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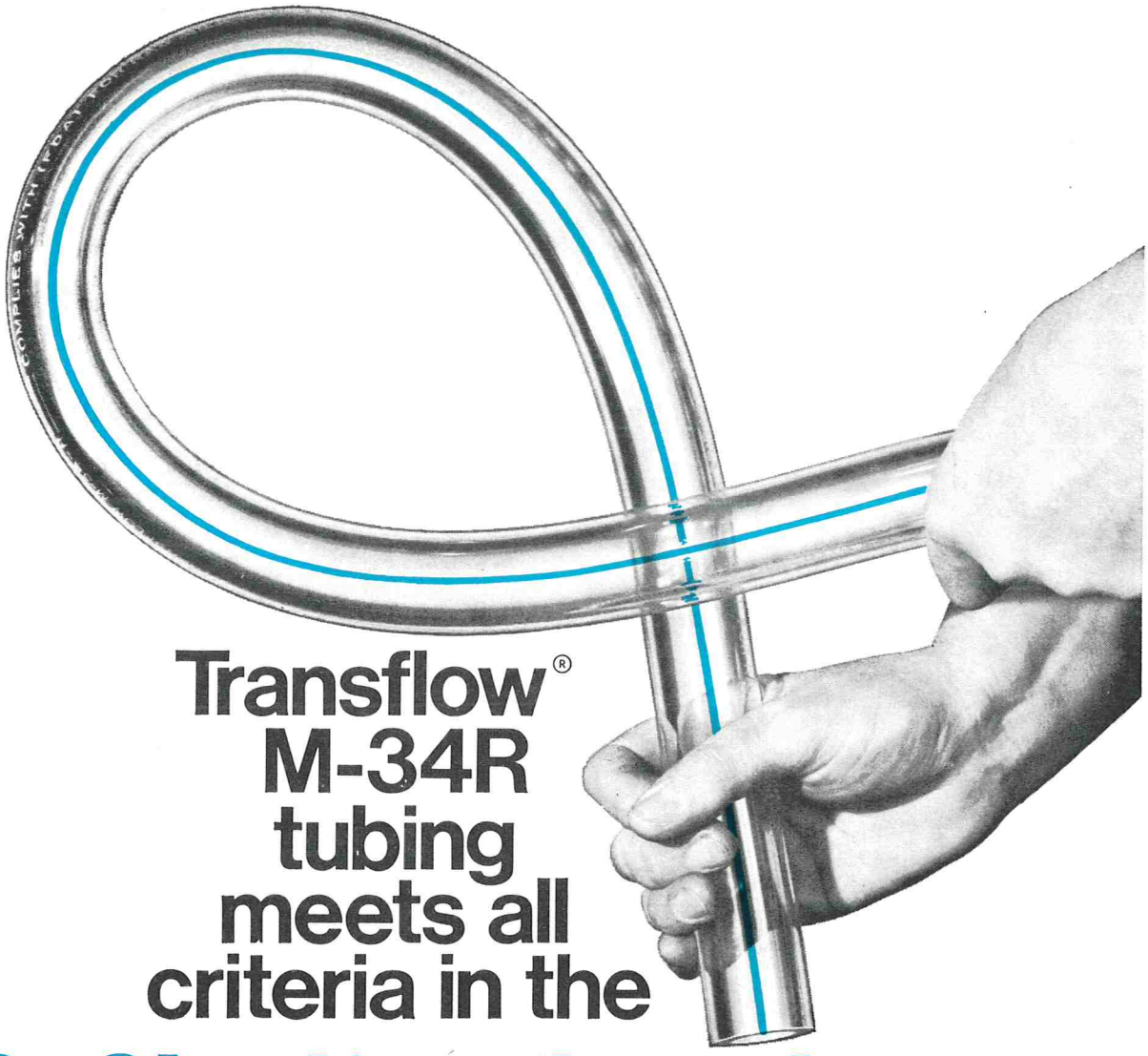

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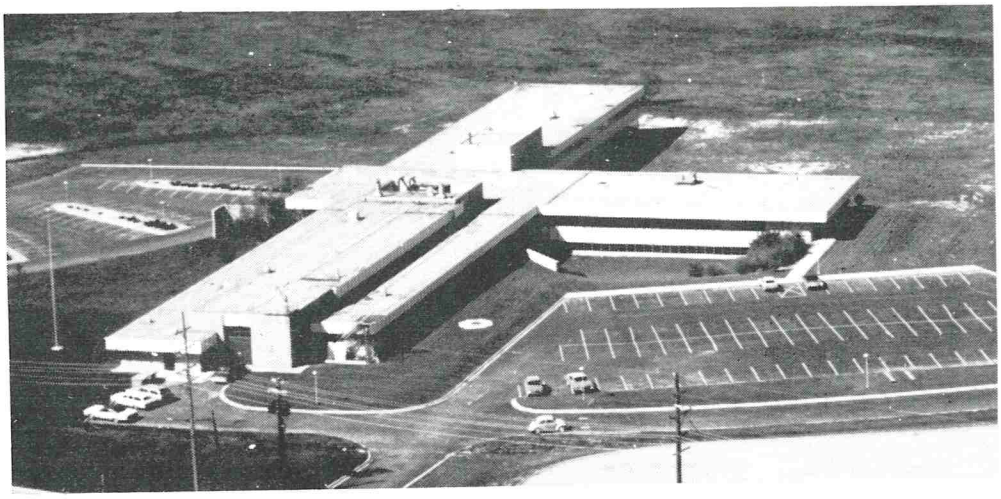

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Flow Behavior of Concentrated Suspensions of Yeast Grown on Sauerkraut Waste[1]

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(Received for publication September 30, 1974)

ABSTRACT

The flow behavior of suspensions of yeast (*Candida utilis*) grown on sauerkraut waste was studied as a function of concentration at 25.8 C. Suspensions containing 5.8, 21.7, 22.6, 23.6, 24.7 and 27.0% dry solids behaved as Newtonian fluids. The viscosity of these suspensions increased exponentially with the concentration of dry solids. A 29.2% suspension showed time dependent shear-thinning behavior. The non-Newtonian characteristics of the suspension could be described by both the Power law and the Casson flow models.

Over 10 million cases of sauerkraut are packed annually in the U.S. with New York State producing about 3.5 million. Presently some of the wastes from sauerkraut are a source of water pollution. Quantitative data on sauerkraut wastes based on an extensive survey of two sauerkraut factories were presented recently (*5*) and subsequently, it has been shown that the wastes can serve as a favorable substrate for food yeasts (*6*). For production of drum-dried yeast on a pilot scale it was necessary to employ concentrated suspensions obtained by continuous centrifugation of the original suspension containing 5.8% dry solids. This study of the flow characteristics of yeast suspensions was undertaken primarily to aid in design and operation of equipment for production of dried yeast.

A second objective of this study was to add to the apparently limited information available on viscosity characteristics of yeast suspensions. Two important aspects of the present study are: (*a*) suspensions of yeast grown and harvested under known conditions were employed, and (*b*) a narrow gap concentric cylinder viscometer was used for accurate determination of the viscosity behavior. The first factor is important because the viscosity behavior of a suspension could be influenced by conditions under which the yeast was grown, harvested, and subsequently handled; while the second is important for accurate description of materials such as suspensions which exhibit non-Newtonian behavior, especially yield stress.

Several rheological studies have been published on protein concentrates extracted from yeast, whose aim was to aid dope formation and spinnability. A list of some of the publications and patents can be found in a recent paper on the rheology of protein concentrates (*9*).

[1]*Approved by the Director of the New York State Agricultural Experiment Station for Publication as Journal Paper No. 2110.*

Because the present study is with whole yeast suspensions rather than extracted protein solutions, the literature on the protein concentrates will not be discussed here. Viscosity data on yeast suspensions (*Saccharomyces cerevisiae* in buffer solutions) obtained with an Ostwald capillary viscometer have been reported (*1*). In that study, relatively low concentration suspensions were employed and the highest Newtonian viscosity reported was about 20% higher than that of water. The viscosity behavior of suspensions of a commercial baker's yeast (*S. cerevisiae*) was studied with a Brookfield LVT viscometer (*10*). The yeast, purchased in the form of a pressed cake was diluted with distilled water to obtain suspensions of different concentrations. Suspensions below 10% dry solids were reported to be Newtonian fluids, while those between 10 and 21% dry solids were found to be pseudoplastic non-Newtonian fluds. It was reported that the suspensions did not exhibit yield stress.

MATERIALS AND METHODS

Candida utilis NRRL Y-900 was cultivated in a 6-liter fermentor at 26 C for 24 h on sauerkraut waste which contained the following expressed as milligrams per liter: BOD, 24,000; total acid as lactic, 19,900; Kjeldahl nitrogen, 1,100; total phosphorous, 192; and NaCl, 26,500. Methods of analysis indicated earlier (*5*) were employed in these determinations. A stock yeast concentrate was produced by centrifugation and lower concentrations were obtained by dilution with the effluent. The dry solids content of the suspensions was determined by evaporating 10-g samples at 105 F for at least 12 h.

Viscosity measurements were made with an Epprecht Rheomat 15, a concentric cylinder viscometer (Contraves, A. G., Zurich, Switzerland) which is also known as the Drage viscometer (*16*). The viscometer, which has been described in detail by Van Wazer et al. (*16*), allows the determination of the torque exerted over the inner cylinder at 15 different rotational speeds. Measurements were made at 25.8 C and the temperature was controlled by means of a constant temperature bath. Measuring system MS-O was employed for the original suspension containing 5.8% dry solids, system MS-A was employed for suspensions containing 21.7, 22.6, 23.6, 24.7, and 27.0% dry solids, and system Ms-Cc was employed for the 29.2% dry solid suspension.

The appropriate measuring system was chosen so that measurable torque readings could be obtained. In general torque readings lower than about 10 scale divisions were discarded. With each sample, measurements were made by first employing rotational speeds in increasing order. At each rotational speed the torque reading was noted 1 min after the drive motor was turned on. Also during this time, the motion of the torque indicator was observed. A stable position of the indicator meant that the sample had no measurable time-dependent flow behavior. In the sample that did exhibit time-dependent behavior, torque readings were noted first in the ascending order of rotational speeds and then in the descending order.

For fluids that exhibited Newtonian behavior the shear stress and the shear rate were deduced from the tabulated values supplied by the manufacturer, while for the non-Newtonian suspension the true magnitude of the shear rate was obtained by correcting the tabulated Newtonian shear rate. The correction method (*2*) is based on a power type relationship between the speed of rotation and the torsion wire deflection, and the radii of the inner and outer cylinders. Data reduction was performed on an IBM 1800 digital computer.

RESULTS AND DISCUSSION

The original suspension containing 5.8% dry solids was a mobile Newtonian fluid. Linear regression analysis of log RPM vs. log torque data of the suspensions containing 21.7, 22.6, 23.6, 24.7 and 27.0% dry solids indicated that they were also Newtonian fluids. The correlation coefficient for these suspensions was greater than 0.9996. Also, at these concentrations, extrapolating the RPM vs. torque data failed to show measurable values of yield stress. The Newtonian behavior of the suspensions was observed over wide ranges of shear rates. The ranges of shear rates and the measuring systems employed are given in Table 1.

TABLE 1. *Measuring system and range of shear rates for Newtonian suspensions*

Dry solids (%)	Measuring system	Range of shear rate, sec^{-1}
5.8	MS-O	766 to 1774
21.7	MS-A	88.4 to 702.3
22.6	MS-A	88.4 to 532.1
23.6	MS-A	67.2 to 399.0
24.7	MS-A	67.2 to 303.0
27.0	MS-A	11.2 to 67.2

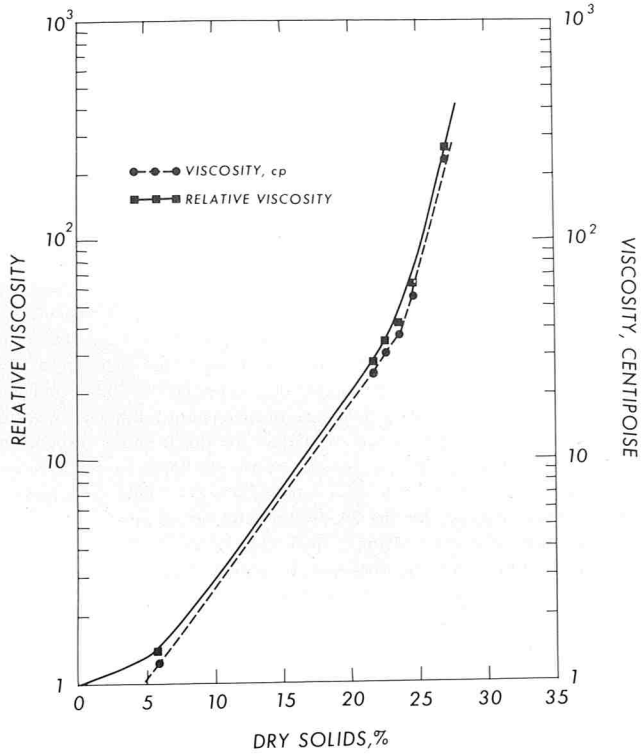

Figure 1. *Influence of concentration on the viscosity of Newtonian suspensions.*

Two curves are shown in Fig. 1, one relating viscosity and concentration, and the other relative viscosity and concentration. The straight line segments in Fig. 1 indicate that the viscosity of the Newtonian suspensions is exponentially related to the concentration. However, the exponential dependence on concentration is not uniform over the entire range of concentration; it is higher over the range 23.6 to 27.0% than over the range 5.8 to 23.6%. Equations [1] and [2] describe the relationship between the viscosity (μ) and the % dry solids content (c) over the concentration ranges of 5.8 to 23.6% and 23.6 to 27.0%, respectively.

$$\mu = 0.415\, e^{0.190c} \qquad [1]$$

$$\mu = 2.07 \times 10^{-4}\, e^{0.511c} \qquad [2]$$

In these equations, the viscosity μ is expressed in centipoises and the concentration c as % dry solids.

For the sake of comparison and for obtaining a better insight into the concentration dependence of the viscosity of the yeast suspensions, it is worthwhile considering a few biological suspensions and rigid sphere suspensions that have been studied extensively. For tomato concentrates (*7*) and concentrated passion fruit juice (*17*), power-type relationships were found between viscosity and concentration. Such relationships were not found to be satisfactory for yeast suspensions. There are numerous studies, which deal with the effect of concentration on viscosity of suspensions containing rigid spheres such as glass beads. Several equations describing the dependence of viscosity on the concentration of solids (expressed as volume fraction of solids) have been proposed and these range in complexity from the relatively simple linear equation of Einstein to more complex equations such as that of Lee (*11*). However, it appears that none of these equations is satisfactory over a wide range of concentration (*11, 12*). Another drawback of all the equations is that they are applicable only for fluids which exhibit Newtonian behavior. For non-Newtonian pseudoplastic fluids, encountered frequently, such behavior is found only at extremely low and extremely high shear rates.

Direct use of the equations developed for rigid sphere suspensions to describe the viscosity-concentration relationship of the yeast suspensions is not possible not only because the use of volume fraction would be meaningless but also because the yeast cells are not spherical and rigid. In fact, the inadequacy of the equations for rigid sphere suspensions even for very low concentration yeast suspensions was demonstrated (*1*). However, the studies with rigid sphere suspensions point out certain similarities between the rigid sphere suspensions and yeast suspensions. One similarity is that above a certain concentration of solids, there is a large increase in viscosity when the concentration of solids is increased by a small magnitude. For yeast suspensions we note that the viscosity increased by only about 50%

when the dry solids content was increased from 21.7 to 23.6%; however, there was more than a 6-fold increase in the viscosity when the solids content was increased from 23.6 to 27.0%. In the case of a suspension containing glass beads (4 to 44 μ, diameter) in a mineral oil, Nicodemo et al. (*12*) found that the viscosity increased from 2.1 to 7.6 poises when the solids content, expressed as volume fraction, was increased from 0 to 0.2; however, increasing the volume fraction from 0.2 to 0.4 resulted in an increase in the viscosity from 7.6 to 182 poises. Similar behavior has been documented for other rigid sphere suspensions (*11*). From the studies on rigid sphere suspensions, it appears that small changes in percentage dry solids content of the yeast suspensions represent significant changes in the volume fraction of solids.

To describe the variation in viscosity of rigid sphere suspensions with concentration, semi-logarithmic plots of relative viscosity vs. concentration of solids are employed. The relative viscosity, μ_r, is defined as

$$\mu_r = \frac{\text{viscosity of the suspension}}{\text{viscosity of the liquid medium}} \quad [3]$$

In equation [3], the two viscosities are to be at the same temperature. For yeast suspensions, the liquid is water with a viscosity of 0.878 cp (*13*). The variation of relative viscosity of the yeast suspensions with concentration is also shown in Fig. 1, and the curves for rigid sphere suspensions are also of a similar shape (*11, 12*). Because there is not as yet a single equation that describes the viscosity-concentration relationship for rigid sphere suspensions, it is doubtful that such an equation can be found for the more complex suspensions of yeast. It is felt that equations having exponential form, such as equations [1] and [2], which describe the dependence of absolute viscosity on concentration, are simpler and more useful for practical purposes; it is emphasized, however, that such equations are valid only for the range of concentrations employed.

The 29.2% suspension exhibited time dependent shear-thinning behavior. Shear stress values were obtained first in the ascending order of shear rates followed by descending order. The latter were lower in magnitude. After resting the fluid for 15 min the ascending order data were reproduced (Fig. 2). A fluid exhibiting this kind of behavior is known popularly as "thixotropic." The data taken in descending order of shear rates are close to equilibrium values. This was determined from experiments in which the torque reading was allowed to reach equilibrium.

Over the range of shear rates employed, as shown in Fig. 2 and 3, the viscosity data obeyed both the Power law flow model (equation 4) and the Casson flow model (equation 5). In equations [4] and [5],

$$\tau = K\dot{\gamma}^{n} \quad [4]$$

$$\tau^{0.5} - \tau_0^{0.5} = K_c\dot{\gamma}^{0.5} \quad [5]$$

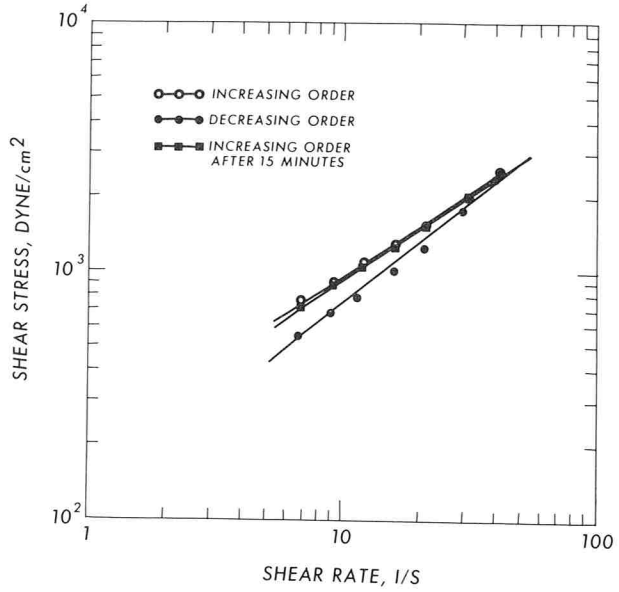

Figure 2. *Thixotropic and Power law flow behavior of 29.2% suspension.*

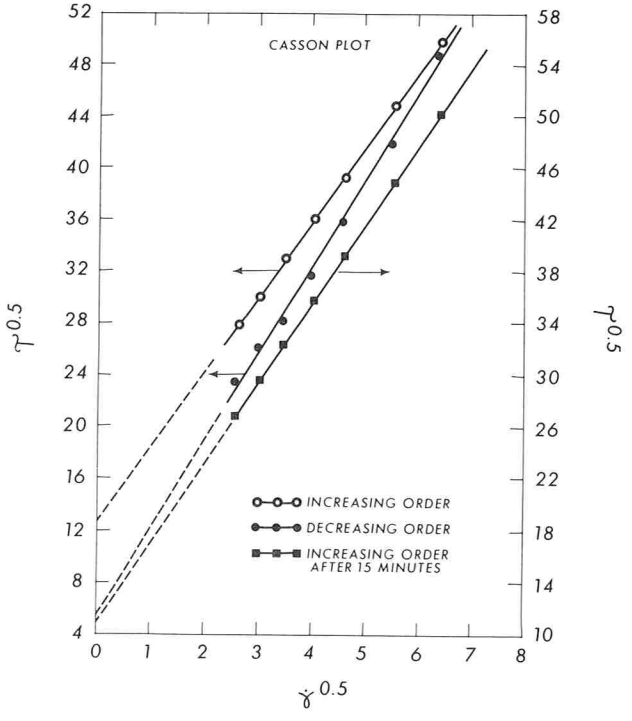

Figure 3. *Casson plot for 29.2% suspension.*

τ is the shear stress, τ_0 is the yield stress, K is the consistency index, n is the flow-behavior index, $\dot{\gamma}$ is the shear rate and K_c is a constant. The correlation coefficient for the Power law model ranged from 0.9939 to 0.9992, while for the Casson model it ranged between 0.9972 and 0.9999. The magnitudes of the Power law constants K and n were 212.6 and 0.653, respectively, for increasing order of shear rate data, and 110.1 and 0.814, respectively, for the decreasing order of shear rate data. Because the magnitude of n is less than 1, the fluid is classified as shear-thinning (pseudoplastic). The

magnitude of the Casson constant K_c was 5.767 and 6.679, respectively, for the increasing and decreasing order of shear rate data.

The applicability of the Power law model to fluid foods and certain applications of it to food processing have been described recently (*8, 14*). The Casson flow model was developed originally to charcterize oil-based suspensions such as printing inks (*3*); however, it was found to be suitable also for describing the flow behavior of foods such as apple sauce and tomato concentrates (*4*) and more recently for mycelial broths (*15*). It has been used successfully for estimating the magnitude of yield stress of biological materials (*4, 15*). The magnitudes of $\tau_0^{0.5}$ were obtained by linear regression analysis of the data $\tau^{0.5}$ and $\dot{\gamma}^{0.5}$, which were then used to compute the yield stress. The yield stress, τ_0, ranged from 160 to 30 dyne/cm² in magnitude, the lower value being for the well sheared sample.

The flow behavior results of this study are in agreement with those of earlier studies in that yeast suspensions at low concentrations are Newtonian in character (*1, 10*), and at higher concentrations they exhibit pseudoplastic behavior (*10*). Quantitative comparison among the studies is not possible because of differences in yeast varieties, growth media, and in one instance various commercial processing operations to which the yeast was subjected. It appears that time-dependent flow behavior and the presence of yield stress in yeast suspensions were not observed in earlier studies, because either narrow gap concentric cylinder viscometers or high concentration suspensions were not employed.

The viscosity data can be used for several processing applications. Pressure drop in process flow lines and pumping power requirements for Newtonian suspensions can be calculated from methods described in handbooks (*13*). Additional information that is rquired includes the flow rate, diameter of the process line, number, size, and type of various fittings, etc. In general the power requirements would be higher for fluids with higher viscosity. For non-Newtonian fluids, equations for pressure drop in pipelines and power requirements for pumping and mixing have been developed for fluids obeying the Power law. These equations (*8*) require knowledge of the Power law constants, K and n in addition to the parameters needed for Newtonian fluids. Suspensions with yield stress should be kept under continuous agitation such that they remain fluid all of the time.

REFERENCES

1. Aiba, S., A. E. Humphrey, and N. F. Millis. 1965. Biochemical engineering, p. 274. Academic Press, New York.
2. Brodkey, R. S. 1967. The phenomena of fluid motions, p. 354, Addison-Wesley, Reading, Massachusetts.
3. Casson, N. 1959. A flow equation for pigment-oil suspensions of the printing ink type. p. 84. *In* Rheology of disperse systems, ed. C. C. Mill, Pergamon Press, New York.
4. Charm, S. E. 1963. The direct determination of shear stress-shear rate behavior of foods in the presence of a yield stress. J. Food Sci. 28:107.
5. Hang, Y. D., D. L. Downing, J. R. Stamer, and D. F. Splittstoesser. 1972. Wastes generated in the manufacture of sauerkraut. J. Milk Food Technol. 35:432.
6. Hang, Y. D., and D. F. Splittstoesser. 1972. Sauerkraut waste: a favorable medium for cultivating yeasts. Appl. Microbiol. 24:1007.
7. Harper, J. C., and A. F. El Sahrigi. 1965. Viscometric behavior of tomato concentrates. J. Food Sci. 30:470.
8. Holdsworth, S. D. 1971. Applicability of rheological models to the interpretation of flow and processing behavior of fluid food products. J. Texture Studies 2:393.
9. Huang, F., and C. K. Rha. 1971. Rheological properties of single-cell protein concentrate: dope formation and its flow behavior. J. Food Sci. 36:1131.
10. Labuza, T. P., D. B. Santos, and R. N. Roop. 1970. Engineering factors in single-cell protein production. I. Fluid properties and concentration of yeast by evaporation. Biotech. Bioeng. XII:123.
11. Lee, D. I. 1969. The viscosity of concentrated suspensions. Trans. Soc. Rheol. 13:273.
12. Nicodemo, L., L. Nicolais, and R. F. Landel. 1974. Shear rate dependent viscosity of suspensions in Newtonian and non-Newtonian liquids. Chem. Eng. Sci. 29:729.
13. Perry, J. H. 1950. Chemical engineers' handbook. McGraw-Hill, New York.
14. Rao, M. A. 1973. Sizing holding tubes of high-temperature short-time pasteurizers for Power law food products. J. Milk Food Technol. 36:181.
15. Roels, J. A., J. Van den Berg, and R. M. Voncken. 1974. The rheology of mycellial broths. Biotech. Bioeng. XVI:181.
16. Van Wazer, J. R., J. W. Lyons, K. Y. Kim, and R. E. Colwell. 1963. Viscosity and flow measurement. A laboratory handbook, p. 129. Interscience, New York.
17. Vitali, A. A., S. M. Roig, and M. A. Rao. 1974. Viscosity behavior of concentrated passion fruit juice. Confructa, Int. J. Technol. Fruit Vegetable Proces. 19: (In press).

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Temperature Equilibration Times of Plate Count Agar and a Comparison of 50 Versus 45 C for Recovery of Raw-Milk Bacteria[1]

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ABSTRACT

Sixty raw milk samples were plated using "Standard Methods" agar tempered to 45 or 50 ± 1 C. The standard plate count was significantly lower with the agar at 50 C. Tempering time (to 44-46 C) of a flask of agar in a water bath was about 5-10 min longer than that of a comparable flask of water. Time required to reach the desired temperature depended upon the volume of agar in the flasks, the number of flasks, and the volume of the water in the bath. Up to an hour of equilibration time may be necessary for newly autoclaved agar to reach the recommended temperature (44-46 C). Insufficient tempering time might cause an excessively high plating agar temperature which might cause a reduction in bacterial counts, especially of a heat sensitive psychrotrophic bacterium.

The extreme heat sensitivity of a psychrotrophic bacterium, *Pseudomonas fluorescens,* was recently shown by Gray et al. (*2*). Exposing cells to 36 C for 2 h resulted in an apparent 99% reduction in plate count. The count, however, increased after 25 C incubation due to repair of the heat-stressed bacteria. Vanderzant and Matthys (*8*) presented some evidence that an increase in pouring-agar temperature from 45 to 50 C resulted in decreased counts of pure cultures of bacteria isolated from dairy products. A 25% reduction in counts was found at 50 C with 6 of 9 cultures tested; however, they provided no statistical treatment for their data. Stapert et al. (*7*) compared Millipore-filtered water samples (receiving no extra heat treatment) to a water-gelatin solution heated to 45 C. The unheated water gave counts 3-4 times that of the heated solution. Comparison with the standard plate count (SPC) indicated that the higher temperature of the agar was responsible for the decreased counts. Mossel and van der Moosdijk (*6*), on the other hand, found no differences between pour plate (agar at 44-46 C) and spread drop counts (agar at room temperature) of 42 food samples. Lawton and Nelson (*5*) used a temperature of 52 C for 7-9 min for partially inactivating cultures of pseudomonads. They observed reductions in counts of 2 to 4 logs. Heather and Vanderzant (*3*) found less than 0.1% survivors when a culture of *Pseudomonads putrefaciens* was heated to 50 C for 1 min.

Standard Methods for the Examination of Dairy Products (*1*) recommends that agar used for the SPC be tempered to 44-46 C before the plates are poured. In this method the temperature of the pouring agar is determined by checking the temperature of a similar flask of water subjected to the same conditions. Because of the possible difference in cooling times of water and agar due to differences in specific heat and viscosity, this method of temperature checking could lead to agar pouring temperatures high enough to destroy some of the heat sensitive bacteria. We chose the temperature of 50 ± 1 C for studying this effect and compared it to the *Standard Methods* temperature of 45 ± 1 C. We also compared the equilibration times of water and agar in identical flasks.

MATERIAL AND METHODS

Methods advocated by *Standard Methods* (*1*) were followed except that agar tempered at 50 ± 1 C was used in addition to the recommended 45 ± 1 C agar. The protocol for the experiments and the analyses were in general similar to those of a previous study (*4*). Sixty samples of raw milk were analyzed by nine analysts. In most instances replicates from the same dilution bottle were plated in duplicate plates. The temperature of the agars was determined by either inserting an alcohol-sterilized and dried thermometer directly in the pouring agar or by inserting a thermometer in a different flask of agar. Separate water baths were used for the two temperatures. A comparison was made of the time required for freshly autoclaved water or agar in similar vessels to reach the recommended temperature of 44-46 C. Temperature readings were made at intervals until the desired temperature was reached.

RESULTS

Statistical analyses of 45 vs 50 C agar

Results of the comparison of agar pour temperatures

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of 45 and 50 C on recovery of raw-milk bacteria are shown in Table 1. Arithmetic means of the plate counts

TABLE 1. *Comparison of 45 and 50 C agar temperature for standard plate counts of raw milk*

Analyst	Milk sample	45 C	50 C
A	1	137[a]	141[b]
	2	29.5	26.8
	3	394	363
	4	230	236
	5	112	101
	6	93	89
	7	11.4	11.1
	8	324	145
	9	9.1	8.5
	10	50	42
arith. mean		139.0	116.3
geom. mean[c]		4.326	4.187
B	11	189	134
	12	3.7	7.1
	13	16	16
	14	3.6	4.3
	15	5.4	5.5
	16	50	48
arith. mean		44.65	35.86
geom. mean		2.694	2.761
C	17	3.5	3.5
	18	22.5	25.1
	19	11.2	11.8
	20	18.2	18.6
	21	13.8	13.8
arith. mean		13.83	14.56
geom. mean		2.461	2.497
D	22	14.4	16.4
	23	13.4	11.9
	24	63	42
	25	3.5	3.9
	26	5.0	4.6
arith. mean		19.76	15.62
geom. mean		2.445	2.361
E	27	109	124
	28	247	138
	29	26.4	22.8
	30	68	72
	31	148	164
	32	97	77
	33	24.0	19.2
	34	8.0	7.1
	35	18.2	14.5
arith. mean		82.74	70.91
geom. mean		3.930	3.791
F	36	20.1	20.6
	37	27.2	25.6
	38	70	72
	39	57	51
	40	6.0	6.6
	41	6.6	6.8
	42	6.5	7.0
arith. mean		27.64	27.18
geom. mean		2.876	2.884
G	43	2.9	2.3
	44	5.8	6.0
	45	7.3	6.8
	46	2.8	2.5
	47	35	25
	48	7.4	6.4
arith. mean		10.21	8.21
geom. mean		1.896	1.752
H	49	10.7	9.8
	50	8.8	6.2
	51	6.3	6.8
	52	22.0	12.8
	53	18.3	15.6
	54	8.5	8.8
arith. mean		12.42	10.00
geom. mean		2.416	2.245
I	55	9.8	9.4
	56	9.4	6.6
	57	6.0	6.0
	58	27.0	13.3
	59	18.6	16.0
	60	8.9	9.4
arith. mean		13.29	10.09
geom. mean		2.451	2.249
All samples			
arith. mean		40.73	34.64
geom. mean		2.833	2.748

[a]Arithmetic mean of four values (2 replicates and 2 duplicate petri dishes); for actual counts multiply by 1000.
[b]Underlined figures denote the higher of the two means.
[c]Natural log.

The temperature variation was ± 0.5 C at most and was checked by inserting thermometer into duplicate flasks of agar or by alcohol sterilizing and inserting in pouring agar.

were lower at 50 C for eight of the nine analysts. The geometric means (log e) were lower at 50 C with six analysts, higher for one (analyst B), and were about the same with the other two analysts. In 34 of 60 milk samples tested, a temperature of 50 C gave lower arithmetic mean counts than 45 C while four samples showed the same counts. Sixteen milk samples gave higher counts at 50 than at 45 C agar pour temperature. The percent differences between the two agar temperatures for each milk sample were calculated, transformed to an arcsin function, and a two-tailed t-test made on the mean of the differences. The results showed the 50 C counts to be significantly lower ($P < 0.05$) than the 45 C counts.

An analysis of variance of the logarithms of plate counts of raw milk samples is shown in Table 2. The highly significant difference between analysts ($P < 0.01$) may have resulted because analysts were from different geographical locations which might in turn have influenced the types of bacteria present in their particular raw milk samples. There was, as expected, a very great difference between samples, but no difference between replicates. The replicates for this experiment consisted of two aliquots from the diluted milk rather than two aliquots from the same raw milk samples. Analysis of the effect of agar pour temperature on plate

TABLE 2. *Analysis of variance of plate count logarithms using 45 or 50 C agar pouring temperatures*

Line	Source of error	df	Sum of squares	Mean square	F	Significant with P <0.05	Significant with P <0.01
A	Analysts	8	316.205	39.5256	4.59	yes	yes
B	Samples/analysts	51	439.143	8.61065	574	yes	yes
C	Replicates/samples	43	0.645017	0.015000	1.17	no	no
D	Temperatures	1	1.017969	1.01796	10.56	yes	yes
E	Temperatures × analysts	8	0.939072	0.117384	1.22	no	no
F	Temperatures × samples/analysts	51	4.91461	0.096365	7.52	yes	yes
G	Temperatures × replicate samples	43	0.550741	0.012808	<1.0	no	no
H	Residual (between plates)	206	3.22949	0.015677			
	Total	411	766.645				

F values calculated from ratios of lines A/B, B/C, C/G, D/F, E/F, F/G, G/H.

TABLE 3. *Analysis of variance of variances of logarithms of plate counts between duplicate petri dishes with agar temperatures of 45 and 50 C*

Line	Source of error	df	Sum of squares	Mean square	F	Significant with P <0.05	Significant with P <0.01
A	Analysts	8	16098.9	2012.36	2.26	yes	yes
B	Samples/analysts	51	45308.8	888.408	1.42	no	no
C	Replicates/samples	43	26853.2	624.493	0.48	no	no
D	Temperatures	1	3464.48	3464.48	3.96	no	no
E	Temperatures × analysts	8	15232.8	1904.10	2.18	no	no
F	Temperatures × samples/analysts	51	44570.2	873.925	0.67	no	no
G	Temperatures × replicate/samples	43	56301.8	1309.34			
	Total	206	207830.12				

F values calculated from ratios of lines A/C, B/C, C/G, D/G, E/G, F/G.

counts showed a highly significant difference between 50 and 45 C (50 was lower). Tests for interactions showed no consistent source of error between temperatures and analysts (line E), but there was a highly significant interaction ($P < 0.01$) between temperatures and milk samples within analysts (line F). However, there was no interaction between agar pour temperatures and replicates (line G). Table 3 shows the analysis of variance of the single-degree-of-freedom variances calculated for the pairs of petri dishes. These indicate homogeneous petri dish variances with each analyst. There was a significant error, however, between analysts.

Time required for agar to reach 45 C

Several analysts reported on their studies of the time required for pouring agar to reach the recommended temperature of 44-46 C. One such study was conducted with a Precision Scientific Co.[9] bath, without agitation, having inside measurements of 10.5 inches by 13.5 inches and containing sufficient water to reach above the freshly melted agar in the flasks (200 ml agar in 250 ml narrow mouth Erlenmeyer flasks). With one flask of agar in the bath, 15-17 min were required to reach 44-46 C; with three flasks 26-28 min; and with five flasks 32-34 min.

Comparison of water and agar tempering times

A comprehensive study of the agar vs water temperature equilibration time was made using two different size water baths (Table 4). The larger one was 12 by 30 by 10 inches containing 32 liters of water with agitation; the smaller bath was 13¾ by 10¾ by 7 inches with 15 liters of water without agitation. Two volumes of agar and water were compared; one was 180 ml in 8-oz medicine bottles; the second was 500 ml in 1000-ml Erlenmeyer flasks. Only two flasks, one of agar, the other of water, were in each water bath at any one time. Temperatures were measured as accurately as possible. Three trials were made under each set of conditions with temperatures being measured as soon after autoclaving as possible (3 min) and at 5-min intervals thereafter. The three trials with bottles containing 180 ml water in the large bath showed that an average of 20 min were required to reach 46 C, while agar bottles required 25 min. The larger volumes (500 ml in 1000 ml Erlenmeyer flasks) in the larger bath cooled to 45 C in an average of 30 min for water and agar flasks. In the smaller bath, water bottles of 180 ml required an average of 40 min while the agar flasks required 50 min. In the bath, the large volumes required 45 min for the water and 50 min for the agar flasks.

TABLE 4. *Time (minutes) for freshly autoclaved water and agar to reach 44-46 C*

Type of fluid	Fluid[a] volume (ml)	Bath[b] size	Trial 1	Trial 2	Trial 3	Ave
Water	180	large	25	20	15	20
Agar	180	large	25	25	25	25
Water	180	small	45	35	40	40
Agar	180	small	50	50	50	50
Water	500	large	30	30	25	30
Agar	500	large	35	30	30	30
Water	500	small	50	45	45	45
Agar	500	small	50	50	50	50

[a]180 ml volumes were in Brockway #12 (8 oz) medicine bottles; 500 ml volumes were in 1000 ml Erlenmeyer flasks.

