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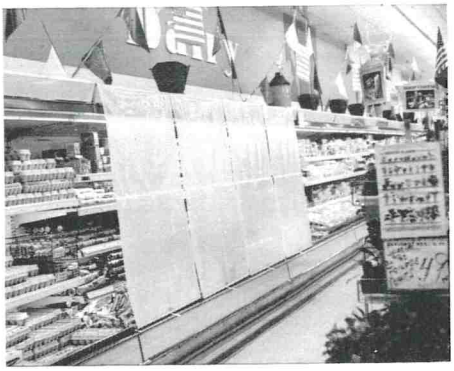

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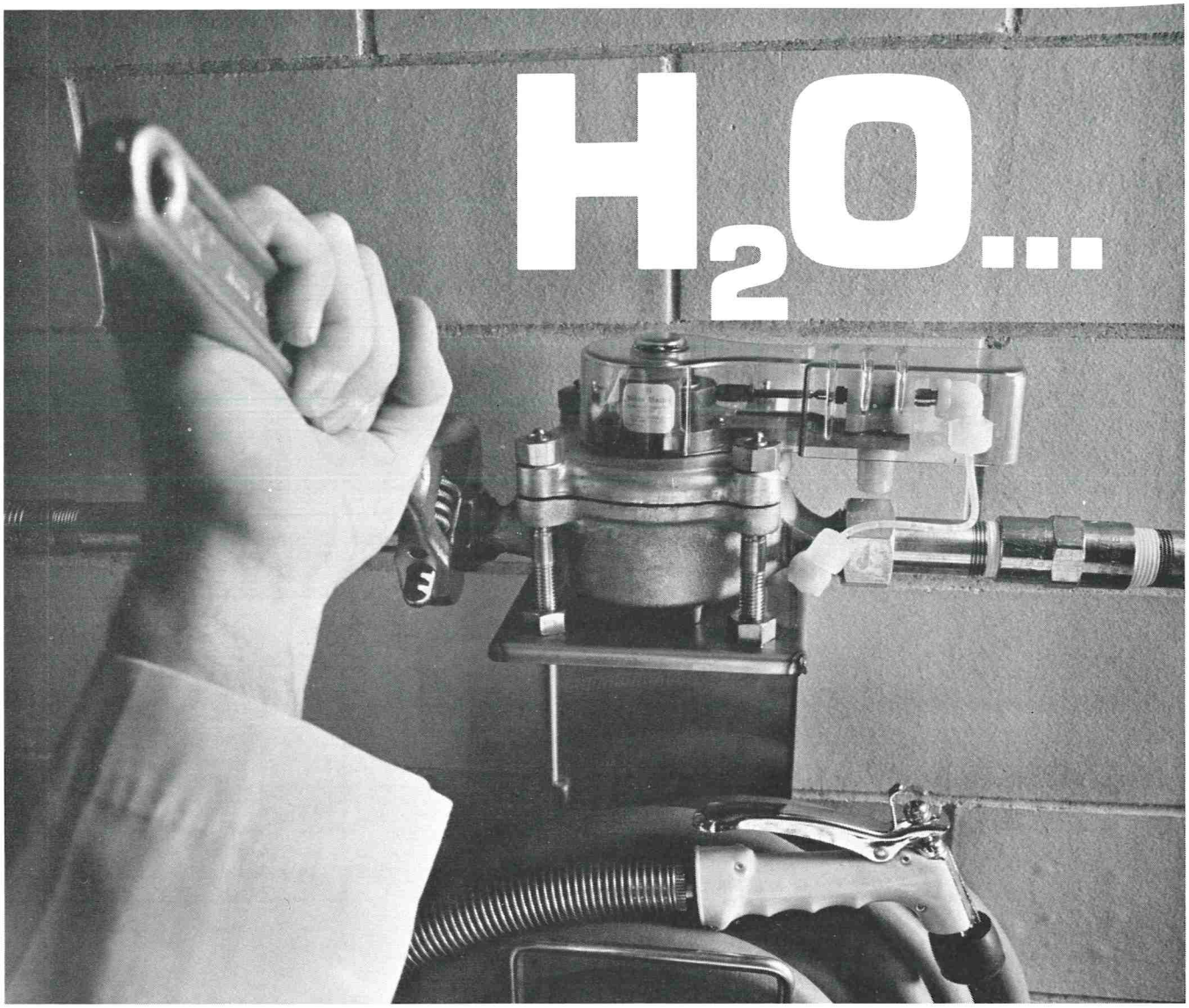

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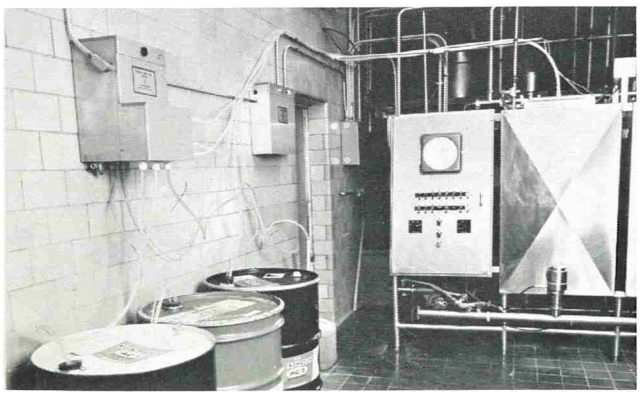

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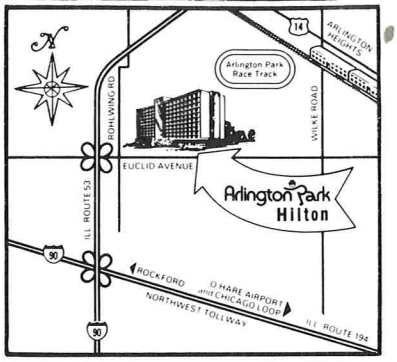

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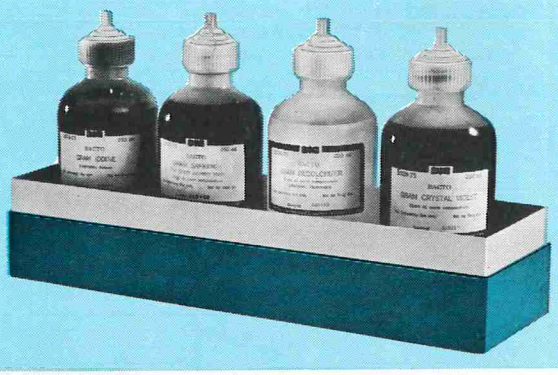

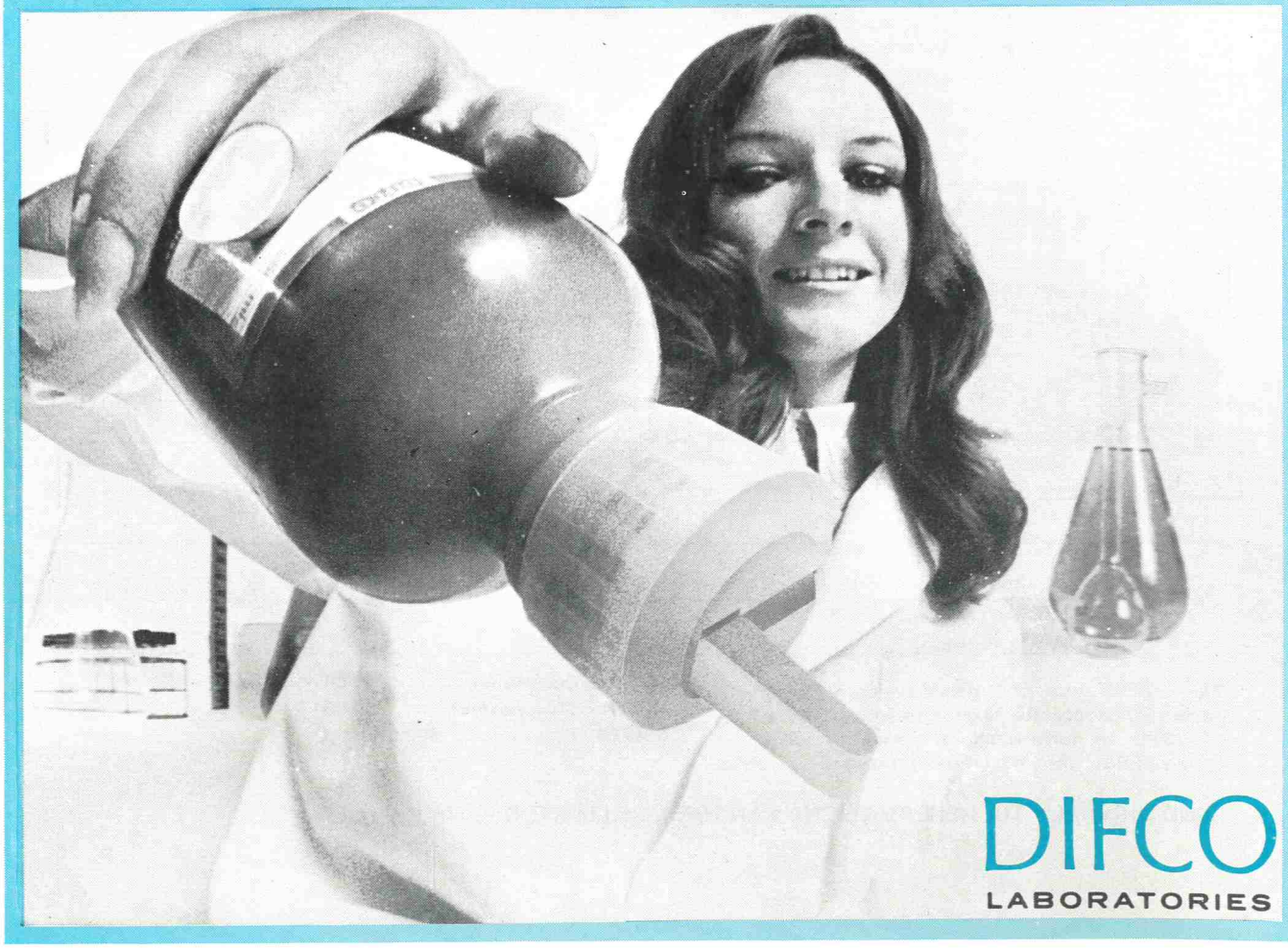

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J. Milk Food Technol. Vol. 39, No. 6, Pages 394-396 (June, 1976)

Comparison of the Milk Quality Gauge (MQG) With the Wisconsin Mastitis Test (WMT)[1]

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Rutgers University, New Brunswick, New Jersey 08903

(Received for publication August 12, 1975)

ABSTRACT

The Milk Quality Gauge (MQG), a cowside, screening test for abnormal milk, is based on observing the rate of flow of warm milk under vacuum through a dense test disk. The MQG, as applied to individual cow and herd milk samples has been evaluated in this study, a filtration time of 15 sec or more being considered a positive test. Results were compared with those obtained by the WMT in which a column height of 20 mm or more was considered a positive test. Of 364 herd samples tested, 97 were positive to MQG and nine to WMT, eight of these latter nine samples being positive for both tests. Of 181 individual cow samples tested, 75 were positive to MQG and 22 to WMT, all of these latter 22 samples being positive for both tests. High sediment content was considered to be a factor contributing to the large number of positive MQG samples. Also, many of the herd milk samples were old, a factor which probably influenced the WMT results obtained on them. When a yellow material on the disk was used as a criterion for a positive test, the MQG was found to agree with the WMT in 16 of the 22 positive WMT results and 175 of the 181 total, individual cow samples.

The California Mastitis Test, Modified Whiteside Test, Catalase Test, and Wisconsin Mastitis Test (WMT) are screening tests which reveal a range of somatic cell levels. The WMT, an objective test, has been demonstrated to give the best indication of somatic cell numbers (*3*) as measured by the direct microscopic cell count (DMSCC), a confirmatory method.

The Interstate Milk Shippers Conference Abnormal Milk Program stipulates that all producer milk be tested at least four times within a 6-month period by one of the four screening tests. In this way, the producer may become aware that cows in his herd are producing abnormal milk even though their affliction is in the sub-clinical stage. Thus, screening tests are playing a major role in milk quality programs today.

Recently, a new means to detect abnormal milk known as the Milk Quality Gauge (MQG) has been introduced (*2*). This test is based on observing the rate of flow of warm milk under vacuum through a dense test disk. Resistance to filtration is considered evidence of abnormal milk, the resistance being due to leucocytes and cell debris. While the test is designed for testing either quarter milk samples or complete individual cow milkings at cowside, its developers believe that it may also be applicable to herd milk samples, Accordingly, the main objective of this research has been to evaluate the MQG as applied to individual cow and herd milk samples.

MATERIALS AND METHODS

MQG

The equipment used in this method was a simple filtration system consisting of a vacuum or receiving chamber with a vacuum connection and milk discharge ports (*2*). A MQG dense test disk (32 mm dia.) was centered on the seat of the receiving chamber which contains four 0.1-inch (2.5 mm) diameter holes. The four-compartment cup containing four 0.2-inch (5.0 mm) diameter holes was then seated firmly over the disk, the holes being aligned with those in the receiving chamber. Warmed (95 F) milk samples (8 ml although 1 oz. is recommended for sediment testing) were transferred to each of the compartments, vacuum was applied, via a water discharge aspirator, and the time for filtration was observed along with the nature of the sediment on the disk.

Wisconsin Mastitis Test (WMT) and Direct Microscopic Somatic Cell Count (DMSCC)

These tests were done in accordance with the procedures found in *Standard Methods for the Examination of Dairy Products*, 13th edition, 1972. The strip reticle counting procedure was used for the DMSCC.

Procedure

Arrangements were made with a local laboratory to obtain herd milk samples. The approximate ages of these samples were noted at the time of pick-up. Samples of the entire milking (unfiltered) of individual cows were obtained at cowside from local herds and, in most instances analyzed within 36 h. All samples were refrigerated continuously from time of pick-up until analysis. They were analyzed by the MQG and WMT tests simultaneously on a maximum of 10 samples. Only samples which yielded positive results by either of the two tests were analyzed by the DMSCC method.

RESULTS AND DISCUSSION

A summary of the MQG and WMT data obtained on herd and individual cow milk samples is shown in Table 1. Of the 364 herd samples, 97 (26.6%) positive results

[1]*Paper of the Journal Series, New Jersey Agricultural Experiment Station, New Brunswick, N.J. 08903. Presented at the 62nd Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Toronto, Ontario, August 11-13, 1975.*
[2]*Present address: Department of Food Science, North Carolina State University, Raleigh.*

TABLE 1. *Summary of MQG and WMT data obtained on herd and individual cow milk samples*

		MQG				WMT		MQG[c]	
No.		Pos.[a]	Avg. time (sec)	Neg.	Avg. time (sec)	Pos.[b]	Neg.	No.	Avg. time (sec)
Herd	364	97	19.4	267	10.6	9	355	8	29.9
Individual cow	181	75	22.9	106	9.0	22	159	22	28.6

[a]Based upon a filtration time of 15 sec or more.
[b]Based upon a column height of 20 mm or more.
[c]Positive for both WMT and MQG tests.

were obtained by the MQG while only nine (2.5%) samples yielded positive WMT results. However, eight of these latter positive tests were also positive for the MQG (Table 2) and, in all instances, a yellow sediment was

TABLE 2. *Herd milk samples demonstrating positive results by both the MQG and WMT*

Sample No.	Age (h)	MQG (sec)	Disk appearance	WMT (mm)	DMSCC (x 10^{-3})
35	48	17	yellow	21	610
54	24	100	yellow	21	879
80	24	17	yellow	20	560
93	24	25	yellow	20	732
98	24	20	yellow	20	673
103	24	27	yellow	29	1,300
173	24	15	yellow	20	489
255	48	18	yellow	22	721

evident on the test disks. All of the DMSCC values were in the expected range, i.e., 500,000 or greater. One must ask why the MQG yielded far more positive tests than the WMT. Two factors are probably responsible for this disparity. Sediment content in the milk does contribute to the MQG results and, undoubtedly, was responsible for several of the positive MQG tests. Second, it is well-known that WMT results are influenced by the age

TABLE 3. *Individual cow milk samples demonstrating positive results by both the MQG and WMT*

Sample No.	Age (h)	MQG (sec)	Disk appearance	WMT (mm)	DMSCC (x 10^{-3})
12	8	22	yellow	20	641
17	8	45	yellow	34	1,700
18	8	30	v. lt. yellow	21	606
20	8	22	yellow	23	742
44	36	18	v. lt. yellow	22	803
61	36	35	yellow	29	—
76	36	20	yellow	21	713
81	36	26	yellow	20	726
88	24	45	yellow	23	906
89	24	21	v. lt. yellow	21	600
91	24	35	yellow	27	1,100
102	24	80	yellow	37	2,600
108	24	100	yellow	33	1,734
109	24	18	yellow	27	1,016
117	48	17	yellow	29	1,513
119	48	17	v. lt. yellow	21	673
120	48	30	yellow	31	1,682
123	48	18	med. sed.	23	819
125	48	16	yellow	37	2,855
132	48	18	yellow	38	3,043
169	36	20	yellow	33	1,823
179	36	18	v. lt. yellow	21	682

of the sample, results diminishing with time. As many of the herd milk samples were 48-96 h old at the time of analysis, it is likely that some samples yielded low WMT results due to the age factor.

