening; inadequate measurement of amount of milk-solids-not-fat, water, and inoculum; improper temperature adjustment at inoculation and control during ripening; lack of measurement into vat; and wet starter rooms and poor equipment.

RIPENING

Frequently, starter is not strained into the vat. Other than over-ripening or under-ripening, there is usually little difficulty attributable to ripening procedures.

COAGULATION

Occasionally, cheesemakers get into the habit of inadequately diluting the color before addition to the vat. This may lead to mottling of the curd through uneven distribution.

Rennet activity is affected by a number of factors, including milk composition and temperature (activity is equally good from 30 to 48 C, 41 C being optimum). Other factors are previous heat treatment, acidity, homogenization, amount and age of milk, the calcium ion content and amount of calcium phosphate and other salts, and additional less important factors. Rennet usage is directly related to moisture control. Milk acidity is extremely important. Too firm a curd at cutting may be too high in moisture; conversely, too soft a curd may become too low in moisture. Properly diluted rennet should always be mixed thoroughly into the milk; following agitation, the motion of the mixed milk should be promptly arrested. Initiation of clotting in milk is a delicate matter; rennet should be permitted to act under optimal conditions. Too frequently, the cheesemaker does not attempt to standardize the length of set but permits this time to vary greatly because of changes in milk, developed acidity, and temperatures.

CUTTING OF THE COAGULUM

The rate of whey drainage is greatly affected by curd size. Uniformity is a virtue to be obtained at all costs in cheesemaking. Probably the most common offense in Cheddar cheese plants is disinterest in immediate replacement of broken wires in curd knives. In second place, is the overwhelming desire

to start stirring the freshly cut curd as soon as possible. This interval should be prolonged as much as possible to permit the freshly cut surfaces to "heal." Lumping of the curd, of course, is not desirable. Vat sides and bottoms should be thoroughly squeeged; valves should be drained and the contents emptied back into the vat at the completion of each step. One of the time-honored sayings of Cheddar cheesemaking is the phrase "Cheese is made in the whey." Moisture control depends in great measure upon the treatment afforded the curd during stirring, cooking, and drawing of whey. Again, uniformity must be emphasized.

COOKING

Common errors occurring during cooking include the application of heat too soon, in too great an amount, too early, and too high. Case hardening of the curd should be avoided; this condition retards moisture loss. Cooking at too high a temperature will retard acid production; many alleged cases of bacteriophage have been proved nothing but cases of inaccurate thermometers.

DRAWING AND PACKING

These processes are relatively simple; the timing should be based upon acidity, desired moisture level, and body characteristics.

CHEDDARING

It is a rather common belief that the quality of Cheddar cheese is assured at the time of packing. This has been phrased as, "When the curd is packed, the cheese is made." However, there are some errors that can be made during cheddaring that affect the quality of the finished cheese. These include the time and height of piling, careless introduction of steam into the jacket, thereby oiling-off the curd, and permitting the curd to become too cool before milling.

SALTING

Too much "forking" during salting causes a greasy curd. The proper addition of the correct amount of salt has a profound effect upon the quality of the cheese. Too much salt "preserves" the cheese so that it breaks down too slowly, if at all, retains a coarse, dead body, and retards flavor development—in some cases permanently. Too little salt encourages equally undesirable effects. Rapid body breakdown, fermented flavors, and gassy, ragged openings may result from uninhibited fermentations. Most work indicates that low-moisture Cheddar cheese should contain approximately 1.75% salt, or at least be within a recommended range of 1.6 to 1.9%. With long-hold cheese, the upper portion of this range is preferred.

PRESSING

The temperature of the curd at pressing should be high enough to permit the curd to fuse and develop a closed rind, but no higher. Normal pressing temperature range is from 30 to 33 C. Pressure should be applied gradually to permit the cheese to drain properly. Some operators stack the hoops three or four high and allow them to drain before pressing.

CONTROL AND RECORDS

While moisture control has been emphasized, little has been said about proper acidity levels to be maintained throughout the make. These, of course, will depend upon the type of cheese being made, long-or short-hold, curing conditions, make schedule, and other factors. In any event, acidity development is an index of bacterial activity and, therefore, is an important indicator of how nearly the cheesemaking operation is following the desired routine. Since certain steps in the manufacture of cheese occur at various desirable acidity levels in the milk or whey, it is necessary to be able to accurately determine the correct titratable acidity. One of the most frequent violations of titrating procedure is the use of too little indicator. Ten drops of a 1% phenolphthalein solution should be added to each 9-ml sample. The amount of indicator added will influence the test results. If too little indicator is used, more base will be required to produce a visible color change. This will result in too large a reading. Good lighting also is important. Individual cheesemakers and their helpers should be made to realize the importance of the acidity test. Steps should be taken to assure that differences are minimized between titration techniques and results of different operators. If pH measurements are made, they should be correctly and promptly run.

Scientific control is essential. Cheesemaking does not permit the taking of moisture samples throughout the process, and the cheesemaker must rely upon acidity or pH values and his sense of touch. Since the latter control measure is but poor at best, it is necessary that a complete make record be kept. All times, temperatures, acidities or pH values, and other pertinent data should be recorded at every step. This information, plus the fat and moisture test for each vat, will give the cheesemaker and manager a record of what has happened. If the records are studied, trends in behavior of the milk, starter, and curd from day to day will be revealed. Sudden changes in moisture content provide a sharp, clear warning of trouble —either present or impending.

TRAINING

Perhaps the best and quickest way to avoid difficulties in cheesemaking is to provide sufficient, continuing, and effective on-the-job training. Frequently, this is lacking in older, established plants. More often, it is lacking in new plants. We take pride in our work only when we know what we are doing and when we feel that we have contributed to a significant degree to the over-all effort. Would this be possible with a plant helper who did not understand even the rudiments of the properties of rennet or starter culture?

As cheesemaking becomes more complex, this requirement will become greater. With proper understanding, plant efficiency can be increased, savings realized, and problems reduced. Management should seek out inventive, openminded people. Resistance to change cannot be tolerated. Training is imperative, and so is supervision. The utility of scientific and technical knowledge lies in what can be done with it. Cheese plants are right in the middle of a period that, not only reuires change, but is forcing change.

MONTANA'S ABNORAL MILK PROGFAM

HERB BALLOU

Dairy and Milk Inspection Division
Livestock Sanitary Board, Helena, Montana

We are committed to follow and comply with the agreements of the National group in our participation in the Interstate Milk Shippers Program. The Abnormal Milk Program adopted at the Interstate Milk Shippers Conference, calls for *THREE* distinct phases over the next three years. These phases are the minimum requirements set forth for each state to follow.

Over a year ago, a statewide meeting was called by Earl Peace, Dairy Extension Specialist at the Montana State University, and by Dr. J. W. Safford, State Veterinarian, to alert the dairy industry people as to what the Abnormal Milk Program would consist of over the next few years. At the time, the Industry set up their own voluntary program, and the plant laboratories reported their results to the Montana Livestock Sanitary Board. Approximately fifty percent of the Grade A milk supply was involved in this program.