[b]Large bath was Blue M Model MW 1130 A-1, measuring 12" × 30" × 10", and contained 32 liters of water with agitation by means of a paddle at 20 strokes per minute. Small bath was a Thelco Model 83, measuring 13¾" × 10¾" × 7", and contained 15 liters of water without agitation.

[9]*Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.*

Effect of degree of loading of bath on tempering time

One collaborator studied the tempering time of a water bath filled to capacity with 12 milk dilution bottles of agar or with two bottles. Twelve bottles required 50 min in one trial and 67 min in another while two bottles required 24 min to reach the recommended temperature.

Agar setting time

To determine the length of time the raw milk bacteria would be subjected to possibly deleterious temperatures, we determined the time required for the agar plates to solidify enough to prevent sliding when tilted. The average solidification time for 45 C agar was 3.2 min and for 50 C agar it was 4.4 min.

DISCUSSION

Our results showed a highly significant reduction in raw milk bacterial counts using an agar pouring temperature of 50 instead of 45 C. This underscores the need for carefully checking agar temperatures before plates are poured. Although the agar setting times indicated that the bacteria were in contact with the warm pouring agar for a maximum of about 3 min, it is possible that even then there was considerable damage to the psychrotrophs (*2, 3*). It would be best to check the temperature of each agar flask before pouring although this is inconvenient and might lead to contamination. As a guide, a tempering time of 1 h would appear to be adequate for proper temperature equilibration. Temperatures should be checked in one of the agar flasks although the differences in tempering times we observed were not in general very different from water flasks (10 min after the water flasks reached the desired temperature would insure that the agar flasks were also in the proper range). Reheating agar and water flasks at the same time in an autoclave would insure that each started out at nearly comparable temperatures before placing in the water bath. Some consideration in future editions of Standard Methods should also be given to the volume of agar relative to the water bath volume as a guide to laboratories in preparing pouring agar.

CONCLUSIONS

(*a*). Use of a pouring agar temperature of 50 C resulted in lower standard plate counts, thus confirming reports of other workers.

(*b*). Water in flasks cools more rapidly than does agar in flasks although assurance that the agar is in pouring range may be had by using the agar no less than 10 min after the water in a flask reaches 45 C.

(*c*). The most important considerations in agar tempering are the size of the bath, the degree of loading, and agitation of water in the bath.

REFERENCES

1. American Public Health Association. 1972. Standard methods for the examination of dairy products, 13th ed.
2. Gray, R. J. H., L. D. Witter, and Z. J. Ordahl. 1973. Characterization of mild thermal stress in *Pseudomonas fluorescens* and its repair. Appl. Microbiol. 26:78-85.
3. Heather, C. D., and C. Vanderzant. 1957. Effect of temperature and time of incubating and pH of plating medium on enumerating heat-treated psychrophilic bacteria. J. Dairy Sci. 40:1079-1086.
4. Huhtanen, C. N., A. R. Brazis, W. L. Arledge, E. W. Cook, C. B. Donnelly, R. E. Ginn, J. N. Murphy, H. E. Randolph, E. L. Sing, and D. I. Thompson. 1970. Effect of dilution bottle mixing methods on plate counts of raw-milk bacteria. J. Milk Food Technol. 33:269-273.
5. Lawton, W. C., and F. E. Nelson. 1955. Influence of sublethal treatment with heat or chlorine on the growth of psychrophilic bacteria. J. Dairy Sci. 38:380-386.
6. Mossel, D. A. A., and A. van der Moosdijk. 1963. The practical significance of the allegedly unusually low heat resistance among psychrotrophic organisms for the microbiological examination of cold-stored proteinaceous foods. J. Appl. Bacteriol. 26:X.
7. Stapert, E. M., W. T. Sokolski, and J. I. Northam. 1962. The factor of temperature in the better recovery of bacteria from water by filtration. Can. J. Microbiol. 8:809-810.
8. Vanderzant, C., and A. W. Matthys. 1965. Effect of temperature of the plating medium on the viable count of psychrophilic bacteria. J. Milk Food Technol. 28:383-388.

Items of Interest for Those Planning to Attend the 62nd Annual IAMFES Meeting

1. We have been in touch with both Canadian and U.S.A. Immigration Offices and obtained the following details. It was suggested that members and friends from U.S.A. carry with them:
   a) Birth Certificate or a Naturalized Citizenship Certificate.
   b) A brochure or program details about the 62nd Annual Meeting.
   c) Your local U.S.A. Customs Office should be contacted for latest information about the amount and type of duty free purchases that can be made in Canada.
2. For those flying to Toronto, we would recommend that you use the Airport Limousine bus from the Airport which will take you directly to the Royal York Hotel. At present the fare is $2.50 one way.
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Recovery of Streptococci from a Variety of Foods: A Comparison of Several Media[1]

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ABSTRACT

Nine liquid and solid media used to isolate and/or enumerate streptococci were evaluated using 30 food samples. Streptococci were recovered from all of the samples surveyed. Azide Blood agar gave the highest recovery of streptococci and also allowed the observation of hemolytic reactions. Streptosel, KF streptococcus, and m-Enterococcus agars were also used, with the latter giving the lowest recovery among the agars. Azide Dextrose, KF, and Streptosel broths gave similar recoveries from most samples. Confirmation of Azide Dextrose isolates in Ethyl Violet Azide broth lowered streptococcal estimates by several log cycles. When isolates from both agar and broth cultures were subjected to further testing, *Lactobacillus* and *Staphylococcus* were often encountered, thus indicating the need to confirm representative numbers of colonies even if they are from selective media.

Food microbiologists have been involved in the isolation and enumeration of streptococci from foods for many years. The primary reason for this activity has been due to the status of enterococci and the Lancefield Group D streptococci as fecal indicators. Group D streptococci include the enterococci (*Streptococcus faecalis* var. *zymhogenes, S. faecalis* var. *liquefaciens, Streptococcus durans* and *Streptococcus faecium*), and *Streptococcus bovis* and *Streptococcus equinus.* The organisms are often termed "fecal streptococci" although this terminology has been questioned by some investigators (*5*). Due to the resistant nature of many of these organisms, particularly the enterococci, they are enumerated to estimate the effectiveness of sanitation procedures and as an index of general microbiological quality. Their role in food-poisoning is somewhat questionable (*2*).

In addition, streptococci other than fecal streptococci are of major importance to man, e.g., the beta-hemolytic streptococci (Lancefield Group A) associated with scarlet fever and septic sore throat. The role of foods in transmission of such organisms is of interest in that only a limited amount of work has been done in this area.

In a comprehensive review on the effects of various media ingredients on recovery and growth of streptococci from foods, Hartman et al. (*6*) concluded that further work in this area should be directed toward a comparison of media and methods using the same samples or subsamples. Because of the divergence of observations by workers involved with isolation and enumeration of streptococci, isolation and quantification of streptococci in natural materials of many types was recommended.

Lee and Koburger (*7*) examined 109 samples of foods and found that 34% of meat and fish samples contained beta-hemolytic streptococci. Vegetables, dairy products, and miscellaneous food items were negative for beta-hemolytic streptococci. Ground meats and sausages that were neither smoked nor cooked were always positive for beta-hemolytic streptococci. Pavlova et al. (*8*) reported on the selectivity of five media containing different classes of inhibitory agents to isolate fecal streptococci from feces, sewage, and foods. The lowest incidence of fecal streptococci was observed with m-Enterococcus and Azideorbitol agar, while KF and Pfizer Selective Enterococcus agar gave the highest recoveries. Most foods examined were precooked frozen seafoods and yielded low numbers (approximately 10^2/g) of fecal streptococci. The need for further investigation of naturally occurring samples in lieu of laboratory cultures was emphasized because of the possible effects of environmental stress on natural microbial populations. Such stresses undoubtedly influence the isolation and enumeration of all microorganisms.

The present work was undertaken to: (*a*) evaluate liquid and solid media that are commercially available and in wide usage by both regulatory and quality control laboratories for recovery and enumeration of streptococci and (*b*) determine the distribution of hemolytic streptococci in a variety of foods.

MATERIALS AND METHODS

Food samples

Thirty food samples were obtained from stores in Gainesville, Florida, or from departmental experimental inventories. Samples included meat products, both raw and further processed, vegetables, and dairy products.

Sample preparation

Fifty-gram samples of the foods were placed in sterile Blendor jars and diluted with 450 g of 0.1% peptone diluent and blended at low speed. Serial dilutions were prepared by transferring 11-ml aliquots into 99 ml 0.1% peptone water dilution blanks. Plates were poured in duplicate and incubated at 35 C for specified periods. When liquid media were used, a 3-tube Most Probable Number (MPN) procedure was utilized. For Azide Dextrose broth and Ethyl Violet Azide (EVA), APHA procedures were adhered to (*1*).

[1]*Florida Agricultural Experiment Stations Journal Series* No. 5567.

Media used

The following commercial media were used: (*a*) solid media: Azide Blood agar base (BBL), m-Enterococcus agar (BBL); KF Streptococcus agar (Difco); Tryptic Soy agar (Difco); Streptosel agar (BBL); Plate Count agar (Difco); (*b*) liquid media: KF Streptococcus broth (Difco); Streptosel broth (BBL); Azide Dextrose broth (BBL); Ethyl Violet Azide broth (BBL).

Hemolytic reactions

Transfers were made of representative colonies from the various media onto Tryptic Soy agar (Difco) containing 5% sheep blood (heparin treated) for observation of hemolytic reactions. The Azide Blood agar base contained 5% sheep blood and the hemolysis observations were made directly on these plates without transfer.

Identification of isolates

Representative numbers of colonies ($\sqrt{n}$, where n = number of colonies on plates with 30-300 colonies) were selected from the various media used and subjected to further identification procedures. Isolates were screened using the gram stain and catalase test and then subjected to further testing which consisted of precipitin reactions (Groups A-O; Difco) and Sherman's criteria for enterococci (growth at 10° and/or 45 C; at pH 9.6, and in 0.1% methylene blue). Not every colony was subcultured for further identification since this was considered to be beyond the scope of the present work. However, numerous colonial types were isolated and characterized as streptococci. No attempts were made to identify species.

RESULTS AND DISCUSSION

Streptococci were recovered from all samples surveyed in this study. Data from both solid and liquid media are in Table 1. Recoveries varied considerably among the

TABLE 1. *Recovery of Streptococci by Various Solid and Liquid Media*

Sample	Count per gram (solid media[a])				
	PCA[b]	AZB	ST	KF	ME
Ground beef	5.8×10^6	2.1×10^5	7.5×10^4	2.3×10^4	1.1×10^4
Ground beef w/veg protein	2.6×10^7	7.4×10^5	3.3×10^5	5.4×10^3	1.5×10^3
Fresh sausage, mild	—[e]	—	—	2.4×10^2	9.0×10^1
Fresh sausage, mild[c]	6.9×10^7	—	—	7.3×10^6	1.2×10^2
Fresh sausage, hot	6.9×10^5	—	—	4.1×10^3	1.6×10^3
Fresh sausage, hot[c]	3.3×10^7	—	—	4.5×10^4	3.9×10^3
Frozen sausage	4.2×10^4	—	—	1.9×10^2	5.0×10^1
Frozen sausage[d]	2.6×10^4	—	—	2.7×10^2	6.5×10^1
Frozen imported lean beef	2.1×10^4	9.4×10^2	—	—	—
Bologna	5.3×10^7	—	5.1×10^7	7.3×10^6	4.3×10^2
Bologna[c]	—	9.3×10^8	4.5×10^8	6.1×10^8	8.8×10^7
Head cheese	1.3×10^7	4.4×10^6	3.4×10^6	1.8×10^6	2.5×10^4
Chorizo sausage	7.6×10^5	—	—	5.0×10^0	0
Chorizo sausage[c]	1.8×10^7	—	—	5.9×10^2	0
Liver cheese	4.0×10^4	2.5×10^4	1.3×10^4	6.1×10^3	1.0×10^2
Cooked, smoked sausage	1.0×10^4	4.0×10^1	1.5×10^1	1.5×10^1	0
Cooked, smoked sausage[c]	4.5×10^3	3.0×10^1	1.0×10^1	1.5×10^1	5.0×10^0
Cooked salami	4.9×10^8	2.5×10^5	2.7×10^4	4.0×10^3	5.0×10^0
Fresh mullet fillets	—	5.0×10^2	2.0×10^1	9.0×10^1	0
Frozen Indian shrimp	7.1×10^4	1.9×10^4	5.6×10^3	1.5×10^3	5.4×10^2
Frozen fish sticks	5.3×10^3	1.3×10^3	1.6×10^2	2.5×10^1	0
Chicken pot pie	2.0×10^3	2.1×10^2	1.7×10^2	4.0×10^1	6.0×10^1
Chicken a la king	5.8×10^3	5.9×10^2	8.5×10^1	6.5×10^1	5.5×10^1
Beef pot pie	8.0×10^3	2.9×10^3	1.6×10^3	8.2×10^2	1.1×10^1
Fresh green beans	5.1×10^7	1.1×10^6	4.0×10^5	2.1×10^5	2.3×10^3
Green beans[c]	1.7×10^7	1.6×10^6	1.3×10^5	9.3×10^4	3.1×10^4
Fresh broccoli	1.5×10^8	5.1×10^4	7.2×10^4	1.4×10^3	1.0×10^1
Fresh lettuce	1.9×10^6	8.0×10^1	0	0	0
Plain yogurt	2.0×10^2	0	0	0	0
Yogurt/peaches	0	0	0	0	0

Sample	Count per gram (liquid media[a])			
	AD	ST	KF	AD - EVA
Ground beef	1.1×10^5	4.6×10^4	4.6×10^4	2.4×10^4
Ground beef w/veg protein	1.1×10^5	4.6×10^4	1.1×10^5	2.3×10^2
Fresh sausage, mild	9.3×10^1	—[e]	—	—
Fresh sausage, mild[c]	$>1.1 \times 10^5$	—	—	$>1.1 \times 10^5$
Fresh sausage, hot	2.3×10^3	—	—	7.5×10^1
Fresh sausage, hot[c]	$>1.1 \times 10^5$	$>1.1 \times 10^5$	—	9.3×10^1
Frozen sausage	4.3×10^2	—	—	4.3×10^2
Frozen sausage[d]	4.3×10^2	—	—	4.3×10^2
Frozen imported lean beef	—	—	—	—
Bologna	$>1.1 \times 10^5$	$>1.1 \times 10^5$	$>1.1 \times 10^5$	$>1.1 \times 10^5$
Bologna[c]	$>1.1 \times 10^6$	$>1.1 \times 10^6$	$>1.1 \times 10^6$	—
Head cheese	$>1.1 \times 10^6$	$>1.1 \times 10^6$	4.6×10^5	2.8×10^3
Chorizo sausage	4.3×10^2	—	—	3.6×10^0
Chorizo sausage[c]	$>1.1 \times 10^5$	$>1.1 \times 10^5$	—	9.3×10^1
Liver cheese	2.4×10^4	4.3×10^3	2.3×10^3	0
Cooked, smoked sausage	4.3×10^1	2.3×10^1	3.6×10^0	3.0×10^0
Cooked, smoked sausage[c]	2.3×10^1	2.3×10^1	3.6×10^0	0
Cooked salami	1.1×10^5	7.5×10^3	0	1.5×10^1
Fresh mullet fillets	2.4×10^2	2.4×10^2	0	7.3×10^0

TABLE 1. (*continued*)

Frozen indian shrimp	4.3×10^2	2.3×10^2	2.3×10^2	2.3×10^2
Frozen fish sticks	2.3×10^3	2.3×10^3	$>0.5 \times 10^1$	0
Chicken pot pie	4.3×10^2	2.3×10^2	1.1×10^1	1.5×10^2
Chicken a la king	4.3×10^2	2.3×10^2	2.3×10^1	7.5×10^1
Beef pot pie	2.3×10^3	1.5×10^3	9.3×10^2	2.1×10^2
Fresh green beans	$>1.1 \times 10^5$	$>1.1 \times 10^5$	4.6×10^4	3.9×10^2
Green beans[c]	4.6×10^5	9.3×10^4	1.5×10^5	9.3×10^1
Fresh broccoli	9.3×10^2	9.3×10^3	4.3×10^1	1.5×10^1
Fresh lettuce	0	0	0	0
Plain yogurt	2.3×10^1	2.3×10^1	0	0
Yogurt/peaches	2.3×10^1	2.3×10^1	0	3.0×10^0

[a]Code for media: *Solid media* – PCA = Plate Count Agar; AZB – Azide Blood Agar Base + 5% Sheep Blood; ST = Streptosel Agar; KF = KF Streptococcus Agar; ME = m-Enterococcus Agar; *Liquid media* – AD = Azide Dextrose Broth; ST = Streptosel Broth; KF = KF Streptococcus Broth; AD – EVA = Azide Dextrose Broth confirmed in Ethyl Violet Azide Broth.
[b]Total Aerobic Plate Count (35 C)
[c]After 1 week of refrigerated storage (4 C)
[d]After 1 h of thawing at room temperature (24 C)
[e]Sample not submitted for analysis

media used. With the solid media, data reported are counts of colonies that were representative of streptococci on the particular medium involved. For example, with KF agar, numerous white colonies were often present but only the brick red to pink colonies were counted (as per manufacturer's recommendations). Representative numbers of these typical colonies were subjected to the identification procedures described above. Occasionally streptococci were recovered from a given sample by one medium but not another, e.g., cooked salami or fresh mullet fillets.

Azide Blood agar gave the highest recovery of streptococci and had the added advantage of allowing observation of hemolytic reactions without further subculturing. In a few instances, hemolytic staphylococci were found but were distinguishable from hemolytic streptococci by colonial morphology and production of catalase. Interference from gram-positive rods was never a problem.

Of the remaining agars tested, Streptosel agar was second in recovery to Azide Blood agar, followed by KF Streptococcus agar with m-Enterococcus agar giving the lowest recovery of the four media. Azide Blood agar and Streptosel agar are intended for isolation and/or enumeration of streptococci in general; whereas, the last two are primarily intended for the fecal streptococci or the enterococcus group. In cases where enterococci comprised essentially all of the streptococcal population, selection of the appropriate medium would make a significant difference in results obtained. There were many instances when isolates from KF agar or ME agar failed to yield typical enterococcus responses to Sherman's criteria and were also not classified as Group D streptococci with the precipitin tests. Care should be exercised in interpreting counts obtained from selective media if confirmatory tests are not used. In this regard, recent publications by Facklam et al. (*3, 4, 5*) have dealt with this problem in primarily clinical microbiological analyses. Their work has shown that if one is interested in differentiating the enterococci from other group D streptococci, a 6.5% salt tolerance test will suffice; i.e., group D enterococci will tolerate the salt broth whereas group D non-enterococci will not.

When liquid media were used in 3-tube MPN analyses for streptococci, there were frequent differences in recovery or estimated numbers of streptococci when either Azide Dextrose broth, Streptosel broth, or KF broth was used. When EVA broth was used as a confirmatory broth for Azide Dextrose broth, as recommended by the American Public Health Association (*1*), estimates of enterococci were often several log cycles lower than the Azide Dextrose broth estimate.

As was true with the solid media, isolates from MPN tubes that were subjected to further characterization demonstrated the presence of organisms other than streptococci. Numerous *Lactobacillus* and a few *Staphylococcus* strains were found capable of survival in the broths. Precipitin tests also indicated that streptococci, other than Group D, were present in the samples taken from Azide Dextrose, KF, and ST broth. Precipitin tests on isolates from EVA, however, were nearly always positive for Group D, indicating that EVA does a fairly effective job of screening out the other groups of streptococci.

Some difficulty was encountered in evaluating the effectiveness of the capillary-tube preparations for the precipitin tests. More clear-cut precipitin formation was achieved when a slide agglutination method was used. Groups A, C, and D gave the most distinct reactions.

A wide distribution of hemolytic streptococci was noted. Both alpha and beta hemolytic reactions were noted and approximate percentages of each were determined by counting hemolytic colonies and reporting this count as a percentage of the total streptococcal count (Table 2).

Isolation of streptococci on Azide Blood agar plates indicates that use of this medium for recovery of streptococci from food samples would be preferred, particularly when it is desirable to observe hemolytic responses. AZB counts were higher than those for Streptosel agar and it was relatively easy to differentiate

TABLE 2. *Percentages of Hemolytic Streptococci* (a and β) *in Various Foods*

Sample	Streptococcal count[a]	a (%)	β (%)
Ground beef	2.1×10^5	16	9
Ground beef w/veg protein	7.4×10^5	87	0
Frozen imported lean beef	9.4×10^2	4	23
Bologna[b]	9.3×10^8	37	0
Head cheese	4.4×10^6	65	31
Liver cheese	2.4×10^4	45	1
Cooked, smoked sausage	4.0×10^1	0	0
Cooked, smoked sausage[b]	3.0×10^1	33	0
Cooked salami	2.5×10^5	0	0
Fresh mullet fillets	5.0×10^2	0	0
Frozen Indian shrimp	1.9×10^4	30	1
Frozen fish sticks	1.3×10^3	49	0
Chicken pot pie	2.1×10^2	76	0
Chicken a la king	5.9×10^2	5	0
Beef pot pie	2.9×10^3	18	0
Fresh green beans	1.1×10^6	20	24
Green beans[b]	1.6×10^6	11	2
Fresh broccoli	5.1×10^4	82	9
Fresh lettuce	8.0×10^1	0	0

[a]Total Streptococcal Count based on data from Azide Blood agar plate count.
[b]After 1 week of refrigerated storage (4 C).

the non-streptococci on the surface of AZB. KF agar counts were close to those of Streptosel agar and were not comprised of just fecal streptococci indicating that care should be taken when interpreting such counts. Of the liquid media used, Azide Dextrose broth showed maximum recovery but Streptosel and KF broths often provided similar recoveries.

When Streptosel agar and KF agar recoveries were compared to their broth counterparts, the data indicated that agar estimates were 21 and 285% greater than broth (MPN) estimates.

A statistical evaluation of the results obtained from solid media is given in Table 3. From these data it is evident that recoveries on all solid media were significantly different ($P = 0.01$) from one another and that there was a highly significant interaction between solid media used and samples tested. Comparisons of geometric means of counts obtained from the samples by the solid media tested showed that a 100-fold difference between recoveries from KF and ME agars, with KF giving the higher counts. For AZB and ST agars, the former showed an 18-fold greater recovery of streptococci.

TABLE 3. *Analysis of variance for solid media used in streptococcal analyses*[a]

Source	Difference	Mean square
Media (M)	3	21.9216
Samples (S)	17	34.8384
M × S	51	.7314
Error	72	.0593

[a]Computed for those samples where all solid media were used.

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REFERENCES

1. American Public Health Association. 1966. Recommended methods for the microbiological examination of foods. 2nd ed. APHA, New York, N.Y.
2. Deibel, R. H., and J. H. Silliker. 1963. Food-poisoning potential of the enterococci. J. Bacteriol. 85:827-832.
3. Facklam, R. R. 1973. Comparison of several laboratory media for presumptive identification of enterococci and group D streptococci. Appl. Microbiol. 26:138-145.
4. Facklam, R. R., and M. D. Moody. 1970. Presumptive identification of group D streptococci: The bile-esculin test. Appl. Microbiol. 20:245-250.
5. Facklam, R. R., J. F. Padula, L. G. Thacker, E. C. Wortham, and B. J. Sconyers. 1974. Presumptive identification of group A, B and D streptococci. Appl. Microbiol. 27:107-113.
6. Hartman, P. A., G. W. Reinbold, and D. S. Saraswat. 1966. Media and methods for isolation and enumeration of the enterococci. Adv. Appl. Microbiol. 8:253-289.
7. Lee, I., and J. A. Koburger. 1970. Incidence and identification of some beta-hemolytic streptococci in foods. J. Milk Food Technol. 33:323-325.
8. Pavlova, M. T., F. T. Brezenski, and W. Litsky. 1972. Evaluation of various media for isolation, enumeration and identification of fecal streptococci from natural sources. Health Lab. Sci. 9:289-298.

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Status of DDT in Milk From Cows Fed Apple Pomace

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ABSTRACT

Two groups of five dairy cows each were fed an identical ration, with the exception of a 10% substitution of dried apple pomace (containing 0.06 ppm DDT) for corn. Milk samples were collected periodically for 15 weeks, and composite samples for each group were analyzed for milkfat and DDT. There was no significant difference in DDT content of milkfat between the control group and the experimental group, nor was there a significant correlation between milkfat percentage and DDT.

Restrictions in recent years on the use of DDT [1, 1, 1-trichloro-2,[2-bis-(p-chlorophenyl)-ethane] in apple orchards have reduced its concentration in apple pomace to negligible quantities.

Previously, extrapolation of DDT data derived from feeding trials showed that a detection threshold of 0.01 ppm in milk resulted from a theoretical feed concentration of 0.8 ppm or its experimental equivalent, 0.5 ppm (*5*). Another study used lower concentrations and showed that small but nevertheless definite amounts of DDT were transferred to milk (*4*), and still another study, that secretion of the residue in milk began about 56 days after low-level DDT contamination of feed (*2*).

The present study was initiated to determine the effect on milk of current levels of DDT in commercial, dried apple pomace (hereafter called pomace) whose DDT content has been found to be 0.12 ± 0.08 ppm (6), when fed at a rate of 10% of the dairy concentrate.

METHODS

Feeding and sampling

Ten lactating cows were divided into two groups of five each, and were fed identical diets for 15 weeks, with the exception of a 10% substitution of pomace (7% moisture) for corn. This rate amounted to a consumption of 2 lb. of pomace per cow per day. Just before the start of the test, milk was collected from each cow. Composite samples were prepared for each group, and were analyzed for DDT and milkfat. Thereafter, on the evening of every 6th day, milk was collected and combined with an equal volume from the morning of every 7th day in a 7-day cycle. From these combined samples, a weekly composite sample for each group was made and analyzed for DDT and milkfat. For the 15th week, each individual sample from the pomace-fed cows was assayed in addition to the composite sample. DDT and milkfat in milk from two commercial, unrelated sources were also assayed, as were selected ingredients in the ration.

Analysis

The AOAC methods of analysis (*1*) were used. Milkfat content was determined by the Babcock method, and DDT was assayed by gas chromatography on a Tracor model MT 220 gas chromatograph, equipped with a ^{63}Ni detector and a 6 ft × 1/8 inch O.D., glass column, packed with 3% OV-1 on Chromosorb W. The oven temperature was held isothermally at 200 C.

Soluble carbohydrates were extracted from pomace in hot water and measured by anthrone, using glucose standards. Protein was determined by the Kjeldahl procedure.

RESULTS AND DISCUSSION

The DDT content found in the weekly composite samples, and a summary of the statistical treatment [Student's method for paired variates (*3*)] are presented in Table 1. There was no significant difference between

TABLE 1. *DDT content (fat basis) in weekly composite samples of milk*

Week no.	Control ration (X^1) (ppm)	Pomace ration (X^2) (ppm)
1	0.070	0.070
2	0.052	0.050
3	0.025	0.076
4	0.068	0.068
5	0.063	0.070
6	0.070	0.068
7	0.098	0.058
8	0.057	0.069
9	0.053	0.063
10	0.059	0.060
11	0.064	0.055
12	0.067	0.058
13	0.075	0.040
14	0.054	0.058
15	0.035	0.054

$\bar{D} = (\bar{X}^2 - \bar{X}^1) = 0.007$

$s_D = \sqrt{\frac{\Sigma(D_i - \bar{D})^2}{n-1}} = 0.0212$

$s_{\bar{D}} = \frac{s_D}{\sqrt{n}} = 0.00560$

$t = \frac{\bar{D}}{s_{\bar{D}}} = 1.228$

For $\upsilon = 14$, $t_{0.05} = 2.145 > 1.228$

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values in milk from the pomace-fed cows and control cows. Also, the residue in milk from pomace-fed cows (0.061 ppm) was less than that which was found in commercial milk (Table 2).

TABLE 2. *DDT content (fat basis) of commercial milk and selected experimental feed ingredients*

Items	Concentration (ppm)
Commercial milk No. 1	0.15
Commercial milk No. 2	0.12
Apple pomace	0.064
Hay silage	0.013
Corn	0.011

The proximate analysis of pomace gave the following results: moisture, 7%; soluble carbohydrates, 34%; crude fiber, 25%; ether extract, 8%; and crude protein, 6%. It would appear, therefore, to be a good, partial substitute for corn and roughage. At a 10% level, its contribution to the total DDT intake was 0.0064 ppm of concentrate. Corn, at 50% of the concentrate, contributed 0.0055 ppm, and at a 6:4 ratio of roughage to concentrate, hay silage contributed 0.020 ppm (Table 2). Thus, 10% pomace would appear to have no greater influence than other ingredients on the DDT content of milkfat.

The coefficient of linear correlation between milkfat and DDT content (Table 3) for the five separate milk

TABLE 3. *Milkfat and DDT in milk from cows fed apple pomace for 15 weeks*

Breed	Milkfat (%)	DDT (ppm)
Holstein	2.7	0.10
Holstein	3.0	0.14
Brown Swiss	4.5	0.08
Jersey	4.3	0.08
Jersey	4.6	0.06

samples from pomace-fed cows, at the end of the 15th week, was calculated to be –0.800, a non-significant value ($\nu = 3$, $p \leq .05$). However, as the trend of data in Table 3 indicates, the lower percentage of milkfat in Holstein milk will result in higher relative concentrations of DDT, since the arithmetic derivation on a fat basis will yield a larger unit value (ppm) for lower percentages of milkfat.

CONCLUSION

On the basis of this study, resuming the feeding of apple pomace to dairy cows at a level of 10% of the concentrate should be a safe practice, barring extraneous contamination with DDT and other residues. The past unacceptability of apple pomace as a feed ingredient should therefore be reconsidered.

Prudent resumption in feeding apple pomace would have important implications for the dairy and apple processing industries, and from the standpoint of waste disposal, for the environment.

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REFERENCES

1. AOAC. 1970. Official methods of analysis. 11th ed. P.O. Box 540, Benjamin Franklin Station, Wash., D.C. 20044.
2. Gyrisco, G. G., L. B. Norton, G. W. Trimberger, R. F. Holland, P. J. McEnerney, and A. A. Muka. 1959. Effects of feeding low levels of insecticide residues on hay to dairy cattle on flavor and residue in milk. J. Agr. Food Chem. 7:707-711.
3. Snedecor, G. W., and W. G. Cochran. 1967. Statistical methods, 6th ed. The Iowa State University Press, Ames, Iowa. 99 ff.
4. Williams, S., P. A. Mills, and R. E. McDowell. 1964. Residues in milk of cows fed rations containing low concentrations of five chlorinated hydrocarbon pesticides. J. Ass. Off. Anal. Chem. 47:1124-1128.
5. Zweig, G., L. M. Smith, and S. A. Peoples. 1961. DDT detectable (in milk). California Agr., March:13.
6. Walter, R. H., J. B. Bourke, R. M. Sherman, D. L. Downing, Elmer George, Jr., A. B. Karasz, and Roger Pollman. 1975. J. Milk Food Technol. 38: (In press).

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Prevention of Churning During Shipment of Dairy Herd Improvement Milk Samples [1]

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ABSTRACT

Polystyrofoam shipping containers used in some DHI central milk testing programs were inadequate to keep fifty 28.3-ml milk samples below 10 C for 1 h when transferred into air at 25 C. Initial milk temperature was 4.4 C. Forty samples in a modified shipping container, packed with 850 g of ice, were at 17 C in 24 h. Unless refrigerated shipment can be assured, samples in plastic bags, sealed to eliminate air, should be adopted to minimize fat losses from churning as measured by a Mark III Milko-Tester. Fat losses increased with increasing air space in containers, absence of dichromate preservative or phosphate additives, and agitation above 10 C. Churned fat losses were greater in mishandled Jersey milk (high fat) than in Holstein milk (low fat) samples.

Several states have experienced unexplained fat losses in Dairy Herd Improvement (DHI) samples during shipment to central test facilities (Ray Schooley, American Jersey Cattle Club, letter dated Aug. 6, 1974). The losses, particularly evident in Jersey milk samples, were apparently more related to shipping conditions than to instrument calibration. The Milko-Tester (MT), used in most central test facilities, correlates well with classical reference methods when properly calibrated and it offers greater precision (*8*). Shipe (*9*) observed the MT to bias results in favor of breeds giving low fat milk. However, the magnitude of the differences he noted was not as great as fat losses claimed by Jersey breeders during the emergence of the problem. Conversely, Al-Omar et al. (*2*) found the MT Mark II biased in favor of breeds giving high fat milk. Shepard (Frank D. Murrill, USDA, ARS, letter dated June 7, 1974) also claimed that the MT, if biased at all, favored breeds giving high fat milk. When instrument calibration has been properly maintained, bias problems over 0.1% have not been demonstrated. This suggested that other factors were of greater significance in contributing to the high fat losses reported on DHI samples.

Minzner and Kroger (*7*) studied "apparent" fat losses attributable to physiochemical and bacteriological activities. Preserved milk samples of high and low test retained their original MT fat test values for 7 days at 27 C. This exceeds the normal sample shipment time in DHI programs. Thus, the losses examined in this paper were considered more related to physical churning and will be discussed as churned fat losses. Fat that has been churned or oiled-off is generally unavailable for turbidity measurement by the MT even when prepared for test using standard methods (*1, 5, 6*).

Breeds giving high fat milk are affected most severely with fat losses in central laboratory testing (*4*). British workers related the losses in central laboratory testing to churning or oiling-off during agitation and shipment at ambient temperatures. They restored the original fat test value through the use of a special homogenizer before introduction to the MT (*4*). Since homogenization is impractical with small samples and because homogenization affects MT readings (*9*), other remedies are required. Kroger et al. (*6*) reduced churning by cooling samples below 10 C and incorporating sodium metaphosphate. The Pennsylvania State DHI program currently uses plastic bags in which the air space has been eliminated (H. C. Gilmore, Penn State Univ., letter dated March 5, 1974). This prevents churning even though no attempt is made to precool or refrigerate the samples. DHI programs in many states use rigid plastic containers holding 28.3 ml of sample and packed into polysyrofoam-insulated shipping containers. This study was initiated to determine the degree of churning associated with the use of these sample and shipping containers.

MATERIALS AND METHODS

Milk samples

Fresh, refrigerated raw milk was obtained from local Jersey and Holstein dairies and stored at 4.4 C for a maximum of one day before use. The milk sample was well mixed by pouring back and forth between two containers and dispensed into 28.3-ml ungraduated rigid plastic containers (Thunderbird Container Corp., El Paso, Texas 79912[5]). The containers were filled to various levels, with and without 0.5-grain $K_2Cr_2O_7$ preservative tablets (C5158N, NASCO, Fort Atkinson, Wisconsin 53538) and sodium metaphosphate and then adjusted to appropriate temperatures. NASCO Whirl-Pak plastic bags (B679N), 169.8 ml, containing preservative tablets were also filled with 84.9 ml of milk. The air space was eliminated and the bags tied to simulate the shipping system used by the Pennsylvania State DHI program. The 56.6-ml Whirl-Pak bags (B991) were also evaluated.

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Fat testing

All samples were tested on a Mark III MT after tempering to 40 C and mixing. Instrument calibration was monitored by doing the Babcock test on a series of samples with from 2 to 6.5% fat. In addition, high- and low-fat samples were regularly transported under refrigeration to other MT laboratories for comparison. Calibration adustments were in accordance with AOAC procedures (*1*).

Sample treatment

Fifty sample containers were loaded into styrofoam shipping continers obtained from the Wahington State DHI laboratory, Burlington, Wash., 98233. Temperature of the samples was monitored during storage of shippers in 4.4 C and 10 C walk-in refrigeration boxes and at 23-28 C ambient temperatures. Samples were removed at intervals from the center of the shipping container and evaluated with a thermometer. Samples were violently agitated on a Garver Babcock bottle shaker operating at 350 cycles/min for intervals up to 3 h. Reagent grade chemicals were used.

Temperature gradient study

A temperature gradient bar was calibrated to give 25 sample tubes a temperature range of from 8 to 23 C. Slightly over 3 ml of Jersey milk were placed in each capped tube and incubated for 5 h. Every 15 min each tube was removed, agitated at top speed on a Vortex mixer for 15 sec, and replaced in the gradient bar pocket. Duplicate fat analyses were done on the MT after tempering samples to 40 C.

RESULTS AND DISCUSSION

Effect of additives and temperature

Churned fat losses as measured on the Milko-Tester and as affected by temperature, fill of container, and sodium metaphosphate addition are included in Table 1.

TABLE 1. *Effect of temperature, fill of container, and phosphates upon Milko-Tester fat test following milk sample agitation for 2 h. Initial fat test of Jersey milk taken from a bulk tank was 3.5%. $K_2Cr_2O_7$ preservative tablet in all samples.*

Sample distance from top	Metaphosphate content	Fat Test	
		< 10 C	26-28 C
(cm)	(%)	(%)	(%)
0	0	3.5	3.5
0.3	0	3.4	3.3
0.6	0	3.4	3.3
1.3	0	3.4	3.0
0.3	2	3.5	3.4

Each value represents a mean of three samples tested in duplicate. Below 10 C slight losses were noted. Greater losses occurred at 26 to 28 C with increasing air space in the containers. Completely filled containers sustained no losses. Since these differences were low, subsequent agitation periods were extended to 3 h to assure a greater degree of churning and thus make differences more evident.

A sample of Jersey milk with 5.3% fat was agitated with different levels of phosphates and with or without a preservative tablet (Table 2). Following agitation at 10 C the test values were higher when preservative and phosphates were added. When the preservative tablet was left out, a 0.2% fat loss was indicated. Much higher fat losses were indicated when samples were churned at ambient temperatures. No improvement was found when more than 0.5% phosphates were incorporated. Phosphates retarded churning but did not adequately prevent it.