There were 181 individual cow samples evaluated of which 75 (41.4%) yielded a positive MQG result and 22 (12.2%) a positive WMT result. All of the samples which yielded a positive WMT result also had a positive MQG result (Table 3). Again, the DMSCC data for these samples were in the expected range. As these samples were relatively fresh (8-48 h) in comparison to the herd milk samples, the data are considered more reliable. They were subjected to further analysis in an effort to improve the WMT:MQG correlation. Figure 1

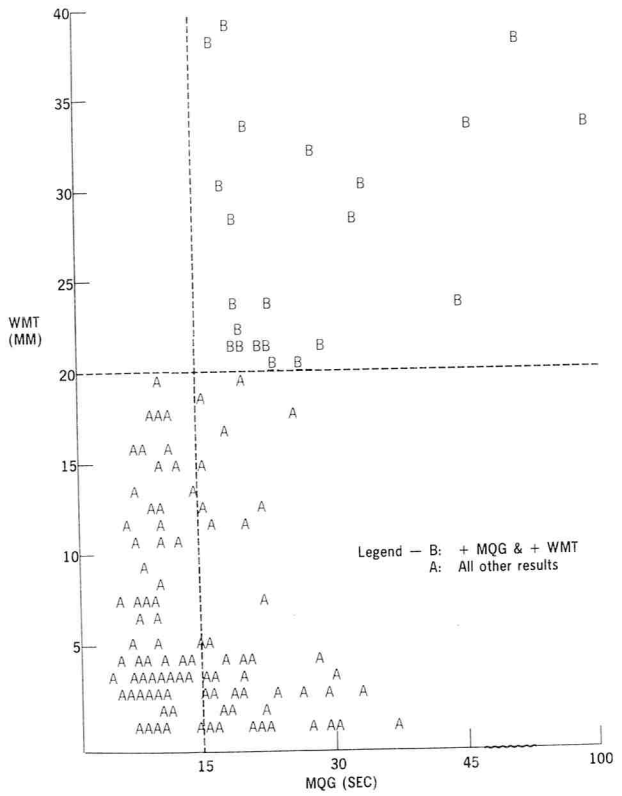

Figure 1. *Computer printout of WMT vs. MQG (time measurement) results for 181 individual cow milk samples.*

demonstrates very clearly that the MQG, based on a filtration time of 15 sec or more, yielded far too many false positive tests in relation to the WMT to be considered a reliable index of abnormal milk. However, if the presence of a yellow-colored material on the disk is used as a criterion for indicating abnormality, a great improvement results (Fig. 2). In this instance, the MQG was found to agree with the WMT in 16 of the 22 positive WMT results and 175 of the 181 total samples. These data substantiate those of Choi and Forster (*1*) who observed that the yellow-colored material appearing on sediment disks was closely related to other indices of mastitis.

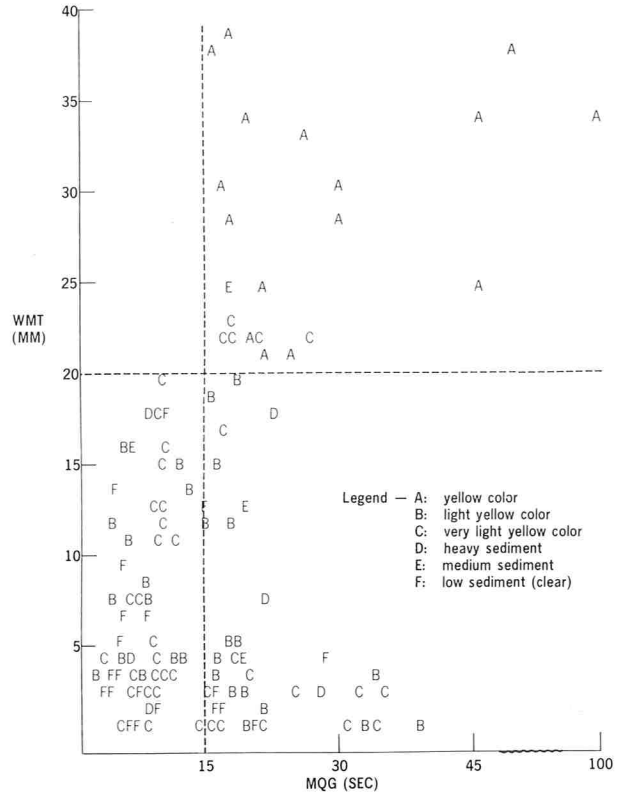

Figure 2. *Computer printout of WMT vs. MQG (color measurement) results for 181 individual cow milk samples.*

CONCLUSIONS

The results of this research reveal that the MQG has potential as a screening test for detecting abnormal milk from individual cows. However, the observation of yellow material on the filter disk appears to be a much better criterion for a positive test than the rate of filtration due to the large number of false positives observed when the latter criterion is utilized.

ACKNOWLEDGMENT

The authors express their gratefulness to Dr. William H. Swallow, Department of Statistics and Computer Science, for his assistance in the computer analyses.

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J. Milk Food Technol. Vol. 39, No. 6, Pages 397-400 (June, 1976)

Effect of Copper on Ascorbic Acid Content, Redox Potential and Development of Oxidized Flavor in Milk of Cows and Buffaloes

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(Received for publication April 29, 1975)

ABSTRACT

The effect of copper on ascorbic acid content, redox potential (E_h) of milk, and development of oxidized flavor is discussed. Oxidized flavor appeared in cow milk only after a marked rise in E_h due to the oxidation of ascorbic acid, whereas in buffalo milk oxidized flavor developed without any appreciable change in E_h. In buffalo milk, copper at low concentrations failed to cause sufficient oxidation of ascorbic acid to effect an increase in E_h. The E_h of cow milk began to rise appreciably when more than half of the ascorbic acid normally present in milk was oxidized. The pro-oxidant action of ascorbic acid in cow milk at concentrations normal to milk is not merely due to conversion of copper to cuprous form; it is suggested that the activation energy involved in this conversion is an important factor in the lipid oxidation which follows. Ascorbic acid did not promote copper-catalyzed oxidation of buffalo milk as it is not involved in the reversible oxidation.

Contamination by copper promotes development of oxidized flavors in milk. Further, it was shown by several workers (*5, 7, 22*) that addition of copper to cow milk causes an increase in redox potential (E_h). The data of Tracy et al. (*19*) indicate a definite relationship between oxidized flavor and redox potential of milk and cream containing added copper. Greenbank (*7*) studying numerous cow milk samples, found that those exhibiting a relatively great increase in E_h after addition of copper were susceptible to oxidized flavor formation, while those with slight or no increase in E_h did not develop the flavor sufficiently to be detected by organoleptic methods. Thurston (*18*) showed that salts of copper raised the E_h of milk but felt that E_h is of no value in predicting the possible behavior of milk as regards its tendency to become tallowy. Similarly, Swanson and Sommer (*17*) found that development of oxidized flavor was not dependent on the change in redox potential caused by metals. However, in their experiment milk with the highest content of reduced ascorbic acid showed less increase in E_h on addition of copper and did not develop any oxidized flavor. The increase in redox potential caused by copper in cow milk is rather sharp and after it has reached a new high, it continues to remain nearly constant for a considerable period of storage. This increase in potential does not take place until the reduced ascorbic acid in the milk has been almost oxidized (*17*). The oxidation of ascorbic acid in turn, is correlated to copper concentration in milk (*1*).

It was of interest to study if the observations just described also hold for buffalo milk. Not much information is available on the poise of buffalo milk, though it is known that buffalo milk is richer than cow milk in ascorbic acid (*3*). There are reports that buffalo milk is more resistant to copper induced oxidation than cow milk (*3, 20*). El-Hagarawy (*3*) suggested that the slower oxidation of fat in buffalo milk is related to the higher content of ascorbic acid. Our preliminary studies showed that addition of ascorbic acid to cow milk to bring its level to that in buffalo milk did not retard oxidized flavor development. The work herein reported provides further data that may be useful in interpretation of E_h in relation to the keeping quality of milk.

MATERIALS AND METHODS

Milks from individual animals were obtained from the herds maintained at the institute farm. The cow herd consisted of Jersey, Tharparkar and cross-bred animals, while the buffaloes were of Surti breed. The animals selected were all in the middle stage of lactation. Samples were drawn directly into glass bottles thoroughly cleaned with nitric acid and repeatedly washed with glass-distilled water. Extreme care was taken to prevent metal contamination of the samples. While transferring samples to laboratory, undue exposure to light was avoided, and the experiment started within 30 min after milking.

Samples were pasteurized in glass bottles. The samples were first heated in boiling water bath to 63 C; immediately after samples attained this temperature, bottles were removed to another water bath maintained at that temperature and held for 30 min. After this holding time, bottles were removed to a cold water bath for immediate cooling. Storage of samples was done in a refrigerator at 5 ± 1 C.

Cupric sulfate or cuprous chloride was added to the samples before or after pasteurization, as the experiments warranted. The concentration of copper solution was such as to give a concentration of 1 ppm in either form when 1 ml was added to 100 ml of milk. Copper contents of the samples were determined using Dithizone as described by Mulder et al. (*12*). All samples tested had less than 0.1 ppm of natural copper content.

The E_h of samples was determined using a Polymetron pH meter, type 42 B, with a platinum electrode and a calomel half cell. Before measurement the platinum electrode was thoroughly cleaned in hot nitric acid solution to which a pinch of sodium nitrite was added, rinsed several times with glass distilled water, and dried before insertion into milk samples. The instrument was standardized before the start of a series of readings and checked intermittently as the determination

proceeded. Each sample was in contact with the electrodes for at least 5 min or until a constant reading was obtained.

Ascorbic acid was estimated by titration with 2-6-dichlorophenol indophenol by the method given by Sommer (*16*) modified as follows to obtain blank readings in milk. Samples were exposed to ultraviolet radiation instead of the addition of copper. This procedure was adopted since it was found that copper could not completely oxidize ascorbic acid in buffalo milk in a short time where as exposure to UV radiation caused its complete oxidation both in cow and buffalo milks.

Milk fat oxidation was followed by thiobarbituric acid values as given by King (*9*). Absorbance value of 30×10^{-3} was found to indicate oxidized flavor.

RESULTS AND DISCUSSION

Cupric ion, a strong electron acceptor and a potent oxidizing agent, tends to raise E_h in milk. On the other hand, milk bacteria through the use of available oxygen in the medium as well as by elaborating reducing substances in the course of their metabolism generally lower the potential. This influence of bacteria on E_h is a function of their population and activity. The interference due to bacteria can be kept to a minimum in studies on the effect of copper on E_h by addition of penicillin G sodium salt. However, it was felt more appropriate to consider the increase in E_h due to copper alone under normal conditions rather than the actual E_h attained by the sample under controlled bacterial activity. Table 1 gives the increase in redox potential of raw milk caused by different concentrations of copper during 96 h of storage. The values given are results of 11 samples tested. From data in the table, it is seen that copper caused a marked increase in redox potential in cow milk. The final increase in potential was independent of the concentration of added copper. Swanson and Sommer (*17*) reported a smaller increase in E_h with low concentrations of copper probably because they studied potential increase only up to 72 h of storage. From Table 1 it is evident that the time required for the E_h to attain the highest level observed is dependent on the amount of added copper.

TABLE 1. *Effect of cupric copper on E_h and ascorbic acid content of raw cow milk*[a]

Hours after addition of copper	ppm of Copper added				
	0.25	0.50	0.75	1.0	2.0
1		3 ± 2[b]	3 ± 2	5 ± 2	10 ± 3
		5 ± 2[c]	11 ± 3	21 ± 4	52 ± 3
2	2 ± 1	3 ± 2	5 ± 2	9 ± 2	43 ± 4
	4 ± 2	8 ± 2	21 ± 5	40 ± 4	77 ± 3
3	3 ± 1	4 ± 2	7 ± 2	15 ± 4	79 ± 5
	6 ± 2	15 ± 4	34 ± 4	52 ± 4	97 ± 3
4	4 ± 2	4 ± 2	10 ± 4	33 ± 6	86 ± 6
	9 ± 4	24 ± 4	50 ± 4	74 ± 5	97 ± 3
8	5 ± 2	8 ± 2	35 ± 4	76 ± 5	86 ± 6
	16 ± 4	40 ± 3	69 ± 4	88 ± 5	98 ± 2
24	12 ± 5	46 ± 6	80 ± 6	84 ± 6	86 ± 6
	52 ± 4	70 ± 4	92 ± 3	96 ± 4	98 ± 2
48	53 ± 6	83 ± 6	82 ± 7	84 ± 6	85 ± 6
	79 ± 4	90 ± 5	96 ± 4	96 ± 3	97 ± 3
72	73 ± 6	86 ± 5	84 ± 6	85 ± 6	85 ± 6
	87 ± 5	96 ± 3	96 ± 2	96 ± 3	97 ± 3
96	84 ± 6	85 ± 6	85 ± 6	86 ± 7	86 ± 6
	96 ± 4	96 ± 3	96 ± 2	96 ± 4	97 ± 3

[a]Values expressed as mean of 11 values ± standard deviation.
[b]Values in top line of each set of two lines are change in Eh in mu.
[c]Values in bottom line of each set of two lines are percent of ascorbic acid that was oxidized.

The E_h of cow milk begins to rise appreciably when more than half the ascorbic acid normally present in milk is oxidized. This observation should be considered in the light of the report of Krukovsky and Guthrie (*11*) that lipid oxidative reactions are initiated more rapidly in milk when the ratio of ascorbic to dehydro-ascorbic acid is approximately 1:1 or lower. Copper catalyses conversion of ascorbic acid to dehydro-ascorbic acid (*23*) and it is seen from Tables 1 and 2 that when more than half of the ascorbic acid is oxidized by copper, that is, when the ratio of ascorbic to dehydro-ascorbic acid is lower than 1:1, the redox potential begins to rise, thereby creating more favorable conditions for lipid oxidation reactions leading to off flavors. Hence the study of E_h gives an explanation for the observation made by Krukovsky and Guthrie (*11*). Samples to which no copper was added showed a slight decrease in E_h during storage, as expected, due to bacterial activity. These samples developed no off-flavors even after 96 h of storage. Based on these observations, samples with shorter time lag for potential increase may be expected to develop oxidized flavors early. When copper was added to cow milk samples before pasteurization, there was an instantaneous rise in E_h. The samples, to which copper was added after pasteurization, had a certain lag period before the E_h started to rise. In this latter instance, however, it was noted that the lag period (Table 2) was less than that of raw milk with the same amount of copper (Table 1). From the study of E_h, these pasteurized samples should be expected to develop oxidized flavor early. This was found true under both conditions of pasteurization (Table 3). However, at higher concentrations of copper

TABLE 2. *Effect of cupric copper on E_h and ascorbic acid content of pasteurized cow milk*[a]

Hours after addition of copper	ppm of Copper added				
	0.25	0.50	0.75	1.0	2.0
1		3 ± 2[b]	5 ± 2	7 ± 2	20 ± 4
	5 ± 2[c]	8 ± 3	13 ± 3	21 ± 5	51 ± 6
2	2 ± 1	3 ± 2	6 ± 2	15 ± 4	59 ± 5
	9 ± 3	13 ± 4	31 ± 5	50 ± 7	84 ± 7
3	3 ± 1	4 ± 2	8 ± 3	55 ± 6	71 ± 5
	15 ± 3	21 ± 4	42 ± 4	76 ± 4	94 ± 4
4	4 ± 2	5 ± 2	21 ± 4	72 ± 6	78 ± 5
	20 ± 3	29 ± 5	62 ± 7	93 ± 5	97 ± 4
8	5 ± 2	11 ± 3	57 ± 6	82 ± 6	83 ± 6
	35 ± 5	51 ± 6	84 ± 6	95 ± 4	96 ± 4
24	35 ± 3	78 ± 5	79 ± 5	82 ± 5	84 ± 6
	57 ± 7	89 ± 4	94 ± 4	95 ± 4	96 ± 4
48	69 ± 5	83 ± 4	82 ± 6	85 ± 5	84 ± 6
	90 ± 3	93 ± 5	95 ± 5	95 ± 4	96 ± 4
72	85 ± 6	84 ± 5	84 ± 6	84 ± 5	84 ± 6
	94 ± 4	95 ± 5	96 ± 5	95 ± 4	96 ± 4
96	86 ± 5	85 ± 4	84 ± 5	86 ± 5	85 ± 5
	95 ± 2	95 ± 4	96 ± 5	95 ± 4	96 ± 4

[a]Values expressed as mean of 11 samples ± standard deviation.
[b]Values in top line of each set of two lines are change in Eh in mu.
[c]Values in bottom line of each set of two lines are percent of ascorbic acid that was oxidized.