Early in September of 1967, this group of industry people were called together again to discuss the results of their mastitis program and to outline a Statewide Regulatory Program to follow. It was rather surprising and encouraging, that some of the producer representatives favored the elimination of the three phase system as recommended in the Interstate Milk Shippers Program, and were in favor of getting directly into Phase Three, thus eliminating the gradual escalation portion of the program. It was suggested that the initial program by the Regulatory Agency be set at a maximum of 500,000/ml leucocyte count.

Presently, the Abnormal Milk Program requires that all Grade A milk be analyzed for pesticides, leucocytes and other adulterants. Producer raw milk will be tested for leucocyte level at least eight times per year. Milk in excess of 500,000/ml or 20/mm on the Wisconsin Mastitis Test scale, shall be considered as abnormal. Owners of such milk will be sent a Notice of Noncompliance on the two of the last four consecutive method. When three of the last five counts are in noncompliance, the cows responsible for the high leucocyte counts must be quarantined from the milk supply. It will require a veterinarian to make determinations as to what cows are to be quarantined. The cows under quarantine shall be returned to the herd when their milk supply bcomes normal.

Montana will incorporate all three phases into one. With a realistic understanding of the program by all concerned, we are looking for quality improvement, greater return to the dairyman, and a continued consumer acceptance of a safer and more wholesome fluid milk supply. Our people feel that with a progressive quality improvement in the fliud milk supply, the consumer will be deterred from the imitation products.

THE PUBLIC HEALTH LABORATORY AND OUR ENVIRONMENT[1]

ROBERT P. MACFATE

Division of Laboratories,

Chicago Board of Health, Chicago, Illinois, 60602

A review of a recent compilation of vital statistics reveals that man's life expectancy is greater than that of any of his ancestors. However, he would live still longer, and even in more abundant health, if all of the presently known principles of preventive medicine, hygiene and environmental health could be fully applied. It has been estimated that at least ten years could be added to the average life-span in the United States of American, if full use could be made of the scientific knowledge now available.

While the problems of war are entering our every day life, it may be heartening to consider, for a while, not the ways in which man's ingenuity has brought him to the atomic era but, instead, how his inventiveness has brought him nearer that utopia where health is bountiful and death is postponed to the biological limit.

PUBLIC HEALTH

The history of Public Health is the story of man's endeavors to protect himself and his community against communicable disease. Over the years, to this base, has been added the attempt to protect man against any part of his environment that will prove antagonistic to his well-being.

In primitive communities, down to the middle ages, society's defense against sickness, physical or mental, was to isolate or destroy the patient. Later came the endeavor to heal the sick, so as to prevent them from infecting or harming their fellow men or becoming a burden on society.

This was followed by the establishment of positive protection of the healthy from the dangers of disease by the use of such safe-guards as quarantine, vaccination, hygiene and sanitation. This was the era of preventive medicine.

However, just as the political concept of the "person" has been replaced by that of the "nation", so in public health has the concept of the "individual" been replaced by that of "society".

Our views have become broader. What is to be done must be done for the welfare of the community and not the individual alone. The activities must be taken out of our individual selves and made to encompass our entire environment. And thus Environmental Health was developed.

ENVIRONMENTAL HEALTH

The concept of environmental health is not of recent origin. The general acceptance of environmental sanitation goes back to more than a hundred years ago. In the 1850's, in London, Edwin Chadwick was appointed Commissioner, to study the operation and administration of the Poor Laws.

Health conditions among the poor were deplorable. Chadwick's Report gave vivid details of existing conditions, and made a serious effort to correlate these conditions with variations in mortality rates and economic status.

Most significant, however, the Report presented with dogmatic clarity, a plausible epidemiological theory that fitted many of the known facts. Disease was based in filth. Permitting human and animal excretions to collect, not disposing of garbage, improper care of food animals, and lack of personal hygiene, was directly responsible for most of the ills that affected the poor.

From these facts, Chadwick derived the principles on which sanitary reform and community health action have been based since that time. Great Britain was first to institute such reforms, then the United States, and finally the rest of the World.

To the early public health workers, these principles constituted the law and the gospel of community health action and, for the most part, they are as valid today as they were when first enunciated.

The Report proved beyond any doubt that disease, especially communicable disease, was related to filthy environmental conditions: due to lack of drainage, proper water supply, and means of removing refuse from houses and streets. From that time, filth was no longer simply a matter for private disgust. It was raised to the status of an important public enemy of the community health.

Environmental health covers every facet of our every day life. Studies are made and service is ren-

[1]Presented at the 25th Spring Conference of the Associated Illinois Milk Sanitarians, Elgin, Illinois, on May 22, 1967.

dered under three general areas: Food, Institutions, and the External Environment.

Under the subject of Food are all studies related to food establishments, including wholesale and retail stores, food plants, bakeries, dairy farms, milk plants, hotels, restaurants, taverns, school cafeterias, and the like.

Under Institutions are included orphanages, hospitals, nursing homes, boarding homes, child-play schools and child-care facilities.

Considered under External Environment are water, wells, sewage, septic tanks, air pollution, insects, rodents and nuisances.

Certainly this is a formidable list when it comes to planning the work to be done.

First Laboratory Studies

At the time of the above studies, there were no laboratories in which to test the theories that had been propounded. It was not until after 1860 that Pasteur conducted a series of experiments which were important, not only in incontrovertibly refuting the doctrine of spontaneous generation of bacteria, but also in establishing the principles of scientific investigation which have influenced bacteriologic research since his time.

In 1882, only 85 years ago, Robert Koch isolated the tubercle bacillus and eventually established a set of requirements which a bacterium must fulfill before it could be accepted as the cause of a specific infectious disease. These were known as Koch's postulates. These requirements were: *(a)* the organism must be found always in association with the disease; *(b)* it must be isolated in pure culture; *(c)* it must reproduce the disease in animals, such as guinea pigs and rabbits; and *(d)* it must be recovered in pure culture from the experimentally infected animals.

Studies of this nature finally provided proof for many of the theories that were proposed following public health studies.

Increased Scope of Health Studies

Much progress has been made in public health and environmental health since the early days referred to above. Originally concerning itself only with infectious diseases, public health studies have expanded to include everything that may affect the health of the public as a whole. In the same way, laboratory studies have expanded. For example, the laboratory discipline of Bacteriology is now called Microbiology and includes: Bacteriology, Virology, Parasitology, Mycology and other studies.

In the meantime, the mysteries of Alchemy had developed into the science of Chemistry. By a long tortuous path, we have finally come to the Wonder of the Ages, the Atomic Era. Accompanying this progress has been the further development of the sciences of mathematics, physics, astronomy, and all of the varieties of present day knowledge. Most of the fundamentals of these sciences have been discovered and tested in our many laboratories. Each, in its own way, has contributed to the knowledge of our environment.

We now find that not only must we avoid bacterial infection, in our attempt to live a long and happy life, but must also take into consideration chemical poisoning, radiation and many other hazards. Each of these has had its dramatic moments, and history is filled with stories of achievements like those referred to above concerning Pasteur and Koch.