Different levels of phsophates were added to a sample of Jersey milk containing 6.93% fat (Table 2). Churned

TABLE 2. *Effect of temperature and phosphates upon Milko-Tester fat test following milk sample agitation for 3 h. Initial fat test of Jersey milk taken from a bulk tank was 5.3%. All samples down 0.6 cm from container top.*

$K_2Cr_2O_7$ preservative tablet	Metaphosphate content	Fat test	
		10 C	26-28 C
	(%)	(%)	(%)
+	2	5.7	4.8
+	1	5.6	4.9
+	0.5	5.7	4.8
+	0.1	5.5	4.2
+	0.05	5.5	4.0
+	0.0	5.5	3.3
−	0.0	5.1	3.1

fat losses were not reduced by adding phosphates if a preservative tablet was added and low temperatures were maintained during agitation. Churning was not a problem even when the containers were filled 1.27 cm below the cap. Conversely, when the preservatives were left out half of the fat was not measured by the instrument after agitation at 26 C. The earlier fat increase (Table 2) could not be explained. However, all

TABLE 3. *Effect of phosphates and temperature upon Milko-Tester fat test following milk sample agitation for 3 h. Initial fat test for Jersey milk taken from a bulk tank was 6.93%. All samples down 0.6 cm from container top.*

$K_2Cr_2O_7$ preservative tablet	Metaphosphate content	Fat test	
		4 C	26 C
	(%)	(%)	(%)
+	3	6.83	5.58
+	2	6.90	5.47
+	1	6.99	4.34
+	0.5	7.02	5.15
+	0	7.00	4.07
−	0	6.89	3.48

samples had measurable fat contents at least equal to their original values if the temperature was below 10 C during agitation, even when large air spaces were present.

Sample temperature changes within the shipper

When four complete shipping containers each with fifty 28.3-ml milk samples at 40 C, were stacked, strapped, and placed in a 10 C refrigerator, samples were only down to 30 C in the middle shipping containers after 5 h. Samples in one complete shipping container placed in the 10 C environment dropped only to 24 C during the same interval. When only the 50 samples and the shipping container inner rack were transferred either to a water bath at 10 C or to the refrigerator at 10 C, where air circulation was allowed, samples dropped to below 16 C in 1 h and reached 10 C in approximately 2.5 to 3 h. Fifty milk samples at 40 C in the assembled shipping container reached 16 C in 5 h after transfer to 4.4 C. Samples in the shipping container inner rack dropped to 4.4 C in 3.5 h. The time required to drop the temperature would retard delivery of samples to the shipping point. Most rapid chilling occurred when the shipping container inner rack and samples were packed in ice. The temperature dropped from 40 C to below 8 C in 1 h and reached 4.4 C in less than 2 h.

The polystyrofoam shipping container must prevent significant temperature increase during agitation and shipment to avoid churning of fat in samples. After cooling of the samples to 4.4 C, shipping containers and samples were held at 26 C. Samples initially at 4.4 C were above 10 C in 50 min, above 19 C in 3.5 h, and were at ambient temperature (26 C) in less than 18 h. Thus, if 10 C is the critical temperature to prevent churning (*6*) shipping containers with samples must be agitated for no more than 1 h after transfer to ambient conditions. This is unreasonable with the present 24 to 48 h shipping time intervals.

Shipping samples in iced packages should provide a superior alternative to present practices. When ten sample containers were filled with water (300 g), frozen, and placed longitudinally at 4.4 C in shipping container inner rack pockets the entire contents were at ambient temperatures within 20 h. When 625 g crushed ice were put into 169.8-ml NASCO Whirl-Pak bags and the bags placed at each end of the inner rack, which had been cut down to allow only 40 samples per shipper, samples were at 13 C in 20 h and 16 C in 24 h. Water was frozen in 169.8-ml NASCO plastic bags and five bags (850 g ice) were placed in the modified shipping container with 40 samples at 4.4 C and the shipping container was held at 23 C. In 19 h the temperature was 14 C, with some ice still present. The temperature was 17 C in 24 h. Shipping containers could be loaded with more ice to extend the time-temperature relationship; however, shipping costs would be increased thereby and number of samples per shipping container would be reduced. If such containers were held over long weekends in postal facilities the ice would disappear and defeat the purpose of icing. Shipping containers might be redesigned to sustain such delays. This would, however, increase the ratios of tare to sample weight.

Critical temperature to prevent churning

A temperature gradient bar allowed definition of the range though which churning occurs. The results (Table 4) indicated that most samples experienced 0.15 to 0.30% losses up to 18 C, with three showing higher losses between 10 C and 18 C. Over 18 C the losses went above 1%, reaching 2% at 22.7 C. This confirmed the observations of others that temperatures must be maintained below 10 C (*6*).

TABLE 4. *Effect of temperature and agitation upon Milko-Tester fat test. Three-milliliter samples were distributed in a 8-23 C temperature gradient bar and agitated for 15 sec on a Vortex mixer every 15 min for 5 h before test. Initial fat test of Jersey milk was 5.59%.*

Average temperature	Fat test	Apparent loss	Average temperature	Fat test	Apparent loss
8.1C	5.38%	0.21%	16.3C	5.35%	0.24%
8.7	5.40	0.19	17.2	5.39	0.20
9.6	5.44	0.15	17.7	5.29	0.30
—	—	—	18.4	4.64	0.95
10.8	5.03	0.56	18.9	4.57	1.02
11.2	5.40	0.19	19.5	3.72	1.07
12.0	4.98	0.61	20.1	3.84	1.72
13.0	5.32	0.27	21.1	4.37	1.22
13.7	4.60	0.99	21.3	3.99	1.60
14.4	5.36	0.23	21.9	3.69	1.90
15.3	5.38	0.21	22.1	3.80	1.79
15.8	5.29	0.30	22.7	3.60	1.99

Air-free plastic bags vs. rigid containers

Jersey milk, testing 3.5% fat, was distributed at 84.9 ml per 169.8-ml NASCO Whirl-Pak bag and tied to eliminate air. Sixteen such bags were positioned with the tied end up in a cardboard partitioned container. They were agitated at 24 C for 3 h at maximum Babcock shaker speed. Each bag was tempered to 40 C, opened to include air, reclosed, mixed by manual shaking, and milk was tested. Three of the bags were ruptured during shaking and the contents were lost. (The smaller 56.6-ml bag used in some DHI programs would probably not be as vulnerable to rupture.) The mean test on twelve of the remaining samples was 3.32%, for a churned fat loss of 0.22%. Aliquots of the 3.5% fat milk sample were dispensed in the rigid 28.3-ml containers, filled to within 0.3 cm of the top, and subjected to identical agitation conditions. The mean test on 15 of the 16 containers was 1.33% for a fat loss of 2.21%.

Several 56.6-ml Whirl-Pak bags were filled with 7.42% fat milk and agitated for 3 h at 24 C and at the half speed setting on the shaker to minimize bag rupture. The fat test means were 7.58, 7.60, and 7.54% after 1, 2, and 3 h of agitation.

These observations reaffirm the value of the Pennsylvania State DHI program involving use of unrefrigerated samples in air-free plastic bags and shipped at ambient temperatures.

Breed-dependent churning

Samples of Jersey and Holstein milks, averaging 6.95% and 3.81% fat on triplicate tests, were distributed into the rigid sample containers. Preservative tablets were added and the samples were agitated on the Babcock shaker for 3 h at 23 C. The mean test for 12 containers was 2.81% for the Jersey and 1.95% for the Holstein milk samples. The fat test recovery ratios were only $2.81/6.95 = 0.40$ and $1.95/3.81 = 0.51$.

Rigid sample containers were filled, 12 with a 6.72% fat Jersey milk and 12 with a 3.65% fat Holstein milk sample. The samples were agiated on the Babcock shaker, removed, warmed, and tested at 20-min intervals. The data (Table 5), indicated that the initial proportion of churned fat lost was comparable with both breeds until beyond 100 min of agitation. The Jersey milk samples ultimately lost a higher proportion of fat than did the Holstein samples. The actual fat test reductions were of greater magnitude with the Jersey samples that had initially tested highest. Losses in 20 min were greater for both breeds than occurred when air-excluded plastic pouch samples had been agitated for 3 h.

The risk of obtaining inaccurate tests due to shipping unrefrigerated samples in the styrofoam shipping containers with inflexible sample containers is great enough to suggest that this system should not be used even for milk from cows of the low fat breeds. The system

TABLE 5. *Effect of breed upon Milko-Tester fat test during agitation of milk samples for 3 h at 23 C. Rigid 28.3-ml containers were filled 0.3 cm from top.*

Total agitation time (min)	Breed			
	Jersey		Holstein	
	Test (% fat)	Ratio[a]	Test (% fat)	Ratio[a]
0	6.72	1.00	3.65	1.00
20	5.91	0.88	3.14	0.86
40	5.51	0.82	2.99	0.82
60	4.97	0.74	2.74	0.75
100	4.03	0.60	2.48	0.68
140	3.56	0.53	2.74	0.75
160	3.02	0.45	2.26	0.62
180	2.49	0.37	1.72	0.47

[a]Fat test percentage following agitation divided by fat test percentage before agitation.

should definitely not be used during the warmer months of the year or in warm climates. Unless temperature control can be assured by adequate icing or refrigerated shipment, plastic bags without air provide the best available solution to the problem. Attempts to fill the 28.3-ml containers without leaving any air space resulted in spillage and difficulty in mixing and preparation for testing.

If low temperatures are required for sample integrity with samples for payment (*3, 5*) it is logical that similar holding conditions should be used for maintaining DHI sample quality. However, if the preservative tablet is effective and no air is trapped in the container, it appears possible to obtain tests free from negative churning bias even under normal ambient temperatures (*7*). Occasional high test results after agitation of air-excluded containers prompts the need for additional investigation.

ADDED IN PROOF

Additional confirmation of these observations was obtained from tests conducted on a 60-cow Jersey herd. Split samples were distributed into glass jars for control Babcock testing, into 28.3-ml rigid plastic containers in styrofoam shipping containers and into 56.6-ml plastic bags in cardboard shipping containers. Samples were cooled during sampling and shipped at winter ambient conditions. The Babcock samples were tested without shipment. One quarter of the samples were tested without shipment. One quarter of the samples were tested immediately upon receipt at the central laboratory. The balance were agitated 5 h before test. The Babcock control test mean was 4.5%. The means of the unagitated samples in bags and rigid containers were 4.6 and 4.0% respectively. The means of the agitated samples were 4.3 and 1.5% respectively.

ACKNOWLEDGMENTS

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REFERENCES

1. A.O.A.C. 1970. Methods of analysis. 16.058-16.063. Assoc. of Offic. Anal. Chem., Wash. D.C.
2. Al-Omar, M. E., R. H. Gough, and J. H. Gholson. 1974. Effect of milk sample condition on replicate milkfat tests analyzed by a Milko-Tester Mark II. J. Milk Food Technol. 37:372-376.
3. Barnum, H. J. 1972. Bulk Milk sampling: a combined dairy industry and regulatory function. J. Milk Food Technol. 35:410-413.
4. Harding, F., and J. L. Morris. 1973. Reconstitution of churned or "oiled" milk samples for instrumental determination of fat content. J. Soc. Dairy Technol. 26:91-93.
5. Hausler, W. J., Jr. Editor. 1972. Standard methods for the examination of dairy products. 13th ed. Amer. Public Health Assoc., Washington, D.C.
6. Kroger, M., D. T. Landis, R. J. Dewar, and H. C. Gilmore. 1968. Churning in raw milk samples and its effect on the fat test. J. Dairy Sci. 51:1776-1780.
7. Minzner, R. A., Jr., and M. Kroger. 1974. Physicochemical and bacteriological aspects of preserved milk samples and their effect on fat percentage as determined with the Milko-Tester. J. Milk Food Technol. 37:123-128.
8. Shannon, C. W., J. T. Cardwell, and L. C. True. 1973. Correlation studies of milkfat content as measured by the Milko-Tester, Babcock, and Mojonnier methods. J. Milk Food Technol. 36:92-93.
9. Shipe, W. F. 1972. Current status of the Milko-Tester. J. Dairy Sci. 55:652-655.

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Rapid Enumeration of Psychrotrophic Bacteria in Raw Milk by the Microscopic Colony Count[1]

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ABSTRACT

Psychrotrophic bacteria were enumerated in raw milk by the microscopic colony count and counts were compared with those obtained by standard methods. Incubation conditions were 7 C for 10 days for the standard procedure and 7 C for 48 and 72 h or 21 C for 13.5 and 16.5 h for the microscopic colony count. Though each of the microscopic colony counts differed from the standard psychrotroph count ($P < 0.01$), there was a good general relationship in each instance (correlation coefficients ≥ 0.94). Wide variations in ratios of microscopic colony counts to standard psychrotroph counts made interpretations difficult. Generally, microscopic colony counts after 48 and 72 h at 7 C were less than standard psychrotroph counts. Incubation at 21 C yielded microscopic colony counts that were similar to standard psychrotroph counts for most samples; however, some microscopic colony counts at this temperature were substantially higher than the standard psychrotroph counts.

The current edition of *Standard Methods for the Examination of Dairy Products* (*2*) recommends enumeration of psychrotrophic bacteria by the plate method; plates are incubated at 7 C for 10 days. The long incubation time is a decided disadvantage and several investigators have proposed other methods (*11, 13*). One of the most reliable rapid methods (*10*) still requires incubation for 4 days.

In 1915, Frost (*5*) developed a procedure to enumerate viable bacteria in milk in about 6 h. The method involved spreading 0.1 ml of a milk-agar mixture over 4 cm² on a glass slide, incubating the slide in a moist chamber, drying, staining, and counting the micro-colonies with a microscope (*6*). This method was included in the third (*1*) to fifth editions of *Standard Methods for the Bacteriological Examination of Milk,* but not as a standard procedure. Results obtained with the method for enumerating bacterial populations in milk have been published (*3, 4, 8, 9*). Thomas (*13*) makes reference to a limited investigation by Druce who used the microscopic colony count for enumerating psychrotrophs; incubation was for 72 h at 7 C.

This investigation was conducted to determine the value of the microscopic colony count as a rapid method for enumeration of psychrotrophic bacteria.

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MATERIALS AND METHODS

Milk samples

Grade A raw milk was obtained from individual producers and from commingled supplies. Samples were stored in ice and examined within 24 h after collection.

Psychrotroph counts

The standard psychrotroph count was done according to *Standard Methods for the Examination of Dairy Products* (*2*).

The microscopic colony count was made with equipment similar to that used by Frost (*7*) and manufactured by Central Scientific Co., Chicago. Equipment consisted of a warm table, a guide for marking 2 × 2 cm areas, and a moist incubation chamber. The method was conducted as follows: (*a*) The warm table was adjusted to 45 C and a tube of melted standard methods agar and a tube containing 2 ml of the milk sample were placed in openings of the warm table. (*b*) Glass microscope slides (25 × 75 mm) were marked with two 4 cm² areas, sterilized by flaming, and laid upon the warm table to cool to 45 C. (*c*) Two milliliters of the melted agar were added to the 2 ml of warmed milk and, after mixing on a vortex mixer, 0.1 ml of the mixture was transferred by pipette to each of the 4 cm² areas on a slide and spread evenly over the area with an appropriately-shaped wire. The slide was removed from the warm table to solidify the agar. (*d*) After solidification of the agar, slides were placed in the moist chamber which was held in an incubator at the selected temperature. (*e*) After incubation, slides were dried on the warm table heated to 95 C. (*f*) Dried slides were stained with thionin blue (*7*) and micro-colonies were counted with the low power objective (16 mm) of a wide-field microscope (147 fields/4 cm²). Sixteen fields were counted on each duplicate preparation.

Statistical analyses

Bacterial counts per milliliter of milk were expressed as $\log_{10}$. Where no colonies were observed in any microscopic field in two 4-cm² areas, the count was recorded as < 180/ml (1/16 of the microscopic factor for one 4-cm² area). Where no colonies were observed in one 4-cm² area only, the count was recorded as less than the count recorded on the duplicate area. An arbitrary value equal to one-half the "less than" value was used for calculation and plotting.

RESULTS AND DISCUSSION

Geometric means for microscopic colony counts conducted at 7 C for 48 and 72 h or at 21 C for 13.5 and 16.5 h and standard psychrotroph counts are given in Table 1. Analyses of variance showed that all microscopic colony counts differed from the standard psychrotroph count ($P > 0.01$). Some difference in counts would be expected because variations are encountered when several portions of the same sample are plated by standard methods.

Initial investigations with the 7 C microscopic colony count showed that the ratio between a count at 24 h and

TABLE 1. *Geometric means for microscopic colony and standard psychrotroph counts*

Count	No. of samples	Mean count/ml
Microscopic colony, 7 C for 48 h	79	4.33×10^3
Microscopic colony, 7 C for 72 h	79	5.81×10^3
Standard psychrotroph	79	1.37×10^4
Microscopic colony, 21 C for 13.5 h	34	9.49×10^3
Microscopic colony, 21 C for 16.5 h	34	1.08×10^4
Standard psychrotroph	34	7.05×10^3

a count at 48 h varied considerably. There was a trend, with exceptions, for the ratio to be greatest with low-count samples and to become less as the count increased. Because of wide variations in counts at 24 h, this incubation time was not investigated extensively.

Microscopic colony counts done at 7 C for 48 and 72 h also showed variations. Increases in this period of 2- to 3-fold were not uncommon, particularly with low count samples (< 10,000/ml) and some increases of greater magnitude were recorded. With a few high-count samples, a slight decrease in count was recorded as incubation time was increased from 48 to 72 h. Increase in colony size may have masked some colonies. Data indicated that little, if any, increase in count resulted with incubation times longer than 72 h at 7 C.

Initial investigations with incubation at 21 C indicated that the time required to give a count equivalent to the standard psychrotroph count varied from sample to sample. With most samples, incubation for < 12 h underestimated the psychrotroph count, and incubation beyond about 20 h generally gave a count in excess of the standard psychrotroph count. The two incubation times used (13.5 and 16.5 h) were selected because they spanned the period of best agreement among most samples.

The relationship between the standard psychrotroph count and the microscopic colony count done at 7 C for 48 h is illustrated in Fig. 1. The relationship between the standard psychrotroph count and the microscopic colony count conducted at 21 C for 13.5 h is illustrated in Fig. 2.

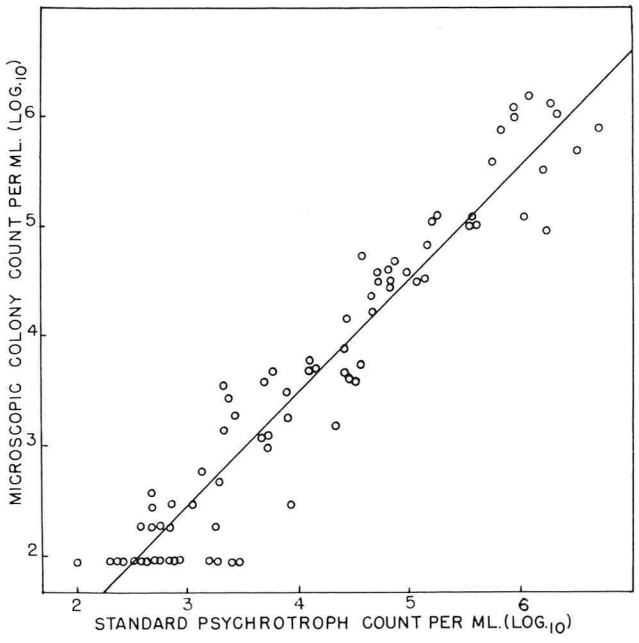

Figure 1. *Relationship between the microscopic colony count (incubation at 7 C for 48 h) and standard psychrotroph count. Regression line is shown.*

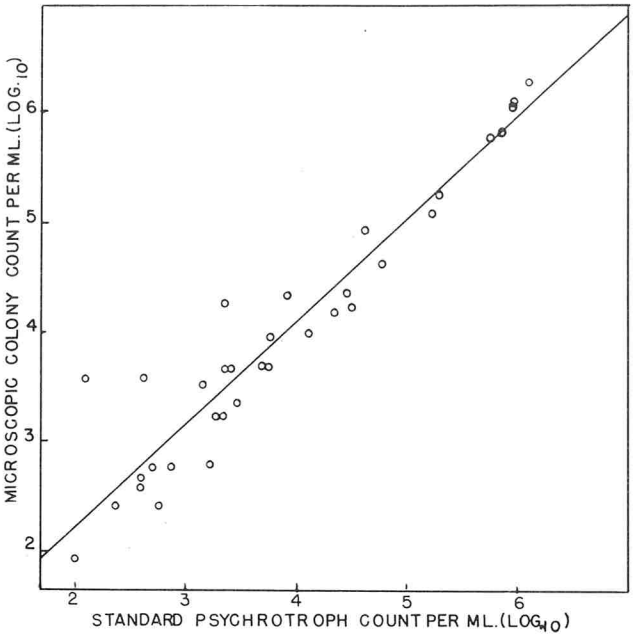

Figure 2. *Relationship between the microscopic colony count (incubation at 21 C for 13.5 h) and standard psychrotroph count. Regression line is shown.*

Relationships for microscopic colony counts conducted at these temperatures for longer periods (7 C for 72 h and 21 C for 16.5 h) were similar to those shown for the shorter times in Fig. 1 and 2, except that the microscopic colony counts were usually slightly higher (note means in Table 1). Regression equations and correlation coefficients for the relationships between microscopic colony counts and standard psychrotroph counts are given in Table 2.

TABLE 2. *Regression equations and correlation coefficients for the relationships between microscopic colony counts (y) and standard psychrotroph counts (x). (Data expressed as $\log_{10}$)*

Microscopic colony count (Incubation temperature and time)	Regression equation	Correlation coefficient
7 C for 48 h	$y = -0.640 + 1.0339\,x$ $x = 0.923 + 0.8835\,y$	0.96
7 C for 72 h	$y = -0.471 + 1.0240\,x$ $x = 0.704 + 0.9119\,y$	0.97
21 C for 13.5 h	$y = 0.404 + 0.9285\,x$ $x = -0.003 + 0.9683\,y$	0.95
21 C for 16.5 h	$y = 0.560 + 0.9023\,x$ $x = -0.071 + 0.9720\,y$	0.94

Though the data in Table 2 indicate that good general relationships existed between the microscopic colony counts and the standard psychrotroph counts, an examination of Fig. 1 and 2 shows that caution would be

TABLE 3. *Percentage distribution of samples with observed microscopic colony count (OMC): standard psychrotroph (SPsyC) ratios (expressed as a percentage with arithmetic counts) in aribitrary ratio categories. Also, distributions for regression corrected microscopic colony counts (RCMC): SPsyC ratios and mean interval factor corrected microscopic colony count (FCMC) : SPsyC ratios.*

Microscopic colony count. Incubation temperature and time		Percent of samples in each ratio category													
		0-20	21-40	41-60	61-80	81-100	101-120	121-140	141-160	161-180	181-200	201-300	301-500	500-1000	>1000
7 C, 48 h	OMC : SPsyC	25	33	15	14	3	4	4	3	0	0	0	0	0	0
	RCMC : SPsyC	5	10	9	6	15	10	5	11	6	4	10	6	1	0
	FCMC : SPsyC	8	9	10	11	10	7	12	9	3	8	6	6	1	0
7 C, 72 h	OMC : SPsyC	19	25	19	16	8	4	3	3	3	0	1	0	0	0
	RCMC : SPsyC	3	9	13	14	9	9	9	10	6	5	9	4	1	0
	FCMC : SPsyC	3	10	15	14	8	10	8	10	5	6	6	3	3	0
21 C, 13.5 h	OMC : SPsyC	0	6	12	3	21	21	9	3	6	3	9	0	6	3
	RCMC : SPsyC	0	6	15	35	9	12	3	6	0	6	0	0	6	3
	FCMC : SPsyC	0	6	18	32	12	9	3	6	0	6	0	0	6	3
21 C, 16.5 h	OMC : SPsyC	0	6	0	9	15	21	3	15	3	3	18	0	6	3
	RCMC : SPsyC	3	3	12	32	15	6	6	9	6	0	0	0	6	3
	FCMC:SPsyC	3	3	15	29	15	6	9	6	6	0	0	0	6	3

required in the quantitative interpretation of a given microscopic colony count. The interpretative problem arises from the wide variation in the ratio between the microscopic colony count and the standard psychrotroph count. The distribution of these ratios for each microscopic colony count method is given in Table 3. With microscopic colony counts done at 7 C, the ratios varied from < 20 to > 200, with a ratio of < 60 for 60% or more of the samples. Some of the microscopic colony counts conducted at 7 C exceeded the standard psychrotroph count; in general, this occurred only with high count samples. When microscopic colony counts were incubated at 21 C, the ratio varied from < 20 to > 1000. Samples that gave very high microscopic colony counts at 21 C relative to the standard psychrotroph count (ratios > 200) represented the main limitation to the use of this temperature.

To equate the microscopic colony counts with the standard psychrotroph count, appropriate regression equations were applied to the microscopic colony count. However, this procedure merely maintained, or even accentuated, the variation in ratios as illustrated by the distributions of the regression-corrected ratios of microscopic colony counts to standard psychrotroph counts given in Table 3.

Since a regression equation would be cumbersome for routine correction of microscopic colony counts, a set of correction factors was derived for microscopic colony counts in given ranges (0 to 10^3/ml, 10^3 to 10^4/ml, etc.). The mean count in each range was used to calculate the factor. The mean interval correction factors are given in Table 4. As expected, values obtained with these factors agreed closely with those obtained with the actual regression equations, as indicated by the ratio distributions given in Table 3.

TABLE 4. *Mean interval correction factors calculated from the appropriate regression equation, for equating microscopic colony counts within given intervals to the standard psychrotroph count*

Microscopic colony count interval	Count used for factor calculation	Factors for microscopic colony counts with the following incubation conditions			
		7 C 48 h	7 C 72 h	21 C 13.5 h	21 C 16.5 h
$0 - 1 \times 10^3$	5.0×10^2	4.06	2.92	0.81	0.71
$1 \times 10^3 - 1 \times 10^4$	5.5×10^3	3.07	2.36	0.75	0.66
$1 \times 10^4 - 1 \times 10^5$	5.5×10^4	2.35	1.93	0.70	0.63
$1 \times 10^5 - 1 \times 10^6$	5.5×10^5	1.79	1.58	0.65	0.59
$1 \times 10^6 - 1 \times 10^7$	5.5×10^6	1.37	1.29	0.60	0.55

Some modifications of the microscopic colony count procedure were investigated with the aim of increasing the counts obtained at 7 C. In the mixing of milk with standard methods agar, the medium is diluted one-half. However, doubling the nutrient content of the medium did not increase the counts, nor did additional agitation of the sample. Use of a medium with a lower solidifying temperature has been claimed to give greater recovery of psychrotrophs with the plate method (*12*). However, a medium prepared with the nutrients of standard methods agar plus 2 g agar and 15 g carrageenan per liter did not give increased counts when used at a pouring temperature of 35 C. The carrageenan stained deeply and made counting of micro-colonies difficult. Some pure cultures of psychrotrophic bacteria held in milk at 45 C showed loss of viability with continued holding. For example, loss of viability of five psychrotrophic bacteria, representing several genera, was as follows: after 5 min at 45 C losses were 0, 0, 18, 12, and 75%; after 10 min at 45 C losses amounted to 0, 30, 20, 22, and 80%. Because of a possible loss of viability, the sample should be mixed with agar as soon as it is adjusted to 45 C, and the mixture placed on a slide. Eugonagar, a medium richer in nutrients than standard methods agar, did not give substantially higher counts than did standard methods agar.

Though precise reasons for low microscopic colony counts, in comparison to standard psychrotroph counts, were not established, correction factors in Table 4 suggest that growth rate may have been a primary factor since the microscopic colony count to standard psychrotroph count ratio became narrower as the counts increased. This fact was especially evident with the counts incubated at 7 C for 48 h. It would be expected that in a high-count sample, more of the bacteria would be actively growing at the time of plating than in a low-count sample.

Some suggestions for prospective users of the microscopic colony count method are: (*a*) The temperature of the sample, or sample-agar mixture should not exceed 45 C and the holding time at this temperature should be as short as possible. (*b*) Drying of the film on the slide must be kept to a minimum during preparation and incubation. (*c*) Prepared slides must be cooled to the incubation temperatures within a short period (< 1 h). (*d*) Unless psychrotroph counts are very high ($> 2 \times 10^5$), dilution of sample is not necessary. If dilution is necessary, dilutions should be made in sterile milk to maintain uniform medium composition.

The microscopic colony count is a useful procedure for enumerating viable bacteria. To enumerate psychrotrophic bacteria, incubation at 7 C for 48 h is suggested. The accuracy of the method on very low-count samples can be improved by scanning the entire area of the smear. Such a procedure is not difficult with a wide-angle, 16 mm, objective. An incubation temperature of 7 C was considered to provide a more relaible count of psychrotrophic bacteria than 21 C because it did not give grossly elevated counts.

REFERENCES

1. American Public Health Association. 1921. Standard Methods for the bacteriological examination of milk. 3rd ed. Amer. Public Health Assoc., Boston.
2. American Public Health Association. 1972. Standard methods for the examination of dairy products. 13th ed. Amer. Public Health Assoc., Washington, D.C.
3. Barkworth, H. 1936. Van Oijen modification of the Frost little plate method. J. Dairy Res. 7:244-257.
4. Bryan, C. S., M. V. Scheid, M. D. Neuhauser, B. L. Gilbert, and G. J. Turney. 1942. A comparative study of the Frost little plate and standard plate methods for the bacteriological examination of milk, cream and ice cream. J. Dairy Sci. 25:827-835.
5. Frost, W. D. 1915. Rapid method of counting bacteria in milk. Science 42:255-256.
6. Frost, W. D. 1916. Comparison of a rapid method of counting bacteria in milk with the standard plate method. J. Bacteriol. 19:273-287.
7. Frost, W. D. 1921. Improved technic for the micro or little plate method of counting bacteria in milk. J. Infect. Dis. 28:176-184.
8. Hatfield, H. M., and W. H. Park. 1922. A study of the practical value of the Frost little plate method in the routine colony count of milk samples. Amer. J. Public Health 12:478-487.
9. Humphriss, E. L. E. 1950. A modified Frost little plate method for routine bacteriological control of milk. Part 2. Dairy Indus. 15: 420-422.
10. Juffs, H. S. 1970. A four-day count for psychrotrophs. Aust. J. Dairy Technol. 25:30-32.
11. Luck, H. 1972. Bacteriological quality tests for bulk-cooled milk. Dairy Sci. Abstr. 34:101-122.
12. Parker, E. T., J. B. Bernsteinas, and J. H. Green. 1968. Increased recovery of psychrotrophic bacteria by the use of a new medium with lower solidifying temperature. Appl. Microbiol. 16:1794.
13. Thomas, S. B. 1969. Methods of assessing the psychrotrophic bacterial content of milk. J. Appl. Bacteriol. 32:269-296.

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Effect of Thawing on Growth of *Staphylococcus Aureus* in Frozen Convenience Food Items[1]

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ABSTRACT

The appearance of convenience food items in the supermarkets and their consumption by wide segments of population has increased in recent years, and hence a study was done on presence of *Staphylococcus aureus* in these items and possible growth of the organism during defrosting of the foods. Using different selective agar media (Baird-Parker, Vogel-Johnson, Tellurite-polymyxin-egg yolk, and Mannitol-salt), various commercially available food items were examined. All suspected cultures were confirmed by coagulase test and Gram stain. Their enterotoxigenicity was also examined. Thawing the products at room temperature for 12 h generally resulted in a two-log increase in *S. aureus* population. Presence of *S. aureus* was observed in 18.3% of beef and poultry products; 12.5% of seafood products; and 8.3% of ready-to-eat frozen desserts. Most isolates produced types A or B enterotoxins. Of four selective agar media used, Baird-Parker was most efficient in isolating coagulase-positive staphylococci from frozen foods.

Consumption of frozen convenience food items has been increasing during the past several years. Such an increase has resulted largely from the trend for the consumer to eat out, lack of sufficient time to prepare food, and the role of women in the working force. At the same time, the consuming public has become more aware of the quality of food products and has raised many questions as to their safety and quality.

Microbiological quality of frozen food items has been studied by various investigators and some have reported the presence of *S. aureus* in certain of these food items (*1-4, 6, 7*). However, the effect of possible defrosting of the product during transportation or at supermarkets or by the consumer on growth of *S. aureus* has not been fully investigated. Hence this study was done to determine the presence of as well as changes in the population of *S. aureus* in frozen convenience food items being thawed at room temperature.

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MATERIALS AND METHODS

A total of 480 commercially produced frozen food items used in this study were purchased from local supermarkets. Four different brands of beef, poultry, fish, and frozen ready-to-eat dessert products were examined. Whenever possible six packages of the same brand having the same code number were selected.

Each frozen package was aseptically opened for sampling (0 h), then sealed, placed at room temperature (26 ± 2 C) and examined at 3-h intervals for 12 h. Both MPN and direct plating on selective agar media (Baird-Parker, Vogel-Johnson, Tellurite-polymyxin-egg yolk, and Mannitol-salt) were used for detection of *S. aureus* according to the procedure outlined by Thatcher and Clark (*10*). All suspected isolates were confirmed by coagulase test and Gram stain. Enterotoxigenicity of isolated cultures was determined using capillary tube assay (*5*).

RESULTS AND DISCUSSION

Preliminary results revealed that most frozen convenience foods which were contaminated with *S. aureus* had counts of over 100/g. Enumeration and isolations were therefore done using direct plating and four selective agar media. Since only Baird-Parker and Vogel-Johnson media resulted in highest recovery, results obtained from these two selective media are reported.

Table 1 presents the level of contamination and the changes in population of *S. aureus* in various frozen beef

TABLE 1. *Effect of thawing (at room temperature) on growth of* S. Aureus *in frozen convenient beef and poultry products*

Number of samples examined	Product	Percent positive samples	Ave. counts per gram			
			Baird-Parker plates		Vogel-Johnson plates	
			Thawing time (h)			
			0	12	0	12
24	Beef dinner	12.5	4.3×10^2	6.2×10^4	3.5×10^2	2.8×10^4
24	Turkey entree	8.3	3.0×10^2	5.1×10^4	1.3×10^2	6.8×10^3
24	Chicken A La King	45.8	6.2×10^4	3.9×10^6	8.3×10^3	4.2×10^4
24	Beef pie	12.5	4.3×10^4	8.6×10^5	3.1×10^3	6.2×10^4
24	Sliced turkey	12.5	6.2×10^2	7.2×10^4	5.2×10^2	4.3×10^3
24	Turkey pie	25.0	3.9×10^3	7.8×10^5	2.0×10^2	8.6×10^4
24	Veal parmagian	16.6	6.2×10^3	9.1×10^5	1.3×10^3	4.0×10^4
24	Sliced beef & gravy	12.5	5.1×10^3	1.2×10^5	3.5×10^3	3.0×10^4
24	Salisbury steaks	4.1	8.3×10^2	6.0×10^4	1.5×10^2	4.9×10^3
24	Chicken pie	33.3	9.3×10^4	3.3×10^6	5.8×10^3	5.3×10^4

and poultry products defrosted at room temperature. The first significant increase (two log) in population of *S. aureus* was noticed after most samples were thawed for 12 h. Products most frequently contaminated with *S aureus* were chicken a la king (45.8%), chicken pies (33.3%), and turkey pies (25.0%). Generally, higher percentages of poultry products were contaminated with *S. aureus* as compared to the beef products. This supports the recent findings of Surkiewicz et al. (*9*) who showed that frozen sliced or diced poultry and gravy products generally had more *S. aureus* contamination than did frozen sliced or diced meat and gravy.

The initial contamination level of most seafood products was higher than those noted with poultry and beef products (Table 2). With the exception of tuna pot pie samples, the initial *S. aureus* counts of the other four seafood products ranged from 3.1×10^4 to 7.0×10^4 cells per gram. Breaded fish sticks and french fried shrimp samples were shown to be the most contaminated products. Prolonged thawing at room temperature resulted in a two-log increase in counts of all samples. After this period, highest counts were obtained from breaded oysters (9.2×10^6/g) and breaded fish cakes (8.9×10^6/g).

Fresh bakery products such as cream pies, custard-filled puffs, and eclairs are well known for their association with staphylococcal food poisoning (*11*). Surkiewicz (*8*) surveyed the frozen cream pie industry and found that 3.5% of cream pie samples contained coagulase-positive staphylococci. Our results showed that 16.6% of the cheesecake samples and 8.3% of coconut custard and chocolate eclairs were contaminated with *S. aureus* (Table 3). When these products were defrosted at room temperature, a two-log increase in counts of cheesecake, chocolate eclairs, and custard pies became evident. Both lemon meringue and strawberry cream pies showed average initial contamination of less than 1000/g which gradually increased to 5.7×10^3 and 3.8×10^3 after 12 h of defrosting. During the same period, counts for cheesecake samples reached the high level of 9.2×10^6 per gram. However, when the same products were thawed at 5 C for the same time, no appreciable increase in population of *S. aureus* was observed (Table 4).

Pure culture studies of the coagulase-positive staphylococci revealed that all isolates produced either type A or type B enterotoxin.