TABLE 3. *Increase in TBA value ($A \times 10^{-3}$) due to addition of cupric copper to cow milk*

Hours after addition of copper	Treatment	Increase in TBA values[a] due to addition of Copper at ppm concentrations of					
		0	0.25	0.50	0.75	1.0	2.0
24	R[b]		7 ± 3	13 ± 5	21 ± 5	26 ± 6	32 ± 6
	AP[c]		19 ± 4	32 ± 4	32 ± 4	37 ± 4	48 ± 6
	PA[d]		15 ± 5	24 ± 6	33 ± 9	44 ± 11	55 ± 11
48	R	3 ± 2	18 ± 6	33 ± 10	40 ± 8	45 ± 9	65 ± 7
	AP	6 ± 2	35 ± 6	42 ± 4	47 ± 6	53 ± 5	67 ± 7
	PA	6 ± 2	32 ± 10	41 ± 8	49 ± 10	56 ± 10	74 ± 10
72	R	8 ± 3	33 ± 10	49 ± 10	62 ± 10	73 ± 8	81 ± 10
	AP	10 ± 4	43 ± 10	53 ± 10	66 ± 10	73 ± 9	79 ± 11
	PA	10 ± 4	50 ± 10	58 ± 9	69 ± 11	76 ± 9	80 ± 9
96	R	11 ± 4	56 ± 10	65 ± 11	76 ± 11	89 ± 9	98 ± 12
	AP	13 ± 5	61 ± 11	69 ± 10	78 ± 9	89 ± 8	98 ± 13
	PA	13 ± 5	66 ± 11	76 ± 10	84 ± 11	91 ± 9	99 ± 11

[a]Values expresses as mean of 11 samples ± standard deviation.
[b]R—Addition of Copper to raw milk.
[c]AP—Addition of copper to raw milk followed by pasteurization.
[d]PA—Addition of copper after pasteurization.

and longer duration of storage samples treated with copper after pasteurization show higher TBA values than the samples treated with copper before pasteurization. This anomaly could probably be due to factors such as differences in the distribution and status of copper in the two sets of pasteurized samples. The influence of the stage of addition of copper on its distribution in milk and its effect on flavor development will be reported in another paper.

Ascorbic acid, a strong reducing agent promotes oxidative deterioration of cow milk at normal concentrations (*2, 10, 11, 13, 14, 21*). According to Smith and Dunkley (*14*) ascorbic acid promoted oxidation by reducing copper to the cuprous state. The presence of copper in the cuprous form was stated to be more potent in bringing out lipid oxidation resulting in off flavors. These authors had come to this conclusion based on their observation that neocuproine, a specific cuprous copper chelating agent could prevent oxidized flavor development in cow milk. If the presence of copper in the cuprous state is the only factor responsible for enhanced lipid oxidation in milk by ascorbic acid, then samples to which cuprous copper is added should develop oxidized flavors earlier than when cupric copper is added. Potencies of cupric and cuprous forms of copper were compared by adding both to milk samples at concentrations of 1 ppm. It was noted that oxidized flavor developed early in samples to which cupric copper was added (Table 4). This means that accelerated oxidation in presence of ascorbic acid is not entirely due to conversion of copper to cuprous state. It is our view that the role of activation energy acquired during the reversible oxidation of ascorbic acid in milk (*15*) is also an important factor in the subsequent lipid oxidation. That copper brings about only the reversible state of oxidation of ascorbic acid has already been reported (*23*). This may explain why catechol, which is as effective as ascorbic acid in reducing copper to cuprous form (*4*), is not so effective as ascorbic acid in promoting oxidation of milk (*14*).

With buffalo milk the conditions for oxidation appear to be different. Copper, even at high concentrations, is not in a state to cause appreciable oxidation of ascorbic acid, (Tables 5, 6, 7). This could be due to differences in the binding and distribution of copper in buffalo milk (*20*). Higher content of casein present in buffalo milk (*6*) which has been shown to act as a deterrent in the

TABLE 4. *Comparative effect of cuprous and cupric forms of copper on TBA value of milk*

Hours after addition of Copper	Milk sample	Increase in TBA values[a] of samples enriched with one ppm of	
		Cuprous Copper	Cupric Copper
48	Cow, raw	29 ± 6	45 ± 9
	Cow, pasteurized	38 ± 6	56 ± 10
	Buffalo, raw	17 ± 3	19 ± 6
	Buffalo, pasteurized	29 ± 6	34 ± 8
96	Cow, raw	78 ± 7	89 ± 9
	Cow, pasteurized	80 ± 10	91 ± 9
	Buffalo, raw	49 ± 8	58 ± 11
	Buffalo, pasteurized	77 ± 8	91 ± 9

[a]Values expressed as mean of 11 samples ± standard deviation.

TABLE 5. *Effect of cupric copper on E_h and ascorbic acid content of raw buffalo milk*[a]

Hours after addition of copper	ppm of Copper added				
	0.25	0.50	0.75	1.0	2.0
1	—	—	2 ± 1[b]	2 ± 1	3 ± 1
	—	—	3 ± 2[c]	3 ± 2	5 ± 2
2	—	—	3 ± 1	3 ± 2	3 ± 1
	3 ± 2	3 ± 2	3 ± 2	3 ± 2	6 ± 2
3	—	—	3 ± 1	3 ± 2	3 ± 1
	3 ± 2	4 ± 2	4 ± 2	4 ± 2	7 ± 2
4	—	—	3 ± 1	4 ± 2	5 ± 2
	4 ± 2	4 ± 2	4 ± 2	4 ± 2	9 ± 2
8	—	3 ± 1	3 ± 1	5 ± 1	6 ± 2
	5 ± 2	5 ± 2	5 ± 2	7 ± 2	12 ± 4
24	—	—	4 ± 2	5 ± 1	7 ± 2
	7 ± 2	7 ± 2	8 ± 3	16 ± 5	31 ± 5
48	—	—	5 ± 2	6 ± 1	16 ± 3
	8 ± 3	8 ± 2	10 ± 2	20 ± 3	47 ± 5
72	—	—	5 ± 2	6 ± 2	20 ± 3
	9 ± 4	9 ± 2	13 ± 3	21 ± 4	51 ± 4
96	—	—	5 ± 2	6 ± 1	29 ± 5
	10 ± 3	11 ± 3	14 ± 3	22 ± 3	55 ± 6

[a]Values expressed as mean of 11 samples ± standard deviation.
[b]Values in top line of each set of two lines are change in Eh in mu.
[c]Values in bottom line of each set of two lines are percent of ascorbic acid that was oxidized.

TABLE 6. *Effect of cupric copper on E_h and ascorbic acid content of pasteurized buffalo milk*[a]

Hours after addition of copper	ppm of Copper added				
	0.25	0.50	0.75	1.0	2.0
1	—	—	2 ± 1[b]	2 ± 1	3 ± 1
	—	—	33 ± 2[c]	3 ± 2	8 ± 2
2	—	—	3 ± 1	3 ± 2	3 ± 1
	3 ± 2	3 ± 2	3 ± 2	4 ± 2	11 ± 3
3	—	—	3 ± 1	3 ± 2	3 ± 1
	5 ± 2	5 ± 2	5 ± 2	7 ± 2	14 ± 3
4	—	—	3 ± 1	4 ± 2	5 ± 2
	6 ± 2	8 ± 2	8 ± 3	9 ± 2	17 ± 4
8	—	—	3 ± 1	5 ± 1	6 ± 2
	7 ± 3	10 ± 2	13 ± 4	14 ± 3	20 ± 3
24	—	—	4 ± 2	5 ± 1	8 ± 3
	10 ± 2	12 ± 3	16 ± 4	17 ± 3	30 ± 4
48	—	—	5 ± 2	6 ± 1	15 ± 3
	14 ± 4	14 ± 2	17 ± 3	21 ± 3	48 ± 3
72	—	—	5 ± 1	6 ± 1	22 ± 2
	15 ± 2	15 ± 3	22 ± 3	24 ± 5	51 ± 6
96	—	—	5 ± 2	6 ± 1	27 ± 5
	15 ± 3	20 ± 4	23 ± 3	28 ± 4	54 ± 5

[a]Values expressed on mean of 11 samples ± standard deviation.
[b]Values in top line of each set of two lines are change in Eh in mu.
[c]Values in bottom line of each set of two lines are percent of ascorbic acid that was oxidized.

oxidation of ascorbic acid by copper (*8*) could also be a factor. In buffalo milk, as the reversible oxidation of ascorbic acid was not pronounced, copper catalysed lipid oxidation was not promoted by ascorbic acid. This may, in part, explain why copper is less potent in buffalo milk in bringing about oxidative deterioration.

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TABLE 7. *Increase in TBA value ($A \times 10^{-3}$) due to addition of cupric copper to buffalo milk*

Hours after addition of copper	Treatment	Increase in TBA values[a] due to addition of copper at ppm concentrations of					
		0	0.25	0.50	0.75	1.0	2.0
24	R[b]		3 ± 2	5 ± 3	9 ± 3	12 ± 4	14 ± 3
	AP[c]		6 ± 3	7 ± 2	10 ± 4	13 ± 4	14 ± 4
	PA[d]		8 ± 3	8 ± 3	9 ± 3	12 ± 3	14 ± 4
48	R	3 ± 2	9 ± 3	16 ± 6	18 ± 6	19 ± 6	36 ± 10
	AP	6 ± 3	15 ± 4	18 ± 4	25 ± 4	27 ± 6	38 ± 6
	PA	6 ± 3	18 ± 4	24 ± 6	31 ± 6	34 ± 8	49 ± 10
72	R	7 ± 3	17 ± 5	24 ± 9	31 ± 9	45 ± 10	58 ± 8
	AP	8 ± 3	18 ± 4	27 ± 7	39 ± 7	56 ± 6	60 ± 8
	PA	8 ± 3	28 ± 6	42 ± 8	50 ± 9	61 ± 10	72 ± 11
96	R	9 ± 3	30 ± 8	42 ± 11	47 ± 11	58 ± 11	72 ± 8
	AP	13 ± 4	36 ± 8	47 ± 6	55 ± 7	67 ± 8	77 ± 9
	PA	13 ± 4	51 ± 9	61 ± 9	64 ± 11	91 ± 9	92 ± 9

[a]Values expressed as mean of 11 samples ± standard deviation;
[b]R—Addition of copper to raw milk
[c]AP—Addition of copper to raw milk followed by pasteurization
[d]PA—Addition of copper after pasteurization.

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Bacteriological Analysis of Ground Beef[1]

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ABSTRACT

A survey on the bacteriological quality of ground beef in Maryland was conducted to provide information relating to establishment of bacterial standards on fresh meats. One hundred forty samples were obtained at the retail, processor, and slaughter-processor levels. Retail samples yielded the highest bacterial numbers. The mean coliform, fecal coliform, and *Escherichia coli* count per gram, for all samples, was 200, 10, and 5, respectively. The mean total aerobic plate count per gram was 7.9×10^6 (28 C) and 2.0×10^6 (35 C). Forty-three percent of all the samples analyzed exceeded 50 fecal coliform per gram, while 18% exceeded a total aerobic plate count of 1.0×10^7 per gram.

Several studies regarding the bacteriological quality of ground beef in different geographical areas have been published (*2,5,7-10,14,18,20*). Some of these previous investigations were in response to possible quality standards on ground beef.

The State of Oregon currently has bacterial standards on ground beef, and other states and local jurisdictions are contemplating the need for such regulation (Table 1). A bill proposing bacterial standards on fresh meats was introduced in the Maryland House of Delegates during 1975 and hearings held. "If any fresh meat sold for human consumption contains a bacteria count of more than five million per gram, or a fecal coliform count of more that 50 per gram, it shall be deemed adulterated. However, ground beef may contain a bacteria count not exceeding ten million per gram." (*12*). This proposed bill was not enacted during the 1975 session. However, interest in bacterial standards has been renewed due to the salmonellosis outbreak attributed to ground beef in Maryland during 1975 (*19*).

This study was designed to determine the bacteriological quality of ground beef in Maryland, and to generate data which could serve as a source of information regarding the establishment of possible quality standards on fresh meats.

MATERIALS AND METHODS

Samples

Approximately 2-lb samples of ground beef were obtained from retail outlets (53), processors (47), and slaughter-processors (40). Processors ground the beef, but did not have slaughtering facilities. Samples were placed in polyethylene bags and transported to the laboratory in crushed ice. If the samples were not analyzed the same day, they were refrigerated at 2 C and analyzed the following day.

Sample preparation

Thirty grams of meat were weighed into a sterile blender containing 270 ml of peptone water (0.1%w/v) and mixed for 3 min at low speed. Meat used in the frozen shelf life study was aseptically reground in a sterile meat grinder. Thirty-gram samples were placed in 12 sterile beakers and covered with aluminum foil. On days 0, 1, 2, and 6 of storage (–15.5 C) three beakers were removed and the meat analyzed in triplicate.

Bacteriological examination

All media were obtained from Baltimore Biological Laboratories, (BBL), Cockeysville, Maryland. Total aerobic plate counts (TPC) were determined using Standard Methods Agar (SMA) with incubation at 28 and 35 C for 72 and 48 h, respectively. Most probable number (MPN) coliform, fecal coliform, and *Escherichia coli* per gram were determined as follows: appropriate dilutions were inoculated into a three-tube series of Lauryl Sulfate Broth (LSB) with incubation at 35 C for 24 and 48 h. Positive LSB tubes (gas) were transferred to Brilliant Green Bile

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TABLE 1. *Current microbiological criteria on ground beef*[a]

State	Status	Total plate count x 10^6/g	coliform/g	Fecal coliform/g	*Escherichia coli*/g	Staph/g	Coagulase positive staph/g
Georgia	guideline	5.0	1,000	—	0	500	100
Idaho	guideline	3.0	50	—	—	0	—
Maryland	proposed standards	10.0	—	50	—	—	—
Massachusetts	guideline	0.05	50	—	—	—	—
New York	guideline	1.0	—	—	3	—	—
North Dakota	proposed standards	5.0	—	—	50	0	—
Oregon	standards	5.0	—	—	50	—	—
Rhode Island	guideline	1.0	200	—	—	—	—
Utah	guideline	3.0	100	—	—	—	—
Virginia	guideline	10.0	—	—	100	—	100
Washington	guideline	5.0	—	—	50	—	—
Wyoming	guideline	1.0	—	—	—	—	—

[a]These data were summarized from responses to a request to all the states by Mr. Y. D. Hance, Secretary, Maryland State Department of Agriculture.

Broth 2% (BGBB) and EC broth, with incubation at 35 C for 48 h, and 45.5 C ± 0.05 for 48 h, respectively. Positive BGBB tubes were used to calculate coliforms per gram, and positive EC tubes were used to calculate fecal coliforms per gram. *E. coli* per gram was determined by streaking positive EC tubes on Levine's Eosin Methylene Blue Agar (EMB), and observing typical colonies following incubation at 35 C for 24 h.

The presence of *Salmonella* was tested for by selective enrichment in Selenite-Cystine Broth, incubated at 35 C for 24 h, followed by streaking on Brilliant Green Agar (BGA) and Salmonella-Shigella Agar (SS) with incubation at 35 C for 18-24 h. Suspect isolates were tested using *Salmonella* polyvalent "O" antisera (BBL).