The Public Health Laboratory

The public health laboratory now holds the key to much of the work performed by a department of health. Actually, the laboratory holds the same position in respect to a health department that a kitchen does to a home.

The activities of a home, to a great extent, revolve about the kitchen. Every day, each person in the household partakes of the food and nourishment prepared there and thus is able to continue his own particular work.

In like manner, the activities of a health department revolve about the laboratory. Every day, each person or each division in the health department receives some information, or some report, or some analysis from the laboratory which permits that person, or that division, to carry on his own particular activities.

The laboratory is a service department, whose function it is to assist the other divisions of a health department to perform the various tasks allotted to them.

New Instruments

It is of interest to note the changes that are taking place in the equipment used, especially in the chemistry laboratory. The expansion of the varieties of complex instruments has been phenomenal. The application of electronics, and other physical principles, has permitted the construction of a variety of instruments, such as the spectrophotometer, which now permits the measurement of minute quantities of chemicals heretofore discoverable by only a few highly-trained scientists. In the past 40 years, the scope of the analytical laboratory has simply exploded. The capabilities of these laboratories have increased more than a hundred fold.

Some 40 years ago, solutions were made and com-

pared, gases were measured, items were weighed, and the microscope permitted the examination of small particles. Light was analyzed by passing it through a prism and the electric current was studied.

Today, weighing is done automatically with far greater precision than could be achieved heretofore.

The sensitivity of the microscope has been greatly multiplied by the electron microscope, where electron beams with wave lengths, thousands of times shorter than visible light, are utilized in place of ordinary light, thus allowing much greater magnification.

The properties of solutions are now studied in great detail. In the analysis of solutions for the concentration of a specific chemical, many reactions are carried on producing a colored product that colors the solution in proportion to the amount of the chemical present. The color produced in these solutions is compared to the color of a standard solution, where the concentration of the chemical is known. A visible color, in such an analysis, can be produced by a remarkably small amount of material. The color is measured in an apparatus known as a colorimeter, or more properly, a photometer.

It was the invention of this colorimeter that started the extensive analyses now done on blood, urine, spinal fluid, and other specimens from the human and animal body. In its use, one problem developed. The colors of the solutions were compared in the colorimeter with the eye. When the eyes became tired, the comparison and matching of colors was not performed accurately.

Again, our scientists came to the rescue. An instrument was devised where a beam of light was passed through each solution and the amount of light absorbed, depending on the concentration of the color, was measured. The light passing through the solution activated a photo-electric cell, producing an electric current that was registered on an ammeter. Here, eye fatigue was not involved and more accurate measurements were made. Later, it was found that light of different wave lengths, that is, light of different colors, was selectively absorbed by the colored solutions, to a greater or lesser extent, depending on the composition or color of the solution to be measured.

The spectrophotometer was then devised, where the light used was passed through a prism, and only the wave length most greatly absorbed was used to pass through the solution to be measured. The selectivity and accuracy of the determination was greatly enhanced.

The apparatus has been further expanded to use ultra-violet and infra-red light where a large number of complex chemicals can be detected and measured, some of which are almost completely invisible to the naked eye.

NEW PROCEDURES

Advances have been made in other laboratory procedures. Our physical chemists have found that, during certain titrations, the electrical potential of a solution changes. Just as litmus changes color and indicates the end point of a titration, so the electrical potential is measured, and when it reaches a certain value, the analyst knows the reaction has come to completion. This can be done despite the color or cloudiness of the solution. Such titrations permit the analysis of blood and other materials that otherwise could not be analyzed as quickly and accurately by other methods. It also permits the analysis of minute quantities of many materials which heretofore have been impossible to detect.

This, the ability to determine the presence and amount of very small quantities of chemicals, has been the basic aim of many of the recent advances in analytical instrumentation.

An interesting study is that of chromatography, which has aided immensely in our study of pesticides and pesticide residues. Chromatography is a system of analysis which allows the separation of mixtures of compounds into their component parts. It is called chromatography because the technic was originally used to separate mixtures of pigments. Now, the method is used to separate a variety of substances, or to concentrate them in phases different from those in which they were originally present. This method is based on the phenomenon of "adsorption", in which use is made of matter in the colloidal state.

In simple chromatography, a tube or column, with a stopcock at the bottom, is filled with an adsorbing medium, such as alumina, magnesia, silica or paper pulp. The specimen to be examined is dissolved in a non-aqueous solvent. The solution is poured over the adsorbent in the column. As the fluid flows down through the column, some of the compounds may be adsorbed at various levels. Others may pass through the adsorbing medium and flow out at the bottom of the tube. Different compounds travel down the tube to different levels and generally form distinct and separate bands. With care, each compound can be dissolved out of the column separately. Or the column of adsorbent can be removed from the tube and cut into pieces, thus separating each band of adsorbed material.

A modification of the procedure is paper chromatography. Here a small drop of the material to be examined is placed on a strip of filter paper near one end. The end of the paper nearest the specimen is placed in a suitable solvent, usually a water- organic solvent mixture. As the solvent flows along the paper, each substance in the mixture will move with the solvent at a definite rate, peculiar to its own

chemical structure. After a time, the various components of the mixture will occupy different positions along the filter paper and may thereby be individually identified and measured. Here we are not analyzing according to our usual chemical technics. We are not identifying elements or chemical groups. Rather, we are identifying complex compounds by their physical properties—the rate at which they move through the paper and solutions used. They can be identified by first using a known solution and comparing this with the results obtained from the unknown solution. Paper chromatography is used extensively in the detection of many chemicals, insecticides, drugs and poisons.

A further modification of this technic is known as gas chromatography. Gas chromatography is analogous to paper and column chromatography, except that a steady flow of a gas, such as helium, is used instead of a solvent. Two or more columns are packed with special adsorbents which will retard the flow of one or more gases and permit others to continue through the medium. The gases are detected and measured by the difference in thermal conductivity of the helium or other carrier gas. Gas chromatography is used for the determination of gases in whole blood, serum and expired air, and for the detection and measurement of insecticides and other compounds.

FOOD ADDITIVES

For many years, small amounts of chemicals have been added to foods to enhance the flavor, add a new flavor, color the food, prevent the formation of mold, etc. It was known that some of these materials in very large amounts would cause cancer if fed to animals. However, so far as experimental studies had been made, the very small amounts of the chemicals used in foods were considered to have no effect on the human being.

However, due to careless use of many of these compounds, and the use of compounds not fully tested, several years ago, Congress passed a law stating that these materials, no matter how little was used, could not be added to food materials if they caused cancer in animals in any amount.

This created a variety of serious problems. First of all, the use of the materials was important in the preparation of the commercial product. Secondly, the analysis of foods for these chemicals was in a rather elementary state. The compounds were used in such small quantities that it was almost impossible to analyze for them quantitatively. And thirdly, all manufacturers were given a time limit in which they had to have their products tested and approved by the Food and Drug Administration. Every plant laboratory, commercial analytical laboratory and experimental pharmacologist was inundated with work. Extensive animal studies had to be performed and, in most cases, these had to cover a span of at least two years. For a time, everything was in confusion.