It should be noted that although initial numbers of coagulase-positive staphylococci in most of the products

TABLE 2. *Effect of thawing (at room temperature) on growth of* S. aureus *on frozen convenient seafood products*

Number of samples examined	Product	Percent positive samples	Ave. counts per gram			
			Baird-Parker plates		Vogel-Johnson plates	
			Thawing time (h)			
			0	12	0	12
24	Breaded fish sticks	20.0	3.4×10^4	8.2×10^6	6.2×10^3	3.0×10^4
24	French fried shrimp	16.6	7.0×10^4	1.6×10^6	3.0×10^3	2.1×10^4
24	Breaded fish cakes	8.3	3.8×10^4	8.9×10^6	2.3×10^2	5.0×10^4
24	Breaded oysters	12.5	3.1×10^4	9.2×10^6	9.1×10^2	3.6×10^4
24	Tuna pot pie	4.1	6.3×10^2	7.7×10^4	2.8×10^2	4.4×10^3

TABLE 3. *Effect of thawing (at room temperature) on growth of* S. aureus *on frozen ready-to-eat frozen desserts*

Number of samples examined	Product	Percent positive samples	Ave. counts per gram			
			Baird-Parker plates		Vogel-Johnson plates	
			Thawing time (h)			
			0	12	0	12
24	Cheesecakes	16.6	3.8×10^4	9.2×10^6	1.5×10^3	3.1×10^4
24	Coconut custard pie	8.3	4.3×10^3	4.2×10^5	3.0×10^3	8.1×10^4
24	Lemon meringue pie	9.1	8.6×10^2	5.7×10^3	9.1×10^2	4.3×10^3
24	Strawberry cream pie	4.1	5.9×10^2	3.8×10^3	3.8×10^2	2.1×10^3
24	Chocolate eclairs	8.3	9.1×10^3	6.2×10^5	6.8×10^2	5.2×10^4

TABLE 4. *Effect of thawing (at 5 C) on growth of* S. aureus *on frozen ready-to-eat desserts*

Number of samples examined	Product	Percent positive samples	Ave. counts per gram			
			Baird-Parker plates		Vogel-Johnson plates	
			Thawing time (h)			
			0	12	0	12
24	Cheesecakes	20.0	1.7×10^4	3.2×10^4	3.8×10^2	2.4×10^2
24	Coconut custard pie	4.1	2.8×10^3	9.2×10^3	1.8×10^3	8.9×10^3
24	Lemon meringue pie	4.1	4.9×10^2	4.6×10^2	1.2×10^2	3.5×10^2
24	Strawberry cream pie	4.1	3.9×10^2	3.1×10^2	2.1×10^2	4.9×10^2
24	Chocolate eclairs	12.5	3.2×10^3	8.2×10^3	9.2×10^2	5.2×10^2

examined were low, counts after 12 h of incubation at room temperature reached as high as 9.2×10^6 cells per gram (Tables 2 and 3). Such prolonged thawing which resulted in an increase in numbers of *S. aureus* could possibly lead to production of enterotoxins which would be impossible to eliminate by ordinary cooking practices. Although it is the responsibility of the food industry to, by good manufacturing practices, prevent contamination of food products with microorganisms that are a hazard to public health, the consumer should also share the responsibility in preventing microbiological food borne illnesses by carefully handling food, practicing hygiene, and closely following cooking and other directions provided by the manufacturer.

REFERENCES

1. Abrahamson, A. E., L. Buchbinder, J. Guekel, and M. Heller. 1959. A study of frozen precooked foods: their sanitary quality and microbiological standards for control. Assoc. Food Drug Off. U.S. 23:63-72.
2. Alexander, D. C., and F. Tittizer. 1971. Bacteriological studies on meat pies and frozen prepared dinners. Can. J. Comp. Med. 35:5-11.
3. Buchbinder, L., V. Loughlin, M. Walter, and G. Dangler. 1949. A survey of frozen precooked foods with special reference to Chicken A La King. J. Milk Food Technol. 12:209-214.
4. Canole-Parola, E., and Z. J. Ordal. 1957. A survey of the bacteriological quality of frozen poultry pies. Food Technol. 11:578-582.
5. Fung, D. Y. C., and J. Wagner. 1971. Capillary tube assay for staphylococcal enterotoxins A, B and C. Appl. Microbiol. 21:559-561.
6. Gunderson, M. F., H. W. McFadden, and T. S. Kyle. 1954. The bacteriology of commercial poultry processing. Burgess Publ. Co., Minneapolis, Minn.
7. Ross, A. D., and F. S. Thatcher. 1958. Bacterial content of marketed precooked frozen foods in relation to public health. Food Technol. 12:369-371.
8. Surkiewicz, B. R. 1966. Bacteriological survey of the frozen prepared food industry. 1. Frozen cream pies. Appl. Microbiol. 14: 21-26.
9. Surkiewicz, B. F., M. E. Harris, and R. W. Johnston. 1973. Bacteriological survey of frozen meat and gravy produced at establishments under federal inspection. Appl. Microbiol. 26:574-576.
10. Thatcher, F. S., and D. S. Clark. 1968. Microorganisms in food. University of Toronto Press, Toronto, Canada.
11. U.S. Dept. of Health, Education and Welfare. Public Health Service. Foodborne outbreaks annual summaries 1961-1968. National Communicable Disease Center. Atlanta, Ga.

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Status of DDT in Apple Pomace for Dairy Feeding

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ABSTRACT

The DDT content of apple pomace in New York State has been found to be well below FDA's action limit of 0.5 ppm in and on livestock feed ingredients. In view of the uncertainty of DDT transfer to milk at such low concentrations in feeds, recommendations on its use as an energy source in dairy rations can only be based on actual feeding studies. These have been preceded by residue analyses which have established the probable, perennial limits of DDT in pomace at 0.18 ± 0.08 ppm.

Feeding apple pomace to livestock was once a common practice throughout the United States, but this was discontinued upon the discovery of prohibitive concentrations of DDT [1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane] in it (*12*), which has precluded its legal use in dairy feeding.

Occurrence of DDT in bovine milk and body-fat, as a result of its presence in feeds, is well documented (*2, 6, 9, 16*). Following its decline in agricultural use during the 1960s, Wilson et al. (*17*) and Rumsey et al. (*12*) fed apple processing waste containing the residue in trial rations. They discovered that the fat of waste-fed cattle contained more DDT than did that of cattle not fed waste. However, a review of the literature will show that there is divided opinion on the critical concentration in feeds at which the residue ceases to accumulate in milk. This discrepancy is suspected to be partly due to the different sensitivities of the various analytical methods. Harrison et al. (*7*) concluded that 1 ppm or less of DDT in forage would not result in a detectable amount in milkfat. Zweig et al. (*18*) detected the residue in milk at a level of 1 ppm in the feed, but they were unable to do so when the amount was reduced to 0.5 ppm or less. Somewhat in conflict with these reports was the claim of Laben et al. (*9*) that 0.5 ppm of DDT in alfalfa hay will result in an accumulation in milkfat of greater than 0.5 ppm but less than 1 ppm.

A concentration of 0.3 ppm (fat basis) is generally considered to be the practical limit of detection of DDT in fatty foods like milk by the most sensitive method available (*15*). The Food and Drug Administration (FDA) recognized the difficulty of analyzing raw agricultural products for these small amounts of residue, and has therefore set an action level of 0.5 ppm of DDT (including DDE and TDE) on hay and feed-grain (*5*).

Four years have elapsed since the curtailment of unrestricted spraying of DDT in the United States, and interest in the current status of apple pomace is again being expressed. Therefore, this study was conducted to ascertain the present concentrations of DDT in apple pomace, before recommendations for possible use in the dairy regimen.

METHODS

Preparation of apple pomace

Apples from the 1971, 1972, and 1973 harvests, which were grown in the experimental orchards of this Station (Geneva, New York), and in a distant, privately-owned orchard (Sodus, New York), were picked directly from trees, labeled according to their source and stored at 32 F, pending conversion to pomace. Periodically, samples from each source were crushed by a hammermill and pressed. The extracted juice was held for DDT assay, and the solids were air-dried (≤7% moisture). Samples from each source were also hand-peeled to a depth of 1-2 mm. The peels (including peduncles, and remnants of calyx and stamens) and calyx tissue (including receptacle, seeds, and vascular bundles) were blended separately in a Waring Blendor with a small amount of solvent and were air-dried.

Solvent extraction

Quadruplicate samples of dried, whole apple pomace and pomace from the peels and calyx, 100 g per sample, representing one set, were extracted by first blending each sample with 1000 ml of water-acetone solvent (3:7), then transferring the mixture to a screw-capped, gallon jar. The volume of solvent in each jar was increased by adding 1500 ml of water-acetone. One sample in a set was fortified with 1.0 ml of an acetone solution of purified DDT which contained 10^{-4} g/ml. This was added to the mixture just before blending was terminated. Each set of gallon jars containing the blended mixture was mechanically tumbled end-over-end for 18 h, after which, the solvent in each jar was removed by decantation. The solids were rinsed with 600 ml of water-acetone. The combined volume of solvent for each sample was reduced by aspiration to about 300 ml, and this concentrate was transferred to a large (3 liter) separatory funnel, whereupon, 1500 ml of water was added. The aqueous phase was extracted twice with a total of 1200 ml of n-hexane. The hexane extract was dried with 75 g of anhydrous sodium sulfate, and stored at 20 F for 3 h, to precipitate as much of the suspended apple constituents as possible. The clarified solution was evaporated further to 75-100 ml in preparation for clean-up with a Florisil column. The eluate was evaporated to 40 ml which was then

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transferred to a 50-ml volumetric flask, and made up to 50 ml with hexane. Pomace was also extracted according to the AOAC method for products containing 5-15% sugar (*1*), but using 1000 ml of solvent per 100 g.

One thousand grams of apples were tumbled with 1500 ml of acetone for 18 h, to dissolve the surface concentration of DDT. The acetone was decanted, evaporated to 300 ml, and 1200 ml of water were added to it in a separatory funnel before extraction and treatment, as previously described. Rice hulls were blended and extracted in a manner similar to the AOAC procedure. Five hundred milliliters of apple juice were extracted with two 350-ml volumes of hexane and thereafter, treatment of the extract was as previously described. Soil samples (2¼inches × 1¾ inches) were collected from under the peripheral branches of the trees in the Geneva orchard from which the apples were picked. The A_{00} and A_0 horizons (14) (2½ inches × 1¼ inches) in each sample were separated from the mineral fraction (2½ inches × ½ inch) and were combined into one organic fraction. Similarly, the mineral fractions of all samples were combined. The two fractions (organic and mineral) were air-dried, and triplicate samples of 100 g of each were prepared for DDT analysis by the procedure outlined in the Shell manual of methods (*13*). One sample of the organic fraction and one of the mineral fraction were fortified with 5.0×10^{-4} g of DDT.

Analysis

The final concentrate (50 ml) from apple pomace, apple surfaces, rice hulls and soil was analyzed for DDT on a Tracor model MT 220 gas chromatograph, using a ^{63}Ni detector, and fitted with a 6 ft × 1/8 inch O.D., glass column, packed with 3% OV-1 on Chromosorb W. The oven temperature was held isothermally at 200 C. Measurements were made either by the standard curve or internal standard technique, depending on the extent of peak resolution shown on the gas chromatogram.

Independently, apple pomace from commercial sources throughout New York State was analyzed for DDT by the New York State Food Laboratory at Albany for the period 1971-1973. The basic, gas chromatographic method outlined in the Pesticide Analytical Manual (*15*) was used, with the major modifications of small samples (5 lb.) and a single eluant (50% ethylether-hexane). The gas chromatographic column was 4 ft × 1/8 inch, O.D., aluminum, packed with 10% DC-200 + 15% QF-1 on 80/100 mesh Gas Chrom Q. Oven temperature was 200 C.

RESULTS AND DISCUSSION

New York State is a major producer of apple pomace (*4*), and a survey of scattered DDT data for apples, which accumulated from the work of several researchers in this department throughout the period, 1959-1967, is summarized in tabular form (Table 1) for the purpose of

TABLE 1. *Range of concentrations of DDT in apples for the period, 1959-1967*

Items	Concentration (ppm)
Whole apples (surface)	0.3-11.8
Apple pomace (dried)	2.8-50
Apple juice	0 - 1.6

contrast with the current status. Considering the wide variations found, the lowest and highest recorded values for the items listed are reported. The perennially high levels in the pomace were the single cause for its preclusion from livestock feeding. Excessive amounts of pulp with an inclusion of surface DDT undoubtedly may have accounted for the presence of the residue in juice.

Since the Station orchards are known not to have been sprayed with DDT for at least 5 years, apple pomace made from the 1973 Station apples is an accurate standard by which to judge the current status of DDT in commercial pomace. This and the variation in concentration over the 3-year period are reported in Table 2. At least 12 samples from each source were

TABLE 2. *Concentrations of DDT[1] in apple pomace in New York State for the period, 1971-1973*

Source	1973 No. of samples	1973 ppm	1971-1973 No. of samples	1971-1973 ppm
Station orchard[2]	8	0.06	24	0.18 ± 0.08
Private orchard[3]	8	0.09	24	0.08 ± 0.03
Commercial sources	39	0.06	69[4]	0.12 ± 0.08

[1]Including TDE and DDE
[2]Geneva, New York
[3]Sodus, New York
[4]Containing rice hulls

analyzed in a given year. The modifications of the AOAC method for DDT in high-sugar samples were necessary to overcome difficulties which were encountered in the extraction. Acetone-water was a more exhaustive solvent for removing material from pomace. However, the DDT concentration found with this extractant was similar to that of the AOAC method, indicating the maximum possible extraction in the latter instance. Recovery was from 60-80% in pomace, to 100% in soil. Corrections were made accordingly. There was no evidence of the presence in pomace of TDE, or of more than the generic level of DDE (9% of p, p′-DDT), except for isolated instances in commercial pomace where the concentrations of these metabolites reached 30-100% of p,p′-DDT. Nevertheless, from Table 2, the indication is that, regardless of the source, the levels of DDT and metabolites in apple pomace are well below the FDA's action limit of 0.5 ppm. In view of the uncertainty of DDT transfer to milk from feeds which contain this residue at such low concentrations, an appraisal of the suitability of apple pomace for dairy feeding can only be made by feeding studies which also take into account other residues.

TABLE 3. *Miscellaneous DDT concentrations for the period, 1971-1973*

Items	No. of samples	ppm
Rice hulls (domestic)		
1970	8	0.5
1971	8	0.1
1972	8	0.3
Orchard soil (Geneva, N.Y.)		
Organic fraction	2	1538
Mineral fraction	2	44
Whole apples	4	0.07
Apple peels	4	0.04
Apple calyx	4	0.03
Apple juice	4	0.00

Table 3 lists the remaining data which were obtained in this study. The high concentrations of DDT in the Station orchard soil is in agreement with those found by Kuhr et al. (*8*) for apple orchards throughout New York State. Judging from the DDT content of rice hulls which are obtained from within the United States for use as a filter-aid in juice extraction, it would appear that this

ingredient should be continually monitored. However, its normal usage level (0.5-1.0% of apples) would seem to preclude it as a major contributor to the DDT found in apple pomace.

The DDT residue in apples appears to be evenly distributed across the epidermis. Since the experimental orchard has been a physically static environment for more than 20 years, and DDT sprays were voluntarily discontinued after the 1968 season, the DDT residue in Station apples does not appear to be a function of the orchard-air concentration, also because of the rapid accumulation of vegetation over the orchard-soil surface for the duration of the growing season. It may be a systemic function of the soil-plant relationship. It is necessary to state here that DDT is not known to translocate en masse to aerial plant organs from soil, but trace amounts of chlorinated hydrocarbons do migrate into vegetative parts, depending on soil concentration, soil type, etc. (*3, 10, 11*). If these current levels of DDT are indeed, systemic, an equilibrium value of $\leq$0.5 ppm in apple pomace will persist for decades.

REFERENCES

1. AOAC. 1970. Official methods of analysis. 11th ed. P.O. Box 540, Benjamin Franklin Station, Washington, D.C. p. 479.
2. Avens, A. W., H. E. Nadler, J. G. Matthysse, and D. J. Lisk. 1965. Persistence of DDT in milk and body fat-a case report. 69th Ann. Proc. U.S. Livestock Sanitary Ass., p. 287.
3. Beestman, G. S., D. R. Keeney, and G. Chesters. 1969. Dieldrin translocation and accumulation in corn. Agronomy J. 61:390-392.
4. Downing, D. L. 1971. Environmental problems in the food processing industry. Cornell Univ. Conf. on Agricultural Waste Management, p. 20.
5. Food and Drug Administration, U.S. Dept. of H.E.W., P.H.S. memorandum: Current action levels for pesticide residues, June 9, 1972. Administrative guideline no. 7420.09(F), transmittal no. 73-1 (1/1/73).
6. Gyrisco, G. G., L. B. Norton, G. W. Trimberger, R. F. Holland, P. J. McEnerney, and A. A. Muka. 1959. Effects of feeding low levels of insecticide residues on hay to dairy cattle on flavor and residues in milk. J. Agr. Food Chem. 7:707-711.
7. Harrison, D. L., J. N. Collett, and C. J. Christie. 1965. DDT residues on pasture and in the butterfat of cows grazing pasture treated with DDT prills and DDT/superphosphate. New Zealand J. Agr. Res. 8:223-231.
8. Kuhr, R. J., A. C. Davis, and J. B. Bourke. 1974. DDT residues in soil, water and fauna from New York apple orchards. Pesticides Monitoring J. 3/4:200-204.
9. Laben, R. C., T. E. Archer, D. G. Crosby, and S. A. Peoples. 1966. Milk contamination from low levels of DDT in dairy rations. J. Dairy Sci. 49:1488-1494.
10. Lichtenstein, E. P. 1959. Absorption of some chlorinated hydrocarbon insecticides from soils into various crops. J. Agr. Food Chem. 7:430-431.
11. Lichtenstein, E. P., and K. R. Schultz. 1960. Translocation of some chlorinated hydrocarbon insecticides into the aerial parts of pea plants. J. Agr. Food Chem. 8:452-456.
12. Rumsey, T. S., K. P. Bovard, S. M. Shepherd, and B. M. Priode. 1969. DDT residues in beef cows fed apple pomace. J. Anim. Sci. 28:418-424.
13. Shell Chemical Company. 1964. Manual of methods for the determination of residues of Shell pesticides.
14. U.S.D.A. Soil Survey Manual. Staff Agr. Handbook No. 18: p. 175.
15. U.S. Department of Health, Education and Welfare, Food and Drug Administration. Pesticide analytical manual. Vol. I. Methods which detect multiple residues. Sec. 2.31(A):3. Washington, D.C.
16. Williams, S., P. A. Mills, and R. E. McDowell. 1964. Residues in milk of cows fed rations containing low concentrations of five chlorinated hydrocarbon pesticides. J. Ass. Off. Anal. Chem. 47: 1124-1128.
17. Wilson, L. L., D. A. Kurtz, J. H. Ziegler, M. C. Rugh, J. L. Watkins, T. A. Long, M. L. Borger, and J. D. Sink. 1970. Accumulations of certain pesticides in adipose tissues and performance of Angus, Hereford and Holstein steers fed apple processing wastes. J. Anim. Sci. 31:112-117.
18. Zweig, G., L. M. Smith, S. A. Peoples, and R. Cox. 1961. DDT residues in milk from dairy cows fed low levels of DDT in their daily rations. J. Agr. Food Chem. 9:481-484.

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The Effect of the Antibiotic, Everninomicin, on Control of *Clostridium Botulinum* in Foods

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ABSTRACT

Everninomicin was evaluated for its effect on *Clostridium botulinum*. Enrichment broth tests with *C. botulinum* Type A demonstrated the effectiveness of 1 ppm Everninomicin in inhibiting exotoxin formation in sporulation broth. Tests using Everninomicin in selected foods demonstrated: Type A exotoxin formation was inhibited by 10 ppm Everninomicin D in inoculated corn; formation of Type E exotoxin was inhibited by Everninomicin D at 1 ppm in inoculated fish; Type A exotoxin formation was not inhibited in sausage by 1 ppm or 10 ppm Everninomicin D. However, when used in ground beef, Type A exotoxin formation was inhibited by 8.0 ppm of Everninomicin B.

Botulism is a relatively rare disease, which is frequently fatal. It is a neuroparalytic syndrome generally caused by ingestion of a food containing exotoxin produced by *C. botulinum*. Six types of *C. botulinum* are recognized on the basis of their production of antigenically distinct exotoxins. Four of these, A,B, E, and F, are the principle causes of the disease in man. Types C and D are usually associated with botulism in birds and mammals, respectively. Since the spores are widely distributed in nature, the opportunity for contamination of foods exists almost everywhere (*10, 20-38*).

The mortality rates of botulism are high, often reaching 60% (*16-19*). Several outbreaks have been attributed to commercially prepared foods (*16*). Examples are the 1960 outbreaks due to vacuum-packed smoked fish and frozen chicken pies (*11*) and the 1963 outbreaks caused by smoked white fish and canned tuna fish (*12*). The canned soup (*39*) and mushroom incidents (*25*) have focused attention on the need for methods of control.

C. botulinum is able to form heat-resistant spores. The vegetative cells are destroyed by 15 min of boiling and the exotoxins may be destroyed by heating to 80 to 85 C (*13*). However; it is important that sufficient time and temperature are used to permit penetration by this heat into the center of the mass, or exotoxin present may still remain in a contaminated food. Spores may require 5 h of boiling for destruction. This is approximately equivalent to a D_{12} thermal process obtainable at various higher temperatures and shorter times (*15*). Specific processes and products require individual studies to determine the D value to insure protection against *C. botulinum*. Preparation of some products precludes the degree of heat treatment necessary to kill spores of *C. botulinum*. Potential consumer hazard may stem from: (*a*) presence of preformed exotoxin, and (*b*) presence of viable spores in the product. Presence of spores in some foods may not constitute a hazard if the food is maintained frozen and prepared as recommended by the manufacturer (*8*). A major consideration in food poisoning arises from consumer misuse of a product after it has been prepared (*7*).

Everninomicin, an antibiotic from *Micromonospora carbonacea*, (*1, 14, 41-43*) is effective in inhibiting the activity of some species of harmful bacteria. Everninomicin D and Everninomicin B are major components of the complex. They were supplied by Dr. M. Weinstein of the Schering Company of Bloomfield, N.J. Work done on laboratory animals demonstrated no systemic toxicity and oral administration did not result in significant blood levels of the antibiotic showing lack of absorption (*1*). Everninomicin D and B have different, but closely related structures.

Modern technology of new convenience foods makes it necessary to evaluate microbiological safeguards against food spoilage and the risk of botulism (*39*). This is necessary because of the increased number of raw materials being processed under a wider variety of processing condition using new packaging materials and techniques.

The objectives of these experiments were (*a*) to determine the effects of Everninomicin against the spores of *C. botulinum* Type A, and (*b*) to determine whether Everninomicin would prevent *C. botulinum* Types A and E exotoxin formation in selected foods. This laboratory undertook to determine whether Everninomicin inhibits formation of exotoxins by *C. botulinum* (*6*).

MATERIALS AND METHODS

The work was conducted using an enrichment medium, and corn, fish, pork, and beef. Strains of *C. botulinum*, their maintenace, spore production and methods of producing exotoxin-free spore suspensions were obtained from U.S. Public Health Service Laboratories in Cincinnati, Ohio (*2*).

Enrichment broth

The first phase was conducted with enrichment broth to determine the effects of 1.0 and 10.0 ppm Everninomicin D against spores of *C. botulinum* Type A, strain "corn." A suspension containing viable and exotoxin-free spores of *C. botulinum* Type A was prepared. Concentrations of 1 and 10 ppm Everninomicin D were incorporated

into separate tubes of Duff's Enrichment Medium (*4, 5*) containing 1000 spores of *C. botulinum*. All tubes were incubated at 30 C under a 10% CO_2 – 90% N_2 atmosphere for 12 days. Six replicates of all suspensions were prepared and heated to and held at 85 C for 15 min immediately before incubation. Heated tubes were incubated with the unheated tubes and served as controls.

Food studies

Four types of foods were selected on the basis of their history in outbreaks of botulism: corn, fish, sausage, and beef. The corn, sausage, and beef samples were inoculated with spores of *C. botulinum* Type A. Fish samples were inoculated with *C. botulinum* Type E, strain "VH." Levels of 1 and 10 ppm Everninomicin D were used to determine if Everninomicin would prevent exotoxin formation in corn, fish, and sausage samples. Beef samples were tested with 8 ppm of the sodium salt of Everninomicin B. Food samples were prepared and inoculated in the following manner.

Corn. Nine 100-g samples of vacuum-pouch packed frozen corn in butter sauce were prepared according to package directions which require the consumer to place the frozen food package in boiling water, heat the water until it boils again, and hold the boiling water and package at a boil for 15 min. Measurements of the internal temperature of the four food products prepared in this manner did not drop below 85 C.

Individual samples were blended for 5 min with an aqueous suspension containing 4×10^6 spores of *C. botulinum* Type A. One set of six tubes contained 1 ppm Everninomicin D and the second set of six samples contained 10 ppm Everninomicin D. Six 50-g portions of the blended sample were placed into individual plastic pouches. The pouches were flushed with nitrogen, heat sealed, and the samples incubated for three weeks at 10 C, followed by 7 weeks at room temperature, and 3 weeks incubation at 32 C to induce *C. botulinum* exotoxin formation.

Fish. Seven 102-g samples of vacuum-pouch packed frozen cod fish in butter sauce were prepared according to the label recipe. Samples were blended for 5 min with an aqueous spore suspension containing 9.2×10^5 spores of *C. botulinum* Type E and 1 and 10 ppm Everninomicin D. Packaging was identical to that used for corn. Incubation was at 5 C for 12 weeks.

Sausage. A total of 675 g of commercially available frozen sausage links were immersed and boiled in water for 15 min. Two 225-g samples of sausage were mixed with a *C. botulinum* Type A spore inoculum containing 3.2×10^6 spores and 1 and 10 ppm Everninomicin D, respectively. Replicates of six tubes for each concentration of Everninomicin were used. Packaging was identical with that used for corn. Incubation was 10 C for 10 weeks followed by 2 weeks at room temperature.

Beef. Two different samples of Everninomicin were obtained for this work. The original sample had been labeled "Everninomicin D, 1600 units per milligram." This original sample was insoluble in water. The second sample was labeled "sodium salt of Everninomicin B," (*43*) and was rated at 1011 units per milligram. This sample was soluble in water.

A 200-g sample of sterilized beef was blended for 15 min with an aqueous suspension containing 2.4×10^6 spores of *C. botulinum* Type A and 8 and 16 ppm of the sodium salt of Everninomicin D. Six portions of approximately 33 g each were heat sealed in a nitrogen atmosphere in polymer bags and incubated at room temperature for 12 days.

The various incubation times and temperatures were used in an effort to encourage product spoilage and exotoxin formation. Testing for exotoxin was necessary at interim times. All analyses for *C. botulinum* exotoxin were performed by standard mouse inoculation procedures (*2*).

RESULTS

After 5 days incubation, all tubes were analyzed for *C. botulinum* exotoxin. Exotoxin was detected in each of the control tubes. Control tubes contained enrichment broth and the viable spores of *C. botulinum,* but did contain Everninomicin. Exotoxin was not detected in any tube which contained Everninomicin D, either heated or unheated, at the 1 or 10 ppm level.

In foods

Corn. Analyses for exotoxin were made after 2, 4, 8, 12, and 13 weeks. Exotoxin was detected in the 1 ppm Everninomicin D sample and in the inoculated control which did not contain Everninomicin, but not in the samples containing 10 ppm Everninomicin D. Enrichment of the samples after two weeks demonstrated viable spores in the samples containing 1 and 10 ppm Everninomicin D.

Fish. Analyses for exotoxin were done after 2, 4, 8, and 12 weeks. No exotoxin was detected after 2 weeks in either the control or at the 1 and 10 ppm levels. The control was an inoculated fish sample which did not contain Everninomicin. After 4 weeks incubation, exotoxin was detected in the control, but not at the 1 and 10 ppm levels of Everninomicin D. The same results were obtained after eight and twelve weeks of incubation.

Sausage. Analyses for exotoxin were made after 2, 4, 5, 8 weeks at 10 C and 12 weeks at room temperature. Exotoxin was detected in control samples as well as at the 1 and 10 ppm levels of antibiotic. Viable spores were demonstrated in all samples after 2 weeks of incubation. These concentrations of Everninomicin D did not prevent formation of exotoxin in sausage.

Beef. Samples were analyzed for exotoxin after 1 week incubation. Exotoxin was detected in control samples, but not in the Everninomicin B protected samples. After 12 days, further samples were analyzed producing similar results. Enrichment of the samples demonstrated that the spores were still viable and toxigenic when removed from the effects of the Everninomicin B.

DISCUSSION

As claimed in the issued patent (*6*), and as demonstrated in this work, protection is afforded by use of small quantities of Everninomicin, added to specific foods subjected to inoculation with spores of *C. botulinum.* The antibiotic may be infused directly into the food or added to a diluent and blended. The diluent permits more accurate measurement of the antibiotic and more uniform distribution throughout the food.

The mode of action of Everninomicin in preventing food spoilage is not known. Examination of contaminated foods treated with Everninomicin has shown the presence of viable *C. botulinum* spores, indicating that the antibiotic is not sporicidal. It is most probable that the antibiotic kills vegetative cells which would produce exotoxin, or alternately it may prevent spore germination or outgrowth. It is also possible, but less likely, that Everninomicin may inactivate the exotoxin as it is produced. Further investigation will be required to determine the mode of action.

CONCLUSIONS

(*a*) Everninomicin has the demonstrated ability to interfere with *C. botulinum* Types A and E exotoxin formation in specific foods and in sporulation broth.

(*b*) Within the limited scope of this work, the effectiveness of Everninomicin was demonstrated to control exotoxin formation under the following conditions: (*aa*) sporulation broth by 1 ppm, (*bb*) corn samples by 10 ppm, (*cc*) fish samples by 1 ppm, (*dd*) beef samples by 8 ppm.

(*c*) Spores are not killed by Everninomicin, as evidenced by recovery of viable spores after established enrichment techniques are employed.

REFERENCES

1. Black, J., R. Calesnick, F. G. Falco, and M. J. Weinstein. 1965. Pharmocological properties of everninomicin D. Antimicrobial agents and chemotherapy-1964. p. 38-46.
2. Crisley, F. D. 1963. The demonstration of botulinus toxins and *Clostridium botulinum* in foods. Examination of food for enteropathogenic and indicator bacteria: Review of Methodology and Manual of Selected Procedures. From the Milk and Food Reseach Section, Milk and Food Branch, Division of Environmental Engineering and Food Protection. U.S. Dept. Health, Education and Welfare, Public Health Service. 1963.
3. Deatherage, F. E. 1957. Method of preserving meat, U.S. Patent No. 2,786,768.
4. Duff, J. T., C. G. Wright, J. Klerer, D. E. Moore, and R. H. Bibler. 1957. Studies on immunity to toxins of *Clostridium botulinum.* I. A simplified procedure for isolation of type A toxin. J. Bacteriol. 73:42-47.
5. Duff, J. T., C. G. Wright, and A. Tavinsky. 1956. Activation of *Clostridium botulinum* type E toxin by trypsin. J. Bacteriol. 72: 455-460.
6. Haas, G. J., and N. F. Insalata. 1971. Botulinum exo-toxin inhibited foods. U.S. Patent No. 3,607,311.
7. Insalata, N. F., and I. Raab. 1970. The industrial significance of the low temperature microbiological preservation of foods. J. Milk Food Technol. 33:555-556.
8. Insalata, N. F., J. S. Witzeman, and J. H. Berman. 1970. The problems and results of an incidence study of the spores of *Cl. botulinum* in frozen vacuum pouch-pack vegetables. Developments in Industrial Microbiol. 11:334.
9. Insalata, N. F., J. S. Witzeman, and J. H. Berman. 1970. The problems and results of an incidence study of the spores of *Clostridium botulinum* in convenience foods. Health Lab. Sci. 7:54.
10. Lewis, K. H., and K. Cassess, Jr. (eds.) 1964. Botulism: proceedings of a symposium. PHS Pub. No. 999-FP-1, U.S. Dept. Health, Education and Welfare, Public Health Service, Cincinnati, Ohio. p. 15.
11. Lewis, K. H., and K. Cassell, Jr. (eds.) 1964. Botulism: proceedings of a symposium. PHS Pub. No. 999-FP-1. U.S. Dept. Health, Education and Welfare, Public Health Service, Cincinnati, Ohio. p. 35-36.
12. Lewis K. H., and K. Cassell, Jr. (eds.) 1964. Botulism: proceedings of a symposium PHS Pub. No. 999-FP-1, U.S. Dept. Health, Education and Welfare, Public Health Service, Cincinnati, Ohio. p. 36-37.
13. Lewis, K. H., and K. Cassel, Jr. (eds.). 1964. Botulism: proceedings of a symposium. PHS Pub. No. 999-FP-1. U.S. Dept. Health, Education and Welfare, Public Health Service, Cincinnati, Ohio. p. 75-78.
14. Luedemann, G. M., and B. C. Brodsky. 1965. *Micromonospora carbonadea* sp. n., an everninomicin-producing organism. Antimicrobial agents and chemotherapy-1964. p. 47-52.
15. Tanner, F. W. 1953. Food-borne infection and intoxications, 2nd ed. Garrand Press. p. 533.
16. U.S. Dept. Health, Education and Welfare. 1899-1967. Botulism in the United Staes, Review of cases. Public Health Service, National Communicable Disease Center, Atlanta, Georgia. p. 3.
17. U.S. Dept. Health, Education and Welfare. 1899-1967. Botulism in the United States, Review of cases. Public Health Service, National Communicable Disease Center, Atlanta, Georgia. p. 27-30.
18. U.S. Dept. Health, Education and Welfare, 1970. National Communicable Disease Center, Foodborne outbreaks, Annual Summary, Atlanta, Georgia. p. 22.
19. U.S. Dept. Health, Education and Welfare, National Communicable Disease Center. 1969. Foodborne outbreaks. Annual Summary, Atlanta, Georgia. p. 21.
20. U.S. Dept. of Health, Education and Welfare Public Health Service. 1974. Type B Botulism-Alaska. Morbidity and Mortality. 23:2.
21. U.S. Dept. of Health, Education, and Welfare, Public Health Service. 1974. Botulism-Alabama. Morbidity and Mortality. 23:90,95.
22. U.S. Dept. of Health, Education, and Welfare, Public Health Service. 1973. Botulinal toxin in a commercial food product-United States. Morbidity and Mortality. 22:57-58.
23. U.S. Dept. of Health, Education, and Welfare, Public health Service. 1973. Morbidity and Mortality. 22:62.
24. U.S. Dept. of Health, Education, and Welfare, Public Health Service. 1973. Probable botulism-Oklahoma. Morbidity and Mortality. 22:71.
25. U.S. Dept. of Health, Education, and Welfare, Public Health Service. 1973. Botulism Type B in commercially canned mushrooms-United States. Morbidity and Mortality. 22:87.
26. U.S. Dept. of Health, Education, and Welfare, Public Health Service. 1973. Botulinal toxin in commercially canned mushrooms—United States Morbidity and Mortality. 22:115.
27. U.S. Dept. of Health, Education, and Welfare, Public Health Service. 1973. Follow-up On botulinal toxin in commercially canned mushrooms—United States. Morbidity and Mortality. 22:123.
28. U.S. Dept. of Health, Education, and Welfare, Public Health Service. 1973. Botulism-West Virginia, Pennsylvania. Morbidity and Mortality. 22:161-162.
29. U.S. Dept. of Health, Education, and Welfare. Public Health Service. 1973. Type A botulism-Idaho, Oregon. Morbidity and Mortality. 22:218-223.
30. U.S. Dept. of Health, Education, and Welfare, Public Health Service. 1973. Type A botulism-Idaho. Morbidity and Mortality. 22: 240.
31. U.S. Dept. of Health, Education, and Welfare, Public Health Service. 1973. Botulism traced to commercially canned marinated mushrooms-Canada. Morbidity and Mortality. 22:241-242.
32. U.S. Dept. of Health, Education, and Welfare, Public Health Service. 1973. Probable botulism-Maryland. Morbidity and Mortality. 22:255-256.
33. U.S. Dept. of Health, Education, and Welfare, Public Health Service. 1973. Type B botulinal toxin in commercially canned mushrooms-United States. Morbidity of Mortality. 22:315.
34. U.S. Dept. of health, Education, and Welfare. Public Health Service. 1973. Probable botulism-Washington. Morbidity and Mortality. 22:317-318.
35. U.S. Dept. of Health, Education, and Welfare. Public Health Service. 1973. Type B botulism-Kentucky. Morbidity and Mortality. 22:350.
36. U.S. Dept. of Health, Education, and Welfare, Public Health Service. 1973. Wound botulism-Colorado. Morbidity and Mortality. 22:395.
37. U.S. Dept. of Health, Education, and Welfare, Public Health Service. 1973. Botulism-Kentucky. Morbidity and Mortality. 22:417-418.
38. U.S. Dept. of Health, Education, and Welfare, Public Health Service. 1973. Type A botulism-California. Morbidity and Mortality. 22:425-426.
39. U.S. Dept. Health, Education and Welfare, Public Health Service, 1971. Morbidity and Mortality. 20:302.

40. U.S. Dept. Health, Education and Welfare, Public Health Service, 1971. Morbidity and Mortality. 20:356-358.
41. Wagman, G., G. M. Luedemann, and M. J. Weinstein. 1965. Fermentation and isolation of Everninomicin D. Antimicrobial agents and chemotherapy-1964. p. 38-46.
42. Weinstein, M. J., G. M. Luedemann, E. M. Oden, and G. H. Wagman. 1965. Everninomicin, a new antibiotic complex from *Micromonospora carbonadea.* Antimicrobial agents and chemotherapy-1964. p. 24-32.
43. Weinstein, M. J., Wagman, G. J., et al. 1966. Purification and biological studies of everninomicin B. Antimicrobial agents and chemotherapy-1965. p. 821-827.

AOAC Publishes New Statistical Manual

The Association of Official Analytical Chemists (AOAC) has scheduled publication of the "Statistical Manual of the AOAC" by W. J. Youden and E. H. Steiner for April 1975. This book is intended for use in the statistical analysis of the results of interlaboratory collaborative tests, such as those required by the AOAC before its adoption as official of analytical methods for agricultural products, foods, beverages, drugs, cosmetics, color additives, and other commodities important in public health.