RESULTS AND DISCUSSION

Ground beef survey

The bacteriological analysis of 140 ground beef samples is summarized in Table 2. Samples obtained from slaughter-processors had consistently lower counts than samples obtained from processors or from retail markets. Retail samples had the highest counts. The average coliform, fecal coliform, and *Escherichia coli* count per gram was 200, 10, and 5, respectively. The average total aerobic plate count (TPC) per gram was 7.9×10^6 (28 C) and 2.0×10^6 (35 C). These data are presented as $\log_{10}$ numbers in Table 2.

TABLE 2. *Summary of the bacteriological analysis of 140 ground beef samples*

Samples	Most probable number per gram			Total plate count per gram	
	Coliform	Fecal coliform	*Escherichia coli*	at 28 C	at 35 C
Retail					
mean	2.8[b]	2.3	2.3	7.0	6.8
SD[a]	0.7	0.9	0.9	0.7	0.8
range	0.8-3.0	1.0-3.0	1.0-3.0	5.2-8.6	4.9-8.3
Processor					
mean	2.4	0.9	0.5	6.9	6.3
SD	0.9	1.6	1.7	0.7	0.8
range	0.6-3.0	0.0-3.0	0.0-3.0	4.3-7.5	4.2-7.5
Slaughter-processor					
mean	1.9	0.8	0.5	6.8	6.0
SD	1.4	1.8	1.7	0.6	0.7
range	0.0-3.0	0.0-3.0	0.0-3.0	5.0-7.5	4.5-7.0
All samples					
mean	2.3	1.0	0.7	6.9	6.3
SD	1.1	1.7	1.7	0.7	0.8
range	0.0-3.0	0.0-4.0	0.0-3.0	4.3-8.6	4.2-8.3

[a]Standard deviation.
[b]Log_{10} of counts.

Coliforms, fecal coliforms, and *E. coli* were not detectable in 3, 20, and 24% of the samples, respectively. All of the retail samples contained fecal coliforms, while 80% of processor and 75% of slaughter-processor samples contained fecal coliforms.

Retail samples, therefore, reflected the highest bacterial content and the lowest bacterial quality. This is undoubtedly due to increases in handling, processing, and time from slaughter. *Salmonella* was not isolated from any of the samples.

Applying the proposed Maryland bacterial limits to our data and other published surveys, the percent of samples that would be in violation was calculated (Table 3). Another, more recent, survey (*16*) reported that 24% of their samples exceeded a TPC of 1×10^6/g. One could conclude that the proposed limits are too restrictive.

TABLE 3. *Percent of samples that would exceed proposed standard*

Reported by	Number of samples	Percent that exceeded	
		Fecal coliform count of 50/g	Total plate count of 1×10^7/g
Ladiges et al. (*11*)	150	50-60	15-20 (32 C)[a]
Duitschaever et al. (*3*)	87[b]	76 (coliform)	64 (32 C)
Rogers and McCleskey (*15*)	96	60 (coliform)	52 (37 C)
Kirsch et al. (*9*)	20	—	35 (30 C)
Weinzirl and Newton (*20*)	44	—	50 —
Elford (*4*)	41	—	5 (37 C)
Carl (*2*)	—	16[c] (1974) 9 (1975)	
This study			
(all samples)	140	43	18 (35 C) 49 (28 C)
retail	39	86	36 (35 C) 59 (28 C)
processor	45	33	13 (35 C) 51 (28 C)
slaughter-processor	56	39	7 (35 C) 46 (28 C)

[a]Number in parentheses in temperature of TPC incubation.
[b]Included are only the samples described as "hamburger packaged."
[c]*E. coli* counts in excess of 50/g.

Incubation temperature for total aerobic plate count

An incubation temperature of 35 C has been recommended for fresh foods and meats (*13, 17*). A comparison of the counts obtained at 28 C versus 35 C suggests, however, that the 28 C count recovers more bacteria and probably better reflects the spoilage flora (psychrotrophic). The American Public Health Association (*1*) recommends an incubation temperature of 21-22 C for meat and meat products for this reason. Assuming a bacterial limit of 10^7/g, 25 of 140 samples (18%) would have been in violation using the TPC obtained at 35 C. If the TPC obtained at 28 C were used, almost 50% of the samples (69 out of 140) would have been in violation (Table 3). Total plate counts were not compared at 21-22 C, because of laboratory restrictions. However, the counts at 28 C would probably compare more favorably with those at 21-22 C, than at 35 C. The point being, that the choice of incubation temperature would be a significant consideration in establishing a standard.

Correlation between counts

Total bacterial numbers, whether determined at 28 or

TABLE 4. *Correlation between bacterial counts on ground beef*

	MPN coliform	MPN fecal coliform	MPN *E. coli*	Total plate count at	
				28 C	35 C
	r values[1]				
MPN coliform	—	0.190	0.195	0.556	0.233
MPN fecal coliform	0.190	—	0.923	0.089	-0.006
MPN *E. coli*	0.195	0.923	—	0.109	0.025
TPC at 28 C	0.556	0.089	0.109	—	0.503
TPC at 35 C	0.233	-0.006	0.025	0.503	—
	r^2 values				
MPN coliform	—	0.040	0.038	0.309	0.054
MPN fecal coliform	0.040	—	0.852	0.008	0.000
MPN *E. coli*	0.038	0.852	—	0.012	0.001
TPC at 28 C	0.309	0.008	0.012	—	0.253
TPC at 35 C	0.054	0.000	0.001	0.253	—

[1]Coefficient of correlation.

35 C, did not correlate well with any of the coliform analyses (Table 4). Actually, there was very little correlation between the 28 C and the 35 C TPC. As would be expected, most of the organisms detected by the fecal coliform MPN procedure were *E. coli*, as these two counts correlate quite well (Table 4).

Shelf life studies

To determine what effect freezing or refrigerating samples might have on cell numbers, samples were held at -15.5 C and at 5.4 C for up to 7 days before analysis. Variation and fluctuation in counts during storage were frequent, even in those meat samples that were reground (see methods) before storage (Fig. 1 and 2). Counts

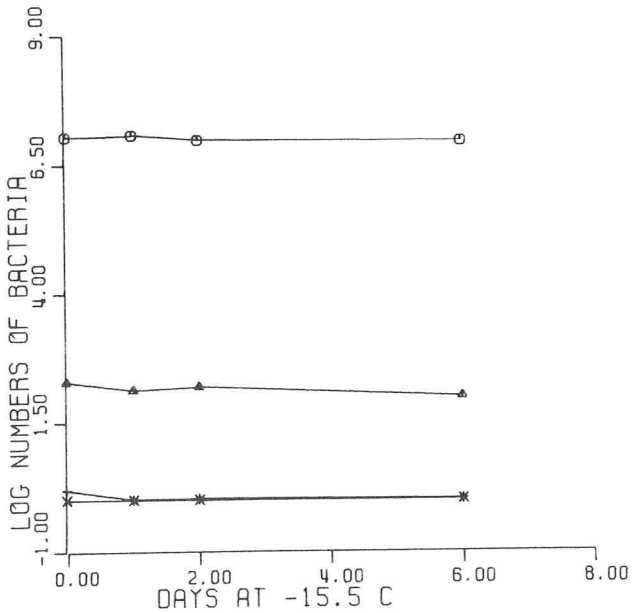

Figure 1. *Total plate count*(o), *coliform counts* (Δ), *fecal coliform count* (+), *and* Escherichia coli (X) *per gram in hamburger during frozen storage.*

decreased much less dramatically during frozen storage than would be anticipated, considering the susceptibility of the coliforms to freezing. It would be desirable to obtain more data on the effect of freezing samples before analysis. Oregon (14) has reported a low percent of samples in violation of its *E. coli* standard (an interim report). We had suspected that a possible explanation was that they were analyzing frozen samples. However, our data suggest that this may not be the case. The Total Plate Counts (28 C) did increase with storage at 5.4 C. Some of our samples (survey) were held at 2 C overnight, before analysis. There is the possibility that bacterial numbers increased during that time.

Consideration for bacterial standards

Persons contemplating the establishment of microbial criteria should first consult the paper by Elliot (*5*) regarding the difference between, and the significance of: microbiological standards, microbiological limits, microbiological specifications, and microbiological guidelines. With an understanding of these terms, and

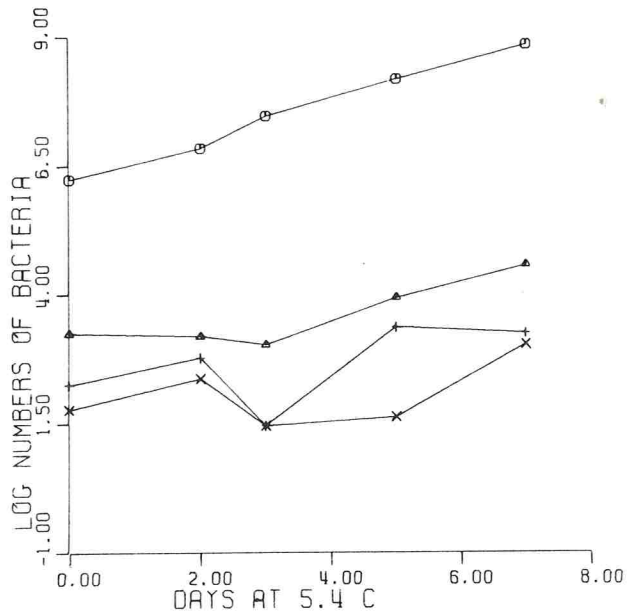

Figure 2. *Total plate count* (o), *coliform count* (Δ), *fecal coliform count* (+), *and* Escherichia coli (x) *per gram in hamburger during refrigerated storage.*

the procedures suggested, any criterion established would be more meaningful. In addition, two papers on the controversial success of the Oregon program (*2,22*) present a good overview of the situation, as seen by the Oregon State Department of Agriculture (*2*), and by a retail chain (*22*).

Secondly, a microbiological criterion should reflect both the consumer's risk and the producer's risk (*7*). This point is reemphasized by Winslow (*22*) and Goepfert and Kim (*6*). It would be difficult not to consider a perishable product, such as ground beef, which consistently has high bacterial counts, a high isolation rate of coagulase positive staphylococci, a high isolation rate of fecal coliforms, and a high isolation rate of *Clostridium perfringens*(*10*), as not being a potential consumer risk or a problem in food-borne outbreaks. However, a recent survey by the Center for Disease Control (CDC) (*18*) reported that ground beef, cold cuts, and frankfurters are relatively infrequently associated with outbreaks reported to CDC. In addition, the inability of several common food-borne pathogens to proliferate in ground beef (*6*) supports the seemingly low frequency of outbreaks attributed to ground beef. It appears, that the consumer's risk is quite small, or at least hard to define and quantitate. This is probably true for the producer's risk, except that laboratory analyses of ground beef do suggest that a large quantity of ground beef would be considered adulterated if the proposed standards became law. In addition, violation of a law (standard) might result in a retail manager going to jail (*22*). To quantitate the potential loss of money to producers, and time spent in enforcement, is difficult. Thirdly, there is limited evidence (*2,3,20*) to suggest that the standards would tend to improve the quality of ground beef at the retail level.

Rogers and McCleskey (*15*) suggested that the bacterial population in ground meat reflects the bacteriological quality of the meat used for grinding, the cleanliness of equipment, and the time and temperature of storage. Considering the comparison of technological developments, improved animal health, inspection programs, the efforts of sanitarians, and the vastly improved refrigeration capacity of today, to say 50 or 60 years ago, one might predict that the quality of ground meat has improved during that time. Duitschaever *et al*, (*3*) concluded, however, that the quality of the samples he analyzed were similar to those reported in surveys dating back as far as 1914. His only basis for comparison was a TPC and in some instances coliform counts. It is impossible to compare current "quality" to previous surveys on other important criteria, including : numbers of pathogens and numbers of food-borne outbreaks attributed to these meat products, without data.

An increasing awareness among consumers, the demand to be informed, and a confusion over the significance of a high number of bacteria in some foods, provided the pressure for adopting bacterial criteria for ground beef. Conversely, the inability to correlate cell numbers in ground beef to a possible health hazard and organoleptic quality, and the seemingly low risk to the consumers (*6,18*) of such products, appear to influence a hesitation toward adopting bacterial standards on ground beef.

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Bacterial, Shelf Life, and Consumer Acceptance Characteristics of Chopped Beef[1]

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ABSTRACT

Several studies were initiated to evaluate factors that exert an influence on bacterial counts, shelf life, and consumer acceptance of commercially processed chopped beef. Bacterial counts in chub packaged (oxygen impermeable film) product underwent only slight increases when evaluated every 4 days over a 20-day storage period. Retail packaging of chopped beef after both 16 and 20 days of chub storage resulted in severely limited shelf life of the retail film wrapped product in terms of bacterial counts, surface discoloration, and flavor. Differences in the anatomical location (chuck, flank, navel, and head) of beef trimmings did not exert a major influence on chopped beef shelf life. However, the use of CO_2 pellets did lower psychrotrophic bacterial numbers in some samples, with subsequently less surface discoloration and improved flavor. Reductions in bacterial counts occurred during the 3-day retail storage in oxygen permeable film only for chopped beef stored in chub form for a total 18 days before the 3-day storage.

Merchandising and distribution patterns for fresh meat products are currently undergoing change in the meat packing and processing industries. Traditionally, much of the retail cutting and packaging of fresh meats has been done in retail stores. However, establishment of centralized fabrication and distribution plants by many retail chains has resulted in mass production of fresh meat products in one location with little or no meat processing at the retail level. Therefore, meat products such as chopped or ground beef are initially processed at locations some distance from the retail establishment where they are sold.

The process of chopping and grinding enables bacteria present on the meat surface to be distributed throughout the product. Thus, the ultimate shelf life of ground beef depends on the bacterial level of the trimmings, sanitary conditions during processing, and time and temperature of processing and storage. Rogers and McClesky (*5*) noted that bacterial levels in ground beef at the time of retail sale were indicative of the history of the product. A recent study of Duitschaever et al. (*1*) indicated that bacterial levels of ground beef processed under current techniques were similar to those reported 60 years ago. Surkiewicz et al. (*7*) found, at the time of manufacture, 76% of 74 sets of raw beef patties had aerobic plate counts of 1,000,000 or fewer/g.

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Since processing operations and the condition of raw ingredients can exert such an influence on the ultimate shelf life of the product, it is important to identify the conditions and ingredients necessary to produce prolonged shelf life in chopped and ground beef products.

MATERIALS AND METHODS

Two studies were initiated to evaluate factors that exert an influence on the shelf life and consumer acceptance of chopped beef products. In both studies, chopped beef refers to product produced from a 681-kg capacity food chopped after five revolutions of the chopper bowl. In the first study, ten 4.54-kg chubs of chopped beef packaged in oxygen impermeable film were obtained during a day's production from a commercial processor and evaluated for psychrotrophic bacterial counts, muscle surface discoloration, and flavor after 0, 4, 8, 12, 16, and 20 days of storage at 2 C. This product was manufactured from trimmings obtained from U.S. Choice, Good, and Utility beef carcasses and was processed to contain 15% fat. Scoring systems for surface discoloration and degree of objectionable flavors are depicted in Table 1. After each storage interval in chub form, two chopped beef samples were removed from each chub package using sterile disposable gloves and placed in oxygen permeable retail film for an additional 3 days of 2 C storage before further bacterial and consumer acceptance evaluations. Surface discoloration scores were assigned by a two-member panel, while flavor assessments were made by a six-member panel after 2.54-cm thick samples were cooked for a constant 30-min period in an oven maintained at 158 C. Psychrotrophic bacteria were enumerated with Plate Count agar following incubation of petri plates at 5 C for 10 days. Before plating, 10-g samples were blended with 90 ml of sterile Butterfields phosphate buffer and then appropriately diluted.