In feeding experiments, for example, using rats and dogs, the animals might be fed for two years. Then, on detailed study, it might be found that the animals appeared physically, clinically and anatomically normal, with say, for example, only a slight but definite increase in the size of the liver.

Was the chemical, fed to the animal, the cause of the enlargement of the liver? And just what did it mean, anyway? Even the physiologists, biochemists and pathologists might not know. Except that they have a profound conviction that increased liver weight forbodes no good.

Since that time, many chemicals have been proved harmless and are now in use again. Others are still under study.

PESTICIDES

A very controversial subject has been the use of many of the pesticides, especially the chlorinated hydrocarbons. We shall not go into the pros and cons of this matter except to say that without some of them, farmers would have no crops.

On the other hand, we are vitally concerned as to the effect of these compounds on our own systems. Do they affect the liver, do they accumulate in the body, and what is the effect of such accumulation?

As mentioned above, one of the methods used for the analysis of pesticide residues is that of gas chromatography. This procedure requires expensive equipment. More of a problem, however, is to find trained chemists, or chemists who can be trained to use the apparatus. The field is very limited since industry is able to pay salaries far more attractive than those that can be paid by most health departments. However, the point to be emphasized is that we now do have procedures that can be used to determine this contamination of our environment.

Further, there are a large number of pesticides, and the field is growing every day. The ultimate aim, of course, is to find a pesticide that will continue to kill the pests, that will not poison animal life, that will have no effect on man, and will not accumulate in the body. Such studies are being made by workers in environmental health.

BACTERIAL CONTAMINATION OF FOODS

Another laboratory discipline, important in environmental health studies, is Bacteriology.

One of the most serious problems, identified only

in recent years, has been the spread of the disease salmonellosis. It is caused by species of the bacterial genus *Salmonella.* The disease is common in reptiles, birds, mammals and man. The clinical symptoms vary from a mild enteritis to a rapidly fatal septicemia.

Salmonella typhosa is a highly specialized member of the genus or group. It infects man, only, and produces typhoid fever. The control of typhoid fever is perhaps the greatest triumph of organized preventive medicine.

However, beyond this, there are over 1000 different types of salmonellae. S. *typhimurium*, originally isolated from mice, in which it produces mouse typhoid, is distributed widely throughout the animal kingdom. It is probably the most frequent cause of salmonella food poisoning (salmonellosis) in man, although many other types are found. In fact, all salmonellae can cause any one, or all three, of the phases of the disease, which are: *(a)* the food poisoning phase, with vomiting and diarrhea; *(b)* the enteritic phase, with inflammation of the intestines; and *(c)* the septicemic phase, with generalized infection in the blood and organs of the body.

Salmonellae are distributed widely in nature. Drinking water and the water of lakes, rivers and swimming pools may be contaminated by animals and man. Contaminated milk and drinking water originally constituted the chief causes of epidemic intestinal infections, but these sources have been eliminated, almost completely, by modern sanitary engineering. Salmonellae in milk and milk products may be destroyed by pasteurization. Contaminated water can be made safe by boiling or chlorination.

All meats, poultry and eggs, including dried eggs, should be considered as potential sources of the infection. Before being served as food, they should be heated to temperatures high enough to kill salmonellae.

Perhaps the most common source of salmonellosis is the human carrier who contaminates the food during its preparation, or after it is cooked. Oily salad dressings, cake fillings and pastries are well adapted for the transmission of the organisms from the carrier to the victim. Therefore, the detection and elimination of carriers among food handlers is one of the major activities of all well-organized public health units.

Recent studies have shown salmonellae to be present in some instant non-fat dry milk powders, dried yeast, dried cocoanut, carmine red (a food color), rendering plant products and other materials. Prompt detection by various health departments and the Food and Drug Administration causes early removal of these contaminated products from the market.

Food may be contaminated in commercial processing, such as in the preparation of dried milk. Pasteurized milk, in which any salmonellae have been destroyed, may be again contaminated during the drying step of the process. An epidemiologic study may be made, but often this presents problems, such as when the milk is dried in one state, compounded in another, and sold all over the country.

Tracing the source of the infection is not an easy matter. Sometimes the workers in a plant are infected. This infection may be spread through the product. In other cases the workers may be the first victims rather than the source of the infection.

The presence of organisms in animal feed may maintain a potential reservoir of infection from animal to animal, and animal to man.

In recent years, it would appear that infections by salmonellae have become more frequent, or at least they are now being reported more frequently. This increased incidence is probably due to more accurate and more detailed laboratory testing. Many times the infection is slight, producing only a little diarrhea, and thus is relatively unnoticed. Of course, the infection may have been caused by other intestinal pathogens, or other conditions.

When such infections are studied and salmonellae found, the organism should be typed serologically. Most species of Salmonella have been given specific names, i.e. from where they were found, the animal. the city, the name of the person first isolating the organism, the name of the person infected, etc. Thus, we have S. *amsterdam, S. london, S. denver, S. georgia, S. infantis, S. thompson, etc.*

The question might arise, since all these organisms can cause the disease, why bother about such a detailed identification? However, specific identification of the organism is essential to enable the epidemiologist to trace the source of the infection. It is important to know if a person was infected by a human carrier who prepared the food, or by infected food, such as a turkey, for example.

A carrier may not necessarily spread the infection, providing his personal hygiene is of the highest order. Without complete identification of the organism, he might be falsely accused as the cause of another person's illness, whereas the source may be actually in the food itself. Of course, if the species isolated from a carrier and that found in the food are of the same type, then no determination can be made as to which source caused the infection.

This is just one phase of the attention paid to the bacterial contamination of food. Other bacterial food-borne illnesses may be caused by *Clostridium botulinum, Staphylococcus aureus, Escherichia coli,* species of *Shigella* and *Clostridium perfringens.* Each

presents a series of problems in the study of environmental sanitation.

SUMMARY

Thus, we find that public health in general, and environmental health in particular, lean heavily on the findings of the various scientific laboratories. Without laboratories, progress would be slow and laborious. The same old arguments would arise and scientific study would be impossible.

The public health laboratory assists with newly identified health problems. In fact, in many cases, the laboratory is required to identify the problem and then serves as a guide in its correction.

From the laboratory side, those working in public health laboratories derive much satisfaction from their labors. Long and irregular hours at the laboratory bench are cheerfully accepted when the results of the study will help to bring man to a longer and healthier life.

ASSOCIATION AFFAIRS

RESOLUTIONS ADOPTED AT IAMFES 1967 ANNUAL MEETING

The following resolutions were unanimously adopted by the membership at the 54th Annual Meeting of the International Association of Milk, Food and Environmental Sanitarians at Miami Beach, Florida, on August 16, 1967:

RESOLUTION NO. 1

WHEREAS: The 54th Annual Meeting of the International Association of Milk, Food and Environmental Sanitarians was held in Miami Beach, Florida, August 14 to 17, 1967, in the Americana Hotel at the invitation of the Florida Affiliate; and
WHEREAS: The Local Arrangements Committee of the Affiliate provided excellent facilities in the conduct of the meeting;
THEREFORE BE IT RESOLVED; that the Association extend its sincere appreciation to the Florida Affiliate of the International Association of Milk, Food and Environmental Sanitarians, and that the Secretary be instructed to transmit this resolution to the President of the Florida Affiliate.