AOAC's new manual consists of a composite, with revisions, of the popular AOAC monograph, "Statistical Techniques for Collaborative Tests," by the late W. J. Youden and a companion volume, "Planning and Analysis of Results of Collaborative Tests," written by E. H. Steiner and originally published in 1974 by the British Food Manufacturing Industries Research Association, who have assigned their copyright to the AOAC. The Youden monograph was specifically written as a "do-it-yourself" manual for those who are at home in the analytical laboratory but who have little or no experience with formal statistics. It presents simple and flexible statistical techniques, using examples related to familiar questions concerning the significance of apparent differences among results. Youden's discussion goes into detail concerning the planning, presentation, and interpretation of the results of interlaboratory collaborative tests of analytical methods with a minimum of mathematics. The Steiner manual, intended for those who are already familiar with the fundamentals covered by Youden, presents guidelines for the planning of collaborative tests and introduces the analysis of variance technique with examples of how it may be used in the analysis of interlaboratory tests. Analysis of variance is a powerful statistical tool which separates the total variation of a set of data into components due to different factors (samples, laboratories, analysts, etc.) incorporated as a result of the design of the experiment. These individual components are usually examined by further statistical techniques to determine if the factors they represent make a significant contribution to the total variation of the experimental design.

Highlights of the contents of the Youden portion of the new AOAC statistical manual include sections on the responsibilities of the analyst, error in an analytical result, importance of and statistical test for systematic error, the two-sample chart, sampling laboratory populations, minimum requirements for a collaborative study, measuring precision with and without duplicates, measuring standard deviation of data, two-sample techniques with duplicates, precision and accuracy, the unit block, and coefficient of variation. Other important topics discussed include missing values, outliers, the ranking test for laboratories, the ruggedness test for procedures, confidence limits, control charts, and sampling considerations.

Steiner's discussion includes sections on the preliminary check of a method, participating laboratories, distribution of samples, replicate analyses, the reporting form, reduction of data to standard form, elimination of extreme results, homogeneity of experimental variation, analysis of variance of adjusted data, repeatability and reproducibility, replicate analyses and confidence limits, application to microbiological counts, and the significance of the ruggedness test.

The combined publication of the Youden and Steiner texts will be an important aid to anyone seeking increased understanding of the planning and analysis of the statistical results of collaborative tests. The new 96-page "Statistical Manual of the AOAC" will be available after April 15 for $5.00 from the Association of Official Analytical Chemists, PO Box 540, Benjamin Franklin Station, Washington, DC 20044.

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Bacteriological Quality of Delicatessen Foods: Are Standards Needed?

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ABSTRACT

Surveillance of seven types of delicatessen salads (chicken, egg, ham, macaroni, American potato, shrimp, and tuna) was conducted from March through July, 1974. Liver spread and sandwich spread were included in the sampling. Products were obtained from approximately 150 retail establishments. A separate surveillance of one central production unit and approximately 18 retail establishments, which it supplied, was also conducted. Twenty six percent to 85% of samples of products obtained from general retail outlets had aerobic plate count (APC) values of less than 100,000 per gram. Thirty six percent to 79% had coliform Most Probable Number (MPN) levels of less than 100 per gram, while 83 to 100% contained less than 10 *Escherichia coli* per gram. Some samples of each of the products contained low numbers of coagulase-positive staphylococci. Seventy one per cent to 96% of samples of various products, obtained from a central production unit, yielded APC values of less than 100,000 per gram. Forty five percent to 94% had coliform MPN values of less than 100 per gram. Two of six products contained low levels of coagulase-positive staphylococci in 9% and 2% of the samples examined.

The Wisconsin Milk and Food Conference, just as consumer organizations and regulatory agencies, has long been concerned about bacteriological standards for foods. The Conference Executive Board designated a Food Standards Committee to review the need for, and feasibility of, adopting such standards in Wisconsin.

Microbiological quality standards have been proposed, at the Federal level, for frozen, ready-to-eat cream-type pies and unflavored gelatin (*13*). The former product (banana, coconut, chocolate, and lemon varieties) would be required to meet the following criteria: (*a*) aerobic plate count (geometric mean) of $\leq$50,000 per gram; and (*b*) coliform count (geometric mean) of $\leq$50 per gram. Unflavored gelatin would be required to meet these criteria: (*a*) aerobic plate count (geometric mean) of $\leq$3,000 per gram. (*b*) coliform count (geometric mean) of $\leq$10 per gram.

While foods, of the delicatessen variety, are widely distributed throughout this country, microbiological standards of quality have not been established at the national level. It is true that military branches of the United States and some individual states have specified quality standards. However, the acceptable microbial levels, and justification for their selection, have not received wide publicity.

The Conference Executive Board became aware of an administrative order promulgated by the State of New Jersey. Chapter 12, section 3.1.6, of the State Sanitary Code of New Jersey, decrees that the State Commissioner of Health shall designate types of potentially hazardous foods to be subject to bacteriological standards of sanitary quality. Foods so designated are: chicken salad, chopped chicken liver, coleslaw, egg salad, macaroni salad, potato salad, shrimp salad, tuna salad, and turkey salad.

The following bacteriological limits have been established for these foods in New Jersey: (*a*) aerobic plate count—not more than 100,000 per gram, (*b*) coliform count—not more than 100 per gram, (*c*) coagulase-positive staphylococci—not more than 100 per gram and (*d*) No *Salmonella, Shigella,* or enteropathogenic *Escherichia coli.*

The total number of sales of delicatessen foods is not readily determinable. However, it can be estimated that thousands of servings are dispensed each day in Wisconsin and the number may approach millions per day nationally.

Surveillance data, relative to the bacteriological quality of delicatessen foods, have been accumulated by the Wisconsin State Department of Agriculture and by municipal health departments in Wisconsin over a number of years. Microbiological methodology employed by these various agencies was by no means uniform. Therefore, the Food Standards Committee agreed to design a surveillance program in which standard bacteriological analytical techniques would be uniformly employed.

The committee's goal was to survey delicatessen foods, distributed by retail stores in Wisconsin, in an effort to determine their bacteriological quality at the time they were available to consumers.

In the evolvement of standards it is necessary to have knowledge of the numbers and kinds of bacteria in the foods produced under good manufacturing procedures. Consequently, the committee also chose to survey delicatessen salads produced in one central production kitchen. The same types of salads were also sampled from approximately 18 of the retail outlets supplied by the central kitchen. This production source was not chosen because it was necessarily known to produce salads under good manufacturing practices. Its selection came about, rather, because it was readily accessible to a

regulatory agency and its laboratory facilities. Furthermore, management within the kitchen's corporation was fully cooperative and also interested in determining the bacteriological quality of delicatessen salads as produced at the wholesale level.

Health department laboratories and inspection staffs of the cities of Fond du Lac, Kenosha, Madison, and Milwaukee participated in the surveillance program along with the Wisconsin State Department of Agriculture.

MATERIALS AND METHODS

Types of salads sampled

Chicken, egg, ham, macaroni, American potato, shrimp, and tuna salads were surveyed in this study. Liver spread and sandwich spread were also included because their composition is such that they must be classified as high risk products for growth of bacteria.

Collection of samples

Salads were purchased from, or obtained gratuitously from, approximately 170 retail outlets in Wisconsin. They were procured from refrigerated display counters and, in most instances, were dispensed from bulk containers into retail cartons as a consumer would receive them. Those salads collected from a central production kitchen were transferred from bulk lots, using kitchen utensils, into laboratory sterilized glass jars. All samples were conveyed to the laboratories in approved refrigerated transport cases. Salads were processed upon receipt, when time permitted, or held at 4 C for up to 24 h. Data, included in this report, were derived from a surveillance conducted from March through July, 1974.

Laboratory analyses

Samples were examined for total aerobic bacteria, coliform bacteria, and coagulase-positive staphylococci at all participating laboratories. Populations of the latter two organisms were estimated by Most Probable Number (MPN) procedures. One of the five laboratories also determined the MPN level of *E. coli* in products it received. Methods for preparation of samples and analysis for bacteria types, identified above, and media employed were those prescribed in the *Federal Register* (*13*), and detailed in Sections 41.013 through 41.018, inclusive of *Official Methods of Analysis of the Association of Official Analytical Chemists* (*4*).

A limited examination of the effects of time of storage and temperature of storage on the bacteriological quality of four types of salads was conducted at one of the laboratories. Bulk samples were obtained from a central production kitchen. Upon receipt at the laboratory each sample was divided into eight aliquots. Four of these were stored at 4 C (39.2 F) and the remainder at 10 C (50 F). Preparation of samples and analyses for all organisms, identified above, with the exceptionof *E. coli,* were conducted on an aliquot taken from each storage temperature after 24, 48, 72, and 96 h.

The pH of a portion of each of the samples was determined electrometrically from a slurry prepared at the time of bacteriological analysis.

RESULTS

pH

The ranges of pH values determined for various foods examined appear in Tables 1 and 3. Although not apparent from these tables virtually no correlation was detectable between pH values and aerobic plate count (APC), coliform MPN, or coagulase-positive staphylococcus MPN values.

Microbial quality of general retail samples

Total counts, on eight products sampled, ranged from less than 1,000 to tens of millions per gram (Table 1).

TABLE 1. *Aerobic plate counts of delicatessen foods collected from approximately 150 retail establishments in Wisconsin*[a]

Product	No. samples	pH Range	Geom. mean/g (No. samples)	Percent of samples with bacteria populations/g in following ranges						
				10^7[b]	10^6[b]	10^5[b]	10^4[b]	10^3[b]	$< 10^3$	Sub total $< 10^5$
Chicken salad	38	4.6-6.2	6.5×10^4 (37)	3	24	26	31	16		47
Egg salad	9	5.1-6.6	5.3×10^5 (8)	33	22	11	22	11		33
Ham salad	24	4.4-5.5	2.4×10^5 (24)	17	13	29	29	8	4	41
Macaroni salad	119	4.2-5.7	2.2×10^4 (117)		10	21	24	31	14	69
American potato salad	172	4.1-6.1	1.8×10^4 (171)	2	9	18	27	29	15	71
Sandwich spread	82	4.1-6.0	4.9×10^5 (81)	11	35	28	16	9	1	26
Shrimp salad	46	4.6-5.8	2.7×10^4 (45)	2	4	20	35	37	2	74
Tuna salad	27	4.5-5.9	1.6×10^4 (27)		4	11	37	48		85

[a]Data provided by cities of Fond du Lac, Kenosha, Madison, and Milwaukee Health Departments and Wisconsin State Department of Agriculture
[b]1 - 9.9 × base 10

TABLE 2. *Coliform and coagulase positive staphylococci MPN values/g determined in delicatessen foods collected from approximately 150 retail establishments in Wisconsin*[a]

Product	No. Samples	Percent of samples with coliform MPN/g in following ranges							Percent of samples with coagulase positive staphylococci MPN/g in following ranges			
		10^5[b]	10^4[b]	10^3[b]	10^2[b]	10^1[b]	$< 10^1$	Subtotal $< 10^2$	10^2[b]	10^1[b]	$< 10 - \geq 3$	< 3
Chicken salad	38		3	40	21	18	18	36	3	5	13	79
Egg salad	9			33		33	33	66			22	78
Ham salad	24			17	21	4	58	62			8	92
Macaroni salad	119	1		10	10	15	64	79		1	7	92
American potato salad	172			21	10	24	45	69		3	7	90
Sandwich spread	82			10	16	28	46	74	1	4	15	80
Shrimp salad	46		2	26	13	30	28	58			11	89
Tuna salad	27			11	15	33	41	74	4	4	4	88

[a]Data provided by cities of Fond du Lac, Kenosha, Madison, and Milwaukee Health Departments and Wisconsin Department of Agriculture
[b]1 - 9.9 × base 10

Some of these foods were prepared in the retail stores from which they were collected. Others were prepared in central production kitchens and transferred to the stores for retail distribution. No consistent pattern of correlation could be established between low counts and either local or central production. At least one sample of macaroni salad, prepared in New York and shipped into Wisconsin for retail sale, had an APC of less than 200 per gram. Approximately 26 to 85%, of the various products sampled, met the New Jersey APC requirement of no more than 100,000 per gram. Five out of eight of the products were found to have mean (geometric) APC values of less than 100,000 per gram.

The coliform MPN values for these eight foods met the New Jersey requirement in from approximately 36 to 79% of the samples (Table 2).

Some of the samples, of each product, contained coagulase-positive staphylococci (Table 2). However, only 3 of 8 products had small percentages of the samples which exceeded the New Jersey requirement.

Microbial quality of centrally produced food

Five varieties of delicatessen foods, produced in a central kitchen, were found to have APC values which met the New Jersey requirement in from approximately 71 to 96% of the samples (Table 3). Samples, of each of these foods, were found to have mean (geometric) APC values of less than 100,000 per gram.

Sandwich spread, was produced in retail stores supplied by the central kitchen being surveyed. Approximately 4% of these samples had an APC of less than 100,000 per gram. This contrasts with a figure of 26% determined for this product collected from general retail stores (Table 1).

Only one sample of liver spread was collected from a retail outlet. Accordingly there are insufficient data to make a meaningful comparison of this product at the wholesale and retail levels.

Other foods (chicken salad, macaroni salad, potato salad, and shrimp salad) collected from retail outlets, supplied by this kitchen, were found to have 0 to 33% of

TABLE 3. *Aerobic plate counts of delicatessen foods collected from one central kitchen and approximately 18 retail outlets which it supplied*[a]

Product	Source	No. samples	pH Range	Geom. mean/g (No. Samples)	Percent of samples with bacteria populations/g in following ranges								
					10^8[b]	10^7[b]	10^6[b]	10^5[b]	10^4[b]	10^3[b]	10^2[b]	$<10^2$	Subtotal $<10^5$
Chicken salad	Central kitchen	56	4.9-5.9	1.1×10^4 (56)			4	23	21	23	27	2	73
	Retail	31	4.5-6.3	1.9×10^7 (28)	16	55	19	10					0
Liver spread	Central Kitchen	12	5.2-6.6	1.7×10^3 (12)				8		50	33	8	91
	Retail	1	6.2	8.4×10^4 (1)					100				100
Macaroni salad	Central Kitchen	50	3.6-5.1	6.3×10^2 (49)				4	2	32	34	28	96
	Retail	30	4.1-6.0	4.4×10^5 (30)		27	10	30	30	3			33
American potato salad	Central Kitchen	36	4.5-5.6	7.7×10^2 (35)			3	3	14	22	36	22	94
	Retail	47	4.5-5.8	9.7×10^5 (47)		15	36	38	11				11
Sandwich spread	Central Kitchen	—[c]											
	Retail	27	4.2-6.3	9.6×10^5 (26)			55	41	4				4
Shrimp salad	Central Kitchen	59	4.4-6.6	1.0×10^4 (57)			7	22	29	8	24	10	71
	Retail	53	4.6-5.7	5.8×10^6 (53)	7	36	42	7	6	2			8

[a]Data provided by cities of Kenosha, Madison, and Milwaukee Health Departments and Wisconsin State Department of Agriculture
[b]1 - 9.9 × base 10
[c]This product prepared in retail store

TABLE 4. *Coliform and cogulase positive staphylococci MPN values/g determined in delicatessen foods collected from one central kitchen and approximately 18 retail outlets which it supplied*[a]

Product	Source	No. samples	Percent of samples with coliform MPN/g in following ranges								Percent of samples with coagulase positive staphylococci MPN/g in following ranges			
			10^6[b]	10^5[b]	10^4[b]	10^3[b]	10^2[b]	10^1[b]	$<10^1$	Subtotal $<10^2$	10^2[b]	10^1[b]	$<10-\geq 3$	<3
Chicken	Central	56		2	3	18	32	20	25	45			9	91
	Retail	31		16	23	55	6			0	7	19	26	48
Liver spread	Central Kitchen	12					8	25	67	92				100
	Retail	1				100				0				100
Macaroni	Central Kitchen	50			4		2	4	90	94				100
salad	Retail	30	7		7	26	40	17	3	20	3		7	90
American potato salad	Central Kitchen	36			3	5	14	17	61	78				100
	Retail	47		11	19	53	11	4	2	6			4	96
Sandwich spread	Central Kitchen	—[c]												
	Retail	27			4	22	4	37	33	70			4	96
Shrimp salad	Central Kitchen	59		2	10	22	15	17	34	51			4	98
	Retail	53	9	11	15	60	4			0		4	4	92

[a]Data provided by cities of Kenosha, Madison, and Milwaukee Health Departments and Wisconsin State Department of Agriculture
[b]1 - 9.9 × base 10
[c]This product prepared in retail store

the samples which yielded APC values of less than 100,000 per gram (Table 3).

The coliform MPN and coagulase-positive staphylococcu MPN values for foods, sampled at a central kitchen and retail outlets it supplied, are shown in Table 4. As is true in comparison of APC values, these data reveal much higher populations of, and higher percentages of samples with, coliform bacteria in foods collected at retail outlets.

Escherichia coli

One of the cooperating laboratories examined foods it recieved for the presence of *E. coli* (Table 5). Approximately 92 to 100% of the samples, of each of eight foods, contained less than 100 *E. coli* per gram; the percentage of samples which contained less than 10 per gram ranged from 83 to 100.

TABLE 5. Escherichia coli *MPN values/g in delicatessen salads collected from 137 retail outlets*

Product	No. samples	Percent of samples with *E. coli* values in following ranges: 10^3[b]	10^2[b]	10^1[b]	Subtotal >10	<10-≥3	<3
Chicken salad	35	3	3	11	17	17	66
Egg salad	6					33	67
Ham salad	20	5			5	5	90
Macaroni salad	105		2	5	7	6	87
American potato salad	139	7	1	4	12	7	81
Sandwich spread	66			9	9	7	84
Shrimp salad	46		2	13	15	15	70
Tuna salad	22			5	5	5	90

[a]Data provided by Wisconsin State Department of Agriculture
[b]1 - 9.9 × base 10

Storage time and temperature vs. microbial populations

Salads of high protein content (chicken and shrimp) had the lowest percentages of samples with an APC of less than 100,000 per gram. In contrast, the highest percentages of samples yielding APC values below this number were found among macaroni salads and potato salads (Table 3). The latter two salads continued to yield relatively low APC values through 4 days of storage at either 4 C (39.2 F) or 10 C (50 F) (Table 6). The ten-fold increase in APC of potato salad held 24 h at 10 C (50 F) as opposed to the sample held at 4 C (39.2 F) may reflect a variation in bacterial distribution in the aliquots. This reason may also account for the ten-fold increase observed after 48 h at 4 C (39.2 F). A more consistent effect of storage time and temperature of storage is apparent in reviewing data relating to high protein salads (chicken and shrimp). In general, a ten-fold, or more, increase in APC was observed with extended storage at a given temperature. A similar magnitude of increase generally occurred in the APC of aliquots of these foods, held at 10 C (50 F) as opposed to 4 C (39.2 F), for a given period.

TABLE 6. *The effect of storage time and temperature of storage on microbial populations in four delicatessen salads*

Chicken Salad

Time stored, (h)	APC/g x 10^3 4 C	APC/g x 10^3 10 C	Coliform MPN/g x 10^3 4 C	Coliform MPN/g x 10^3 10 C	Coag. pos. staph. MPN/g x 10^1 4 C	Coag. pos. staph. MPN/g x 10^1 10 C	pH 4 C	pH 10 C
24	36	460	1.5	28	0.36	1.5	5.2	5.4
48	140	170	9.3	4.3	0.36	2.8	5.1	5.5
72	64	1800	4.3	21	9.3	4.3	5.1	5.6
96	370	15000	43	24	2.3	9.3	5.5	5.4

Shrimp Salad

Time stored (h)	APC/g x 10^5 4 C	APC/g x 10^5 10 C	Coliform MPN/g x 10^3 4 C	Coliform MPN/g x 10^3 10 C	Coag. pos. staph. MPN/g 4 C	Coag. pos. staph. MPN/g 10 C	pH 4 C	pH 10 C
24	2.7	12	2.1	15	<3	<3	5.6	5.7
48	9.5	130	46	93	<3	<3	5.8	5.5
72	230	1400	46	240	<3	<3	5.5	5.2
96	85	1000	460	930	<3	<3	5.5	5.0

American Potato Salad

Time stored (h)	APC/g x 10^4 4 C	APC/g x 10^4 10 C	Coliform MPN/g x 10^3 4 C	Coliform MPN/g x 10^3 10 C	Cog. pos. staph. MPN/g 4 C	Cog. pos. staph. MPN/g 10 C	pH 4 C	pH 10 C
24	4.8	44	11	4.3	<3	<3	5	5.1
48	38	5.9	0.93	4.3	<3	<3	4.8	5.1
72	19	4.3	0.93	0.43	<3	<3	5.1	4.9
96	6.1	6.1	2.4	0.93	<3	<3	5	5

Macaroni Salad

Time stored (h)	APC/g x 10^1 4 C	APC/g x 10^1 10 C	Coliform MPN/g 4 C	Coliform MPN/g 10 C	Coag. pos. staph. MPN/g 4 C	Coag. pos. staph. MPN/g 10 C	pH 4 C	pH 10 C
24	2	4.5	<3	<3	<3	<3	4.5	4.4
48	2.5	5	<3	<3	<3	<3	4.3	4.3
72	2	10	<3	<3	<3	<3	4.3	4.3
96	2	2	<3	<3	<3	<3	4.5	4.6

DISCUSSION

Delicatessen foods which have low APC values are not necessarily safe products; however, they are more likely to be safe than those which have high counts. An APC can provide a means by which food processing can be monitored at specified control points. It can be an aid in determining the effectiveness of thermal processing of cooked components andof their subsequent rapid cooling to temperatures below which bacteria cannot multiply. This bacteriological determination also is potentially useful in monitoring the quality of chilled, and cold rinsed, raw components. Finally, APC monitoring of the finished products, following preparation and at the retail level, would provide evidence relative to maintenance of their bacteriological quality through refrigeration storage.

Presence of small numbers of potentially hazardous organisms in these foods is probably unavoidable. Christiansen and King (*2*) in a survey of salads and sandwiches examined three types (chicken, ham, and potato salads) included in this study, Eight, 7, and 9 samples, respectively, were found to have APC values ranging from 1.2×10^4 to 1.4×10^7, 8.0×10^2 to 8.7×10^6, and 4.0×10^2 to 1.2×10^5 in that order. Sixty percent of chicken salad, 25% of ham salad, and about 45% of

potato salad samples contained coagulase-positive staphylococci; counts ranged from 1.0×10^2 to 8.0×10^4 per gram. Chicken, egg, ham, potato, and tuna salads were included in a survey of restaurant foods by Jopke and Riley (*5*). These yielded APC values ranging from 1.4×10^4 to 1.9×10^6, 2.6×10^4 to 1.1×10^7, 3.7×10^2 to 3.1×10^5, 9.3×10^3 to 1.6×10^6, and 2.1×10^3 to 6.8×10^6 per gram, respectively. Chicken, egg, and potato salads were found to contain coagulase positive staphylococci; up to 3.8×10^2 per gram in the first, 1.0×10^1 per gram in the second, and 2.4×10^2 per gram in the third product. This latter observation is in good agreement with the low levels of coagulase-positive staphylococci detected in foods included in the present survey.

Staphylococci are presumed to be poor competitors with saprophytic organisms in foods (*6*). This claim is supported by studies in which commercial salads of chicken and ham variety were inoculated with 1.0×10^6 coagulase positive staphylococci per gram (*2*). These products were incubated at 4 C (39.2 F) and 37 C (98.5 F). Staphylococci decreased to < 100 per gram in 4 days at 37 C (98.6 F). At 4 C (39.2 F) the staphylococcus counts in chicken salad and ham salad diminished to 5.4×10^4 per gram and 9.0×10^4 per gram, respectively. Conversely, the APC of chicken salad increased from 1.1×10^7 to 5.3×10^8 per gram in 2 days at 37 C (98.6 F); the ham salad A.P.C. increased from 5.0×10^6 to 8.1×10^7 per gram in 3 days at the same temperature.

A more recent study (Keoseyan, Bennett, and Amos, Abstracts Ann. Mtg. A.S.M., E-83, pg 14, 1974) revealed that *Staphylococcu aureus,* type A increased to 1.0×10^7 per gram in 18 h at 37 C (98.6 F) in chicken salad which had been inoculated with 3.5×10^4 organisms per gram; enterotoxin A was detectable at the same time. Uninoculated samples of the same salad yielded APC values, of indigenous organisms, ranging from 8.3×10^7 to 8.1×10^8 per gram after 15, 18, 21, 24, and 27 h at the same temperature. Enterotoxin B was detectable, in inoculated salad, after 24 h; the *S. aureus* population was 9.0×10^7 per gram at that time. It is clear that *S. aureus* not only competes quite well with a considerable population of indigenous microflora in chicken salad, incubated at 37 C (98.6 F), but more importantly it elaborates enterotoxin.

It may not be realistic to expect delicatessen salads to be free of *S. aureus.* However, consumers, producers, retailers, and regulatory agencies have a right to demand assurance that the components and finished products are maintained under conditions which are not conducive to bacterial multiplication. APC monitoring at specified control points, within the manufacturing and distribution chain, can provide evidence of this security.

Six outbreaks of foodborne illness, in which delicatessen type salads were incriminated, occurred in the U.S. in 1972 (*11*). The vehicles and causative agents were: (*a*) chopped liver, *S. aureus;* (*b*) cole slaw, *Salmonella agona;* (*c*) potato salad, *Salmonella kottbus,* and *S. aureus;* (*d*) shrimp salad, *Salmonella infantis;* and (*e*) turkey salad, *S. aureus.*

Potato salad was the vehicle for two outbreaks reported in 1973; one was attributed to staphylococci (*8*), the other to salmonellae (*10*). An outbreak of staphylococcal foodborne illness also occurred, in 1973, in which macaroni salad was involved (*9*).

Data, reported herein and elsewhere, demonstrate that delicatessen salads not only contain abundant bacteria but also are capable of supporting their growth. It is surprising that larger numbers of outbreaks of foodborne illness, due to ingestion of these products, have not been reported. This may be a fortuitous occurrence because of the infrequency with which pathogenic microorganisms gain access to the products. More likely it is because of poor reporting and inadequate investigation of outbreaks.

Principles for establishing microbiological criteria have been defined (*7*). "Microbiological criteria should: (*a*) accomplish what they purport to do, i.e., reduce public health hazards; (*b*) be technically feasible, i.e., attainable under conditions of good commercial practice; and (*c*) be administratively feasible." Although Microbiological limits may not eliminate a public health hazard they will certainly reduce it.

An APC limit of less than 10^5 per gram of delicatessen salads appears feasible among those products sampled from a central production unit (Table 3). Seventy-one percent to 96% of the samples, of five products, were found to comply. However, with the exception of liver spread, no more than 33% of the samples of the same salads, obtained from retail stores supplied by this unit, produced values of less than 10^5 per gram. This observation led to the suspicion that mishandling of the products may have occurred between preparation and retail availability. Conversely, 26 to 85% of the samples, of eight products, obtained from other retail units yielded APC values of less than 10^5 per gram (Table 1). Thus preparation and retail distribution of salads with APC values less than 10^5 per gram is being accomplished, at least partially, at present. However, it is apparent that industry and regulatory agencies must direct attention to manufacturing and handling procedures to ensure release of products which meet even an APC requirement of no more than 10^5 per gram. An APC bacteriological guideline is preferable, at present, to monitor implementation of the necessary improvements in manufaturing and handling practices. Additional data relative to APC values attainable and maintainable, at specified control points, are required to support feasibility of a bacteriological standard.

Potato and macaroni salads maintained low level APC values through 96 h of refrigerated storage (Table 6). The latter product, which was more highly acidified, had lower levels to start. Furthermore, no appreciable increase was detected with increased time of storage at a given temperature or vice versa. Chicken and shrimp salads showed marked increases in APC levels coincident

with an increase of either variable while the other was held constant. High protein salads may require special production methods to reduce bacterial populations more effectively. Higher acidification of the final product might well be considered to supplement refrigeration as a means of inhibiting bacterial growth during storage.

The New Jersey requirement of not more than 100 coagulase-positive staphylococci per gram was met in 96 to 100% of samples, representing eight products, collected from general retail stores. Samples of all salads obtained from central production complied. While only 2 of 5 of these products, obtained from retail outlets which it supplied, were found to contain *S. aureus* in excess of 100 per gram in 7% and 3% of the samples.

No more than 79%, and frequently none, of the samples, of various salads, taken from retail stores met the coliform requirement of no more than 100 per gram. However, 83 to 100% of retail samples of eight different products examined contained less than 10 *E. coli* per gram. Jopke and Riley (*5*) have speculated that residue of soil from, or on, vegetables may account for high coliform populations. Therefore, it may be advisable to establish a requirement of less than 10 *E. coli* per gram rather than no more than 100 coliform per gram. The former index would more specifically reflect freedom from contamination with bacteria of fecal origin and associative pathogens.

The third criterion, administrative feasibility, has not been addressed by the committee to this point. For that matter, neither has a recommendation of microbiological limits or standards been formulated. It remains for that body to review the data contained in this report. It must also wrestle with the mechanics of involing sanctions.

Obviously it is impractical to penalize a manufacturer or retailer for a single high count which may be of spurious origin. The luxury of establishing a mean geometric value for 10 samples is not afforded regulatory agencies unless the samples can be related to a common production lot. This approach would require coding and retail packaging at the production site. The United Staes Public Health Service Grade "A" Pasteurized Milk Ordinance (*12*) might be adapted to enforcement of microbiological limits for delicatessen foods. It states: Whenever two of the last four consecutive samples exceed the bacteria standard a warning letter is to be sent. Such warning is to remain in effect as long as two of the last four samples continue to exceed the standard. Permit or license is subject to revocation, and court action may ensue, should three of the last five consecutive samples fail to meet the standard. This approach to administrative action would require at least four samplings in each 6-month period.

The committee's efforts, to this point, have revealed a glaring infraction of recommended food-handling practices in the one central production unit it surveyed. A passing effort is made to refrigerate freshly prepared salads. That is, deep containers, of 5 to 10-lb. capacity, are placed in refrigerated storage areas; but little attention is paid to the role of container size and its geometry on cooling rates. Dickerson and Read (*3*) report a 14-gal. container of beef stew required 84 h to cool from 46.1 C (115 F) to 10 C (50 F) in a walk-in refrigerator held at 4.4 C (40 F). Their study also pointed to the fact that large containers, due to slow cooling, developed hazardous areas for bacterial growth near the container wall rather than at its geometric center during the first 24 h. The admonition to ensure safety of prepared foods by keeping them hot or cold has been repeated often. Presumably it has been heard but not really understood. Use of shallow trays for salads, no more than 4 inches deep, or rapid transfer of the products into ½ lb. and 1 lb. retail containers at the production plant may be advisable or even essential.

It is even more difficult to understand the failure, of the central kitchen in question, to provide refrigerated transport of delicatessen salads from the production plant to retail outlets. Some shipments may be held in an unrefrigerated truck for 4 h or more. Some deliveries are placed on loading docks several hours before retail personnel arrive to transfer them to walk-in coolers. This neglect of a basic tenet of food handling (proper refrigeration) has been reported to be the number one cause of foodborne illness (*1*).

A charge has been issued to the committee by the Wisconsin Milk and Food Conference Executive Board. Data, contained in this report, are to be evaluated by the committee and jointly considered in the formulation of recommendations. Evidence that standards are needed, from a health hazard, consumer quality, and producer quality aspect, is abundant. It is also evident that delineation of the methodology of delicatessen salad production and accumulation of data relating the microbiological quality attainable and maintainable, while employing good manufacturing procedures, must precede formulation of standards.

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REFERENCES

1. Bryan, F. L. 1972. Emerging foodborne diseases. II. Factors that contribute to outbreaks and their control. J. Milk Food Technol. 35:632-638.
2. Christiansen, L. N., and N. S. King. 1971. The microbial content of some salads and sandwiches at retail outlets. J. Milk Food Technol. 34:289-293.
3. Dickerson, R. W., Jr., and R. B. Read, Jr. 1973. Cooling rates of foods. J. Milk Food Technol. 36:167-171.
4. Horwitz, W., P. Chichilo, and H. Reynolds (ed) 1970. Official methods of analysis of the associationof official analytical chemists. 11th ed. pg 842-844. Association of Official Analytical Chemists, P.O. Box 540, Benjamin Franklin Station, Washington, D.C. 20044.
5. Jopke, W. H., and J. R. Riley. 1968. Microbiology of restaurant-cafeteria prepared food dishes. J. Milk Food Technol. 31:393-397.

6. McCroan, J. E., T. W. McKinley, A. Brim, and W. C. Henning. 1964. Staphylococci in commercial wrapped sandwiches. Pub. Health Reports 79:997-1004.
7. National Academy of Sciences—National Research Council. 1964. An evaluation of public health hazards from microbiological contamination of foods. Pub. No. 1195. National Academy of Sciences, Washington, D.C.
8. U.S. Department of Health, Education, and Welfare, Center for Disease Control. 1973. Morbidity mortality weekly reports. 22:141.
9. U.S. Department of Health, Education, and Welfare, Center for Disease Control. 1973. Morbidity and mortality weekly reports. 22: 285.
10. U.S. Departmentof Health, Education, and Welfare, Center for Disease Control. 1973. Morbidity and mortality weekly reports. 22: 342.
11. U.S. Departmentof Health, Education and Welfare, Public Health Service, Center for Disease Control. 1973. Foodborne outbreaks annual summary 1972. D.H.E.W. Pub. No. (CDC) 74-8185.
12. U.S. Departmentof Health, Education, and Welfare, Public Health Service. 1965. Grade "A" pasteurized milk ordinance. Pub. No. 229, pg 38. U.S. Government Printing Office, Washington, D.C.
13. U.S. Government. 1973. Federal register, 38:20726-20730. U.S. Government Printing Office, Washington, D.C.

AOAC Publishes Updated Aflatoxin Methods Reprint

The Association of Official Analytical Chemists (AOAC) is embarking on a new experiment in the dissemination of validated analytical methods. Four new methods on *Natural Poisons,* approved in October 1974, have been incorporated into Chapter 26 of the 12th edition of *Official Methods of Analysis* and reprinted as a single, convenient, 36 page laboratory manual covering methods for the analysis of foods and feeds for mycotoxins, including aflatoxins, ochratoxins, patulin, and sterigmatocystin, as well as marine toxins and phytotoxins. Commodities covered include peanuts and peanut products, cocoa beans, coconut, copra, and copra meal, corn, cottonseed products, green coffee, pistachio nuts, soybeans, dairy products including fluid and powdered milk, barley, apple juice, wheat, shellfish, and beans. In addition, AOAC methods which have also been approved by other associations, such as the American Oil Chemists' Society (AOCS), the American Association of Cereal Chemists (AACC), and the International Union of Pure and Applied Chemistry (IUPAC), are so designated, so that the user does not need to compare methods line-by-line to determine which are common to other organizations.

This manual is intended to be replaced annually by an updated version. *Natural Poisons* is one of the AOAC's most active areas, and the work of Associate Referees for citrinin, penicillic acid, trichothecenes, and zearalenone bears promise of more mycotoxin methods to come. Previously the laboratory worker has to review the AOAC's primary publication, *Official Methods of Analysis,* and each annual supplement until the appearance of the next edition, 4 or 5 years later, in order to determine the availability of methods. Laboratory directors have indicated that they would prefer to have the AOAC supply an updated version rather than to have their personnel spend the time searching through the "Changes in Methods" supplements and compiling their own. The response to this experimental chapter will determine the availability of future reprints of Chapter 26 and the possibility of providing other chapters in the same format. The booklet will be available after March 15 for $4.00 from the Association of Official Analytical Chemists, PO Box 540, Benjamin Franklin Station, Washington, DC 20044.

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Exposure of Man to Mercury. A review [1,2]

II. Contamination of Food and Analytical Methods

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ABSTRACT

Man is exposed to mercury through every facet of his life, however, for the average citizen the most probable source of toxic levels of mercury would be his food supply. Although most foods contain less than 0.02 ppm mercury, considerable variation occurs depending on the type of food, production techniques, and location. Mercury is concentrated at higher trophic levels of food chains, particularily in aquatic food chains in which concentration factors of hundreds and thousands have been observed. The concentration of mercury in some large fish has been found to exceed the 0.5 ppm tolerance limit of the FDA and the 1.0 ppm limit of the Swedish government. Fifty-seven grams of fish containing 0.5 ppm mercury in the methyl form could be consumed daily without exceeding the joint FAO/WHO recommended weekly tolerable intake of 0.2 mg. In the U.S., Sweden, and Japan the per capita daily fish consumptions are 18, 56, and 88 g, respectively.

Determination of mercury concentrations generally involves colorimetric, atomic absorption or emission spectrometry, neutron activation, or gas chromatography techniques. The sample preparations are often time consuming, subject to numerous sources of error, and complicated by the low concentrations of mercury. Differentiation of mercury compounds usually necessitates selective extraction followed by gas chromatographic analysis.

MERCURY IN FOOD

Man is exposed to mercury through air he breathes, water he drinks, and food he eats. The concentration of mercury in foods has been considered to present the greatest toxicological danger to the average citizen, however, Lu (*152*) reported the average level of mercury in foods is quite low, approximately 0.02 ppm. Considerable variation in the level of mercury in foods occurs, depending on the type of food, the environmental level of mercury in the area where the food is produced, and the use of mercury containing compounds for agricultural and industrial production of the food. Pathways by which mercury levels in foods may be increased are numerous, becoming more complex as the length of the food chain increases.