TABLE 1. *Scoring systems for surface discoloration and objectionable odors*

Surface discoloration	Objectionable flavors
7 = No surface discoloration	4 = No objectionable off-flavor
6 = <10% surface discoloration	3 = Slightly detectable off-flavor
5 = 10-25% surface discoloration	2 = Moderately detectable off-flavor
4 = 25-50% surface discoloration	
3 = 50-75% surface discoloration	1 = Strongly detectable off-flavor
2 = 75-90% surface discoloration	
1 = >90% surface discoloration	

In the second study, 4.54-kg chubs of chopped beef were obtained after the product had been processed according to the following four batches; batch 1-81.8% chuck meat, 18.2% flanks and navels; batch 2-77.6% chuck meat, 17.2% flanks and navels, 5.2% CO_2 pellets; batch 3-45.4% chuck meat, 25.4% lean flanks, 18.2% lean navels, 11.0% head

meat; batch 4-43.1% chuck meat, 24.1% lean flanks, 17.2% lean navels, 10.4% head meat, 5.2% CO_2 pellets. The CO_2 pellets were added to create a product with a lower temperature at the point of packaging into the chub. Generally, addition of CO_2 pellets resulted in a 7 C reduction in product temperature at the point of packaging. Bacteriological, shelf life, and consumer acceptance studies were made using the procedures outlined in the first study. Data were analyzed using analysis of variance (6) and the mean separation technique of Duncan (2).

RESULTS AND DISCUSSION

Only a slight increase in psychrotrophic bacterial numbers occurred during the first study for chub-packaged product as a result of the 20-day storage period (Table 2). Thus, the chub packaging system, while not a complete vacuum package, may have helped keep the increases in the prevalent bacteria low. Generally, a substantial increase in bacterial numbers occurred after chopped beef was placed in retail packages (Table 3), however, the differences between the various total days in storage were nonsignificant (P>.05).

TABLE 2. *Bacterial counts in chub packaged chopped beef after various storage periods*

Days of storage in chub package	Bacterial counts[ab]
0	3.8
4	4.0
8	4.3
12	4.8
16	4.4
20	4.8

[a]Mean bacterial counts (log_{10}) per gm determined from total plate count agar.
[b]Mean values for days in storage are not significantly different (P > .05).

TABLE 3. *Shelflife and consumer acceptance characteristics in retail packaged chopped beef after various storage periods*

Days of storage		Bacterial counts[a]	Surface discoloration scores	Flavor scores
In chub package	In retail film			
0	3	4.9^{b}	4.8^{b}	3.2^{b}
4	3	4.8^{b}	5.0^{b}	2.6^{bc}
8	3	4.8^{b}	4.2^{b}	3.1^{b}
12	3	5.1^{b}	2.7^{c}	2.6^{bc}
16	3	5.8^{b}	1.7^{cd}	1.6^{cd}
20	3	5.4^{b}	1.1^{d}	1.9^{cd}

[a]Mean bacterial counts (log_{10}) per g determined from Plate Count agar.
[bcd]Means in the same column bearing the same superscripts are not significantly different (P > .05).

Subjective color evaluations on retail packaged ground beef indicated that a 3-day retail storage period after 12, 16, and 20 days of chub storage was more conducive to greater (P<.05) lean discoloration that storage in retail packages after 0, 4, and 8 days of chub storage (Table 3). Samples stored in chubs for 16 days turned unacceptable in surface color in about 1 day, while samples from chubs held in storage for 20 days exhibited undesirable surface color in less than 1 day of retail storage. Sensory panel scores for flavor in samples stored in chub form for 0, 4, 8, and 12 days followed by 3 days of retail storage were generally acceptable. However, retail packaged product stored for 16 and 20 days in chub form before 3 days of retail display possessed between moderate and strong amounts of off flavor. Many samples had a sour taste which could have been due to growth of lactic acid producing bacteria. It would appear from the color and flavor data that 12 days of chub storage may be the maximum under the processing conditions employed.

Bacterial counts in chub-packaged chopped beef obtained during the second study are presented in Table 4. Since product for batch two was not obtained at the time of processing, statistical analyses were done only on the remaining three storage times. With the exception of batch three, significant (P>.05) increases in psychrotrophic counts occurred with each additional 6 days of storage. Batch three which contained trimmings from four different sources, but with no CO_2, yielded higher (P<.05) bacterial counts after 6 days of storage than the other three formulations of chopped beef. In comparing the lower bacterial counts for batch four versus batch three, there is some indication that the addition of the CO_2 pellets may have been beneficial in keeping the bacterial levels down when chuck meat, lean flanks, lean navels, and head meat were included in the formulation. Bacterial counts after 18 days of storage were similar for all batches. Bacterial examination of the raw muscle ingredients indicated slightly higher psychrotrophic levels for head meat than for chuck, flank, and navel trimmings. Bacterial swab samples taken on the chopped beef processing equipment failed to show any item to be a major source of contamination. Likewise, bacterial counts on equipment did not appreciably increase during 8-h production periods, once production had begun.

TABLE 4. *Bacterial counts in chub packaged chopped beef as related to formulation of trimmings, CO_2 usage and storage time*[a]

Days of storage in chub package	Batch[b]			
	1	2	3	4
0	2.5	—	4.6	3.7
6	4.9^{c}	4.7^{c}	6.1^{d}	4.6^{c}
12	5.0^{d}	5.9^{d}	6.0^{d}	5.6^{d}
18	6.5^{e}	6.6^{e}	6.5^{de}	6.6^{e}

[a]Mean bacterial counts (log_{10}) per g determined from Plate Count agar.
[b]Formulations for the batches are as follows: batch 1-81.8% chuck meat, 18.2% flanks and navels; batch 2-77.6% chuck meat, 17.2% flanks and navels, 5.2% CO_2 pellets; batch 3-45.4% chuck meat, 25.4% lean flanks, 18.2% lean navels, 11.0% head meat; batch 4-43.1% chuck meat, 24.1% lean flanks, 17.2% lean navels, 10.4% head meat, 5.2% CO_2 pellets.
[cde]Means on the same line or in the same column bearing the same superscripts are not significantly different (P > .05).

Bacterial counts for retail packaged chopped beef

TABLE 5. *Bacterial counts in retail packaged chopped beef as related to formulation of trimmings, CO_2 usage and storage time*[a]

Days of storage		Batch[b]			
In chub package	In retail film	1	2	3	4
0	3	3.7	—	5.4	3.1
6	3	5.6^{c}	3.9^{d}	6.5^{e}	5.0^{c}
12	3	6.6^{e}	6.5^{e}	6.6^{e}	6.1^{e}
18	3	5.4^{c}	5.6^{c}	5.4^{c}	6.5^{e}

[a]Mean bacterial counts (log_{10}) per g determined from Plate Count agar.
[b]Formulations for the batches are footnoted in Table 4.
[cde]Means on the same line or in the same column bearing the same superscripts are not significantly different (P > .05).

followed similar trends to that observed for the chub packaged product (Table 5). After 6 days of chub storage and 3 days of retail storage, bacterial counts tended to be lower in batches that were processed with CO_2 pellets (batch two versus batch one and batch four versus batch three). However, after 12 days of chub and 3 days of retail storage, differences in bacterial counts between batches were nonsignificant (P>.05). It is interesting to note, that for all batches, storage for 18 days in chub form followed by 3 days storage in retail film produced lower bacterial counts than those found in the chub-packaged product after 18 days of storage before the 3-day retail display (Table 4). These reductions in bacterial counts may be due to development of unfavorable conditions in the products such as acid production. Reddy et al. (*4*) found lactic acid producing bacteria to have a pronounced inhibitory effect on growth of gram negative bacteria in ground beef.

TABLE 6. *Surface discoloration scores for retail packaged chopped beef as related to formulation of trimmings and CO_2 usage*

Batch[a]			
1	2	3	4
2.2	2.5[b]	1.4[c]	3.0[b]

[a]Formulations for the batches are footnoted in Table 4.
[bc]Means on the same line bearing the same superscripts are not significantly different (P > .05).

Scores assigned by a two member panel for surface muscle discoloration are presented in Table 6. Only the scores assigned at the end of the 3-day period are presented. The differences between the 6-, 12-, and 18-day chub storage times were nonsignificant (P>.05) after 3 days of retail display and thus only mean values for the batches are shown in Table 6. When averaged across storage time, batches two and four displayed significantly (P<.05) less surface discoloration than batch three. These results somewhat paralleled those noted for bacterial growth in Tables 4 and 5. While no differences were observed in surface discoloration attributable to storage time, chopped beef product held in chub form for at least 6 days was incapable of achieving 3 days of retail storage from the standpoint of color. Jay (*3*) and Reddy et al. (*4*) reported improvements in ground beef color scores after 3 and 5 days of refrigerated storage, respectively.

Sensory panel scores assigned to cooked samples stored for 6 days in chub form followed by 3 days in retail film were significantly (P<.05) higher (less off-flavor) than scores given to samples stored 18 days in chub and 3 days in retail packages (Table 7). Differences between batches and the interaction of batch formulation with storage time were not statistically (P>.05) important sources of variation. While not statistically significant, batch four (CO_2) samples were rated more desirable in flavor than batch three (no CO_2) after every storage interval.

TABLE 7. *Sensory panel scores for objectionable flavors in retail packaged chopped beef as related to storage time*

Days of storage		
In chub package	In retail film	Panel scores
0	3	3.3
6	3	2.9[a]
12	3	2.3[ab]
18	3	1.9[b]

[ab]Means bearing the same superscripts are not significantly different (P > .05).

In conclusion, it would appear that storage of chopped beef in chub packages (oxygen impermeable film) for prolonged periods may result in very limited shelf life from the standpoint of bacterial level and sensory values. However, use of CO_2 pellets in product formulation may help extend the shelf life of chopped and ground beef products.

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Proposed Microbiological Standards for Ground Beef Based on a Canadian Survey

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ABSTRACT

Microbiological standards are proposed for ground beef sold in Canada. The proposal is based on a national survey conducted in 1974-75. The proposed standards are: aerobic colony count (35 C), $\leq 10^7$ for non-frozen and $\leq 10^6$ for frozen products; *Escherichia coli*, $\leq 10^2$; *Staphylococcus aureus*, $\leq 10^2$ per g; and *Salmonella*, absent in 25-g portions from each of five subsamples. To accomodate the variable distribution of bacteria between packages in a single lot of product, three-class plan based on a format suggested by the International Commission on Microbiological Specifications for Foods is used.

There is much concern by the general public about the microbial quality of ground meat. There is no doubt that the microbial quality of ground meat can be improved by changes in methods of production, but there is considerable doubt that the expense of very great improvement would provide a suitable cost/benefit ratio to the consumer (*19*).

The use of trimming from aged beef (*2*) and deboning and grinding (*5*), especially in non-refrigerated areas (*17*) results in a higher bacterial population in ground meat than in unground meat. Sanitation is economically profitable (*3*), but many species of bacteria on meat grow well at normal refrigerator temperatures (*1, 3*). Thus, to minimize growth after grinding, the ground meat must be at temperatures of −1 C to + 1 C pending sale (*3*) and to prevent growth, it must be frozen. Decreasing the temperature will, of course, increase the cost of refrigeration.

Bacteria and parasites that are capable of causing disease of humans may be naturally present in beef, and some are not detectable by methods used during inspection by veterinarians at time of slaughter. Such organisms do not grow in products that are adequately refrigerated and they are destroyed by cooking until the center of the meat is no longer pink (about 70 C; C. Davidson, personal communication). A recent study of epidemiological data indicates that adequately cooked beef has not been the cause of food-borne disease unless it has been mishandled or recontaminated after cooking (*16*). On the other hand, consumption of raw or grossly undercooked ground beef has resulted in outbreaks of toxoplasmosis (*15*) and salmonellosis (*8*) and is not recommended by competent authorities in public health. Nevertheless, there are some concerns about the microbiology of raw ground meat (*6, 7*). As a result, the Health Protection Branch of Health and Welfare Canada conducted a survey of ground beef during 1974-75. This report presents some results of the study and proposes microbiological standards for the product. A more detailed report of the survey will be published elsewhere.

METHODS

The survey was conducted from December 1974 to March 1975. Both non-frozen and frozen products were examined.

Non-frozen ground beef was purchased from 218 retail stores in 18 urban areas and their suburbs across Canada according to a predetermined plan. Three types of products (>15-30% fat, ≤15% fat, ground beef with soya) were purchased and three types of stores (supermarket, corner store, meat specialty store) served as sources of product. One sample consisting of five subsamples (each weighing at least 225 g) of a single type of product was obtained from each store. About 50% of the samples consisted of five prewrapped packages from display units while the other samples were made up of five subsamples, each of approximately 0.5 lb., taken from bulk preparations. The samples were transported in insulated boxes containing refrigerant and analyses were usually done within 3 h after purchase. Temperature readings of the product were taken at time of purchase and at time of arrival at the laboratory.

The frozen product was purchased as patties (preformed portions ready for cooking) from 59 retail stores and 59 takeout hamburger specialty restaurants in the same urban areas as the non-frozen product. Each sample consisted of five subsamples. For the retail stores, each subsample consisted of a pre-wrapped package containing at least two frozen patties while for the hamburg specialty restaurants a subsample was at least two patties taken from the same carton. The samples were kept frozen until analysed-usually within 1 day of collection.

Bacteriological examinations were done according to Acceptable Methods (*12*) of the Health Protection Branch. The examinations were: aerobic colony count at 35 and 21 C; presumptive and confirmed coliforms and fecal coliforms using a 5-tube MPM procedure, coagulase-positive *Staphylococcus aureus*, and *Salmonella* sp. Each sub-sample was examined individually.

[1]*Food Directorate*
[2]*Field Operations Directorate*

RESULTS AND DISCUSSION

Non-frozen product

The number of non-frozen samples analyzed, subdivided by type of store and type of product is presented in Table 1. In total, 1090 subsamples from 218 samples were individually examined. Results are considered in the context of both subsamples and samples.

TABLE 1. *Types of stores in the survey and types of non-frozen ground beef examined*

Type of store	Number of stores	Number of subsamples[a]		
		15-30% Fat[b]	≤15% Fat	Soya
Supermarket	164	440	120	80
Corner store	38	195	40	0
Meat specialty store	16	185	30	0
Total	218	820	190	80

[a]A sample consists of five packages (subsamples) of a single product available in a single store at the time of purchase.
[b]Canadian regulations (B.14.015) permit a maximum of 30% fat in ground beef and 15% fat in lean ground beef (9); soya is a mixture of ground beef and soya protein.

The variation in microbiological content of subsamples within a sample and the effect of methodology used are important considerations in developing standards. A comparison of aerobic colony count (ACC) at 35 C and 21 C for two typical samples (A and B) is shown in Table 2 and illustrates these two considerations. The variation in ACC (35 C) between subsamples of Sample A was greater than 7-fold; for Sample B, 2.5-fold. The ACC's at 21 C were higher than those at 35 C; for Sample A the difference between means was 2-fold, for Sample B, 10-fold. It is probable that the high counts in Sample B resulted from growth of psychrotrophs during refrigerated storage. Growth of psychrotrophs in Sample B could account for the much higher ACC's at 21 C than at 35 C and also for the greater uniformity between subsamples when ACC's were incubated at 21 C.

TABLE 2. *Comparison of Aerobic Colony Count obtained at 35 C and 21 C*[a]

Sample	Sub	ACC X 10^{-6}	
		35 C	21 C
A	1	1.2	3.9
	2	0.7	1.2
	3	0.5	1.0
	4	0.5	1.6
	5	3.6	8.5
	Mean	1.3	3.2
B	1	16.0	130.0
	2	7.7	94.0
	3	11.0	130.0
	4	16.0	90.0
	5	6.4	97.0
	Mean	11.4	108.2

[a]Temperature of meat at time of purchase was 2-4 C and 3 h later, at time of analysis it was 2 C.

Classification of data obtained from a survey into arbitrarily assigned groupings is useful in examining such data in the context of setting standards. This is done in Tables 3-6. The data have been grouped for ACC's obtained at two temperatures: 35 C and 21 C (Table 3). When petri dishes were incubated at 35 C, 87.8% of 1090 subsamples of non-frozen ground beef had ACC's of $\leq 10 \times 10^6$, but when duplicate petri dishes were incubated at 21 C only 47.2% had ACC's of $\leq 10 \times 10^6$. Clearly, the temperature of incubation for the ACC is important and must be specified for methods and for standards that are used in regulatory activities. The effect of type of product on the ACC at 35 C is shown in Table 4. The product with ≤15% fat contained a higher percentage of subsamples with lower bacterial populations, probably the result of more severe trimming of suface fat and use of more expensive cuts of meat for this product. The distribution of subsamples according to their content of fecal coliforms and *S. aureus* are shown in Tables 5 and 6, respectively. Combining results for all three types of products, 75.6% contained ≤100 fecal coliforms and 90.7% contained ≤100 *S. aureus* per g. The mixture of meat with soya appears to be microbiologically inferior to the other two products,

TABLE 3. *Differences in Aerobic Colony Count of non-frozen ground beef using two incubation temperatures*[a]

Arbitrary grouping of ACC (X 10^{-6})	Aerobic Colony count at:			
	35 C Percent of subsamples		21 C Percent of subsamples	
0.0- 0.5	35	(35)[b]	6.1	(6.1)
>0.5- 1.0	13.1	(48.1)	5.1	(11.2)
>1.0- 10.0	39.7	(87.8)	36.0	(47.2)
>10.0-100.0	11.6	(99.4)	44.7	(91.9)
>100.0-	0.6	(100)	8.1	(100)

[a]1090 subsamples (218 samples, each of five subsamples).
[b]Brackets contain cumulative percentages.