RESOLUTION NO. 2

WHEREAS: In that Congress has now made funds available for additional research by U. S. D. A. and because of the vital and pressing need for new studies in the prevention and control of mastitis in dairy herds;
BE IT RESOLVED: That the International Association of Milk, Food and Environmental Sanitarians convened at its 54th Annual Meeting, hereby directs the Secretary to request of the Secretary of Agriculture to approve the immediate start of research in this important matter.

RESOLUTION NO. 3

WHEREAS this Association believes that uniformity in the labeling of dairy products is a necessary and vital goal for all persons connected with the industry regardless of whether they be regulatory or industry representatives; and
WHEREAS the work of the National Labeling Committee in providing a voluntary state model regulation for the labeling of fluid milk and milk products represents the first major step to achieving uniformity on a nationwide basis; and
WHEREAS the National Labeling Committee's guidelines represent sound and reasonable requirements which will adequately safeguard the consumer;
THEREFORE BE IT RESOLVED that the International Association of Milk, Food and Environmental Sanitarians fully endorses the labeling provisions contained in the National Labeling Committee's Model Labeling Regulation; and
BE IT FURTHER RESOLVED that this Association through its membership will actively support the adoption of this voluntary model regulation in the fifty states.

RESOLUTION NO. 5

WHEREAS: IAMFES has been engaged in negotiations through an ad hoc committee with a similar committee from NAS; and
WHEREAS: This committee has developed a tentative set of by-laws as a starting point for a new organization; and
WHEREAS: These tentative by-laws and the intent of this endeavor has been discussed by means of the presidential address and a special open meeting at the 54th Annual Meeting of the IAMFES;
THEREFORE BE IT RESOLVED: That the Association at its 54th Annual Meeting, assembled, endorse the principles of the merger plan and direct the Executive Board to further investigate the possibility of merger between the two organizations and to develop a set of by-laws to be presented for approval by members of the IAMFES.

Resolution No. 4 pertaining to Standardized Identification of the Sanitarian was tabled.

KENTUCKY ASSOCIATION LAUNCHES NEW NEWSLETTER

At the recent Affiliation Council meeting at Miami Beach one of the many matters "kicked around" was the problem of communication. It was universally agreed that it was of vital importance in the life of a state organization to keep up the interest of current members and to try to reach prospective members through some satisfactory news medium. Contrary to Mark Twain's famous commentary to the effect that nobody does anything but talk about the weather, the Kentucky Association of Milk, Food and Environmental Sanitarians has embarked on a laudable enterprise of periodic publication of an interesting and informative newsletter.

In his opening message in the first issue, President Ed Napier appoints each and every member to an "Action Committee" and charges everyone with

an equal responsibility for the success of the communications medium. Of course, the Kentucky Association still has its standing Membership and Publicity Committees but Ed Napier evidently believes that the organization can thrive best if everyone pitches in to achieve a successful assembling and dissemination of news on milk, food and environmental sanitation around the State of Kentucky.

The first issue lists the state Officers and Directors and also the make-up of the Publicity and Membership Committee proposing a plan for expansion of membership through a contest system with prizes. It announces 1968 as "The Year for New Members."

Items of interest to Kentucky sanitarians follow, including the appointment of a new Assistant Extension Professor of Dairy Technology at the state university, the need for a state meat inspection program, a salute to the dairy fieldman and his responsibilities and some personal notes on member activities.

An interesting full length article on the subject of "Food Sample Collection" features this first edition. While the material was presented as a paper at the Summer Regional Conferences throughout the state, the message is made available to all members who should be universally concerned with the technique of sample collection and analysis.

The news letter concludes with a list and description of late summer and fall meetings of interest to sanitarians not only in the state but in adjacent states. This includes official agency meetings and industry and university sponsored programs as well.

This initial issue of the newsletter bound in an attractive cover is a noteworthy effort and deserves full support of the membership to further the welfare of the Kentucky organization.

LOUIS ARRAGONI JOINS ASSOCIATED GROCERS

Dr. Louis Arrigoni became executive vice-president of Associated Grocers, Inc., Seattle, effective October 1, 1967. Dr. Arrigoni has been associated in increasingly important position with Consolidated Dairy Products Company of Seattle since 1949, having served during the past eight years as a member of their broard of directors and vice-president. According to Mr. E. E. Pedersen, President of Consolidated, who made the announcement relative to the change, Dr. Arrigoni has been a moving force in the successful expansion of his company operations here in the United States and in the Far East.

Mr. Willard Rhodes, President of Associated Grocers, Inc., said "We have known Dr. Arrigoni since 1949 when he first became associated with Consolidated, and we know how difficult it is for him to sever his relationship completely with a firm with which he has so long and successfully ben connected. However, the broad management experience in the food industry he brings with him is a great asset to our firm, and we are extremely pleased to have him with us in a top management position."

Louie Arrigoni has been an active member of IAMFES and of the Washington affiliate for a number of years.

BEN ZAKARIASEN RETIRES

Ben M. Zakariasen, Manager of Laboratories and Field Services for Land O'Lakes Creameries, Inc., Minneapolis, Minnesota, who has been directly connected with and part of the dairy industry all his life, retired October 1, 1967.

Ben graduated from School of Agriculture in 1921, earned the Bachelor of Science degree in 1925 and the Master of Science degree in 1937 from the University of Minnesota. Appointed in 1924 as chemist for Land O'Lakes Creameries, Ben started a one man laboratory in a corner of the company warehouse. Tiny as it was, some were dubious about the expenditure. This one man laboratory, at the state contained little apparatus except instruments needed to test butter samples for butterfat, salt and moisture content.

Soon his department was caught up in a problem that lent impetus to an expansion which has been going on ever since. Mold organisms began showing up in large numbers on the surface of the butter, as much as $1,500 losses per creamery for one month. Very little organized knowledge was available at the time to combat mold in the butter. Dairy manufacturing experts, Professors Macy and Combs from the University of Minnesota were enlisted to cooperate in solving this problem. Mr. Zakariasen conducted thousands of laboratory tests as an integral part of

the research using samples in the creamery from the raw cream to the finished product including the processing equipment.

At that time many creameries still had iron piping thru which cream passed and in which numerous cases this piping could not be taken apart for proper cleaning and sanitizing. Cream was drawn into wooden chuns having wooden roller butter workers with the use of suction developed by steam. Due to the fine cooperation of the cooperative creameries and the Land O'Lakes laboratory, sanitary equipment was installed that could be taken apart and properly cleaned and sanitized resulting in the mold problem being conquered to a point where it is scarcely a minor nuisance instead of a major catastrophe.