Excellent reviews regarding the uptake and movement of mercury in plants were written by Smart (*210*) and D'Itri (*57*). A debated issue has been the translocation of mercury from treated seeds to harvested grains. Westöö (*252*) reported two to three times higher levels of mercury in eggs from chickens fed grain grown from methyl mercury treated seed. Levels of mercury in eggs from chickens fed grain grown from methoxyethyl mercury-treated seed were no higher than noted from chickens fed grain grown from untreated seed (*252*). The study group on mercury hazards (*171*) questioned that the 20 ppm mercury content of the treated seed could contribute significantly to the concentration of mercury in the grain because of the tremendous dilution factor involved. Mercury on the seed would be transported and deposited throughout the leaves, straw, and grain. Furthermore, Saha et al. (*203*) found no significant differences in the content of mercury in wheat or barley grown from untreated seed and seed treated with methyl mercury and ethyl mercury compounds. These authors (*203*) reported increases in the mercury content of grain grown in soil heavily treated with methyl mercury, therefore, proving that absorption and translocation of mercury can occur in plants. Smart (*210*), D'Itri (*57*), and the Study Group on Mercury Hazards (*171*) concluded that translocation following foliar application of mercury compounds may be more significant than translocation from seeds. Residue levels in crops treated with foliar sprays are four to six times higher than crops not sprayed (*57, 171*). Foliar application of mercury compounds to plants was discussed previously with reference to agricultural uses of mercury.

The relatively low levels of mercury in plant tissue may become significant if the crop is part of a long food chain. Biological species have demonstrated an ability to concentrate mercury, the extent of the phenomenon being somewhat dependent upon the form of the mercury involved (*183*). Alkylmercury compounds are concentrated to a greater extent by most species. The report of mercury content of eggs from chickens fed grain grown from mercury treated seed would represent such a food chain (grain to chicken to man) (*252*). Peakall and Lovett (*183*) stated that biological concentration factors for terrestrial food chains were generally on the order of two to three, however, in aquatic food chains, concentration factors of hundreds and thousands were observed.

Goldwater (*93*) illustrated the effects of mercury in the aquatic food chain which may be summarized as follows: (*a*) marine species of plants, algae, and fish concentrate to a higher level than is found in the water; and (*b*) species which feed on this marine life also concentrate the mercury to higher levels than were present in the original organism. Fimreite et al. (*70*) observed increasing concentrations of mercury in aquatic food chains and

[1]*Contribution from the College of Agricultural and Life Sciences.*
[2]*Part I of this review appeared in the May issue of this Journal.*

highest concentrations of mercury were found in the species at the higher trophic levels. Hannerz (*101*) collected specimens of various water plants, algae, moss, and invertebrates. Comparing the ratio of mercury in various tissues to that of the water, concentration factors at approximately 30 days ranged as follows: 3 for the emergent portion of plants to 560 for invertebrates in mercuric chloride treated ponds; 4 for the emergent portion of plants to 1990 for the invertebrates in methoxyethylmercury-treated ponds; 9 for the emergent portion of plants to 4200 for invertebrates in phenylmercury treated ponds; 8 for the emergent portion of plants to 8470 for invertebrates in methyl mercury treated ponds. Hannerz (*101*) also studied the concentration of mercury in various body components of cod and pike which received mercury from water or their food. After two days, the mean concentration of mercury in cod was 388 times that of water and 118 times that of food for methoxyethylmercury, 606 times that of water, and 1107 times that of food for methyl mercury. Pike, after 3 days, had a mean concentration of mercury that was 783 times that of water and 70 times that of food contaminated with methoxyethylmercury, and 750 times that of water and 367 times that of food contaminated with methyl mercury. Johnels and Westermark (*123*) reported the concentration factor of pike is 3000 or more, comparing the concentration of mercury in the tissue to that in the water. They reported a linear relationship existing between the age or weight of the pike and the mercury concentration of the tissues; however, in waters with high mercury pollution, extent of exposure was more influential than age or weight.

The concentration effect of the aquatic food chain also may be extended to species for which fish is the primary diet. Dustman et al. (*59*) reported mercury analyses of Great Blue Heron gave carcass levels of 21.2-23.0 ppm mercury compared to concentrations of 1.8-3.6 ppm mercury in the fish found in their stomachs. In a Common Tern, the carcass level of mercury was 7.5 ppm and the fish found in the stomach contained 3.8 ppm mercury (*59*).

Concentration of mercury in foods

Many studies have been done to determine the mercury content of common food items; however, some of these studies have been discredited after other laboratories analyzing the same food samples found considerably less mercury (*136*). These incidents emphasize the need for development of better methods of analysis, including sampling techniques, for mercury levels in foods. A listing of the mercury concentration of a number of food items determined over a period of years is shown in Table 1. It is interesting to note that the mercury concentration of most of these food items has not changed significantly during this time period. The mercury concentration in food will vary somewhat, depending on the areas in which it is produced. Corneliussen (*50*) and Kirkpatrick and Coffin (*130*) reported the results of a market basket survey from regions of the United States and Canada. Results of mercury analysis done on composite samples of market basket items are presented in Table 2.

It is unfortunate that very few data are available on the chemical form of the mercury which is present in most foods. Methods for differentiating the chemical forms of mercury with reasonable accuracy have been developed only recently and are somewhat more complicated to perform than total mercury analysis. Westöö (*250, 252*) analyzed some food samples for methyl mercury and total mercury; these results are summarized in Table 3. Lu (*152*) stated that the mercury present in most other food items other than fish is believed to be in the form of inorganic mercury.

A major concern has been expressed regarding the concentration of mercury in fish. It is difficult to assess

TABLE 1. *Concentration of mercury in foods.*

	Germany 1934-1938	U.S.[c] 1940	U.S.[d] 1964	Japan[e] 1964
	(ppm Mercury)			
Cereals	.02 – .036[a]	.002 – .006	.002 – .025	.012 – .048
Dairy products				
Milk	.0006 – .004[a]	.003 – .007	.008	.003 – .007
Butter	.07 – .28[b]		.14	
Cheese	.009 – .01[a]		.08	
Egg products				
Whole	.002[a,b]			
White			.01	.08 – .125
Yolk			.062	.33 – .67
Fish	.02 – .18[a]	.0016 – .014	0 – .06	.035 – .54
Fruit	.005 – .035[b]		.004 – .03	.018
Meats	.001 – .067[a]	.0008 – .044	.001 – .15	.31 – .36
Vegetables	.002 – .044[a]	.005 – .025	0 – .02	.03 – .06

[a]Data from reference *217*.
[b]Data from reference *218*.
[c]Data from reference *89*.
[d]Data from reference *92*.
[e]Data from reference *84*.

TABLE 2. *Mercury concentrations in U.S. and Canadian foods*

Food class	Boston[1], U.S.	Kansas City[1], U.S.	L.A.[1], U.S.	Baltimore[1], U.S.	Minneapolis[1], U.S.	Van Couver[2], Can.	Halifax[3], Can.
	(ppm Mercury)						
Dairy products	0.02	0.004	0.002	0.002	0.01	0.001	0.002
Meat, fish, poultry	0.05	0.04	0.04	0.01	0.04	0.051	0.028
Grain and cereal	0.03	0.02	0.05	0.03	0.04	0.004	0.002
Potatoes	0.01	0.006	0.02	0.006	0.01	0.001	0.001
Legumes	0.01	0.01	0.01	0.005	0.002	0.001	0.001
Beverages	0.002	0.002	0.002	0.006	0.006	N.D.	<0.001

[1]Samples collected June 1967-Apr. 1968. Reference *50*.
[2]Samples collected in 1970. Reference *130*.
[3]Samples collected in 1971. Reference *130*.

TABLE 3. *Mercury and methylmercury content of some foods*

Food	Total mercury	Methylmercury (% of total mercury)		Reference
	(Limits)	(Limits)	(Average)	
Meats				
Ox	0.075	92	92	250
Hen	0.023-0.051	73-74	74	250
Pork				
Liver	0.096-0.140	68-78	73	250
Liver	0.011-0.049	45-86	67	252
Kidney	0.014-0.086	24-71	50	252
Chop	0.006-0.016	80-100	88	252
Eggs				
Egg yolk	0.010	50-90	70	252
Egg white	0.012-0.025	76-96	89	250
Fish-muscle tissue				
Perch	0.22 -3.25	83-93	89	250
Pike	0.56 -3.35	88-98	94	250
Haddock	0.033-0.052	76-83	80	250
Cod	0.026-0.036	78-85	82	250

an average level of mercury in fish since the concentration of mercury will vary significantly depending on the following: species of fish, diet, age, size, and location in its environment (*187*). The Joint FAO/WHO Committee (*69*) stated that 99% of the world's fish catch has a total mercury content not exceeding 0.5 mg/kg and that 95% probably contains less than 0.3 mg/kg. Tuna has been carefully screened; the average tuna apparently contains less than 0.5 ppm mercury. FDA scientists reported large tuna fish, weighing more than 23 kg, average about 0.25 ppm and smaller tuna weighing less than 12 kg, average 0.13 ppm (*100*). Swordfish is the only species of ocean fish which has been banned from U.S. markets, now being sold only after individual fish certification (*3*). The report by Miller et al. (*164*) indicates that the mercury concentration of these deep sea fish may not have significantly changed in the past 100 years, since museum specimens contain mercury levels similar to that found in current catches.

Fish caught in the coastal waters and inland waterways of Sweden often contain much higher levels of mercury than deep sea fish (*252*). Frequently, these fish were found to contain between 1-5 ppm (*252*). Johnels and Westermark (*123*) reported that a slight elevation of the mercury content of fish can result from runoff after agricultural uses of mercury. More significant increases occur in lakes near industrial areas where aerial fallout may occur. Also, thehighest levels of mercury occurred in lakes where mercury-containing pollutants were added. Bligh (*24*) discussed the mercury concentrations in Canadian freshwater fish; also he reported more than one million pounds of fish were destroyed during the 3- or 4-month period after the Canadians became aware of the mercury problem. Greig and Seagran (*98*) reported that fish from Lake St. Clair and the western basin of Lake Erie contain from 0.4-3.0 ppm mercury, depending on the species. In Lake Huron the mercury content of fish reportedly was lower (*98*). Newberne (*172*) discussed some studies of the mercury concentration of fish in the United States. These reports indicate a substantial number of fish collected from many areas of the United Staes exceed the 0.5 ppm guideline for mercury established for fish by the Food and Drug Administration. Celeste and Shane (*37*) reported that more than 900 samples representing 37 species of fish were collected from 28 bodies of water with known or suspected pollution. About 25% of these samples contained mercury at or above the 0.5 ppm guideline level.

The danger of mercury contained in fish is undoubtedly dependent upon the chemical form in which mercury in fish exists. Westöö (*249, 250*) reported that

nearly all the mercury present in fish existed as methyl mercury. Peterson et al. (*187*) indicated that several studies have found that a significant portion of the mercury in fish may be in the inorganic form and that the ratio of organic mercury to total mercury may not be consistent among or within species. Bache et al. (*5*) reported the percentage of mercury in the methyl form increased in lake trout in relation to the age of the fish. In fish 1 year old, 31-35% of the total mercury was methyl mercury; 67-88% of the mercury in 12-year old fish was methyl mercury. Clarification of the differences is needed. Literature reviews of mercury in fish were published by Newberne (*172*) and Peterson et al. (*187*).

Established tolerance levels for mercury in food

Recognition of the toxic nature of mercury compounds and the awareness of their presence in foods have prompted the establishment of regulations by a number of governments to protect their citizens. Because fish seem to present the greatest threat, this food item has received the most attention. Action has ranged from establishing maximum permissible levels of mercury in fish, to recommending restricted consumption of fish. Based on the results of the Swedish studies by Westöö (*249, 250*), which indicated that most mercury in fish is in one form, methyl mercury; most regulations are expressed in terms of total mercury. Determination of total mercury is easier and probably more reliable than are methods which determine different mercury compounds.

In 1967, the Swedish government established a practical residue limit of 1 mg/kg for mercury in fish. Berglund and Berlin (*13*) explained the basis on which this limit was established. Factors considered were the following: (*a*) concentration of methyl mercury in different foods, (*b*) intake of methyl mercury-contaminated foods by the population, (*c*) relationship of intake and body levels of methyl mercury, and (*d*) toxic levels of methyl mercury for man. Two allowable daily intakes (ADI) were established: 0.1 mg Hg/day based on the estimated mercury intake via fish, and the mercury concentration of the erythrocytes of a healthy subject to which a 10-fold safety factor was added; 0.06 mg Hg/day based on the critical concentration of mercury in the brain, body distribution, excretion rates, and a 10-fold safety factor. Whereas many have criticized the maximum tolerance permitted in fish by the Swedish government, 1 mg/kg, as being too high, Berglund and Berlin concluded this limit is probably appropriate. They stated that the average Swedish citizen would consume the lower ADI if all fish contained 1 mg Hg/kg; however, a large percentage of the fish consumed in Sweden are caught from the ocean and contain less than 0.15 mg Hg/kg. The Swedish government has recommended that the population limit consumption of fish captured from potentially dangerous areas, but establishing a limit of less than 1 mg Hg/kg would have necessitated a ban on commercial fishing in more than half of the Swedish fishing area.

In May 1969, FDA established an action guideline of 0.5 mg Hg/kg in fish and shellfish, a value which was based on the toxicological information and analytical methodology which was available at that time (*37*). Ingestion of 30 μg of methyl mercury daily was considered the highest safe level (*172*). Canada has also established the 0.5 mg Hg/kg level for fish. Goldwater (*93*) reported that the FAO/WHO recommended a maximum level of 0.05 mg Hg/kg in food items other than fish.

If the ADI of 0.06 mg/day is accurate, a daily consumption of 120 g of fish containing 0.5 mg Hg/kg would be safe. If 0.03 mg/day is the maximum safe level, only 60 g of fish containing 0.5 mg Hg/kg would be safe. The 0.06 mg/day ADI, however, is also higher than the recent FAO/WHO recommended provisional tolerable intake. The Joint FAO/WHO Expert Committee on Food Additives in their sixteenth report (*69*) recommended a provisional tolerable intake of 0.3 mg total mercury weekly of which not more than 0.2 mg should be in the methyl mercury form (expressed as mercury). Expressed on a weight basis, tolerable weekly intakes are 0.005 mg/kg body weight for total mercury and 0.0033 mg/kg body weight for methyl mercury expressed as mercury. The Committee based these recommendations on data collected from patients at Niigata, Japan which indicated the lowest mercury levels at the onset of poisoning symptoms were 50 μg/g for hair and 0.4 μg/g for red blood cells. The estimated mercury intake of these patients was 0.3 mg Hg/day, primarily in the methyl mercury form, over a long period.

If the majority of mercury in fish exists as methyl mercury, 57 g of fish containing 0.5 mg/kg could be consumed daily without exceeding the weekly tolerable intake of 0.2 mg methyl mercury. The FAO (*68*) reported the per capita fish consumption was 56 g/day in Sweden, 18 g/day in U.S.A., and 88 g/day in Japan. Mercury poisoning in the U.S.A. does not appear likely unless an individual consumed fish in a large excess to that of the average population.

Finch (*71*) published an evaluation of the mercury intakes that a sample population would incur at different hypothetical guidelines. The evaluation was based on the results of a survey of 1586 households, a total of 4864 persons, who kept accurate records of all fish and shellfish consumption during a 12-month period. Results showed a total of 52 principal kinds of fish had been consumed in varying amounts. Using data from numerous reliable sources, the average mercury level of each type of fish was calculated on the basis of the following hypothetical guideline: (*a*) no limit on the mercury content of fish; (*b*) fish containing more than 1.5 ppm mercury would be removed from the market, (*c*) fish containing more than 1.0 ppm mercury would be removed from the market; (*d*) fish containing more than 0.75 ppm mercury would be removed from the market; and (*e*) fish containing more than 0.5 ppm mercury would be removed from the market. The estimated

TABLE 4. *Summary of intake estimates using data base 1 (μg/day)*[1]

Guideline base	0.1%[2] level	1.0%[2] level	Average level	Intake levels of individual in four highest families			
None	30	17	2.48	31.7	29.2	26.6	26.4
1.5 ppm	26	15	2.28	27.2	25.5	24.1	23.0
1.0 ppm	25	15	2.23	24.6	24.0	23.4	23.0
0.75 ppm	21	15	2.17	23.5	22.9	20.9	20.6
0.5 ppm	20	14	1.99	21.3	20.1	20.0	19.0

[1]From reference *71*.
[2]Intake levels in μg/day exceeded by only 0.1% and 1.0% of the test group.

TABLE 5. *Effect of eliminating swordfish on mercury intakes (μg/day)*[1]

Basis	0.1%[2] level	1.0%[2] level	Average level	Intake levels of individuals in the four highest families			
No guideline	30	17	2.48	31.7	29.2	26.6	26.4
As above but eliminate swordfish	24	15	2.22	26.6	26.4	23.5	23.2

[1]From reference *71*.
[2]Intake levels in μg/day exceeded by only 0.1% and 1.0% of the test group.

mercury intakes which whould have occurred under each hypothetical guideline were calculated for the members of the survey and are summarized in Table 4. Finch also calculated the intakes which would have occurred with no guidelines except that swordfish would be eliminated from the diet. The results are presented in Table 5. Whereas one family would have exceeded the 30 μg Hg/day ADI limit if no restrictions were placed on the mercury content of fish; removing swordfish from their diet would have reduced their mercury intake to less than 30 μg Hg/day. Newberne stated that the group surveyed may not have included individuals such as "Weight Watchers" whose fish consumption was far in excess of the average fish consumer (*172*).

It is recognized that the safety margin for the established tolerance levels of mercury are not as great as those for many other toxic residues. The possibility remains that some manifestations of low level consumption of mercury may not be recognized. Lu (*152*) stated that strict guidelines have not been established in fear of depriving a number of people of an important source of protein as well as bringing undue hardship on people dependent upon the fishing industry.

METHODS OF MERCURY ANALYSIS

The amount of literature on mercury analysis is overwhelming. Smith (*212*) included a bibliography of about 460 papers in his review of the literature on mercury analysis, and stated more articles could have been listed. Reviews of mercury analysis have also been published by D'Itri (*57*), Krenkel (*136*), Fishbein (*72, 73*), the Study Group on Mercury Hazards (*171*), Friberg et al. (*81*), and Lindstedt and Skerfving (*147*). Briefly, the four most common and reliable methods of detection are the colorimetric dithizone procedure, atomic absorption and emission spectrophotometry, neutron activation, and gas chromatography.

The major problem of environmental mercury analysis is the low concentrations of mercury involved. As previously discussed, concentrations in the ppm and ppb range are common. Many of the characteristics of mercury are utilized in the analytical procedures; however, these same characteristics also present problems during other stages of analysis. The strong affinity of mercury for sulfhydryl groups is a basis for colorimetric analysis by the dithizone procedure. Westöö (*250*) used cysteine to form a water soluble cysteine-mercury complex to aid in separating methyl mercury from interfering compounds. Sulfide-impregnated filter pads have been used to collect and concentrate mercury compounds for analysis (*11, 182*). The affinity of mercury for sulfhydryl compounds also necessitates that the mercury be separated from the sample before most methods of detection can be employed. This is most often accomplished by a wet oxidation treatment in an aqueous solution. Total combustion of the sample at elevated temperatures is also employed. Because of the volatility of mercury, extreme care must be taken to avoid losses. Volatility of mercury compounds, however, is an essential characteristic for detection by cold vapor atomic absorption, emission spectrophotometry and gas chromatography.

Gorsuch (*95*) evaluated some wet oxidation techniques and reported that substantial losses of mercury occurred during acid digestion. The Association of Official Analytical Chemists (AOAC) (*4*) recommends the use of nitric and sulfuric acids for oxidation of samples. The apparatus used for this procedure is equipped with a reflux condenser to minimize volatilization losses of mercury. Fatty acids and aromatic compounds, which are not digested by this procedure, should be filtered from the solution before analysis. Some other wet oxidation mixtures employ sulfuric acid and a 50% solution of hydrogen peroxide (*95*), perchloric acid (*166, 195, 211*), potassium permanganate (*7, 146, 232*) and potassium persulfate-potassium permanganate (*115*).

Following digestion, the mercury concentration of the solution may be analyzed directly or the mercury may be concentrated before analysis.

The total combustion technique utilizes high heat to separate mercury from the sample matrix. Because of the volatility of mercury, it is quantitatively vaporized with ease. A high temperature flame to combust the sample was employed (*108, 148, 151*). Also, combustion of the sample in high temperature furnaces was reported (*106, 167*). Vapors of mercury are either collected by some means, or aspirated directly into an atomic absorption cell for detection.

Mercury collection and concentration has been accomplished by the use of sulfide-impregnated filter pads (*11, 182*), deposition of the mercury on the surface of other metals (*29, 49, 145, 189*), collecting the mercury on ion exchange resins (*12, 201*), and extraction of the compound or its derivatives into organic solvents (*250*). Should concentration be necessary, the choice of the method will be somewhat determined by the detection procedure used.

Dithizone colorimetric procedure

Until the last decade, the most routinely used method of mercury analysis was colorimetric or spectrophotometric measurement of the dithizone complex. Two official methods for mercury analysis have been presented, one by the AOAC (*4*) and the other by the Analytical Methods Committee of the Society of Analytical Chemistry (*124*). Both procedures require addition of hydroxylamine hydrochloride to reduce the remaining oxidizing materials from the wet oxidation procedure. By the AOAC procedure, mercury is extracted from the solution by adding dithizone in chloroform. The Analytical Methods Committee recommended use of dithizone in carbon tetrachloride. Since copper also reacts with dithizone and may be present in significant concentrations, AOAC recommended addition of sodium thiosulfate to the chloroform layer. A mercury thiosulfate complex which is water soluble is formed, whereas the copper-dithizone complex is stable permitting copper removal with chloroform. After separation, the aqueous phase containing the mercury thiosulfate complex is again oxidized and the mercury extracted with dithizone in chloroform. The mercury-dithizonate is determined immediately in a spectrophotometer at 490 nm against similarly treated mercury standard solutions. The Analytical Methods Committee recommended nitrite reversion of the mercury rather than the thiosulfate reversion. After reversion, ethylene diaminetetracetic acid (EDTA) is added to bind copper, mercury is then reextracted with dithizone in carbon tetrachloride and the mercury-dithizone measured in a spectrophotometer at 485 nm.

The sensitivity of the dithizone procedure is approximately 0.05 ppm using a 10-g sample (*137*). Because of the low cost, simplicity and sensitivity of colorimetric analysis, the dithizone method has retained some popularity. The time needed for sample preparation and the possibility of interferences from other metals principally copper are disadvantages of the procedure. However, more sensitive methods have been developed.

Neutron activation analysis

The general procedure for neturon activation analysis involves sealing the sample in a quartz or polyethylene vial, irradiating the sample with neutrons to produce isotopes, and measuring the radiations from these isotopes by gamma-spectrometry. Either the ^{197}Hg or ^{203}Hg radioactive nucleotides may be measured. The initial radiation flux of ^{197}Hg is higher, however, ^{203}Hg has a longer half-life [46.57 days compared to 65 h (*240*)] and therefore may offer advantages for determinations over a period of weeks. The techniques of neutron activation analysis may be divided into two classes, nondestructive or destructive analysis, depending upon whether the constituents are separated before measuring the radiations.

Westermark and Sjöstrand (*248*) pioneered use of neutron activation analysis for measuring environmental mercury levels. The nondestructive procedure involves irradiating the sample for 2 to 3 days and detecting the 68-K.e.v. x-quanta and 77-K.e.v. γ-quanta of ^{197}Hg by gamma-spectrometry. Sjöstrand (*208*) reported the procedure had been applied successfully to the analysis of hundreds of samples of various biological and organic materials, but was limited in its sensitivity to 0.1-0.5 ppm for biological materials. Haller et al. (*99*) reported a nondestructive neutron activation procedure in which a germanium-lithium detector was utilized to separate the peaks of the many isotopes of irradiated plant tissues. The advantage of this procedure is that mercury and 14 other elements are determined simultaneously without chemical separation. The lowest concentrations of mercury detected were 13 ppb in pears using a 3.317-g sample and 25 ppb in peas using a 0.463-g sample. The authors reported that the statistical deviation was relatively high in mercury analysis, this being caused by Compton and Bremsstrahlung corrections and the necessity to correct for overlap of selenium and mercury peaks.

Sjöstrand (*208*) described a destructive method for neutron activation analysis which greatly increased sensitivity. Following irradiation, carrier solutions of $HgCl_2$ were added to the sample, and the sample was oxidized by heating in concentrated nitric acid containing 5-10% concentrated sulfuric acid in a closed system. After digestion, a mixture of perchloric acid and aqueous glycine was added, heated, and mercury was collected in the distillate by electrode position on gold foil. The chemical yield of the procedure was determined by increased weight of the foil in proportion to the quantity of carrier mercury added. The mercury content of the sample was determined by measuring the activity of ^{197}Hg and correcting for chemical yield. The sensitivity limit of the procedure was estimated as 0.5 ppb mercury

for a 0.5 g sample irradiated 2 to 3 days in a thermal neutron flux of 10^{12} N/cm^2-second.

Rottschafer et al. (*201*) dissolved irradiated samples of fish in 1:1 concentrated HNO_3-H_2SO_4, diluted the solution with 1 M HCl and added the solution to an ion exchange column. The interfering ions, particularly $^{24}Na^+$ were eluted from the column. The resin to which the mercury remained bound, was transferred to glass vials and counted. Ishida et al. (*114*) eluted interfering metal ions from a DEAE cellulose column with a solution 0.01 M in HCl and 0.01 M in ammonium thiocyanate, then mercury was eluted with 6 M HCl, reduced to elemental mercury and counted.

Brune (*32*) observed that mercury was vaporized from water solutions during the irradiation process. He developed a method which used dry ice cooling to maintain −40 C and prevent vaporization of the mercury (*33*). Brune separated the radioactive mercury from the digest by the isotope exchange technique of Kim and Silverman (*129*). Tiny droplets of liquid elemental mercury were added to the digest after the pH of the solution was partially neutralized, and dissolved mercury isotope was absorbed by the droplets.

Neutron activation is probably the most sensitive method available for mercury analysis. Destructive analysis is more sensitive and probably more reliable than nondestructive analysis (*208*). The primary disadvantages of both neutron activation procedures are cost of equipment, need for highly qualified personnel, and time required for analysis. The Study Group on Mercury Hazards (*171*) mentioned that Californium-246 may be utilized as a neutron source in the future. This would negate the need for nuclear reactors and reduce the cost of analysis.

Spectrophotometry

Atomic absorption spectrophotometry has probably become the most popular method of mercury analysis. Recently developed procedures and equipment for atomic fluorescence analysis also show great promise. Both techniques depend on the ability of mercury atoms to pass between ground and excited energy levels by absorbing or emitting radiation at the characteristic wavelength of 253.7 nm. In atomic absorption analysis the quantity of energy absorbed is measured as a cloud of elemental mercury vapor passes through a beam of ultraviolet light at 253.7 nm and the atoms are raised to an excited energy state. In fluorescence analysis the quantity of fluorescent emission from atoms in the excited energy state is measured at 253.7 nm. Both quantities are proportional to the number of mercury atoms present.

For excitation of mercury atoms to occur, it is necessary that mercury is in the elemental vapor state. In conventional flame atomic absorption, this is accomplished by atomizing a solution of the metal salt directly into a flame where decomposition and vaporization of the salt results in atoms or moledules. A beam of energy at 253.7 nm is passed through the upper regions of the flame and as mercury atoms are produced, energy is absorbed. This technique is regaining some popularity according to Krenkel (*136*) since many of the interferences encountered in wet oxidation techniques are avoided. The sensitivity of the procedure is limited because only a small amount of the sample (estimated at 10^{-5} ml) can be atomized into the optical path at any given moment (*149*). Lindstrom (*148*) stated that the limit of detection was 1 μg of mercury per liter of solution. Hingle et al. (*108*) studied some variables of the flame atomic absorption techniques, and reported that the sensitivity of detection for mercury could be increased by adding reducing or complexing agents to the solution or converting the mercuric ion to the mercurous ion before the solution is atomized into the flame.

Because of the ease by which mercury compounds can be reduced to the elemental vapor phase, techniques known as "cold vapor" and "flameless" atomic absorption spectrophotometry have been developed. By these procedures, mercury vapor is passed through a long path absorption cell which is placed in the path of the light between the energy source and the photomultiplier tube. Although both terms are often used interchangeably, generally "cold vapor analysis" refers to the technique in which mercury ions in solution are reduced chemically to the elemental state. The mercury may then be quantitatively removed from the solution and passed through the absorption cell by aerating the solution. The flameless technique refers to a method by which heat is applied to the sample causing the mercury to vaporize and pass through the absorption cell. Using the later technique, sufficient heat may be applied to reduce all forms of mercury to the elemental vapor state negating the need for chemical treatment. Both techniques offer greater sensitivity than flame analysis, as the mercury concentration of the vapor rather than the solution is detected.

Hatch and Ott (*105*) published a cold vapor technique for the determination of mercury in nickel and cobalt metal and rock samples. The procedure involves acid oxidation of the sample, reduction of the mercuric ions by adding stannous sulfate to a sodium chloride-hydroxalamine sulfate medium containing a portion of the digested sample, and aeration of the mercury vapor directly from the medium through the absorption cell by means of a circulating pump. Hoover et al. (*110*) reported more accurate results were obtained if the reduction-aeration-expulsion cycle was repeated for each solution until the absorbance for the last cycle was near zero. They stated that an equilibrium existed between mercury in vapor and mercury in solution and was dependent upon the volume ratios of each and the sample being analyzed. Moreover, addition of a small amount of hydrazine sulfate facilitated the reduction reaction with stannous chloride. Iskandar et al. (*115*) reported the lower limit of detection for mercury in sediment and soils by their procedure was 0.01 μg Hg per gram of sample. The technique involved digestion of the

sample by nitric and sulfuric acids, oxidation with potassium permanganate and potassium persulfate, reduction of the mercuric ions with stannous chloride, and direct measurement of the mercury content of the vapor aerated from the solution.

Whereas Clarkson and Greenwood (*45*) reported that stannous chloride reduced only the mercury which was in the inorganic form, Magos (*157*) reported that a mixture of cadmium chloride, stannous chloride, and sodium hydroxide added to an acidified homogenate of the sample reduced both organic and inorganic mercury ions.

The "flameless" technique by which the sample is completely combusted, avoids many of the interferences encountered through wet oxidation (*136*). The following procedures for "flameless" atomic absorption analysis of mercury were cited from the Study Group of Mercury Hazards (*171*). Brandenberger and Bader (*29*) burned the sample in a large closed flask in the presence of oxygen. The combustion products were absorbed in dilute acid, and the mercury was electro-deposited from the acid solution onto a copper wire. The copper wire was removed from the solution, placed in a side arm of the absorption cell, and the mercury was vaporized by electrically heating the wire. Lidums and Ulfvarson (*145*) reportedly passed the combustion gases over gold foil which collected the mercury. The gold foil was then heated, causing a rapid vaporization of the mercury into the absorption cell. Using sample sizes of less than 1 g, Lidums and Ulfvarson could detect mercury concentrations below 1 ppb.

Some interferences are encountered when using atomic absorption analysis. Rains and Menis (*195*) reported that anions of all acids affect the release of mercury by the stannous chloride reduction, aeration technique. Iodide, bromide, sulfate, and phosphate cause the greatest reduction in the amount of mercury vaporized. Ballard et al. (*11*) reported that benzene, pyridine, and acetone absorb energy at 253.7 nm. Wenninger (*245*) reported that sulfur dioxide, nitric oxide, nitrogen dioxide, and hydrogen sulfide absorb strongly in the 253.7 nm range.

The atomic fluorescence determination of mercury appears to have many advantages over atomic absorption determinations. Thompson and Reynolds (*231*) stated that the distinct advantages are simplicity since there is no enclosed cell and therefore no fogging of the cell windows, no spurious signal from broad band absorption by organic contaminants, no recirculating system or drying column required, sensitivity is high, and the standard curve is linear over a wide concentration range. Thompson and Reynolds reported that the presence of acetone, ethanol, chloroform, or benzene in the sample solution resulted in a reduction in the peak height recorded for a given concentration of mercury. The reduction, which they believed was caused by quenching of excited mercury atoms by the solvent, was larger when argon was used as the carrier gas than when air was employed.

Corcoran (*49*) reported that this interference could be removed by first separating the mercury by amalgamation, then revaporizing the mercury by heat. In the absence of organic solvents, the peak height with argon was four times greater than that with nitrogen and 35 times greater than that with air (*231*). Muscat et al. (*167*) reported that there was good agreement of results obtained by the reduction-aeration procedure using stannous chloride as the reductant and the furnace combustion technique in which the sample was heated to 800 C. In both methods, mercury was collected on a silver amalgamator, revaporized by heat, and flushed into the fluorescence cell by the carrier gas.

Thomerson and Thompson (*230*) reported that instrumentation is available to permit detection of less than 0.5 mg mercury in the vapor produced by the stannous chloride reduction-aeration technique. He stated that the equipment involved was relatively inexpensive. Corcoran (*49*) made five replicate measurements of 0.5 mg mercury which gave a standard deviation of 16%.

Chromatography

The use of gas liquid chromatography (GLC) has gained wide acceptance for the analysis of organic mercury compounds. Methods which have practical application for analysis of inorganic mercury compounds by GLC have yet to be developed. An excellent review of the literature pertinent to GLC analysis of mercury compounds was recently published by Mushak (*168*).

Most procedures utilize a gas chromatograph equipped with an electron capture detector (*249, 250*), of either the tritium (^{3}H) or radioactive nickel (^{63}Ni) type. Sensitivity approaching 1 ppb is possible with favorable conditions (*250, 251*). For detection to occur with this system, it is necessary that the organomercurial forms an unsymmetrical halide (bromide, chloride, or iodide). Dialkyl or diaryl mercury compounds will not be recorded by an electron capture detector.

Other detectors have been used for GLC analysis of organomercurials. Bache and Lisk (*6*) reported detection of mercurials by emission spectrophotometry in a microwave powered inert gas plasma. With this system, it is possible to detect diorganomercurials also, and the specificity of the detector for mercury compounds is greater than the electron capture detector. Longbottom (*151*) used an atomic absorption system to detect mercury compounds separated by GLC. The organomercury compounds were reduced to the elemental form by the flame ionization detector of the gas chromatograph or by passing the column gases through a combustion furnace. The combustion gases were then pumped directly through the absorption cell for detection.

A number of different liquid phases have been used for GLC analysis of organic mercury compounds. Westöö (*249*) reported good separation was obtained using Carbowax 20 M in a column heated at 130-145 C and

purged with nitrogen. Methyl mercury compounds had the shortest retention time, followed by ethyl mercury, methoxyethyl mercury, and phenyl mercury compounds. Bache and Lisk (*6*) reported that OV-101 slowed elution of the volatile dimethyl mercury. However, dimethyl mercury was eluted after the monomethylmercury compounds. Tatton and Wagstaffe (*229*) reported that the most satisfactory column consisted of 2% polyethylene glycol succinate on Chromasorb G. The order of elution was methyl mercury, ethyl mercury, ethoxyethyl mercury, methoxyethyl mercury, tolyl mercury, and phenyl mercury.

Several authors have noted that the same retention time is observed for the various salts of a particular organomercurial ion. Johansson et al. (*122*) used a combined GLC-mass spectrometer to study the phenomenon, and they concluded that rearrangement of the anions occurred on the column. Dressman (*58*) reported that during GLC analysis of several phenyl mercury compounds, diphenyl mercury and phenyl mercury chloride were formed on the column. The relative amount of each compound varied with the concentration of chloride ions present in the injection block. In view of this problem, Dressman recommended that all aryl mercury compounds be converted to the aryl mercury chloride prior to GLC analysis.

Thin-layer chromatography has received considerable attention for analysis of organic mercury compounds. It has been used to purify organomercury compounds before GLC analysis as well as serving as a qualitative verification of the GLC analysis. Ion exchange and column chromatography have also been used to separate mercury compounds. Use of chromatography procedures for mercury analyses has been extensively reviewed by Fishbein (*72, 73*).

Methods of separation of organomercury compounds

Most procedures for separating organomercury compounds have been based on the methods of Westöö (*249, 250, 251*). After a number of changes, the procedure most commonly employed may be briefly described as follows (*251*) (*a*) Hydrolysis of the sample homogenate is accomplished with hydrochloric acid. For some samples, inorganic mercuric chloride is added to the acidified homogenate. Through binding site competition, this aids in liberating methyl mercury from sulfhydryl groups. For liver samples, the proteins are precipitated with molybdic acid rather than adding mercuric chloride. This procedure was adopted when it was discovered that methyl mercury was synthesized from mercuric ions by the liver (*251*). (*b*) Organic mercury compounds are extracted from the acidified homogenate with benzene. (*c*) To separate organomercury compounds from interfering compounds also extracted by benzene, a water soluble cysteine-organomercury complex is formed by adding an aqueous cysteine salt solution to benzene. (*d*) After separating the aqueous phase, hydrolysis with HC1 is repeated and organic mercury is reextracted with benzene. The final benzene solution is ready for analysis by GLC, thin-layer chromatography (TLC), atomic absorption or emission spectrophotometry after appropriate pretreatment, or neutron activation. Westöö reported greater than 90% recovery of added methyl mercury in samples of fish, egg white, kidney, blood, meat, bile, and moss. Kamps and McMahon (*127*) reported an average recovery of 79.5% in fish samples that the FDA analyzed using this procedure. They reported that losses probably result from partition coefficients which are sufficiently reproducible for a given sample to allow correction factors to be used.

Tatton and Wagstaffe (*229*) reported alkoxyalkyl mercury compounds are very unstable in even dilute acid solutions, therefore, the procedure of Westöö would be unsatisfactory for their analyses. The procedure which they proposed used a slightly alkaline solution of cysteine hydrochloride in isopropanol to extract all organomercurials from the sample homogenate. The extract was washed with diethyl ether or toluene, and finally, the organomercurials were extracted with a diethyl ether solution of dithizone. Recovery of 85-95% of the added organomercury compounds was reported for samples of potatoes, tomatoes, and apples.