TABLE 4 *Distribution of Aerobic Colony Counts (35 C) of non-frozen ground beef*[a]

Arbitrary grouping of ACC (X 10^{-6})	Percentage of subsamples within each group			
	15-30% Fat	≤15% Fat	Soya	All products
0.0- 0.5	32.4	53.2	18.8	35.0 (35.0)
>0.5- 1.0	11.6	19.1	13.7	13.1 (48.1)
>1.0- 10.0	43.5	21.8	43.8	39.7 (87.8)
>10.0-100.0	12.1	5.9	21.2	11.6 (99.4)
>100.0-	0.5	0	2.5	0.6 (100.0)

[a]See Table 3 for footnotes.

TABLE 5. *Fecal coliforms in non-frozen ground beef*[a]

Arbitrary grouping of No. per g	Percentage of subsamples within each group			
	15-30% Fat	≤15% Fat	Soya	All products
0- 20	60.7	66.3	40.0	60.2 (60.2)
>20- 100	17.0	12.1	7.5	15.4 (75.6)
>100- 500	14.1	16.3	22.5	15.1 (90.7)
>500-1,600	4.5	4.7	18.8	5.6 (96.3)
>1,600-	3.7	0.5	11.2	3.7 (100.0)

[a]See Table 3 for footnotes.

TABLE 6. Staphylococcus aureus *in non-frozen ground beef*[a]

Arbitrary Grouping of No. per g	Percentage of subsamples within each group			
	15-30% Fat	≤15% Fat	Soya	All Products
0- 100	92.4	89.5	80.0	90.7 (90.7)
>100- 1,000	6.5	10.5	13.7	7.8 (98.5)
>1,000- 10,000	0.7	0	5.0	1.1 (99.6)
>10,000-100,000	0.2	0	1.2	0.3 (99.9)
>100,000-	0.1	0	0	0.1 (100.0)

[a]See Table 3 for footnotes.

possibly reflecting inadequate practices in rehydrating soya protein and admixing it with ground meat.

The temperature distribution of the ground meat at time of purchase is presented in Table 7. The effect of

TABLE 7. *Temperature of non-frozen ground beef at time of purchase*[a,b,c]

Temperature (°C)	Percentage of samples at temperature ranges indicated	
<0	8.3	(8.3)
>0- 2	15.1	(23.4)
>2- 4	25.5	(48.9)
>4- 6	25.0	(73.9)
>6- 8	15.9	(89.8)
>8-10	7.7	(97.5)
>10-12	2.6	(100)

[a,b]See Table 3 for footnotes.
[c]A few subsamples were frozen. However, they have been included with the non-frozen products because they were packaged in a similar manner.

temperature at time of purchase on the percentage of 30%-fat subsamples meeting arbitrary bacterial limits is shown in Table 8. Temperature appeared to affect the

TABLE 8. *Relationship of temperature of non-frozen ground beef*[a] *at time of purchase to percentage of subsamples meeting arbitrary microbiological limits*

Temperature range	No. of subsamples	Percentage of subsamples meeting each limit		
		ACC (35 C) $\leq 10^7$/g[b]	Fecal coliforms $\leq 10^2$/g[b]	*S. aureus* $\leq 10^2$/g[b]
<5 C	440	90.4	75.2	89.8
5-10 C	340	84.7	81.2	92.6
>10 C	40	77.5	75.0	87.5

[a]820 subsamples with 15-30% fat.
[b]Arbitrary microbiological limits.

ACC: as the temperature at time of purchase increased, the percentage of subsample with an ACC of $\leq 10^7$ decreased. There were no similar trends for fecal coliforms and *S. aureus.*

Frozen product (patties)

In total, 118 samples comprising 590 subsamples of frozen ground meat were examined. The frozen ground meat had lower ACC's than the non-frozen ground meat (compare Tables 9 and 3), but the frozen products were similar to the non-frozen in yielding higher ACC results at 21 than at 35 C. The distributions of fecal coliforms and *S. aureus* counts for the frozen subsamples (not illustrated) were similar to the distributions obtained for the non-frozen product. These results are similar to those obtained by others (*1, 6, 7, 11, 14, 17, 18*).

TABLE 9. *Differences in Aerobic Colony Count of frozen ground beef patties using two incubation temperatures*[a]

Arbitrary grouping of ACC (X 10^{-6})	Aerobic colony count at	
	35 C Percent of susamples	21 C Percent of subsamples
0.0- 0.5	56.8 (56.8)	12.7 (12.7)
>0.5- 1.0	23.7 (80.5)	17.8 (30.5)
>1.0-10.0	18.6 (99.1)	51.7 (82.2)
>10.0-	0.8 (99.9)	17.8 (100.0)

[a]590 subsamples (218 samples, each of 5 subsamples).

Isolation of Salmonella

Salmonella were found in 8/218 non-frozen and 1/118 frozen samples; in some samples all five subsamples contained *Salmonella,* in others only 1 of 5 subsamples contained *Salmonella.* In all, 20 subsamples (each 25 g) contained *Salmonella.* The serotypes isolated were: *S. infantis* (*4*), *S. saint-paul* (*2*), *S. muenster* (*1*), *S. london* (*1*), and *S. anatum* (*1*). Others have found a much higher incidence of *Salmonella* contamination in ground beef (*19*).

Proposed standards

We have chosen for our proposed official method the ACC at 35 C realizing fully that the count at 21 C would be much higher. If we had chosen 21 C, we would have had to increase the standard for the ACC 5- or 10-fold. Because 35 C is used in our laboratories for incubation of coliforms, *Staphylococcus, Salmonella, Clostridium,* and *Bacillus,* and ACC at 35 C is more convenient.

Proposed Canadian standards for raw ground beef with or without admixed vegetable protein are shown in Table 10. For non-frozen products approximately

TABLE 10. *Proposed Canadian standards for raw ground beef with or without vegetable protein*

Determination	Non-frozen	Frozen
ACC (35 C)	10^7/g	10^6/g
E. coli	10^2/g	10^2/g
S. aureus	10^2/g	10^2/g
Salmonella	0[a]	0[a]

[a]Absent in 25 g.

80-90% of subsamples were less than the proposed limits for each of ACC (35 C), *E. coli,* and *S. aureus* and 98% did not contain *Salmonella.* The proposed standards for frozen products are similar to those for non-frozen products except for a lower ACC: $<10^6$. This lower ACC is readily attainable because the ACC does not increase during storage of frozen product, and may even decrease.

Although we determined fecal coliforms, rather than *E. coli* in the survey, we have proposed *E. coli* (IMViC ++−− and −+−−) for our standards because of the more precise definition of the species. According to Geldreich (*10*), over 90% of fecal coliforms from livestock are *E. coli.*

The variability of bacterial populations between subsamples is well recognized, and the International Commission on Microbiological Specifications for Foods

(*13*) advocates use of the three-class plan to accomodate such variability when writing specifications or standards. The parameters of the three-class sampling plan are defined in Table 11 and specific 3-class plans are proposed for non-frozen (Table 12) and frozen (Table 13) ground beef.

TABLE 11. *Definitions of parameters for three-class plan for ground beef*

LOT:	All packages of a single product that have been produced, handled and stored within a limited period under uniform conditions (*13*)
SAMPLE:	Predetermined number of subsamples from lot
n:	No. of subsamples (packages or patties) to be examined
m:	Maximum No. of bacteria per g that are of no concern
c:	Maximum No. of subsamples that can have concentrations between m and M without rejection of the lot
M:	No. of bacteria per g in any one subsample that causes violation of standard

TABLE 12. *Three-class plan for proposed Canadian standards-non-frozen ground beef*

Test	n	c	m	M
ACC (35 C)	5	3	10^7	5×10^7
E. coli	5	3	10^2	5×10^2
S. aureus	5	2	10^2	10^3
Salmonella	5	0	0[a]	0

[a]Absent in 25 g in each of five subsamples.

TABLE 13. *Three-class plan for proposed Canadian standards-frozen ground beef*

Test	n	c	m	M
ACC (35 C)	5	2	10^6	10^7
E. coli	5	2	10^2	5×10^2
S. aureus	5	2	10^2	10^3
Salmonella	5	0	0[a]	0

[a]Absent in 25 g in each of five subsamples.

Enforcement of the proposed regulations will be based on examination all five subsamples constituting a sample. While the foregoing data dealt with individual subsamples, criteria for acceptance (Tables 12 and 13) specify examination of five subsamples per sample. When the data are reconsidered on this basis, the number of *samples* found unacceptable is given in Table 14. A relatively small number of samples (13.2%) would be unacceptable based on ACC, but a greater number (19.6%) would be unacceptable based on excessive *E. coli*. Considering all causes of unacceptability, 40% of non-frozen and 33% of frozen samples would have been uacceptable. Undoubtedly this high percentage of unacceptable samples can be decreased by adequate attention to refrigeration and sanitation. A decided improvement has been noted in Oregon, U.S.A. subsequent to introduction of microbiological standards for ground meat (*4, 20*).

The proposed standards appear to be realistic. They reflect the bacteriological quality of ground meat in Canada and should be attainable by competent manufacturers and retailers. They can be made more or less stringent by changing the values for n, c, m, or M in the sampling plan and will be made more stringent if additional protection for the consumer is required. They should bring about greater attention to rotation of stock, decreased temperature of holding, and improved sanitation where necessary. However, in the context of protection of the consumer, we did not notice that any of the 1680 subsamples of ground meat were organoleptically unacceptable at time of analysis and, as mentioned above, ground meat is rarely the cause of disease.

What happens if a vendor is in violation? There are several regulatory approaches taken by the Department of National Health and Welfare (Table 15). The severity of the action would depend in part on the seriousness of the problem and the past history of compliance by the

TABLE 15. *Regulatory process in case of violation*

1. Education
2. Warning letter
3. Formal hearing
4. Seizure-Feasible only for frozen product
5. Prosecution
6. Any one or more of the above.

TABLE 14. *Number of lots unacceptable by proposed standards*

Type of product	Number of lots sampled	Number of lots unacceptable on the basis of the results of the following tests[a,d]: ACC only	Ec[b] only	Sa only	Salm only	ACC & Ec	ACC & Sa	Ec & Sa	Ec & Salm	ACC & Ec & Sa	ACC & Sa & Salm	Number of lots unacceptable
Non-frozen ground beef												
(a) 15-30% Fat	164	13	29	10	5	1	1	4	1			64 (40)[c]
(b) ⩽15% Fat	38	1	2	3		1		3				10 (26)
(c) Soya	16	2	5	1		1		2	1		1	13 (81)
Total	218	16	36	14	5	3	1	9	2		1	87 (37)
Frozen patties												
(a) Patties from retail stores	59	8	2	5	1	2	1			2		21 (36)
(b) Patties from take-out restaurants	59	7	6	2		2				1		18 (31)
Total	118	15	8	7	1	4	1			3		39 (33)

[a]ACC-Aerobic Colony Count at 35 C; Ec-*E. coli;* Sa-*S. aureus;* Salm- *Salmonella.*
[b]*E. coli* counts were not made; fecal coliform counts were made. For the purpose of this report, fecal coliforms (45 C) are considered to be *E. coli.*
[c]Brackets enclose percentages.
[d]Every test was done on every sample.

vendor. In all, we feel that if regulatory action is required, there are several avenues that may be taken, and the vendor should know what these avenues are. The choice of a specific avenue depends on the circumstances involved.

We again emphasize that the proposed standards will be publicized through our normal procedures and may be revised after discussion with concerned parties. They can not become standards until they are promulgated by Order-in-Council.

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Streptococci in Dried and Frozen Foods

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ABSTRACT

Streptococci were obtained on KF and bile-esculin agars from 209 (57%) samples of a variety of dried foods and from 299 (87%) samples of frozen vegetables. They were not obtained from dried tea, coffee, flavored drinks, or fruits. Numbers per gram were greater in frozen vegetables than in dried foods. Nearly 60% of the 2334 strains isolated were identified as follows: *Streptococcus faecalis* (8.9%), *Streptococcus faecium* (21.1%), *S. faecium* biotype *casseliflavus* (18.9%), *Streptococcus lactis* (2.2%), *Streptococcus bovis* (0.04%), *Aerococcus* (2.7%), and *Leuconostoc* (4.5%). The 1173 strains of unidentified streptococci formed 32 groups when characterized for growth at 10 and 45 C, in broth containing 6.5% NaCl, and in broth adjusted to pH 9.6; and the deamination of arginine. The isolated strains were classified into seven plant streptococcal (PS) types. Unidentified streptococci obtained from foods were as variable in their charcteristics as those obtained from plants. The variety of species obtained indicated that neither KF nor bile-esculin agars were reliable in the selection of *S. faecalis* from foods.

Streptococci have been isolated from frozen vegetables (*1, 2, 13, 28-30*), frozen sea foods (*11, 22*), fish fillets (*26, 33*), frozen orange concentrate (*5*), citrus juice (*21*), barley kernels (*31*), soft drinks (*23*) and wild rice (*6*). Many of the studies have reported the streptococci as being enterococci or have claimed them to be indices of pollution, and some authors have relied upon little more than superficial characterization in designating them as fecal streptococci. Current knowledge of the plant-resident streptococci (*17, 19*), many of which are culturally similar to *Streptococcus faecalis* (*16*), would suggest that the streptococci encountered in these foods may not be of human intestinal origin.

Strains which bear a superficial resemblance to described species of streptococci and strains which are unidentifiable are prominent among the plant-resident streptococci and also among foods (*19, 21, 23, 26, 29*). Splittstoesser et al. (*29*), with fewer than 100 strains obtained from green beans and corn, divided the unidentified streptococci into three groups based on phenotypic descriptions which were subjected to computer analysis.

This study was undertaken to obtain a spectrum of the streptococci occurring in a wide variety of dried and frozen foods, comparable to an earlier study of the streptococci on plants (*19*). During the evaluation of the data it became apparent that the three groups recognized by Splittstoesser et al. do not provide for all of the types of streptococci which were isolated. Consequently, a more comprehensive and functional classification was developed which divides the unidentified streptococci into seven groups.

MATERIALS AND METHODS

Samples

A total of 375 samples of dried foods and 344 samples of frozen vegetables were obtained from distributors, manufacturers, processors, and retailers. Vegetables processed in Tennessee, dried eggs, and dried onions were supplied as line samples. Sequential samples of green beans for three successive days were obtained to determine the consistency or changes in the streptococci with time. Most spice samples were obtained from bulk containers at wholesale distributing centers and from bulk spice users.

Analysis

All dried foods were prepared at 10% homogenates or suspensions in water. Serial dilutions were cultured in azide-dextrose broth (*14*) and plated on KF agar (*12*). Frozen foods were surface-plated serially on KF and bile-esculin (BE) agars (*9*). Where possible, five colonies, if the colonies appeared to be homogeneous, and as many as five colonies of each colony type if mixtures were observed, were selected to MJ (*20*) broth (azide-dextrose broth with azide omitted and brom-cresol-purple added). All incubations were aerobic at 35 to 37 C for 24 to 48 h, unless otherwise indicated.

Identification and characterization of strains

All strains were streaked on tryptic (ase)-soy-4% sucrose agar for purification and for recognization of dextran-producing *Leuconostoc* and *Streptococcus faecium* biotype *casseliflavus* (*19*). All cultures except these were subjected to cultural and physical criteria which have been described (*15, 19, 20*).