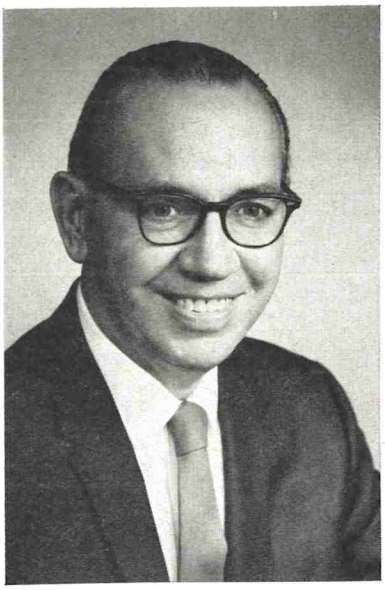
In 1924 scarcely 25% of the butter handled could be sold under the Land O'Lakes brand (U. S. Grade AA or U. S. 93 score). Today approximately 95% of all the butter handled meets this highest standard. Ben's department has played a very important part in bringing about this improvement in quality. His dpartment conducts the only year round butter efficiency contest of its kind in the world based on quality, weights, workmanship, composition and sanitation which has been in existance since 1928. The contest has been a valuable aid in the improvement of efficiency in the plants and the standardization and maintenance of the quality of Land O'Lakes butter.

Mr. Zakariasen is a member of the American Chemical Society, American Dairy Science Assn., Minnesota Dairy Technology Society, International Dairy Congress and the International Assn. of Milk, Food and Environmental Sanitarians. He has served as President of the Minnesota affiliate as well as general chairman of general arrangements committee for the International Annual Meeting in Minneapolis in 1966.

Ben is a committee member of the following trade associations: American Butter Institute (Research); National Cheese Institute (Research); Amrican Dry Milk Institute (Standards); National Milk Producers Federation (Technical); and the Milk Industry Foundation (Technical advisory committee on pesticides). He served on advisory committees for the following government departments: United States Department of Agriculture, Minnesota Department of Agriculture, Dairy Industries Department of the University of Minnesota, Wisconsin Department of Agriculture and State Board of Vocational, Technical and Adult Education.

Mr. Zakariasen is the author and co-author of over 25 technical and non-technical papers dealing with such products as fluid milk, cream, butter, cheese, dry milk and other related products pertaining to problems in production methods, manufacturing techniques, laboratory procedures, dairy plant cleaning and sanitizing methods.

Mr. and Mrs. Zakariasen will reside at the Pellinore Hall apartments, 4250 Galt Ocean Drive, Fort Lauderdale, Florida.



BEN M. ZAKARIASEN RECIPIENT OF ACHIEVEMENT AWARD

Ben M. Zakariasen, Manager, Laboratories and Field Services, Land O'Lakes Creameries, Inc. was the recipient of the Minnesota Sanitarians Association achievement award for his outstanding contributions in the field of quality control.

The presentation was made at the annual sanitarians banquet held Thursday evening, September 14, 1967 at the President Cafe, Minneapolis, by Orlowe M. Osten, secretary of the sanitarians association.

He noted that Zakariasen has often been referred to as "the father of quality work in the dairy industry".

REPORT OF THE COMMITTEE ON BAKING INDUSTRY EQUIPMENT—1967

The Committee has had three meetings with the Baking Industry Sanitation Standards Committee (BISSC) since our 1966 report. Also, since last year, President Elliker has appointed Fred Vitale, Director of Sanitation for the Continental Baking Company, as a member of this Committee.

In our 1966 report this Committee stated that the revision of existing standards should be given high priority. BISSC concurred with the Committee's recommendation and initiated an accelerated revision program. In addition to the usual Fall and Spring meetings held in Chicago, a special meeting was convened in Cincinnati, Ohio, May 16, 17, 18, 1967.

At the Cincinnati meeting this Committee, the U. S. Public Health Service, and committees representing the Ameri-

can Public Health Association and National Association of Sanitarians worked diligently and in close cooperation with representatives from the baking industry in revising a total of eleven standards. The revisions, aside from improving and tightening the standards from a sanitary viewpoint, will be of great benefit to the bakery equipment manufacturers. Their equipment can now be manufactured to conform to BISSC Standards with some assurance that the existing standards will not be radically changed. There will, of course, be future revisions or upgrading of the approved standards as new and different manufacturing methods and processes are initiated.

Standards Published. BISSC will have, effective January 1, 1968, a total of twenty seven (27) Standards published.

Standards Pending. There are, as of this date, nine Standards in various stages of development. With the completion of these Standards a great majority of the equipment used in modern bakeries can be manufactured to comply with a BISSC Approved Standard.

Availability of Standards. This Committee has arranged through BISSC to provide a complete set of revised standards to every interested sanitarian who is a member of the International Association of Milk, Food and Environmental Sanitarians Association.

Standards may be obtained by writing to: Raymond Walter, Executive Sec'y., BISSC, 521 Fifth Avenue, New York 17, N. Y.

COMMITTEE MEMBERS

Vincent T. Foley, *Chairman*,
(Mo. Ass'n.),
City Health Dept.,
Kansas City, Mo.

Louis A. King, Jr.,
(Ill. Ass'n.),
American Institute of Baking,
Chicago, Ill.

A. E. Abrahamson,
(N. Y. Ass'n.),
City Health Dept.,
New York 13, N. Y.

Fred R. Vitale,
(N. Y. Ass'n.),
Continental Baking Co., Inc.,
Rye, N. Y.

Harold Wainess,
(Ill. Ass'n.),
Wainess & Associates,
Chicago, Ill.

REPORT OF THE COMMITTEE ON FROZEN FOOD SANITATION—1967

The objectives of the Committee on Frozen Food Sanitation this year have been as follows:

1. To determine the major problems in Frozen Food Sanitation.
2. To compile a list of reference material on Frozen Food Sanitation.
3. Since there are other organizations having similar interests, the Committee sought to determine if there existed a duplication of effort and if there was a need for this Committee.
4. The Committee sought to determine what projects should be initiated that would be of value to all persons interested in Frozen Food Sanitation.

The Committee has concluded that the following aspects of Frozen Food Sanitation are of major concern:

1. Adequate refrigeration on route delivery vehicles.
2. Inadequate sanitation in the freeze drying process which results in a concentration of bacterial population.
3. Training of personnel in sanitation and personal hygiene.
4. Equipment should be of proper sanitary design and construction.
5. Proper cleaning of display cases, processing equipment, and storage facilities.

The Committee has concluded that the activities may be duplicated by other organizational committees to a degree; however, it is felt that the Committee can serve a useful purpose in resolving some of the existing problems. Following are projects which the Committee proposes to consider:

1. Stimulate both industry and governmental agencies to establish bacteriological standards for frozen foods.
2. Differentiate frozen foods such as fruits and vegetables from prepared frozen foods such as complete dinners.
3. Stimulate and encourage additional study of the freeze dry processes.
4. Consider the adoption of Equipment Standards for the frozen food industry.
5. Compile a list of reference materials and publications related to Frozen Food Sanitation.
6. Provide an exchange of information between state regulatory agencies concerned with frozen food legislation and strive for uniformity between all agencies.