Magos (*157*) reported a method for determining organic mercury, inorganic mercury, and total mercury contents of samples. Treatment of the acidified sample homogenate with a mixture of stannous chloride and cadmium chloride reduced both inorganic and organic mercury compounds to elemental mercury. Then, the total mercury content of the sample was determined by cold vapor atomic absorption spectrophotometry. Treatment of a duplicate sample of the acidified homogenate with stannous chloride only, reduced inorganic mercury to the elemental form but not the organomercurials. Cold vapor atomic absorption analysis of this sample indicated the concentration of inorganic mercury present. Organomercury concentration then is calculated by the difference of total and inorganic mercury concentration. The major advantage of the procedure is that both forms of mercury are determined by relatively the same procedure, however, differentiation of the organic mercury compounds is not possible.

CONCLUSION

Man must live with mercury. It is a natural element which is distributed throughout the various strata of the earth. For toxicological and epidemiological reasons, it is important that man's activities do not significantly alter the natural distribution of mercury. The chemical form of mercury as well as the concentration of mercury influence the toxicologic response. Alkyl mercury compounds, which are likely to be the most dangerous mercury compounds in the environment are produced by chemical reaction. Research has also shown that certain biological species have the ability to synthesize methyl mercury from inorganic forms of mercury. Because the extent to which this synthesis occurs in nature is

unknown, pollution of the environment with any form of mercury must be avoided.

Whereas much information has been compiled on the response of various species to mercury compounds, it is a laborious task to assess the extent of exposure in all sectors of the earth's population, and to determine what ramifications will occur after prolonged exposure to particular forms of mercury. Agencies of various governments have attempted to minimize the danger by imposing tolerance limits for the concentration of mercury in food. These established limits have received criticism for being too lenient as well as for being too strict. To resolve this issue, further research is needed.

REFERENCES

1. Åberg, B., L. Ekman, R. Falk, U. Greitz, G. Persson, and J. O. Snihs. 1969. Metabolism of methyl mercury (^{203}Hg) compounds in man: Excretion and distribution. Arch. Environ. Health 19: 478-484.
3. Anon. 1971. Swordfish banned as U.S. Food-FDA. Food Pro- Paper 713:1-5. U.S. Government Printing Office, Washington, D.C.
3. Amon. 1971. Swordfish banned as U.S. Food-FDA. Food Processing 32(6):14.
4. Association of Official Analytical Chemists. 1970. Official methods of analysis. 11th ed. Association of Official Analytical Chemists, Washington, D.C.
5. Bache, C. A., W. H. Gutenmann, and D. J. Lisk, 1971. Residues of total mercury and methylmercuric salts in lake trout as a function of age. Science 172:951-952.
6. Bache, C. A., and D. J. Lisk. 1971. Gas chromatographic determination of organic mercury compounds by emission spectrometry in a helium plasma. Anal. Chem. 43:950-952.
7. Bailey, B. W., and F. C. Lo. 1971. Automated method for determination of mercury. Anal. Chem. 43:1525-1526.
8. Bailey, E. H., and R. M. Smith. 1964. Mercury, its occurrence and economical trends. U.S. Geol. Surv. Circ. 496, U.S. Government Printing Office, Washington, D.C. 11 p.
9. Bailey, E. H., P. D. Snavely, Jr., and D. E. White. 1961. Chemical analysis of brines and crude oil, Cymric Field, Kern County, California. Geol. Surv. Res. 1961 (Article 398), U.S. Geol. Surv. Prof. Paper 424D:D306-309. U.S. Government Printing Office, Washington, D.C.
10. Bakir, F., S. F. Damluji, L. Amin-Zaki, M. Murtadha, A. Khalidi, N.Y. Al-Rawi, S. Tikriti, H. I. Dhahir, T. W. Clarkson, J. C. Smith, and R. A. Doherty. 1973. Methylmercury poisoning in Iraq. Science 181:230-241.
11. Ballard, A. E., D. W. Stewart, W. O. Kamm, and C. W. Zuehlke. 1954. Photometric mercury analysis: correction for organic substances. Anal. Chem. 26:921-922.
12. Becknell, D. E., R. H. Marsh, and W. Allie, Jr. 1971. Use of anion exchange resin-loaded paper in the determination of trace mercury in water by neutron activation analysis. Anal. Chem. 43:1230-1233.
13. Berglund, F., and M. Berlin. 1969. Human risk evaluation for various populations in Sweden due to methylmercury in fish, p. 423-432. *In* M. W. Miller and G. G. Berg (ed.) Chemical fallout: Current research on persistent pesticides. Charles C. Thomas, Springfield, Ill.
14. Berlin, M. 1963. Renal uptake, excretion, and retention of mercury. II. A study in the rabbit during infusion of methyl and phenylmercury compounds. Arch. Environ. Health 6:626-633.
15. Berlin, M., J. Fazackerly, and G. Nordberg. 1969. The uptake of mercury in the brains of mammals exposed to mercury vapor and to mercuric salts. Arch. Environ. Health 18:719-729.
16. Berlin, M., and S. Gibson. 1963. Renal uptake, excretion, and retention of mercury. I. A study in the rabbit during infusion of mercuric chloride. Arch. Environ. Health 6:617-625.
17. Berlin, M., and S. Ullberg. 1963. Accumulation and retention of mercury in the mouse. I. An autoradiographic study after a single intravenous injection of mercuric chloride. Arch. Envrion. Health 6:589-601.
18. Berlin, M., and S. Ullberg. 1963. Accumulation and retention of mercury in the mouse. II. An autoradiographic comparison of phenylmercuric acetate with inorganic mercury. Arch. Environ. Health 6:602-609.
19. Berlin, M., and S. Ullberg. 1963. Accumulation and retention of mercury in the mouse. III. An autoradiographic comparison of methylmercuric dicyandiamide with inorganic mercury. Arch. Environ. Health 6:610-616.
20. Bertine, K. K., and E. D. Goldberg. 1971. Fossil fuel combustion and the major sedimentary cycle. Science 173:233-235.
21. Bidstrup, P. L. 1964. Toxicity of mercury and its compounds. Elsevier Pub. Co., New York. 112 p.
22. Biram, J. G. S. 1957. Some aspects of handling mercury. Vacuum 5:77-92.
23. Birkhaug, K. E. 1933. Phenyl-mercuric-nitrate. J. Infect. Dis. 53:250-261.
24. Bligh, E. G. 1972. Mercury in Canadian fish. Can. Inst. Food Sci. Technol. J. 5(1):A6-A14.
25. Borg, K., H. Wanntorp, K. Erne, and E. Hanko. 1966. Mercury poisoning in Swedish wildlife. J. Appl. Ecol. (Suppl.) 3:171-172.
26. Bornmann, G., G. Henke, H. Alfes, and H. Möllmann. 1970. Über die enterale Resorption von metallischem Quecksilber. Arch. Toxicol. 26:203-209.
27. Bowen, H. J. M. 1966. Trace elements in biochemistry. Academic Press, New York. 241 p.
28. Bradley, R. L., Jr. 1970. Mercury hearing before the State of Wisconsin Department of Natural Resources. October 12.
29. Brandenberger, H., and H. Bader. 1967. The determination of nanogram levels of mercury in solution by a flameless atomic absorption technique. Helv. Chem. Acta 50:1409-1415.
30. Brown, J. R., and M. V. Kulkarni. 1967. A review of the toxicity and metabolism of mercury and its compounds. Med. Serv. J. Can. 23:786-808.
31. Brubaker, P. E., G. W. Lucier, and R. Klein. 1971. The effects of methylmercury on protein synthesis in rat liver. Biochem. Biophys. Res. Commun. 44:1552-1558.
32. Brune, D. 1966. Low temperature irradiation applied to neutron activation analysis of mercury in human whole blood. Acta Chem. Scand. 20:1200-1202.
33. Brune, D. 1969. Aspects of low-temperature irradiation in neutron activation analysis. Anal. Chim. Acta 44:15-20.
34. Burton, J. D., and T. M. Leatherland. 1971. Mercury in a coastal marine environment. Nature 231:440-442.
35. Byfield, A. 1970. Mercury hearing before the State of Wisconsin Department of Natural Resources. October 12.
36. Cammaroto, V. A., Jr. 1974. Mercury. p. 771-781. *In* U.S. Department of Interior, Bureau of Mines. Minerals Yearbook 1972. Vol. 1. U.S. Government Printing Office, Washington, D.C.
37. Celeste, A. C., and C. G. Shane. 1970. Mercury in fish. FDA Papers 4(9):27-29.
38. Cember, H. 1969. A model for the kinetics of mercury elimination. Amer. Ind. Hyg. Ass. J. 30:367-371.
39. Cember, H., and A. Donagi. 1963. The influence of dose level and chemical form on the dynamics of mercury elimination. XIV International Congress on Occupational Health. Excerpta Med. Int. Cong. Ser. 62:440-442.
40. Chang, L. W., P. A. Desnoyers, and H. A. Hartmann. 1972. Quantitative cytochemical studies of RNA in experimental mercury poisoning. 1. Changes in RNA content. J. Neuropathol. Exp. Neurol. 31:489-501.
41. Chang, L. W., A. H. Martin, and H. A. Hartmann. 1972. Quantitative autoradiographic study on the RNA synthesis in the neurons after mercury intoxication. Exp. Neurol. 37:62-67.
42. Clarkson, T. W. 1971. Epidemiological and experimental aspects of lead and mercury contamination of food. Food Cosmet. Toxicol. 9:229-243.

43. Clarkson, T. W. 1972. Biotransformation of organo-mercurials in mammals. p. 229-238. *In* R. Hartung and B. D. Dinman (ed.) Environmental mercury contamination. Ann Arbor Science Pub. Inc., Ann Arbor, Mich.
44. Clarkson, T. W. 1972. The pharmacology of mercury compounds. Annu. Rev. Pharmacol. 12:375-406.
45. Clarkson, T. W., and M. R. Greenwood. 1970. Selective determination of inorganic mercury in the presence of organomercurial compounds in biological material. Anal. Biochem. 37: 236-243.
46. Clarkson, T. W., and A. Rothstein. 1964. The excretion of volatile mercury by rats injected with mercuric salts. Health Phys. 10:1115-1121.
47. Clarkson, T. W., H. Small, and T. Norseth. 1971. The effect of a thiol containing resin on the gastrointestinal absorption and fecal excretion of methylmercury compounds in experimental animals. Fed. Proc. 30:543. (Abstr.)
48. Cooke, N. E., and A. Beitel. 1971. Some aspects of other sources of mercury to the environment. p. 53-62. *In* J. E. Watkin (ed.) Mercury in man's environment. Proc. Roy, Soc. Can. Symp., Ottawa, Canada.
49. Corcoran, F. L. 1974. Trace mercury determination by atomic fluorescence. Amer. Lab. 6(3):69-73.
50. Corneliussen, P. E. 1969. Residues in food and feed. Pesticide residues in total diet samples (IV). Pest. Monit. J. 2:140-152.
51. Curley, A., V. A. Sedlak, E. F. Girling, R. E. Hawk, W. F. Barthel, P. E. Pierce, and W. H. Likosky. 1971. Organic mercury identified as the cause of poisoning in humans and hogs. Science 172:65-67.
52. Daniel, J. W., and J. C. Gage. 1971. The metabolism by rats of phenylmercury acetate. Biochem. J. 122:24 p.
53. Daniel, J. W., J. C. Gage, and P. A. Lefevre. 1971. The metabolism of methoxyethyl mercury salts. Biochem. J. 121:411-415.
54. Davies, D. J., and A. Kennedy. 1967. The excretion of renal cells following necrosis of the proximal convoluted tubule. Brit. J. Exp. Pathol. 48:45-50.
55. Day, F. H. 1964. Mercury. p. 185-187. *In* F. H. Day. The chemical elements in nature. Reinhold Pub. Corp., New York.
56. Derrybury, O. M. 1972. Investigation of mercury contamination in the Tennessee Valley Region. p. 76-70. *In* R. Hartung and B.D. Dinman (ed.) Environmental mercury contamination. Ann Arbor Science Pub. Inc., Ann Arbor Mich.
57. D'Itri, F. M. 1972. The environmental mercury problem. CRC Press, Cleveland, Ohio. 124 p.
58. Dressman, R. C. 1972. The conversion of phenylmercuric salts to diphenylmercury and phenyl mercuric chloride upon gas-chromatographic injection. J. Chromatogr. Sci. 10:468-472.
59. Dustman, E. H., L. F. Stickel, and J. B. Elder. 1972. Mercury in wild animals, Lake St. Clair, 1970. p. 46-52. *In* R. Hartung and B. D. Dinman (ed.) Environmental mercury contamination. Ann Arbor Science Pub. Inc., Ann Arbor, Mich.
60. Eckert, W. 1970. Statement: Mercury use on Wisconsin golf courses, prepared for Mercury hearing before the State of Wisconsin Department of Natural Resources, October 12.
61. Ehmann, W. D., and J. F. Lovering. 1967. The abundance of mercury in meteorites and rocks by neutron activation analysis. Geochim. Cosmochim. Acta 31:357-376.
62. Ehrlich, R. 1970. How mercury pollutes. Chem. Eng. News 48 (30):18-19.
63. Ekman, L., U. Greitz, G. Persson, and B. Åberg. 1968. Metabolism and retention of methyl-mercurynitrate in man. Nord. Med. 79:450-456.
64. Ellis, R. W., and S. C. Fang. 1967. Elimination, tissue accumulation, and cellular incorporation of mercury in rats receiving an oral does of ^{203}Hg-labelled phenylmercuric acetate and mercuric acetate. Toxicol. Appl. Pharmacol. 11:104-113.
65. Engle, G. T. 1966. Mercury. p. 647-656. *In* U.S. Department of Interior, Bureau of Mines. Minerals Yearbook 1965. Vol. I. U.S. Government Printing Office, Wahington, D.C.
66. Eriksson, E. 1967. Mercury in nature. Oikos (Suppl.) 9:13.
67. Falk, R., J. O. Snihs, L. Ekman, U. Greitz, and B. Åberg. 1970. Whole body measurements on the distribution of mercury-203 in humans after oral intake of methylradiomercury nitrate. Acta Radiol. Therapy Phys. Biol. 9:55-72.
68. FAO. 1972. Food Supply, p. 435-462. *In* N. Erus (ed.) Production yearbook 1971. FAO, Rome.
69. FAO/WHO, Joint Expert Committee on Food Additives. 1972. Evaluation of mercury, lead, cadmium, and the food additives, amaranth, diethylpyrocarbonate, and octylgallate. WHO Food Additive Ser. 1972 (4):1-59.
70. Fimreite, N., W. N. Holsworth, J. A. Keith, P. A. Pearce, and I. M. Gruchy. 1971. Mercury in fish and fish-eating birds near sites of industrial contamination in Canada. Can. Field Naturalist 85:211-220.
71. Finch, R. 1973. Effects of regulatory guidelines on the intake of mercury from fish. The MECCA project. Fish. Bull. 71:615-626.
72. Fishbein, L. 1970. Chromatographic and biological aspects of organomercurials. Chromatogr. Rev. 13:83-162.
73. Fishbein, L. 1971. Chromatographic and biological aspects of inorganic mercury. Chromatogr. Rev. 15:195-238.
74. Fiskesjo, G. 1969. Some results from *Allium* tests with organic mercury halogenides. Hereditas 62:314-322.
75. Fiskesjo, G. 1970. The effect of two organic mercury compounds on human leukocytes in vitro. Hereditas 64:142-146.
76. Fitzhugh, O. G., A. A. Nelson, E. P. Laug, and F. M. Kunze. 1950. Chronic oral toxicities of mercuri-phenyl and mercuric salts. Arch. Ind. Hyg. Occup. Med. 2:433-442.
77. Fleischer, M. 1970. Summary of the literature on the inorganic geochemistry of mercury. U.S. Geol. Surv. Prof. Paper 713:6-13. U.S. Government Printing Office, Wahington, D.C.
78. Friberg, L. 1959. Studies on the metabolism of mercuric chloride and methyl mercury dicyandiamide. A. M. A. Arch. Ind. Health 20:42-49.
79. Friberg, L. 1961. On the value of measuring mercury and cadmium concentrations in urine. Pure Appl. chem. 3:289-292.
80. Friberg, L. T. (Chairman). 1969. Maximum allowable concentrations of mercury compounds. Arch. Environ. Health. 19:891-905.
81. Friberg, L. T. (Chairman). 1971. Report from an expert group. Methyl mercury in fish: A toxicologic-epidemiologic evaluation of risks. Nord. Hyg. Tidskrift (Suppl.)4:1-364.
82. Friberg, L., and G. Nordberg. 1973. Inorganic mercury—a toxicological and epidemiological appraisal, p. 5-22. *In* M. W. Miller and T. W. Clarkson (ed.) Mercury, mercurials, and mercaptans. Charles C. Thomas, Springfield, Ill.
83. Friberg, L., E. Skog, and J. E. Wahberg. 1961. Resorption of mercuric chloride and methyl mercury dicyandiamide in guinea-pigs through normal skin and skin pre-treated with acetone, alkylarylsulphonate and soap. Acta Derm. Venereol. 41:40-52.
84. Fujimura, Y. 1964. Cited from Goldwater, L. J. 1971. Mercury in the environment. Sci. Amer. 224(5):15-21.
85. Gage, J. C. 1961. The distribution and excretion of inhaled mercury vapour. Brit. J. Ind. Med. 18:287-294.
86. Gage, J. C. 1964. Distribution and Excretion of methyl and phenyl mercury salts. Brit. J. Ind. Med. 21:197-202.
87. Ganther, H. E., C. Goudie, M. L. Sunde, M. J. Kopecky, P. Wagner, S.-H. Oh, and W. G. Hoekstra. 1972. Selenium: Relation to decreased toxicity of methylmercury added to diets containing tuna. Science 175:1122-1124.
88. Ganther, H. E., and M. L. Sunde. 1974. Effect of tuna fish and selenium on the toxicity of methylmercury: A progress report. J. Food Sci. 39:1-5.
89. Gibbs, O. S. 1940. Cited from Goldwater, L. J. 1971. Mercury in the environment. Sci. Amer. 224(5):15-21.
90. Goldberg, A. A., and M. Shapero. 1957. Toxicological hazards of mercurial paints. J. Pharm. Pharmacol. 9:469-475.
91. Goldwater, L. J. 1964. Occupational exposure to mercury. The Harben lectures. J. Roy. Inst. Publ. Health Hyg. 27:279-301.

92. Goldwater, L. J. 1964. Cited from Goldwater, L. J. 1971. Mercury in the environment. Sci. Amer. 224(5):15-21.
93. Goldwater, L. J. 1971. Mercury in the environment. Sci. Amer. 224(5):15-21.
94. Goldwater, L. J. 1973. Aryl- and alkoxyalkylmercurials, p. 56-67. *In* M. W. Miller and T. W. Clarkson (ed.) Mercury, mercurials, and mercaptans. Charles C. Thomas, Springfield, Ill.
95. Gorsuch, T. T. 1959. Radiochemical investigations on the recovery for analysis of trace elements in organic and biological materials. Analyst 84:135-173.
96. Grant, N. 1971. Mercury in man. Environment 13(4):2-15.
97. Greenspoon, G. N. 1970. Mercury, p. 639-652. *In* U.S. Dept. of Interior, Bureau of Mines, Mineral facts and problems. 1970 Edition. U.S. Government Printing Office, Washington, D.C.
98. Greig, R. A., and H. L. Seagran. 1972. Survey of mercury concentrations in fishes of Lakes St. Clair, Erie, and Huron, p. 38-45. *In* R. Hartung and B. D. Dinman (ed.) Environmental mercury contamination. Ann Arbor Science Pub. Inc., Ann Arbor, Mich.
99. Haller, W. A., L. A. Rancitelli, and J. A. Cooper. 1968. Instrumental determination of trace elements in plant tissue by neutron activation analysis and Ge(Li) gamma-ray spectrometry. J. Agr. Food Chem. 16:1036-1040.
100. Hammond, A. L. 1971. Mercury in the environment: Natural and human factors. Science 171:788-789.
101. Hannerz, L. 1968. Experimental investigations on the accumulation of mercury compounds in water organisms. Rep. Inst. Freshwater Res., Drottningholm 48:120-176.
102. Hanson, A. 1971. Man-made sources of mercury, p. 22-33. *In* J. E. Watkin (ed.) Mercury in man's environment. Proc. Roy. Soc. Can. Symp., Ottawa, Canada.
103. Haq, I. U. 1963. Agrosan poisoning in man. Brit. Med. J. 5345: 1579-1582.
104. Harada, Y. 1968. Clinical investigations on Minamata disease. C. Congenital (or fetal) Minamata Disease, p. 93-117. *In* M. Kutsuna (ed.) Minamata disease. Study group of Minamata disease. Kumamoto University, Japan.
105. Hatch, W. R. and W. L. Ott. 1968. Determination of submicrogram quantities of mercury by atomic absorption spectrophotometry. Anal. Chem. 40:2085-2087.
106. Hayes, H., J. Muir, and L. M. whitby. 1970. A rapid method for the determination of mercury in urine. Ann. Occup. Hyg. 13: 235-239.
107. Hayes, A. D., and A. Rothstein. 1962. The metabolism of inhaled mercury vapor in the rat studied by isotope techniques. J. Pharmacol. Exp. Therapeutics 138:1-10.
108. Hingle, D. N., G. F. Kirkbright, and T. S. West. 1967. Some observations on the determination of mercury by atomic absorption spectroscopy in an air-acetylene flame. Analyst 92:759-762.
109. Hirota, K. 1969. Distribution of methyl mercury compound in human brain and rat organs, and the effect of D-penicillamine on mercury in rat organs. Clin. Neurol. 9:592-601.
110. Hoover, W. L., J. R. Melton and P. A. Howard. 1971. Determination of trace amounts of mercury in foods by flameless atomic absorption. J. Ass. Offic. Anal. Chem. 54:860-865.
111. Hunter, D., R. R. Bomford, and D. S. Russell. 1940. Poisoning by methyl mercury compounds. Quart. J. Med. 33:193-213.
112. Inoue, K., and S. Aomine. 1969. Retention of mercury by soil colloids. III. Absorption of mercury in dilute phenylmercuric acetate solutions. Soil Sci. Plant Nutr. 15(2):86-91. (Chem. Abstr. 71:80273v).
113. Irukayama, K. 1968. Minamata Disease as a public nuisance, p. 301-324. *In* M. Kutsuna (ed.) Minamata disease. Study group of Minamata disease. Kumamoto University, Japan.
114. Ishida, K., S. Kawamura, and M. Izawa. 1970. Neutron activation analysis for mercury. Anal. Chim. Acta 50:351-353.
115. Iskandar, I. K., J. K. Syers, L. W. Jacobs, D. R. Keeney, and J. T. Gilmour. 1972. Determination of total mercury in sediments and soils. Analyst 97:388-393.
116. Iwata, H., and H. Okamoto. 1970. Coefficients of metals on methyl mercury poisoning. Stencil, Dept. of Pharm. Sci. Univ. of Osaka, Japan. (Japanese).
117. Jalili, M. A., and A. H. Abbasi. 1961. Poisoning by ethyl mercury toluene sulphonanilide. Brit. J. Ind. Med. 18:303-308.
118. Jensen, S., and A. Jernelov. 1969. Biological methylation of mercury in aquatic organisms. Nature 223:753-754.
119. Jernelöv, A. 1969. Conversion of mercury compounds. p. 68-74. *In* M. W. Miller and G. G. Berg (ed.) chemical fallout. Current research on persistent pesticides. Charles C. Thomas, Springfield, Ill.
120. Jernelöv, A. 1972. Factors in the transformation of mercury to methylmercury. p. 167-172. *In* R. Hartung and B. D. Dinman (ed.) Environmental mercury contamination. Ann Arbor Science Pub. Inc., Ann Arbor, Mich.
121. Joensuu, O. I. 1971. Fossil fuels as a source of mercury pollution. Science 172:1027-1028.
122. Johansson, B., R. Ryhage and G. Westöö. 1970. Identification and determination of methylmercury compounds in fish using combination gas chromatograph-mass spectrometer. Acta Chem. Scand. 24:2349-2354.
123. Johnels, A. G., and T. Westermark. 1969. Mercury contamination of the environment in Sweden. p. 221-241. *In* M. W. Miller and G. G. Berg (ed.) Chemical fallout. Current research on persistent pesticides. Charles C. Thomas, Springfield, Ill.
124. Johnson, W. C. (Chairman). Analytical Methods Committee, Society for Analytical Chemistry. 1965. The determination of small amounts of mercury in organic matter. Analyst 90:515-530.
125. Jonasson, I. R., and R. W. Boyle. 1972. Geochemistry of mercury and origins of natural contamination of the environment. Can. Mining Metallurgical Bull. 65(1):32-39.
126. Jones, H. R. 1971. Mercury pollution control. Noyes Data Corp., Park Ridge, N. J. 251 p.
127. Kamps, L. R., and B. McMahon. 1972. Utilization of the Westöö procedure for the determination of methyl mercury in fish by gas-liquid chromatography. J. Ass. Offic. Anal. Chem. 55:590-595.
128. Kessler, R. H., R. Lozano, and R. F. Pitts. 1957. Studies on the structure diuretic activity relationships of organic compounds of mercury. J. Clin. Invest. 36:656-668.
129. Kim, C. K., and J. Silverman. 1965. Determination of mercury in wheat and tobacco leaf by neutron activation analysis using mercury-197 and a simple exchange separation. Anal. Chem. 37: 1616-1617.
130. Kirkpatrick, D. C., and D. E. Coffin. 1974. The trace metal content of representative Canadian diets in 1970 and 1971. Can. Inst. Food Sci. Technol. J. 7:56-58.
131. Kitamura, S. 1968. Determination of mercury content in bodies of inhabitants, cats, fishes, and shells in Minamata District and in the mud of Minamata Bay, p. 257-266. *In* M. Kutsuna (ed.) Minamata disease. Study group of Minamata disease. Kumamoto University, Japan.
132. Klein, D. H. 1972. Some estimates of natural levels of mercury in the environment, p. 25-29. *In* R. Hartung and B. D. Dinman (ed.) Environmental mercury contamination. Ann Arbor Science Pub. Inc., Ann Arbor, Mich.
133. Klein, D. H., and E. D. Goldberg. 1970. Mercury in the marine environment. Environ. Sci. Technol. 4:765-768.
134. Kojima, K., and M. Fujita. 1973. Summary of recent studies in Japan on methyl mercury poisoning. Toxicology 1:43-62.
135. Konrad, J. G. 1972. Mercury contents of bottom sediments from Wisconsin rivers and lakes. p. 52-58. *In* R. Hartung and B. D. Dinman (ed.) Environmental mercury contamination. Ann Arbor Science Pub. Inc., Ann Arbor, Mich.
136. Krenkel, P. A. 1973. Mercury: Environmental considerations, part 1. CRC Critical Rev. in Environ. Control 3:303-373.
137. Kudsk, F. N. 1964. Determination of mercury in biological materials, a specific and sensitive dithizone method. Scand. J. Clin. Lab. Invest. 16:575-583.
138. Kudsk, F. N. 1965. Absorption of mercury vapour from the respiratory tract in man. Acta Pharmacol. Toxicol. 23:250-262.

139. Kudsk, F. N. 1969. Uptake of mercury vapour in blood in vivo and in vitro from Hg- containing air. Acta Pharmacol. Toxicol. 27:149-160.
140. Kudsk, F. N. 1969. Factors influencing the in vitro uptake of mercury vapour in blood. Acta Pharmacol. Toxicol. 27:161-172.
141. Kurland, L. T. 1973. An appraisal of the epidemiology and toxicology of alkylmercury compounds. p. 23-55. *In* M. W. Miller and T. W. Clarkson (ed.) Mercury, mercurials, and mercaptans. Charles C. Thomas, Springfield, Ill.
142. Kutsuna, M. 1968. Historical perspective of the study on Minamata disease. p. 1-4. *In* M. Kutsuna (ed.) Minamata disease. Study group of Minamata disease. Kumamoto University, Japan.
143. Landner, L. 1971. Biochemical model for the biological methylation of mercury suggested from methylation studies in vivo with *Neurospora crassa.* Nature 230:452-454.
144. Leatherland, T. M., J. D. Burton, M. J. McCartney, and F. Culkin. 1971. Mercury in North Eastern Atlantic Ocean water. Nature 232:112.
145. Lidums, V., and U. Ulfvarson. 1968. Mercury analysis in biological material by direct combustion in oxygen and photometric determination of the mercury vapour. Acta Chem. Scand. 22:2150-2156.
146. Lindstedt, G. 1970. A rapid method for the determination of mercury in urine. Analyst 95:264-271.
147. Lindstedt, G., and S. Skerfving. 1972. Methods of analysis. p. 3-13. *In* L. Friberg and J. Vostal (ed.) Mercury in the environment; an epidemiological and toxicological appraisal. CRC Press, Cleveland, Ohio.
148. Lindstrom, O. 1959. Rapid microdetermination of mercury by spectrophotometric flame combustion. Anal. Chem. 31:461-467.
149. Ling, C. 1968. Portable atomic absorption photomete for determining nanogram quantities of mercury in the presence of interfering substances. Anal. Chem. 40:1876-1878.
150. Lofroth, G. 1970. A review of health hazards and side effects associated with the emission of mercury compounds into natural systems. 2nd ed. Swedish Natural Science Research Council, Stockholm, Sweden.
151. Longbottom, J. E. 1972. Inexpensive mercury-specific gas chromatographic detector. Anal. Chem. 44:1111-1112.
152. Lu, F. C. 1974. Mercury as a food contaminant. WHO Chron. 28:8-11.
153. Lucier, G., O. McDaniel, P. Brubaker, and R. Klein. 1971. Effects of methylmercury hydroxide on rat liver microsomal enzymes. Chem. Biol. Interactions 4:265-280.
154. Lundgren, K., and Swensson, Å. 1949. Occupational poisoning by alkyl mercury compounds. J. Ind. Hyg. Toxicol. 31:190-200.
155. Magos, L. 1967. Mercury blood interactions and mercury uptake by the brain after vapor exposure. Environ. Res. 1:323-337.
156. Magos, L. 1968. Uptake of mercury by the brain. Brit. J. Ind. Med. 25:315-318.
157. Magos, L. 1971. Selective atomic absorption determination of inorganic mercury and methyl mercury in undigested biological samples. Analyst 96:847-853.
158. Matsumoto, A., A. Suzuki, C. Morita, K. Nakamura, and S. Saeki. 1967. Preventive effect of penicillamine on the brain of fetal rat poisoned transplacentally with methyl mercury. Life Sci. 6:2321-2326.
159. McAlpine, D., and S. Araki. 1958. Minamata Disease, an unusual neurological disorder caused by contaminated fish. Lancet 1968 (II):629-631.
160. McAlpine, D., and S. Araki, 1959. Minamata disease, Late effects of an unusual neurological disorder caused by contaminated fish. A. M. A. Arch. Neurol. 1:522-530.
161. McCarthy, J. H., Jr., J. L. Meuschke, W. H. Ficklin, and R. E. Learned. 1970. Mercury in the atmosphere. U.S. Geol. Surv. Prof. Paper 713:37-39. U.S. Government Printing Office, Washington, D.C.
162. Miettinen, J. K. 1973. Absorption and elimination of dietary mercury (Hg^{2+}) and methylmercury in man. p. 233-243. *In* M. W. Miller and T. W. Clarkson (ed.) Mercury, mercurials and mercaptans. Charles C. Thomas, Springfield, Ill.
163. Miettinen, J. K., T. Rohola, T. Hattula, K. Rissanen, and M. Tillander. 1971. Elimination of ^{203}Hg-methylmercury in man. Ann. Clin. Res. 3:116-122.
164. Miller, G. E., P. M. Grant, R. Kishore, F. J. Steinkruger, F. S. Rowland, and V. P. Guinn. 1972. Mercury concentrations in museum specimens of tuna and swordfish. Science 175:1121-1122.
165. Miller, V. L., P. A. Klavano, and E. Csonka. 1960. Absorption, distribution, and excretion of phenylmercuric acetate. Toxicol. Appl. Pharmacol. 2:344-352.
166. Munns, R. K., and D. C. Holland. 1971. Determination of mercury in fish by flameless atomic absorption: A collaborative study. J. Ass. Offic. Anal. Chem. 54:202-205.
167. Muscat, V. I., T. J. Vickers, and A. Andren. 1972. Simple and versatile atomic fluorescence system for determination of nanogram quantities of mercury. Anal. Chem. 44:218-221.
168. Mushak, P. 1973. Gas-liquid chromatography in the analysis of mercury (II) compounds. Environ. Health Perspectives (Exp. issue) 4:55-60.
169. Neal, P. A., R. H. Flinn, T. I. Edwards, W. H. Reinhart, J. W. Hough, J. M. Dallavalle, F. H. Goldman, D. W. Armstrong, A. S. Gray, A. L. Coleman, and B. F. Postman. 1941. Mercurialism and its control in the felt-hat industry. U.S. Public Health Serv. Bull. No. 263. U.S. Government Printing Office, Washington, D.C. 132 p.
170. Neal, P. A., R. R. Jones, J. J. Bloomfield, J. M. Dallavalle, and T. I. Edwards. 1937. A study of chronic mercurialism in hatters' fur-cutting industry. U.S. Public Health Serv. Bull. No. 234. U.S. Government Printing Office, Washington, D.C. 70p.
171. Nelson, N. (Chairman). 1971. Hazards of mercury; special report to the Secretary's Pesticide Advisory Committee, Department of Health, Education and Welfare, November 1970. Environ. Res. 4:1-69.
172. Newberne, P. M. 1974. Mercury in fish: A literature review. CRC Critical Rev. Food Technol. 4:311-335.
173. Nishimura, M., N. Urakawa, and M. Ikeda. 1971. An autoradiographic study on the distribution of mercury and its transfer to the egg in the laying quail. Jap. J. Pharmacol. 21:651-659.
174. Nordberg, G. F., and F. Serenius. 1969. Distribution of inorganic mercury in the guinea pig brain. Acta Pharmacol. Toxicol. 27:269-283.
175. Nordberg, G. F., and S. Skerfving. 1972. Metabolism. p. 29-91. *In* L. Friberg and J. Vostal (ed.) Mercury in the environment; an epidemiological and toxicological appraisal. CRC Press. Cleveland, Ohio.
176. Norseth, T. 1969. Studies of intracellular distributionof mercury. p. 408-419. *In* M. W. Miller and G. G. Berg (ed.) Chemical fallout. Current research on persistent pesticides. Charles C. Thomas, Springfield, Ill.
177. Norseth, T. 1971. Biotransformation of methyl mercuric salts in the mouse studied by specific determination of inorganic mercury. Acta Pharmacol. Toxicol. 29:375-384.
178. Norseth, T., and T. W. Clarkson. 1970. Studies on the biotransformation of ^{203}Hg- labeled methyl mercury chloride in rats. Arch. Environ. Health 21:717-727.
179. Norseth, T., and T. W. Clarkson. 1971. Intestinal transport of ^{203}Hg-labeled methyl mercury chloride. Role of biotransformation in rats. Arch. Environ. Health 22:568-577.
180. Novick, S. 1969. Mercury contamination in food, air, and water. Environment 11(4):3-9.
181. Ostlund, K. 1969. Studies on the metabolism of methyl mercury and dimethyl mercury in mice.Acta Pharmacol. 27 (suppl. 1):1-132.
182. Pappas, E. G., and L. A. Rosenberg. 1966. Determination of submicrogram quantities of mercury by cold vapor atomic absorption photometry. J. Ass. Offic. Anal. Chem. 49:782-792.
183. Peakall, D. B. and R. J. Lovett. 1972. Mercury: Its occurrence and effects in the ecosystem. Bioscience 22:20-25.
184. Pekkanen, T. J., and K. Salminen. 1973. Inductive effects of