RESULTS AND DISCUSSION

Foods from which streptococci were obtained

Streptococci were obtained from 209 (57%) samples of dried foods (Table 1) and from 299 (87%) samples of frozen vegetables (Table 2). Some samples of all types of dried foods except the beverages and the fruits contained streptococci. Four of the positive nut samples were coconut. Most of the cocoas and the cocoa mixes did not contain streptococci. Samples of wheat and soy flours and cake and pancake mixes contained streptococci; five samples each of rice, potato, and oat flours did not yield streptococci on culture.

Most samples of each frozen vegetable type except okra contained streptococci. Successive line samples of green beans taken during a single day tended to have the same streptococcal types, but the types of streptococci in green beans sampled on successive days differed. Streptococci of 19 groups according to Sherman's criteria (*25*) were isolated from this vegetable.

TABLE 1. *Recovery of streptococci from dried foods*

Type dried food	Samples: Number	Samples: % Containing streptococci
Beverages: tea, coffee, fruit drinks	8	0
Corn meal, grits, rice, beans, peas	37	22
Cocoas and cocoa mixes	22	23
Dessert mixes	18	11
Eggs	56	98
Wheat, rice, potato, oat, soy flours cake, and pancake mixes	62	24
Fruits	3	0
Milk	6	50
Nuts: peanut, pecan, walnut, coconut	9	55
Onions	42	83
Pastas	23	48
Soups, gravy mixes	13	54
Spices	56	79
Yeast	3	100
Miscellaneous: icings, toppings	7	0
Total	375	
Percent of all samples		57

TABLE 2. *Recovery of streptococci from frozen vegetables*

Vegetable	Samples: Number	Samples: % Containing streptococci
Beans, green	122	98
Beans, lima and butter	28	100
Broccoli and cauliflower	13	100
Corn	7	86
Greens	47	91
Mixed vegetables	17	94
Okra	9	11
Peas, blackeye	46	67
Peas, English	4	75
Peppers	22	77
Potatoes, white	6	67
Potatoes, sweet and yam	8	63
Squash, summer	15	87
Total	344	
Percent of all samples		87

Range in numbers

Fewer than 100 colony-forming units (cfu) were recorded for 29% of the dried foods and spices containing streptococci. More than 1,000 cfu/g were enumerated for 30% of the samples, chiefly the frozen vegetables, and the remainder of the samples ranged between 100 and 999 cfu/g.

Identification, distribution and frequency

The identification or designation, numbers of food samples, numbers of strains of each species, and the foods in which the species were dominant are recorded in Table 3.

Strains conforming to the description of the human type *S. faecalis* were isolated from 20 (2.7%) of the foods, chiefly coconut and frozen vegetables. Strains resembling the human type have been isolated from plants in nature (*16*). In comparison with the distribution on plants (*19*), the frequency in occurrence of strains of *S. faecalis* resembling the human and the plant types, and *Leuconostoc* and *Aerococcus* in dried and frozen foods was low, and that of *S. faecium* and its pigmented biotype was greater. Sixty of the 71 strains of *Streptococcus lactis*, all of which were isolated from frozen vegetables, failed to grow on BE agar. *Streptococcus bovis* was isolated only from dried yeast. Many strains of streptococci which resembled this species were isolated from the foods but did not conform in other attributes, as noted by others (*29*), and these were assigned to the unidentified group.

The unidentified strains

The unidentified strains were homofermentative, occured in pairs and in short chains, produced a final pH 4.6 or less in glucose broth and grew in ethyl violet-azide broth. They were variable in the patterns of fermentation of arabinose, raffinose, melezitose, meliboise, mannitol, and sorbitol; in utilization of citrate; growth on 0.0% potassium tellurite agar; reduction of tetrazolium; growth in milk with 0.1% methylene blue; and production of reactions in litmus milk. Nearly 80% of the strains survived heating to 60 C for 30 min. In all respects they appear to be identical with the plant-resident, unidentified streptococci which have been described (*17*).

Grouping of the unidentified streptococci

When divided according to ability to initiate growth at 10 and 45 C, in 6.5% NaCl broth, in broth at pH 9.6. and for the ability to deaminate of arginine, 32 groups were formed. These groups were then condensed into seven groups termed PS (plant streptococcal) Types. The types are described in Table 4. The condensation should serve as a useful means of dividing the unidentified streptococci until species are recognized on the basis of genetic homology (*10*) and the identifying phenotypic traits for the species are elucidated.

According to this classification, PS Type I comprises those streptococci earlier termed *S. faecium*-like (*15*).

TABLE 3. *Frequency in occurrence and numbers of strains of streptococci and dominant foods*

Species	Food samples: Number	Food samples: %	Strains: Number	Strains: %	Dominant food
Streptococcus faecalis resembling human type	20	2.8	80	3.4	Coconut, frozen corn, potatoes, egg, mixed vegetables
resembling plant type	32	6.3	129	5.5	Dried onion, frozen peppers
Streptoccus faecium	107	21.1	288	12.3	Dried eggs
" biotype *casseliflavus*	95	18.9	425	18.2	Frozen vegetables
Streptococcus lactis[a]	11	2.2	71	3.0	Frozen vegetables
Streptococcus bovis	1	0.1	1	0.04	Dried yeast
Aerococcus	14	2.8	62	2.7	Frozen greens
Leuconostoc mesenteroides	22	4.3	105	4.5	Dried onions, frozen beans
Unidentified streptococci	206	40.4	1173	50.2	

[a]60 of 71 strains failed to grow on BE agar.

TABLE 4. *PS (Plant Streptococcus) types according to adherence to Sherman's characteristics*[a]

PS Type	Strains		Sherman's characteristics
	Number	%	
I	242	21	Positive to all characteristics
II	299	25	Positive to any four characteristics
III	269	23	Positive to any three characteristics
IV	182	16	Positive to any two characteristics
Va	108	9	Positive to one feature: growth at 45 C, in 6.5% salt broth, or in broth at pH 9.6
Vb	54	4.5	Single positive feature: growth at 10 C or deamination of arginine
VI	19	1.5	Negative to all characteristics

[a]Sherman's characteristics: initiation of growth at 10 C and 45 C, in broth with 6.5% NaCl and broth at pH 9.6 and deamination of arginine.

They share traits with *S. faecalis*, and appear to be identical with the Group IV of Splittstoesser et al. (*29*). PS Types II, III, and IV consist of those streptococci which are negative to one, two, or three traits, respectively. Strains of these types may resemble *Aerococcus, S. bovis,* and *Streptococcus equinus,* but they are segregated from these species on the basis of other characteristics. Strains in PS Type IV are those which initiate growth at 45 C or in salt or alkaline broths. Strains negative to these three features usually are identified as *S. lactis*. PS Types Va and Vb accomodate those strains exhibiting but one property; those initiating growth at 45 C or in salt or in alkaline broths are placed into PS Type Va, while strains with responses negative to these tests are placed into Type Vb. Type VI contains those strains negative to all five tests. PS Types Vb and VI occur with low frequency in foods, and have been isolated from mountain streams (unpublished).

Distribution of PS Types among dried and frozen foods

The distribution of the seven PS types in dried and frozen foods is recorded in Table 5. Inclusion of a large number of dried egg samples in the study may account for the many PS Type I strains found in dried foods, and the figure may not be indicative of the normal frequency of occurrence for this type. Types I, II, and III constituted 69% of all strains isolated, and they comprized 82% of the cultures isolated from dried foods and 65% of the strains isolated from frozen vegetables.

TABLE 5. *Distribution of PS types among dried and frozen foods*

PS Type	Dried foods		Frozen foods	
	Number	% strains	Number	% strains
I	150	49	92	11
II	78	25	221	26
III	25	8	244	28
IV	40	13	142	16
Va	13	4	95	11
Vb	3	1	51	6
VI	0	0	19	2

Reactions on BE agar, in litmus milk and hemolysis

Except for the absence of hemolytic strains among PS Type VI, positive and negative reactions were recorded for these tests among all types (Table 6). This is similar to the distribution among the plant-resident streptococci (*17*). Among that group, most of the BE-negative strains hydrolyzed esculin, but did not tolerate the bile salts in the medium. Distribution of strains growing on BE agar suggested that while it may be a useful medium for detecting enterococci in clinical practice (*9*), it should not be relied upon as the sole agent for the selection of enterococci (*4*).

TABLE 6. *Numbers of PS types growing on BE agar, reacting in litmus milk and producing alpha hemolysis*

PS Type	Number strains	Growth on BE agar[a]		Reaction in litmus milk[b]		Alpha hemolysis[c]	
		Number	%	Number	%	Number	%
I	242	236	98	132	55	74	43
II	299	238	80	184	62	76	62
III	269	104	39	107	40	153	33
IV	182	51	28	62	34	81	57
Va	108	11	11	61	56	20	19
Vb	54	16	30	15	28	20	37
VI	19	7	37	11	58	0	0

[a]Incubation to 4 days.
[b]Reduced, acidic reaction with or without curd.
[c]On human blood agar.

Coments on KF agar

KF agar was developed for enumeration of enterococci in water (*12*). It was used subsequently for the recovery of enterococci from foods (*7*). Insalata et al. (*8*) enumerated all red-pigmented colonies appearing on the medium as fecal enterococci. The wide variety of streptococci isolated on this medium during the study, and the occasional recovery of aerococci, pediococci, and lactobacilli, and one colony of *Flavobacter,* suggests that KF agar is an excellent medium for the enumeration of all streptococci in a food sample. Colonial variation among the streptococci on the medium is too slight to enable differentiation; only *Leuconostoc* will produce a distinctive, large, readily recognized colony on KF agar.

CONCLUSIONS

Streptococci in dried and frozen foods bear a close similarity in their many forms to the plant-resident streptococci. They would appear to be present in foods as a direct result of their plant residence. The variation among the streptococci isolated from a single vegetable obtained from a single processor is indicative of naturally occurring, post-blanching contamination (*18*), similar to the contamination by other bacteria. The presently unidentified streptococci similar to those isolated from plant foods may occur in fish foods as well, since strains described by Silverman et al. (*26*) which were obtained from fish fillets seem to conform to PS Type II.

Strains among PS Types I through IV bear a phenotypic resemblance to *S. faecium,* and PS Types Va, Vb and VI to *S. lactis.* If a species is to be constituted on the basis of these sequence homology (*10*), it is possible that many of the presently unidentified strains will prove not to be affiliated with presently recognized species. The homologies of eight selected strains with *S. faecalis, S. faecium,* and *S. lactis* were in most instances between 0 and 13% (*24*). Six of the eight strains were homologous

with each of the three species at this low level. One strain exhibited homology only with *S. lactis* at a level of 25%, and one strain exhibited 37% homology with *S. faecalis,* 52% homology with *S. faecium,* and 18% homology with *S. lactis.* Strains within a species normally exhibit homology at or above 70% (*10*).

Until new species are recognized through studies on homology, the division of the presently unidentified strains into the proposed seven groups should provide a useful and convenient, albeit informal, means of expressing the nature or the type of streptococcus isolated from various sources.

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Comparison of Incubation at 30 and 32 C for 48 and 72 Hours for Enumeration of Raw-Milk Bacteria[1]

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ABSTRACT

Plates incubated for 48 h showed significantly higher ($0.01<P<0.05$) counts at 30 than 32 C (arithmetic means were 5.4% higher; geometric means were 1.5% higher). These higher counts, however, were largely obtained by two of 15 analysts representing 12 of 135 samples. Seventy-two-hour incubation gave significantly higher ($P<0.01$) counts than 48 h (arithmetic means for 72 h were 4.53% higher than 48 h; geometric means for 72 h were 2.58% higher) at both temperatures. There were interaction effects indicating a geographical or personal bias in the results.

Some of the earliest work on comparing incubation temperatures for plate counts of bacteria from raw-milk and milk products was by Pederson and Yale (*18*) who indicated that a 32 C incubation gave higher and more reproducible counts than did 37 C. They also indicated that a ±2 degree variation from 32 C decreased counts by 6% (30 C) and 13%(34 C). These results were obtained using the standard peptone agar then recommended by *Standard Methods for Milk Analysis* (*2*). Yale and Pederson (*24*) later showed that raw-milk plated in a tryptone-glucose skimmilk agar gave higher counts at or slightly below 30 C than at 32 C. The fallacy in translating plate counts from one medium to those obtained with was pointed out by Abele (*1*) in 1939.

More recent work has been with Standard Methods agar (tryptone-glucose-yeast extract) now recommended by *Standard Methods for the Examination of Dairy Products* (*6*). Pure cultures from milk were studied by Lawton and Nelson (*16*). They found that most of the psychrotrophic bacteria grew better at 21 or 25 C than at 5 or 10 C while some grew slower or not at all at 32 C. They did not study growth at 30 C. Nelson and Baker (*17*) showed that higher counts were obtained at 25 C for 3 days or 21 C for 4 days than at 32 C for 2 days. Greene and Jezeski (*10*) also studied several psychrotrophs from creamery water supplies and demonstrated a shorter lag period and more rapid growth at 30 C than at 25 C or lower. Thomas et al. (*23*) showed that thermoduric bacteria from pasteurized milk (30 min at 62.5) were recovered in greater numbers when plates were incubated at 28 C for 4 days than when incubated for 32 C at 2 or more days. They indicated that incubation at 32 C gave increasingly higher counts with the increased duration of incubation.

Pedraja and Mengelis (*19*) also showed that for determining the Standard Plate Count of nonfat dry milk, 3 days incubation at 32 C gave higher counts than 2 days. The effect of incubation for 2 or 3 days was investigated by Babel et al. (*7*). They found no increase in counts for plates incubated at 32 C at 3 days among approximately 40 raw-milk samples tested although they indicated that incubation longer than 3 days at 26 C gave higher counts. Randolph et al. (*20*) however, found no significant advantage for a 27-C incubation temperature for 2 days over that presently recommended. Huhtanen (*12*) found higher (although not statistically significant) counts in 2 days at 30 C than at 32 C. Evidence was presented to show that 33-C incubation gave significantly lower counts that 30 C.

Hartley et al. (*11*) found higher, statistically significant, geometric mean counts at 28 than at 32 C while the effect of two types of agar media (standard methods and eugonagar) seemed to depend on the origin of the milk sample. Pasteurized milk and manufacturing-grade raw bulk tank samples did not show agar

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differences, while Grade A raw-milk counts varied with agar; higher counts were obtained on standard methods agar.

Recently Huhtanen et al. (*13*) showed that a 3-day incubation at 32 C gave significantly higher ($P<0.05$) counts than incubation for 2 days. Roughley et al. (*22*) and Johns and Smith (*15*) showed that incubation for 3 days at 30 and 32 C gave higher counts than 2 days; they also indicated that 30-C incubation gave higher counts than 32 C for raw but not for pasteurized milk.

The basic philosophy for the introduction of new methodology in the introduction to the 12th Edition of *Standard Methods*, edited by Walter (*5*), was that "no new method or modification of an old method should be introduced unless it has undergone careful cooperative testing in several laboratories, with the data available to the committee (on standard methods), and to any other interested parties, preferably by publication in a recognized scientific journal." Some consideration is being given to changing the Standard Plate Count incubation time and temperature from 2 days at 32 C to 3 days at 30 or 32 C. The study reported here was undertaken to determine the validity of any such change using the concepts advanced above.

MATERIALS AND METHODS

Fifteen analysts from the states of Ohio, Minnesota, Tennessee, Virginia, Kentucky, Iowa, Oregon, Arizona, Wisconsin, Texas, and Indiana participated in this study. Each selected his own raw-milk bulk tank samples (135 in all) and assayed them according to *Standard Methods for the Examination of Dairy Products* (*4*). Each analyst was asked to assay a minimum of 12 raw-milk samples. Previous cooperative testing (*13*) had indicated that six samples were adequate for satisfactory statistical results; however, many of the counts of the 12 samples were not used due to spreaders, too few colonies (less than 30), too many colonies (more than 300), or incompleteness due to laboratory accidents. Duplicate petri dishes were used for each experimental condition. Comparisons were made of 30 and 32 C incubation temperatures for 48 and 72 ± 3 h. Statistical analysis was by conventional analysis-of-variance with mixed classification of variables done on ln transformed data.

The anlaysis of variance was, for convenience in computer programing, divided into two parts; one with the results for 48-h incubation at 30 and 32 C (all 15 analysts, 135 milk samples); the other with data from both temperatures and times of incubation (8 analysts, 80 milk samples). The statistical model for the anlaysis was a mixed-variable one with random samples, fixed temperatures of incubation, and random "analysts." Since each analyst assayed different lots of raw-milk and no samples were assayed by more than one analyst, the concept of analyst error was unavoidably confounded with samples. The actual error term used could be due to factors other than differences between analysts per se, such as source of milk samples, geographical locations, etc. With the mixed model, the interaction error for samples and temperatures was used for testing main effects while the residual (between petri dishes) error tested the sample-temperature interaction.