COMMITTEE MEMBERS

Eugene C. Viets, *Chairman*
(Missouri Association),
State Division of Health,
Jefferson City, Mo.

C. P. Orr,
General Foods Corporation,
White Plains, N. Y.

Stephen J. Palmer,
National Association of Frozen Food Packers,
Washington, D. C.

Frank E. Fisher,
(Indiana Association),
State Board of Health,
Indianapolis, Ind.

Eaton E. Smith,
(Connecticut Association),
State Department of Consumer Protection,
Hartford, Conn.

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(act of October 23, 1962; Section 4369, Title 39, United States Code)

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I certify that the statements made by me above are correct and complete.

H. L. Thomasson, Managing Editor

NEWS AND EVENTS

RECORD $4.5 BILLION IN SALE OF PRODUCTS THROUGH VENDING MACHINES

Sales of products through vending machines are expected to reach $4.5 billion in 1967, more than double the $2 billion mark of 10 years ago, according to data released by the National Automatic Merchandising Association (N A M A). Although no major technical breakthroughs were reported in recent years, 1967 sales of vended products will increase by more than $300 million over 1966, the association said. Diversification of vending service firms into in-plant and institutional food service contracting, partly without use of vending machines, was cited as one of the major developments.

Some 1,500 of the 6,000 vending firms now operate their own food production commissaries, and many are expanding into mobile truck catering as well as concession services for the recreation and leisure markets. Sales of commissary-prepared foods, such as sandwiches, salads, pastry and entrees, through coin-operated machines, will total more than $180 million, an increase of nearly 70 per cent in the past five years, according to the N A M A report. Hot and cold beverages, candy and cigarettes still account for 80 per cent of all vending sales. Volume of these products has increased from 7 to 10 per cent annually during recent years.

The association says vending machines accounted for one third of all candy bars retailed in the United States in 1966, averaging 572,220 confection sales per hour around the clock. Total confection sales through vending machines amounted to 5,012,652,000 units in 1966. Vending of canned soft drinks is showing the largest rate of growth, with production of canned beverage machines reaching a record total of 36,870 venders in 1966, a 21-fold increase during the past four years.

The trend toward coffee machines which brew a single cup at a time from fresh grounds also is on the upswing. These machines were produced at three tims the rate of other types of coffee venders last year, the association reported.

FOOD UPDATE PLANS 1968 SEMINAR IN ATLANTA

Executive and technical management of the U. S. food industry is invited to register for a Food Update seminar during the week of February 18, 1968, to be held at the Regency Hyatt Hotel, Atlanta, Ga. The informational forum, by and for the food industry, will probe the broad implications of governmental, educational and scientific factors on food field growth and change. It will also examine the latest thinking in new product development, processing, packaging, marketing and law.

Sponsored by The Food and Drug Law Institute, Inc., and directed by its president, Franklin M. Depew, the program proposes to survey "practices and promises spanning food business activity, and will explore for important ideas to assist the decision-maker".

Food Update seminars previously held in the East, mid-West and West have attracted speakers and participants from major corporations, government, education, agriculture and science. The 1968 southern program will continue this tradition with broad-ranging presentations by experts on current developments that will sharply influence the national industry's future.

Attendance will be held to an intimately-sized group within which staff and enrollees will be able to participate personally and at length. Food Update, which is a non-profit educational activity, has staged six such seminars during recent years.

Information about the program, registration and fees is available from Food Update, The Food and Drug Law Institute, Inc., 205 East 42 Street, New York, N. Y. 10017.

WISCONSIN MASTITIS TEST TRAINING FILM AVAILABLE

A movie demonstrating the Wisconsin Mastitis Test has been released by the University of Wisconsin. The film is designed for the training of labor-

atory personnel and veterinarians in the correct technique for conducting this test.

The importance of continuous refrigeration of samples at 32°-40° F. in obtaining close comparisons with the direct microscopic leucocyte count is stressed. The objective of the film—and the test—is to help achieve nationwide uniformity and accuracy in making estimates of leucocytes in raw milk samples.

Copies of this 11 minute, sound-color film are available for short term rental from the Bureau of Audio Visual Instruction, University of Wisconsin, P. O. Box 2093, Madison, Wisconsin 53701. The BAVI film number is 6812.

Copies may also be purchased from the Office of Information, Motion Picture Service, U. S. Department of Agriculture, Washington, D. C. 20250. Order by title—Wisconsin Mastitis Test—and USDA number: 1224-185. The price is $58 a copy for color or $23 for black and white.

AMERICAN ACADEMY DIPLOMATES HOLD ANNUAL MEETING

The American Intersociety Academy for the Certification of Sanitarians, Inc., held its annual meeting at the Hotel Fontainebleau, Miami Beach, Florida on October 25, 1967.

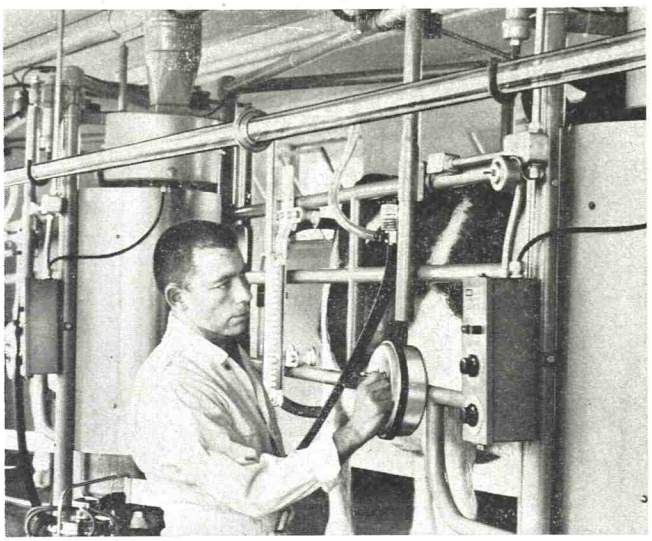
The Academy, incorporated in 1966 certifies professional sanitarians and grants Diplomate status to those who meet high standards of competence and who have demonstrated qualities of leadership in the field of environmental health. Currently, over fifty Professional Sanitarians have earned the honor of Diplomate in the Academy. In addition, some fifteen applications are being reviewed and processed by the Academy's certification committee.

The Secretary of the Corporation is Darold W. Taylor, 2101 Wakefield Street, Alexandria, Virginia. Inquiries and other correspondence dealing with the Academy and its objectives should be addressed to Mr. Taylor.

DAIRY FIELDMEN'S AND DAIRY PLANT SANITATION CONFERENCES TO BE HELD AT PURDUE

J. L. Krider, Head of the Animal Sciences Department at Purdue University has announced two, one-day meetings for dairymen to be held in November 1967. The Dairy Fieldmen's Conference will be held on November 14 and the Dairy Plant Sanitation Conference on November 15, in the Memorial Center at Purdue University. The conferences are sponsored annually in cooperation with the Indiana Dairy Products Association.

Additional information may be obtained by contacting H. F. Ford, Smith Hall, Purdue University, Lafayette, Indiana.