methyl mercury on the hepatic microsomes of mice. Acta. Pharmacol. Toxicol. 32:289-292.
185. Pennington, J. W., and G. N. Greenspoon. 1958. Mercury. p. 771-791. *In* U.S. Department of Interior, Bureau of Mines. Minerals Yearbook 1955. Vol. I. U.S. Government Printing Office, Wahington, D.C.
186. Permanent Commission and International Association on Occupational Health, Task Group on Metal Accumulation. 1973. Accumulation of toxic metals with special reference to their absorption, excretion, and biological half-time. Environ. Physiol. Biochem. 3:65-107.
187. Peterson, C. L., W. L. Klawe, and G. D. Sharp. 1973. Mercury in tunas: A review. Fish. Bull. 71:603-613.
188. Phillips, R., and H. Cember. 1969. The influence of body burden of radiomercury on radiation dose. J. Occup. Med. 11:170-174.
189. Pillay, K. K. S., C. C. Thomas, Jr., J. A. Sondel, and C. M. Hyche. 1971. Determination of mercury in biological and environmental sample by neutron activation analysis. Anal. Chem. 43:1419-1425.
190. Platonow, N. 1968. A studyof the metabolic fate of ethylmercuric acetate. Occup. Health Rev. 20(1):1-8.
191. Platonow, N. 1968. A study of the metabolic fate of methylmercuric acetate. Occup. Health Rev. 20(1):9-19.
192. Potter, S., and G. Matrone. 1964. Effect of selenite on the toxicity of dietary methyl mercury and mercuric chloride in the rat. J. Nutr. 104:638-647.
193. Prickett, C. S., E. P. Laug, and F. M. Kunze. 1950. Distribution of mercury in rats following oral and intravenous administration of mercuric acetate and phenylmercuric acetate. Proc. Soc. Exp. Biol. Med. 73:585-588.
194. Rahola, T., T. Hattula, A. Korolainen, and J. K. Miettinen. 1971. The biological half-time of inorganic mercury (Hg^{2+}) in man. Scand. J. Clin. Lab. Invest. 27 (Supple. 116):77. (Abstr.)
195. Rains, T. C., and O. Menis. 1972. Determination of submicrogram amounts of mercury in standard reference materials by flameless atomic absorption spectrometry. J. Ass. Offic. Anal. Chem. 55:1339-1344.
196. Ramel, C. 1969. Genetic effects of organic mercury compounds: I. Cytological investigations on *Allium* roots. Hereditas 61:208-230.
197. Ramel, C. 1972. Genetic effects. p. 169-181. *In* L. Friberg and J. Vostal (ed.) Mercury in the environment; an epidemiological and toxicological appraisal. CRC Press, Cleveland, Ohio.
198. Ramel, C., and J. Magnusson. 1969. Genetic effects of organic mercury compounds: II. Chromasome segregation in the *Drosophila melanogaster.* Hereditas 61:231-254.
199. Rothstein, A. 1973. Mercaptans, the biological targets for mercurials. p. 68-95. *In* M. W. Miller and T. W. Clarkson (ed.) Mercury, mercurials, and mercaptans. Charles C. Thomas, Springfield, Ill.
200. Rothstein, A., and A. D. Hayes. 1960. The metabolism of mercury in the rat studied by isotope techniques. J. Pharmacol. Exp. Therapeutics 130:166-176.
201. Rottschafer, J. M., J. D. Jones, and H. B. Mark, Jr. 1971. A simple, rapid method for determining trace mercury in fish via neutron activation analysis. Environ. Sci. Technol. 5:336-338.
202. Saha, J. G. 1973. Significance of mercury in the environment. Residue Rev. 42:103-163.
203. Saha, J. G., Y. W. Lee, R. D. Tinline, S. H. F. Chinn, and H. M. Austenson. 1970. Mercury residues in cereal grains from seeds or soil treated with organomercury compounds. Can. J. Plant Sci. 50:597-599.
204. Sand, P. F., G. B. Wiersma, H. Tai, and L. J. Stevens. 1971. Preliminary study of mercury residues in soil where mercury seed treatments have been used. Pest. Monit. J. 5(1):32-33.
205. Shepherd, M., S. Schumann, R. H. Flinn, J. W. Hough, P. A. Neal. 1941. Hazard of mercury vapor in scientific laboratories. J. Res. Nat. Bureau of Standards (U.S.). 26:357-375.
206. Sibbett, D. J., R. H. Moyer, and G. H. Milly. 1972. Emission of mercury from latex paints. Amer. Chem. Soc., Div. Water, Air Waste Chem., General Paper 12(1):20-26. (Chem. Abstr. 80: 18957d).
207. Silberberg, I., L. Prutkin, and M. Leider. 1969. Electron microscopic studies of transepidermal absorption of mercury. Arch. Environ. Health 19:7-14.
208. Sjostrand, B. 1964. Simultaneous determination of mercury and arsenic in biological and organic materials by activation analysis. Anal. Chem. 36:814-819.
209. Skerfving, S., A. Hansson, and J. Lindsten. 1970. Chromosome breakage in humans exposed to methyl mercury through fish consumption. Arch. Environ. Health 21:133-139.
210. Smart, N. A. 1968. Use and residues of mercury compounds in agriculture. Residue Rev. 23:1-36.
211. Smart, N. A., and A. R. C. Hill. 1969. Determination of mercury residues in potatoes, grain, and animal tissues using perchloric acid digestion. Analyst 94:143-147.
212. Smith, R. G. 1972. Methods of analysis for mercury and its compounds: A review. p. 97-136. *In* R. Hartung and B. D. Dinman (ed.) Environmental mercury contamination. Ann Arbor Science Pub. Inc., Ann Arbor, Mich.
213. Smith, R. G., A. J. Vorwald, L. S. Patil, and T. F. Mooney, Jr. 1970. Effects of exposure to mercury in the manufacture of chlorine. Amer. Ind. Hyg. Ass. J. 31:687-700.
214. Snyder, R. D. 1971. Congenital mercury poisoning. New Eng. J. Med. 284:1014-1016.
215. Sollmann, T., and N. E. Schreiber. 1936. Chemical studies of acute poisoning from mercury bichloride. Arch. Intern. Med. 57:46-62.
216. Stewart, D. J. 1970. Mercury hearing before the State of Wisconsin Department of Natural Resources. October 12.
217. Stock, A. E. 1934. Cited from Goldwater, L. J. 1971. Mercury in the environment. Sci. Amer. 224(5):15-21.
218. Stock, A. E. 1938. Cited from Goldwater, L. J. 1971. Mercury in the environment. Sci. Amer. 224(5):15-21.
219. Suzuki, T. 1969. Neurological symptoms from concentration of mercury in the brain. p. 245-257. *In* M. W. Miller and G. G. Berg (ed.) Chemical fallout. Current research in persistent pesticides. Charles C. Thomas, Springfield, Ill.
220. Suzuki, T., T. Miyama, and H. Katsunuma. 1971. Comparison mercury contents in maternal blood, umbilical cord blood, and placental tissues. Bull. Environ. Contamination Toxicol. 5:502-508.
221. Swensson, Å. 1952. Investigations on the toxicity of some organic mercury compounds which are used as seed disinfectants. Acta Med. Scand. 143:365-384.
222. Swensson, Å., K. D. Lundgren, and O. Lindstrom. 1959. Retention of various mercury compounds after subacute administration. Arch. Ind. Health 20:467-472.
223. Swensson, Å., and U. Ulfvarson. 1968. Distribution and excretion of various mercury compounds after single injection in poultry. Acta Pharmacol. Toxicol. 26:259-272.
224. Swensson, A., and U. Ulfvarson. 1968. Distribution and excretion of mercury compounds in rats over a long period after a single injection. Acta Pharmacol. Toxicol. 26:273-283.
225. Takahashi, H., and K. Hirayama. 1971. Accelerated elimination of methyl mercury from animals. Nature 232:201-202.
226. Takeda, Y., T. Kunugi, O. Hoshino, and T. Ukita. 1968. Distribution of inorganic, aryl, and alkyl mercury compounds in rats. Toxicol. Appl. Pharmacol. 13:156-164.
227. Takeuchi, T. 1968. Pathology of Minamata disease. p. 141-228. *In* M. Kutsuna (ed.) Minamata disease. Study group of Minamata disease. Kumamoto University, Japan.
228. Takizawa, Y. 1970. Studies on the Niigata episode of Minamata disease outbreak investigation of causitive agents of organic mercury poisoning in the district along the river Agano. Acta Med. Biol. 17:293-297. (Chem. Abstr. 74:109862m).
229. Tatton, J. O. G., and P. J. Wagstaffe. 1969. Identification and determination of organomercurial fungicide residues by thin-layer and gas chromatography. J. Chromatogr. 44:284-289.
230. Thomerson, D. R., and K. C. Thompson. 1974. Recent develop-

ments in atomic absorption spectrometry. Amer. Lab. 6(3):53-61.
231. Thompson, K. C., and G. D. Reynolds. 1971. The atomic-fluorescence determination of mercury by the cold vapour technique. Analyst 96:771-775.
232. Thorpe, V. A. 1971. Determination of mercury in food products and biological fluids by aeration and flameless atomic absorption spectrophotometry. J. Ass. Offic. Anal. Chem. 54:206-210.
233. Tokuomi, H. 1968. Clinical investigations on Minamata disease. A. Minamata disease in human adult. p. 37-72. *In* M. Kutsuna (ed.) Minamata disease. Study group of Minamata disease. Kumamoto University, Japan.
234. Tryphonas, L., and N. O. Nielsen. 1970. The pathology of Arylmercurial poisoning in swine. Can. J. Comparative Med. 34:181-190.
235. Turekian, K. K., and K. H. Wedepohl. 1961. Distribution of the elements in some major units of the earth's crust. Geol. Soc. Amer. Bull. 72:175-192.
236. Turney, W. G. 1972. The mercury pollution problem in Michigan. p. 29-31. *In* R. Hartung and B. D. Dinman (ed.) Ann Arbor Science Pub. Inc., Ann Arbor, Mich.
237. Ulfvarson, U. 1969. The effect of the size of the dose on the distribution and excretion of mercury in rats after single intravenous injection of various mercury compounds. Toxicol. Appl. Pharmacol. 15:1-8.
238. U.S. Environmental Protection Agency, Office of Air and Water Programs, Office of Air Quality Planning and Standards. 1973. Control techniques for mercury emissions from extraction and chlor-alkali plants. Pub. AP-118. 60 p.
239. Viola, P. L., and G. B. Cassano. 1968. The effect of chlorine on mercury vapour intoxication. Autoradiographic study. Med. Lavoro 59:437-444. (Chem. Abstr. 70:55852e).
240. Weast, R. C. 1974. Handbook of chemistry and physics. 55th ed. CRC Press, Cleveland, Ohio.
241. Webb, J. L. 1966. Mercurials. p. 729-985. *In* J. L. Webb, Enzyme and metabolic inhibitors, Vol. II. Academic Press, New York.
242. Weed, L. A., and E. E. Ecker. 1931. The utility of phenylmercury-nitrate as a disinfectant. J. Infectious Dis. 49:440-449.
243. Weiner, I. M., R. I. Levy, and G. H. Mudge. 1962. Studies on mercurial diuresis: Renal excretion, acid stability, and structure-activity relationships of organic mercurials. J. Pharmacol. Exp. Therapeutics 138:96-112.
244. Weiss, H. V., M. Koide, and E. D. Goldberg. 1971. Mercury in a Greenland ice sheet: Evidence of recent input by man. Science 174:692-694.
245. Wenninger, J. A. 1965. Direct microdetermination of mercury in colar additives by the photometric-mercury vapor procedure. J. Ass. Offic. Anal. Chem. 48:826-832.
246. Werhaw, R. L. 1970. Sources and behavior of mercury in surface waters. U.S. Geol. Surv. Prof. Paper 713:29-31. U.S. Government Printing Office, Washington, D.C.
247. Westermark, T. 1965. Kvicksilverfragan i Sverige, p. 25-76. Kvicksilverkonfersensen. Stockholm, Sweden.
248. Westermark, T., and B. Sjostrand. 1960. Activation analysis of mercury. Int. J. Appl Radiat. Isotopes 9:1-15.
249. Westöö, G. 1966. Determination of methylmercury compounds in foodstuffs: I. Methylmercury compounds in fish, identification and determination. Acta Chem. Scand. 20:2131-2137.
250. Westöö, G. 1967. Determination of methylmercury compounds in foodstuffs: II. Determination of methylmercury in fish, egg, meat, and liver. Acta Chem. Scand. 21:1790-1800.
251. Westöö, G. 1968. Determination of methylmercury salts in various kinds of biological material. Acta Chem. Scand. 22: 2277-2280.
252. Westöö, G. 1969. Methylmercury compounds in animal foods. p. 75-93. *In* M. W. Miller and G. G. Berg (ed.) Chemical fallout. Current research in persistent pesticides. Charles C. Thomas, Springfield, Ill.
253. Williston, S. H. 1968. Mercury in the atmosphere. J. Geophys. Res. 73:7051-7055.
254. Wood, J. M., F. S. Kennedy, and C. G. Rosen. 1968. Synthesis of methyl-mercury compounds by extracts of a methanogenic bacterium. Nature 220:173-174.
255. Worf, G. L. 1970. Mercury Hearing before the State of Wisconsin Department of Natural Resources. October 12.
256. Yoshino, Y., T. Mozai, and K. Nakao. 1966. Biochemical changes in the brain in rats poisoned with an alkylmercury compound with special reference to the inhibition of protein synthesis in brain cortex slices. J. Neurochem. 13:1223-1230.

ERRATA

To LIST OF HOLDERS OF 3-A SYMBOL COUNCIL AUTHORIZATIONS ON FEBRUARY 20, 1975 in March issue of Journal of Milk and Food Technology, Volume 38, No. 3, Pages 159-162.

Additions to List:

08-09 Fittings Used on Milk and Milk Products Equipment, and Used on Sanitary Lines Conducting Milk and Milk Products

260 CREPACO, INC. (5/22/74)
100 CP Avenue, Lake Mills, Wisconsin 53551

255 THE DURIRON COMPANY (1/18/74)
45 North Findlay Street, Dayton, Ohio 45401

22-03 Silo-Type Storage Tanks for Milk and Milk Products

262 DELAVAL COMPANY LIMITED (11/11/74)
113 Park Street South, Peterborough, Ontario K9J 3R8

Deletions from List:

01-06 Storage Tanks for Milk and Milk Products, as Amended

116 Jacob Brenner Company, Inc. (10/8/59)
450 Arlington, Fond du Lac, Wisconsin 54935

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Development and Present Status of FDA *Salmonella* Sampling and Testing Plans[1]

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ABSTRACT

Food containing *Salmonella* is considered adulterated under the provisons of the Food, Drug, and Cosmetic Act. Foods are examined for *Salmonella* in accordance with three specified sampling and testing plans. Use of each sampling and testing plan is determined by the extent of hazard that a food may present. For this purpose, foods are grouped into categories according to risk of hazard likely to be characteristic of the food. Acceptance criteria are specified for foods of each category.

Salmonellosis remains one of the most important communicable disease problems in the United States. In addition, the Food and Drug Administration (FDA) considers food containing salmonellae as adulterated. Specifically, the following taken from the Food, Drug, and Cosmetic Act, Section 402 (a) (*1*) provides the legislative base for this position: "A food is adulterated if it bears or contains any poisonous or deleterious substance which may render it injurious to health."

In 1966 FDA launched an extensive *Salmonella* surveillance program which included intensive testing of processed foods and certain pharmaceutical products of animal origin. this program was prompted by a series of outbreaks, e.g., the outbreak due to *Salmonella new brunswick* in non-fat dry milk and *Salmonella cubana* in carmine red, and others.

These events sharply drew attention to the *Salmonella* problem. It was evident that the problem needed review and assessment in the interest of charting appropriate courses of action toward its control. Accordingly, in 1967, the FDA and U.S. Department of Agriculture jointly requested the National Academy of Sciences to undertake a thorough evaluation of the *Salmonella* problem. The task was assigned to a specifically appointed *Salmonella* Committee of the National Research Council, which filed its report in December 1968. (*2*). In the report particular attention is given to problems of control of salmonellosis as related to food production and processing industries and to activities of government in surveillance and research.

Among other things, the report emphasized that the sale of foods containing salmonellae cannot be condoned; however, recognition must be given to the fact that salmonellae can be found in many products if a sufficient number of tests are made. Further, the report called attention to confusion and uncertainty in the food-processing industry, due to the lack of a definitive sampling and testing plan. The following question was posed: When should we stop testing and conclude that a product is salmonellae-free (which may simply mean that the contamination level is below the sensitivity of the sampling plan and the analytical procedure)?

In recognition of the fact that there is no way to be absolutely certain that a particular lot of non-sterile food is free of salmonellae, the NAS/NRC Committee recommended two specific actions. In brief, these were: (*a*) evolve a realistic assessment of the degree of hazard imposed by various foods, feeds, and drugs; and (*b*) develop sampling plans that will provide adequate assurance that the number of salmonellae present, if any, is below a statistically defined limit that offers minimal hazard to the consumer.

The Committee had suggested a system by which the degree of hazard presented by any food could be assessed. Further, a sampling and testing plan was proposed that could lead to a decision whether to accept or reject a particular lot of food.

After reviewing the NAS-NRC Committee report we began to consider ways of responding to the various Committee recommendations, including the two just mentioned—assessment of degree of hazard and appropriate sampling and testing plan(s). As a consequence, FDA invited Dr. E. M. Foster, Director of the Food Research Institute, University of Wisconsin, to assemble a group of knowledgeable people representing both government and industry to develop a classification system and sampling plan along the lines envisioned by the *Salmonella* Committee. It seemed reasonable to us that such a group could come to some mutual agreement upon these two issues. And, in fact, they did. Their report was submitted to us in October 1970 and was published in March 1971 (*1*). They agreed with the NAS-NRC Committee on the classification system. Table 1 is illustrative of this point. Briefly, three product characteristics determine the degree of potential health

TABLE 1. *Product hazard characteristics*

A	Product contains sensitive ingredient
B	No destructive step during manufacture
C	Liklihood of growth if abused

[1]*Presented at the 74th Annual Meeting of the American Society for Microbiology, Chicago, Illinois, May 12-17, 1974.*

hazard that a food presents: (*a*) The product contains an ingredient that has been identified as a significant potential factor in salmonellosis, in other words, a sensitive ingredient. (*b*) The manufacturing process does not include a controlled step that would destroy salmonellae. (*c*) There is substantial liklihood of microbiological growth if the product is mishandled or abused in distribution or consumer usage.

These three hazard characteristics can be combined into eight different configurations. This is illustrated in Table 2. Categories are defined as follows:

TABLE 2. *Categories of food products based on product hazard characteristics*

Type of food	Hazard characteristic[a] A	B	C	Category
Intended for infants, aged, and infirm	+	+ or 0	+ or 0	I
Intended for general use	+	+	+	II
	+	+	0	III
	+	0	+	III
	0	+	+	III
	+	0	0	IV
	0	+	0	IV
	0	0	+	IV
	0	0	0	V

[a]See Table 1 for explanation of hazard characteristics A, B, and C
+ = Hazard present; 0 = Hazard not present.

Category I—foods that are intended for infants, aged and the infirm, and that contain a sensitive ingredient; the latter criterion recognizes the intention of the Committee *not* to include all foods consumed by these groups, but rather those with a significant *Salmonella* hazard.

Category II—foods with all three hazard characteristics (Sensitive ingredient, no destructive treatment, abuse potential).

Category III—foods with two hazard characteristics.

Category IV—foods with one hazard characteristic.

Category V—Foods with none of the three hazard characteristics.

TABLE 3. *Acceptance plan based on 25 g test units*

Product category	No. of units tested[a] None positive[b]	No more than 1 positive[b]	Significance: 95% probability no more than 1 organism in
I	60 (1500)	95 (2375)	500 g
II	30 (750)	48 (1200)	250 g
III, IV, V	15 (375)	24 (600)	125 g

[a]Numbers in parentheses indicate total grams of sample tested; each test unit = 25 g
[b]As recommended by the committee.

Table 3 illustrates the testing and acceptance criteria recommended to us by the Committee. In essence, this plan is the same as that recommended by the NAS-NRC Committee. Note the three different testing levels—one for each product category. Also, note that a lot may be accepted even though one analytical unit was positive.

As mentioned earlier, the objective here was to have a sampling and testing plan that would provide adequate assurance that the number of salmonellae present, if any, is below a statistically defined limit that offers minimal hazard to the consumer. In the considered judgement of the Committee, this limit is as given in the last column of Table 3, i.e., if the test results provide 95% confidence that the salmonellae contamination level, if any, is no more than 1 in 500 g for Category I; no more than 1 in 250 g for Category II; and no more than 1 in 125 g for Categories III, IV and V.

I should emphasize at this point that the sampling and testing plan recommended by the two Committees would apply to (*a*) processed foods or ingredients, as contrasted to, e.g., raw meats, poultry and fish; (*b*) lots that conform to specific criteria that establish their integrity; (*c*) lots that have been questioned because of the possible presence of salmonellae. The sampling plan was not designed—or intended—to replace routine surveillance operations, including testing, that a food manufacturer or a regulatory agency may employ. *It was intended to be used in arriving at a final decision whether to accept or reject a particular lot in question.* The Committee listed five reasons for questioning a lot, any one of which, as well as others, would make it necessary to seriously consider the acceptability of a product.

What is FDA's position on salmonellae sampling and testing plans? Basically, it is as recommended by the NAS-NRC Committee and the Interagency-Industry Committee, with the following significant exceptions. (*a*) A sampled lot is acceptable only if analyses of all analytical units or composite units are negative for salmonellae. (*b*) Analytical units may be composited. The maximum size of a composite unit is 375 g. The composite unit must consist of a series of 25 g analytical units, the maximum size of the composite being 375 g or 15, 25 g analytical units. (*c*) The acceptance criterion indicated in (*a*) is applicable to any lot of product tested in connection with any of our surveillance or compliance programs—it is not restricted to questioned lots.

TABLE 4. *Definitions*

Sample:	A series of sample units
Sample unit:	100-g Minimum
Analytical unit:	25-g From sample unit
Composite unit:	375-g (15 Analytical units)

Table 4 gives the definitionsof serveral pertient terms, and Table 5 shows the acceptance plan.

Now, a word of explanation about the exceptions

TABLE 5. *Number of analytical units or composite units tested and criteria of acceptance for each product category*

Product category	Anal. units	Total wt. tested	Composite units[a]	No. + [b]	Conc. of salmonellae (No more than:)
I	60	1500 g	4	0	1/500 g
II	30	750 g	2	0	1/250 g
III-V	15	375 g	1	0	1/125 g

[a]375 g each.
[b]Criteria for FDA acceptance.

indicated above. First, we recongize, for example, that one positive result from 95 analytical units or 0 positive from 60 analytical units provides the same probability (95%) that the level of salmonellae given in Table 3 is not exceeded. We feel, however, that acceptance of any lot of food in which salmonellae have been shown to be present is not administratively feasible.

Second, the advantage of compositing analytical units is obvious. To do so, however, requires that no significant decrease in sensitivity of the test for salmonellae would result. We are satisfied that this is the case. There are several published reports that support this position. Third, we have also taken the position that the sampling and testing plan is applicable to any lot of food sampled by FDA. If we sample a lot, we question it; otherwise, why sample it? Fourth, the sampling and testing plan and criteria for acceptance apply to the genus *Arizona* as well as to the salmonellae.

This gives our position on sampling and testing foods for the presence of salmonellae together with pertinent background information.

REFERENCES

1. Foster, E. M., Chairman. 1971. The control of salmonellae in foods: A classification system and sampling plan. J. Assoc. Offic. Anal. Chem. 54:259-266.
2. National Academy of Sciences—National Research Council. Committee on *Salmonella*. 1969. An evaluation of the *Salmonella* problem. National Academy of Sciences, Washington, D.C.

News and Events

Patton Wins Award

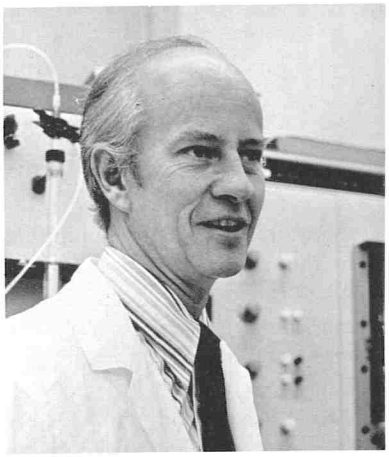
Dr. Stuart Patton, Evan Pugh research professor of agriculture at the Pennsylvania State University, has been named recipient of the 1975 Award for Distinguished Achievement and Service in Agricultural and Food Chemistry of the American Chemical Society.

The award, sponsored by International Flavors and Fragrances, Inc., will be presented at the 170th national meeting of the American Chemical Society in Chicago, Ill., on August 27. Dr. Patton's selection was announced recently at the society's spring meeting in Philadelphia.

The annual selection honors an outstanding researcher who has contributed to knowledge in agricultural and food chemistry. With the award goes a $2,000 cash grant and a plaque.

Dr. Patton is well-known for his studies of the biochemistry of milk and for his work on the role of cholesterol and other fatty materials in atherosclerosis hardening of the arteries. He earlier won the Borden Award for Chemistry of Milk.

Dr. Patton took part in three voyages of the Alpha Helix, a research vessel operated by the Scripps Institution. On two of them, he studied the cholesterol metabolism of Pacific salmon, off British Columbia and



Alaska. On the third, he studied lipids in the milk of elephant seals off the coast of Baja, California.

A 1943 graduate of Penn State, Dr. Patton was named Evan Pugh research professor of agriculture in 1966. This rank honors faculty members for exceptional quality of research publications or creative work, and for significant contributions to the education of students who later achieve recognition. He received the doctor of philosophy degree in dairy science from Ohio State University in 1948.

New Film Available

The Audio-Visual Education Center of the University of Michigan is pleased to announce general distribution of a 16 millimeter, 20 minute color motion picture entitled "THE BIG DIFFERENCE." The film is of special interest to hospitals, nursing homes and medical teaching institutions.

"THE BIG DIFFERENCE" deals with the problems of sanitation versus health care, in attempting to realize every good hospital's goal: The best of patient care while using all available facilities to maximum advantage.

The film is realistically and effectively presented. It is of interest to environmental sanitarians as well as administrators, orderlies and aides. In short, its audience is anyone who is actively concerned with the general well-being of the hospital.

For additional information and fee schedule call 313-763-1232.

3-A Discharges Long Spring Agenda

A review of five new standards was among the 17 items on the spring meeting agenda of the 3-A Sanitary Standards Committees at Hunt Valley, Md., April 8-10, 1975.

The industry meeting, attended by 75 dairy processors, sanitarians and equipment fabricators, examined tentative new standards for cottage cheese vats, conveyors and bins for dry milk and its products, colloid mills for processing liquid products and continuous blenders. Drafts of the proposed standards were studied and returned to task committees with suggestions for changes.

Subject to task committee approval of minor alterations, four projects were cleared by the processor group and transmitted to the sanitarians for action. They are an amendment to the standard for homogenizers and pumps of the plunger type, revision of the standard for frozen dessert and cottage cheese packaging equipment, amendment to the standard for automotive milk transportation tanks used for bulk delivery or farm pick-up, and supplement to the standard for fittings used on milk and milk product equipment. The latter is a revision of the 21-year-old standard for leak protection plug valves for batch pasteurizers.This supplement also adds the same criteria to the sanitary fittings standard.

Two items were completed and will be published before the end of the year. One is an additional amendment to the automotive transportation tank standard and the other is a supplement to the sanitary fittings standard to provide for continuous samplers.

The 3-A program is directed to safeguarding the public health through the development of standards and practices for the cleanability of equipment used in the processing of dairy products and to protecting the product against contamination from the equipment itself or foreign elements of dust, dirt or liquids.

DFISA Members Elect New Board Representatives

Three new directors and four incumbent directors were elected by the membership of Dairy and Food Industries Supply Association to the DFISA board of directors at the association's 56th Annual Meeting at Naples, Fla., April 16-18, 1975.

The new directors are Douglas B. Smith, Owens-Illinois Inc., Lily division, who was elected to fill the container group's vacant directorship for the remaining year of a three-year term; Robert C. Anderson Jr., Anderson Instrument Co. Inc., elected to a three-year term as director of the general commodities and services group, and George J. Remus, Cherry-Burrell Co., division of Paxall Inc., elected to a three-year term as an at-large board member.

Incumbents whose board terms were renewed for three years are Worth Weed, Foote and Jenks Inc., ingredients director, Hase H. Smith, M. G. Newell Co. Inc., jobbers director, and Ralph L. Bjorgen, Norton Co., plastics and synthetics division, and Arthur A. Rogers, C. E. Rogers Co., re-elected at-large representatives.

Rogers was re-elected by the board of directors to a sixth term as DFISA treasurer.

Leaving the board are I. T. Swartwood, Sun Industries Inc., who served as general commodities and services director, and Harry Goff, James Dole Corp., who served as an at-large representative.

New Micropipetting Tips

A new tip-loading system that permits faster, cleaner and safer micropipetting of liquid samples in food and dairy laboratory testing has been introduced by Oxford Laboratories Inc.

New Oxford® SAMPLER® 810Q Organized Tips are designed for use with Oxford® hand-operated pipetting systems (HOPS) without cross-contamination dangers, time-consuming setup labor or the risks of mouth-pipetting, according to William E. Finney, president.

The system provides 1,200 disposable Oxford® Tips in five stacked trays (240 tips each), ready to use with Oxford® SAMPLER® Micropipetting Instruments. Used in this manner, the tips remain untouched to avoid cross-contamination. The compact package is cushioned for rugged use and is completely disposable.

The tips accommodate volumes of 0.005 to 0.2 ml. Other disposable Oxford® Tips, in bulk and individually-wrapped (sterile), are available separately for volumes of 0.005 to 1 ml.

Oxford, a subsidiary of G. D. Searle & Co., manufactures liquid-handling instruments and supplies for clinical, research and industrial laboratories.

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Association Affairs

Dr. W. C. Lawton Appointed

Dr. W. C. Lawton has been appointed General Manager of A & L Laboratories, Inc., it was announced today by President W. I. Van Liere. Dr. Lawton was formerly with Mid-America Dairymen, Inc., as manager of operations in the Northern Division. Prior to his service with Mid-America Dairymen, Inc., he was director of the Quality Control Laboratory, St. Paul, Minnesota.

A & L Laboratories, Inc., has recently completed moving its offices and manufacturing operations to 1001 Glenwood Avenue, Minneapolis, Minnesota. A & L Laboratores, Inc., manufactures detergents and sanitizers for environmental sanitation.

Virginia Association Holds a Dairy Industry Workshop

The Virginia Association of Sanitarians and Dairy Fieldmen sponsored a Dairy Industry Workshop on March 11, 12 and 13, 1975, at the Donaldson Brown Center for Continuing Education in cooperation with the Department of Dairy Science, the Department of Food Science and Technology and the Extension Division of V.P.I. & S.U.

Subject matter covered during the Workshop were; the role of various trade associations connected with the dairy industry at state and national levels, Federal Milk Marketing status in Virginia, nutritional labeling and dating, with a final lecture on the State Milk Commission's activities.

The second day covered the bacterial effects on milk and their prevention, off flavors in milk, and proper water treatment for sanitization of equipment.

Subject matter pertaining to the Water Control Board,

Left to right: J. Carl Bussey, Retiring President; M. R. Cooper, President; L. T. Lester, First Vice-President; Charles Scott, Second Vice-President; W. H. Gill, Secretary-Treasurer; Dale Higginbotham, International Representative.

manufactured milk program, home preservation of foods and the interpretation and enforcement of state laws and regulations completed the program.

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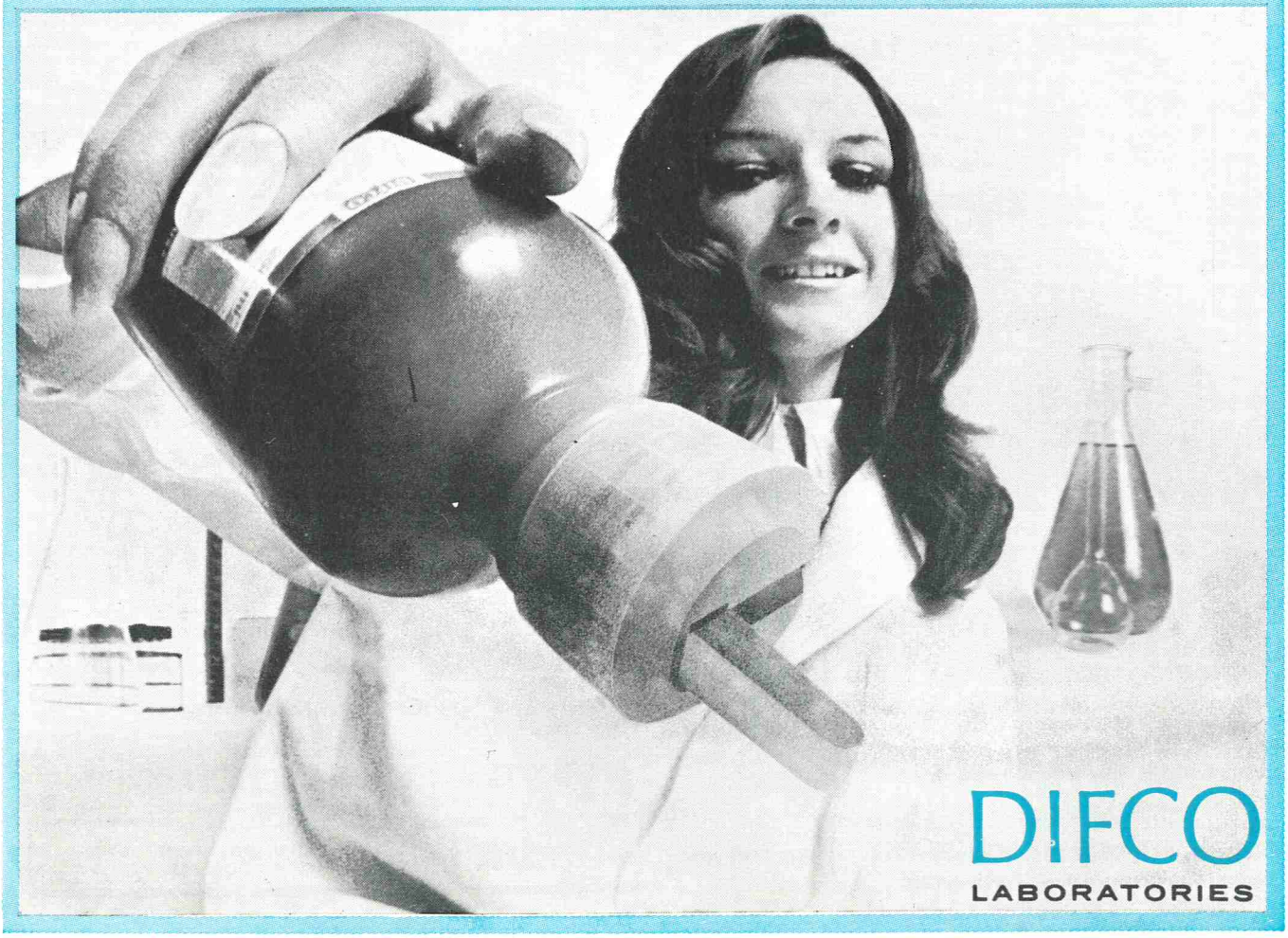

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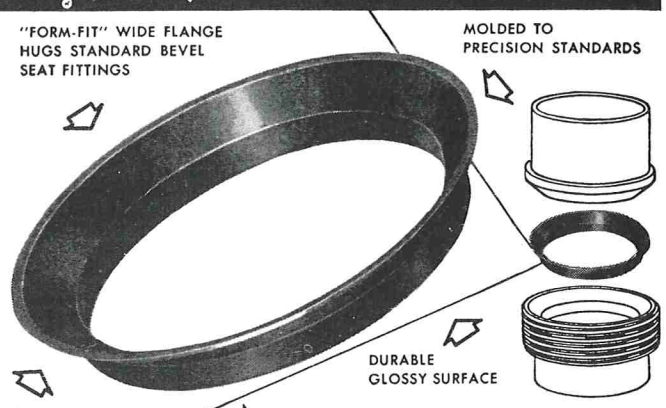

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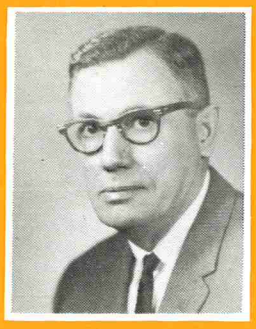
TUBES — PACKED 12 — 4 OZ. TUBES PER CARTON

THE HAYNES MANUFACTURING CO.
CLEVELAND, OHIO 44113

Dairy authorities speak out on better cow milking



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What questions should a dairyman ask before expanding or modernizing?

Here are some of the most basic questions I believe dairymen should ask themselves before they begin a program of modernization or expansion:

What about my future in dairying? Will the changes I make be profitable in the long run? Will the changes be feasible in the short run?

Long run profitability refers to a period of eight to ten years or more. It is usually studied through the use of budgets. In the budgeting process, capital expenditures are prorated over the life of the assets by arbitrary depreciation methods.

Short run feasibility refers to the income-generating ability of a business in a short period of time. It is usually studied through the use of a projected cash flow.

Watch your cash flow

Projected cash inflow and outflow during the period are compared, reflecting payment requirements from credit agencies as well as all normal expenditures. Some business changes are capable of being profitable in the long run *but they are not capable of meeting short run demands for cash, particularly when payment requirements for capital expenditures are concentrated in the early years of an investment.*

Management must expand as herds get larger. More failures are due to lack of management than any other one thing. Hours spent thinking and getting facts will pay off and the manager or owner must assume this responsibility. A manager must have the ability to get the right things done at the right time.

Here are some more questions:

Will my expansion or modernization plan improve the chances and ease of producing a higher quality product? Will it increase the ease of the key jobs associated with dairying? Will it increase the efficiency of labor, investment and/or equipment? Will the modernized set-up provide the minimum movement of men, animals and material? Can this modern dairy farm compete with nonfarm occupations for good labor or manpower?

Do you as a manager or owner have a concern for production, people, quality milk and a desire to make the dairy more profitable? Will you develop a good milking program? One that obtains high production per cow, controls mastitis, produces clean excellent-flavored milk and uses a minimum amount of labor?

Check this planning list

Tomorrow's profitable dairy farm can be expected to be a planned operation. Here are some of the things you will want to consider during the planning phase: 1) Size of herd to be accommodated; 2) Dairy breed; 3) Climate conditions; 4) Topograpy of farmstead, slope, drainage and exposure; 5) Size and productivity of the farm; 6) Feeding program as related to feed storage and equipment for feeding; 7) Existing buildings and equipment that will fit into long range plans; 8) Availability of labor; 9) Capital available for investment; 10) Sanitary regulations or codes or present and potential milk markets; 11) Personal preference of the owner; 12) Water and air pollution regulations.

Planning with pencil and paper prior to the start of construction permits you to analyze combinations of equipment and building arrangements without any expenditure. Visit as many farms as possible and incorporate the good points seen at these farms into your facilities. Use university people and private consulting people.

Unless a dairyman regularly tests production, a pipeline milker can be the best friend that a cull cow ever had. The cull cow strolls in with the good producers, gives only enough milk to color the line, eats almost as much feed as the best cows, and then goes her merry way. A dairyman needs profitable production from every cow. You will call the correct signals and build a profit-winning dairy team if you use production records.

Feeding practices important

Constant attention to feeding practices, breeding for production and herd health during expansion are very important. A well designed, properly installed milking system is essential to proper milking. You can't afford an inadequate or poorly maintained milking system.

Productivity must be the key

The productivity of a resource depends on the amount and kind of other resources with which it is combined. As an example, when capital is substituted for labor, it requires fewer hours of labor per cow. However, unless production increases or more cows can be handled, the machinery and equipment cost will offset the savings in labor. Productivity of a resource then depends on the kind and quality of the resource with which it is combined. Modern technology is closely related then to the substitution of capital for labor and land. It increases the demands on management, emphasizes financial management and increases technical requirements of hired labor.

Finally, all of this modernization should help make dairying more pleasant, as well as more productive for you and your family.

This is one of a series of topics developed by noted Dairy authorities. For a complete set write for a free booklet.