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Five series of sanitary type, air-acuated valves are described in an informative new 40 page Handbook on Flow Control, offered by Tri-Clover Division of Ladish Company. Catalog AFC 66 details usage, installation and application data as well as specifications and dimensions on the TRI-FLO line of stainless steel air valves. Included are both sanitary CIP and take-down models of shut-off, divert, throttling and tank or kettle valves—in a range of sizes from 1 thru 4 inch Tube OD.

An improved actuator, which can be modified on the job to suit function is described. It can also be serviced in the field without special tools and is standard equipment on all valves. A section on automation and control components provides useful information on system layout and proper valve usage. Cutaway photos of each valve style show construction detail, recommended product usage and illustrates valve benefits. Head pressue loss curves are included for each valve series.

The catalague is available from the LADISH COMPANY, Tri-Clover Division, Kenosha, Wisconsin 53140.

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SANITARIAN—General environmental program. Pueblo City-County Health Department. Headquarters, Pueblo, Colorado. Salary range $6,192 to $8,052. Contact Dalton Roberts, Administrative Officer, Colorado State Department of Public Health, 4210 East 11th Avenue, Denver, Colorado 80220.

SANITARIAN—Specializing in milk program for the Colorado State Department of Public Health. Headquarters Denver, Colorado. Salary range $7,920 to $10,608, may start up to $8,724, depending upon qualifications. Requires at least 4 years experience. Contact Dalton Roberts, Administrative Officer, Colorado State Department of Public Health, 4210 East 11th Avenue, Denver, Colorado 80220.

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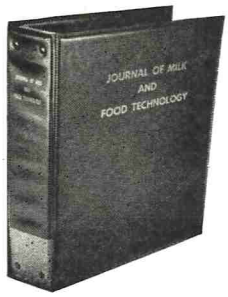

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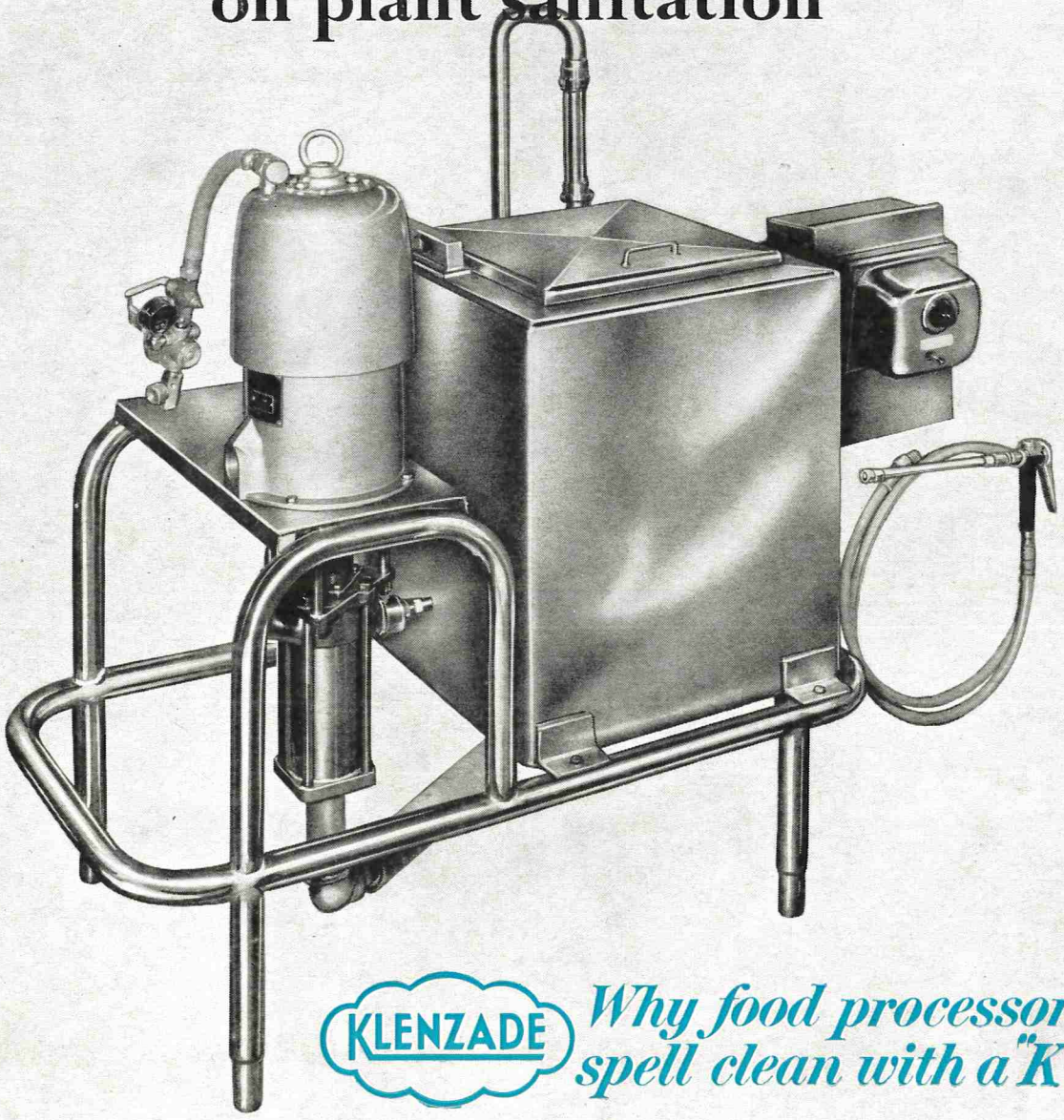

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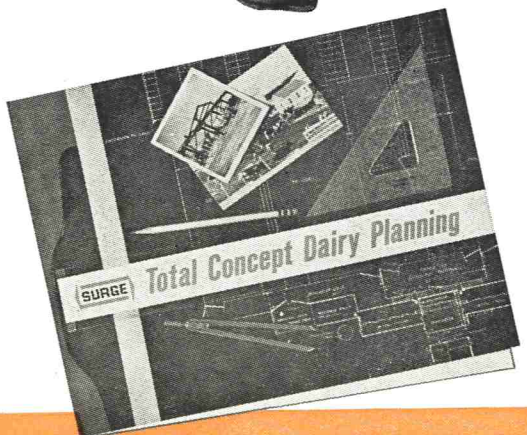
Babson Bros. Dairy Planning Service is staffed by specialized personnel. Skilled draftsmen and realistic survey and layout men combine individual talents to complete practical, workable Dairy Units of any size and for any conditions.

Complete preliminary plans will be drawn to fit individual needs. Dairymen may then work directly with their contractor . . . or if they prefer, Babson Bros. Dairy Planning Service will assume full responsibility . . . Complete construction drawings, construction supervision and operator education makes possible a turn-key Dairy Unit.



For Further Information write:

BABSON BROS.
DAIRY PLANNING SERVICE
A subsidiary of Babson Bros. Dairy Research Service
2100 S. YORK RD. • OAK BROOK, ILL. 60521