The raw data are not given in this report due to their voluminous nature (860 individual plate counts were made), but are available to interested parties.

RESULTS

Means of incubation temperatures

The arithmetic and geometric means are shown in Table 1. Fifteen analysts incubated plates for 48 h at 30 and 32 C while 8 analysts incubated plates for 48 and 72 h at both temperatures. Eight of the 15 analysts incubating plates for 48 h obtained higher arithmetic mean count at 30 than at 32 C (mostly due to analysts 8 and 13); seven obtained higher counts at 32 C. For all 15 analysts, however, the arithmetic means were 5% higher at 30 than at 32 C; the geometric means were 1% higher.

Means of incubation times

All eight analysts of those reporting results for both 48 and 72 h found higher arithmetic and geometric means at 72 h for both temperatures of incubation. For 30 and 32 C, 17% higher arithmetic and 6% higher geometric means were found at 72 than at 48 h.

TABLE 1. *Means of raw-milk plate counts incubated at 30 and 32 C for 48 and 72 h*

Analyst	Number samples tested (total = 135)	Arithmetic mean[a] 48 h 30	Arithmetic mean[a] 48 h 32	Arithmetic mean[a] 72 h 30	Arithmetic mean[a] 72 h 32	Geometric mean[b] 48 h 30	Geometric mean[b] 48 h 32	Geometric mean[b] 72 h 30	Geometric mean[b] 72 h 32
1	14	112	111	114	115	4.48[b]	4.48	4.52	4.52
2	7	14.7	15.9	15.7	16.6	2.47	2.48	2.53	2.53
3	19	28.7	28.8	29.3	31.2	2.76	2.80	2.86	2.86
4	11	10.3	11.0	12.6	11.9	2.14	2.22	2.31	2.30
5	7	63.1	63.0	69.2	67.3	3.55	3.59	3.68	3.70
6	7	15.1	14.0	16.5	15.8	2.52	2.46	2.59	2.57
7	5	10.3	10.7	11.3	10.8	2.27	2.31	2.38	2.33
8	7	174	145	—	—	5.07	4.91	—	—
9	10	59.9	57.1	62.1	58.8	3.49	3.36	3.55	3.45
10	9	26.8	27.1	—	—	3.25	3.24	—	—
11	10	24.5	23.8	—	—	2.57	2.54	—	—
12	8	6.34	5.96	—	—	1.83	1.75	—	—
13	5	73.8	57.9	—	—	4.15	3.90	—	—
14	10	12.3	12.8	—	—	2.33	2.28	—	—
15	6	42.0	43.8	—	—	3.14	3.17	—	—
Average of 15 analysts		44.7	42.4	—	—	3.06	3.03	—	—
Average of 8 analysts		39.3	38.9	45.9	45.8	2.96	2.96	3.15	3.13

[a]Actual counts divided by 1000 and rounded off from 9 significant figures of computer data. Averages are based on computer data and are true overall means.

[b]ln of original counts divided by 1000.

TABLE 2. *Analysis of variance of plate counts incubated for 48 h at 30 and 32 C*

Line	Source	df	ms	F[a]	Significant with P < 0.05	Significant with P < 0.01
A	"Analysts"[b] (a)	14	28.9347	10.1	yes	yes
B	Samples within analysts (s)	120	2.8636	145	yes	yes
C	Temperature (t)	1	0.0792	4.01	yes	no
D	a × t	14	0.0572	2.90	yes	yes
E	s × t	120	0.0198	1.02	no	no
F	Residual	270	0.0194			
	Total	539				

[a]Ratios from lines A/B, B/E, C/E, D/E, E/F.
[b]See text for explanation of analyst error. This table includes data from all 15 analysts with 72-h incubation omitted for analysts 1-7 and 9. A total of 135 milk samples was analyzed.

TABLE 3. *Analysis of variance of plate counts incubated for 48 or 72 h at 30 and 32 C*

Line	Source	df	ms	F[a]	Significant with P < 0.05	Significant with P < 0.01
A	"Analysts"[b] (a)	7	54.8576	8.75	yes	yes
B	Samples within analysts (s)	72	6.2680	652.92	yes	yes
C	Temperature (t)	1	0.0044	0.46	no	no
D	Incubation times (d)	1	1.0010	104.37	yes	yes
E	t × d	1	0.0191	1.99	no	no
F	t × a	7	0.0456	4.75	yes	yes
G	a × d	7	0.0171	1.78	no	no
H	a × t × d	7	0.0105	1.09	no	no
I	s × t	72	0.0298	3.11	yes	yes
J	s × d	72	0.0190	1.98	yes	yes
K	s × t × d	72	0.0096	0.72	no	no
L	Residual	320	0.0133			
	Total	639	1.3228			

[a]Ratios from lines A/B, B through J/K, K/L.
[b]See text for explanation of analyst error. This table includes data from analysts 1-7 and 9 with a total of 80 milk samples.

Analysis of variance for temperatures

The analysis of variance for 48 h is shown in Table 2. The samples, as expected, were highly significantly different. "Analysts" also were significantly different ($P<0.01$). In this analysis, the plate counts at the two temperatures of incubation were significantly different at 5% but not at the 1% level. There was analyst-temperature interaction indicating some geographical or personal bias.

The second part of the analysis of variance with temperatures and both incubation times is shown in Table 3. There was no difference between 30 and 32 C incubation but there was a statistically significant difference between 48 and 72 h incubation. There were also significant analyst-temperature, sample-temperature and sample-days interactions.

DISCUSSION

This study indicates that significantly higher Standard Plate Counts for raw-milk bacteria are obtained when the incubation time is extended to 72 from 48 h. The incorporation of such a change in *Standard Methods for the Examination of Dairy Products* would seem to be warranted if its aim is to determine the greatest possible numbers of total bacteria in raw-milk with the greatest possible precision. The early concept underlying the introduction of standardized methodology for plate counts seemed to be one of detecting potential human pathogens with plates being incubated at 37 C. This concept apparently underwent a metamorphosis with the work of Pederson and Yale (*18*) and Yale and Pederson (*24*), who demonstrated increasing precision and higher counts at 32 C. The eighth edition of *Standard Methods*(*3*) recognized incubation at 32 or 37 C, while the ninth edition recommended only 32 C (*4*).

The rationale behind the Standard Plate Count was discussed by Reinbold (*21*), who indicated that there seemed to be no direct connection between public health and the Standard Plate Count as presently constituted. Barnum (*8*) and Blankenagel (*9*) suggested further that one could not equate poor farm sanitation practices with high bacterial counts in the raw-milk obtained using the Standard Plate Count.

Any change in the Standard Plate Count should be considered only after the inconvenience and cost to the industry have been assessed. A mandatory 72-h incubation would necessitate considerable restructuring of laboratory schedules. An optional 72- or 48-h incubation might be a reasonable compromise and would permit the greatest flexibility in the dairy laboratory.

A change from 32 to 30 C incubation temperature is not warranted solely by the small, questionably significant, differences obtained. A change to 30 C probably would not decrease the Standard Plate Count and would be the same as that adopted by the International Dairy Federation (*14*) for determination of mesophiles in milk. The present tolerance of temperatures for incubators is ±1 C (*6*); a recent study (*12*) indicated that lower counts were obtained at 33 C than 32 C. A similar tolerance at 30 C would assure that

incubators did not reach a possibly deleterious temperature.

The highly significant "analyst" error observed in this study underscores the wisdom in the cooperative testing concept of the 12th edition of *Standard Methods for the Examination of Dairy Products* (5).The results obtained at any one location or by one analyst cannot be a satisfactory basis for changing present methodology.

CONCLUSIONS

Incubation for 72 ± 3 h gave a significantly higher Standard Plate Count than 48 h. There was no significant difference in counts obtained at 30 or 32 C incubation temperature.

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Effect of Oxidation-Reduction Potential upon Growth and Sporulation of *Clostridium perfringens*[1]

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ABSTRACT

Three strains of *Clostridium perfringens* were examined for the influence of oxidation-reduction potential on growth and sporulation. The limiting Eh_7 for growth was +350 mv; the cultures slowly died at this Eh. Sporulation occurred at numbers typical for the individual strains as long as growth occurred.

Little is known about the limiting oxidation-reduction potential (Eh) for sporulation of *Clostridium perfringens.* The limiting redox potential for growth of *C. perfringens* has been defined several times, but little agreement among results is observable (Table 3). The goal of this work was to establish a limiting Eh for both growth and sporulation of *C. perfringens,* to investigate the interrelationships of Eh, pH, growth, and sporulation, and to determine if aeration or Eh poised with an oxidizing agent is more effective as an inhibitor of growth and sporulation of *C. perfringens.*

MATERIALS AND METHODS

The apparatus and the methods for sterilization were described by Tabatabai and Walker (*15*) except for sterilization of the electrodes. Platinum and glass electrodes were sterilized by immersion for 45 min in a solution containing 0.15% sodium hypochlorite acidified with dilute sulfuric acid to approximately pH 5 and then rinsed five times in sterile distilled water before insertion in the electrode vessel.

After assembly of the apparatus and before inoculation of the medium, the vessels were placed in a 40 C water bath and connected to the gas manifold. The flow of nitrogen or mixture of air and nitrogen was adjusted to provide a total flow of 87 ml gas/min/liter of medium. This flow rate was sufficient to keep the medium well mixed. Equilibration of the medium continued until a stable potential was reached, usually after 48 h, for an air-nitrogen mixture or 72-84 h for nitrogen alone.

The pH changes in bacterial cultures were measured in millivolts (mv) rather than pH units to avoid changing the zero setting on the pH meter. Calibration curves of pH at 40 C versus millivolt readings were prepared for each set of pH electrodes by using standard buffers of the following pH: 4.01, 6.85, 6.99, 7.40, and 9.14 (Beckman Co., Fullerton, Calif.).

To check the accuracy of the platinum electrodes, phthalate buffer, pH 4.00, saturated with quinhydrone was used at 40 C; this system has an Eh of +439 mv. Electrodes that varied more than 7 mv from this value were discarded.

The Eh_7 of biological cultures was calculated with the equation developed by Leistner and Mirna (*12*):

$$Eh_7 = E_m + E_{ref} + \frac{RT}{F} 1n(pH_x - 7.0)$$

E_m is the measured potential of the system; E_{ref} is the potential of a saturated calomel electrode; R is the gas constant of 8.315 joules/mole/°C; T is the temperature in degrees absolute; F is Faraday's constant of 96,500 coulombs; pH_x is the measured pH of the system.

Three strains of *C. perfringens* were tested; S-45, isolated from dried beef, was obtained from Herbert E. Hall, Robert Taft Center, Cincinnati, Ohio and strains 1 and 15 were isolated from locally purchased chicken liver and calf liver, respectively.

The organisms were stored in cooked meat medium (Difco) at room temperature. When needed, stock cultures were heat shocked at 75 C for 20 min and used for inocula. One 14-16 h and two 4-h precultures in fluid thioglycolate broth at 37 C followed; 1 ml into 10 ml of fresh medium was used for each transfer. The final 4-h preculture was used to inoculate the vessels containing fluid thioglycolate (Difco) medium, medium of Kim et al. (*9*) or Ellner's medium (*6*). The vessels were incubated at 40 C. Repeated subculturing was done to avoid inoculating spores into the vessels and to assure a healthy inoculum. At least two vessels for each strain were inoculated for each condition tested, and the results were averaged.

The pouch method designed by Bladel and Greenberg (*4*), along with the SPS agar of Angelotti et al. (*2*) without antibiotics, was used to enumerate spores and vegetative cells. Spores were counted after heat shocking at 75 C for 20 min.

RESULTS AND DISCUSSION

Prepurified nitrogen was bubbled through six media to achieve a constant redox potential. Figure 1 shows the Eh_7 patterns of sterile media equilibrated with prepurified N_2. The medium of Kim et al. (*9*) (KCW medium) was chosen for the bulk of the Eh_7 work because it attained a steady Eh_7 quickly (24 h) and maintained this Eh_7 for more than 200 h. Because Ellner's medium did not achieve a constant Eh_7 during equilibration, it was used only for preliminary investigations. Potassium ferricyanide added to sterile KCW medium was slowly reduced; for example, the potential decreased from +500 mv to +350 mv in 50 h when 0.005 *M* potassium ferricyanide was added. Addition of 0.1% sodium thioglycolate to KCW medium was not sufficient to maintain a constant Eh_7; the potential slowly rose as the sodium thioglycolate was

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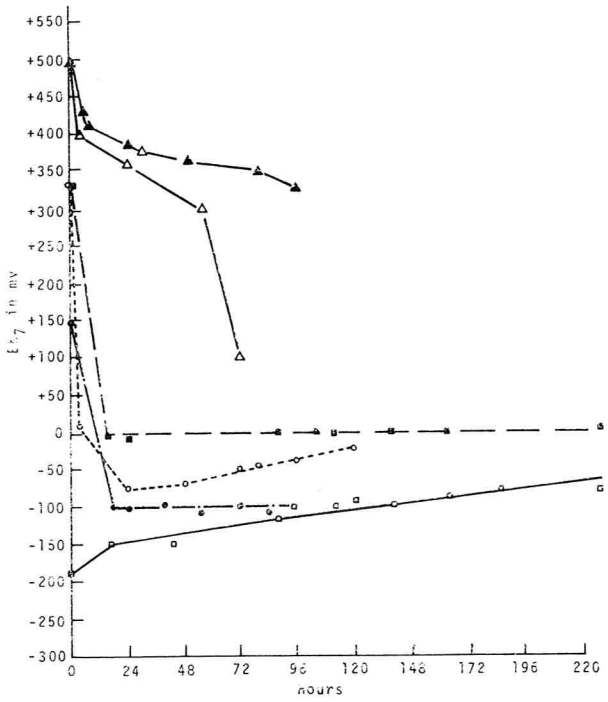

Figure 1. *Eh_7 patterns of sterile media equilibrated with prepurified N_2.* ▲—▲, *KCW medium with 0.005* M *K_3Fe $(CN)_6$*, △—△ *KCW medium with 0.0025* M *K_3Fe $(CN)_6$*, ■——■ *KCW medium,* O-----O *Ellner's medium,* ●—·—● *Fluid thioglycolate medium (Difco), and* □——□ *KCW medium with 0.1% Na thioglycolate.*

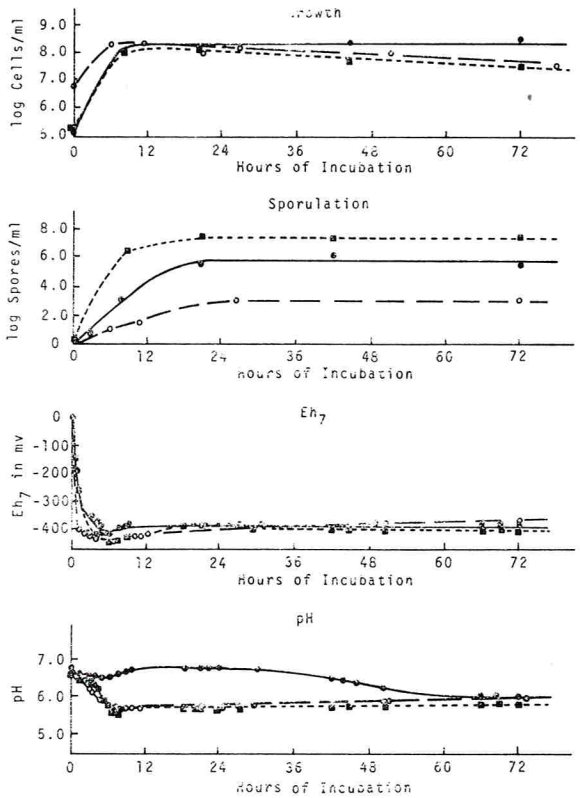

Figure 2. *Patterns of growth, sporulation, Eh_7, and pH for 3 strains of* C. perfringens *in KCW medium under anaerobic conditions.* ●——● *Strain S-45,* O—·—O *Strain 1, and* ■-----■ *Strain 15.*

oxidized. The 0.5% sodium thioglycolate in fluid thioglycolate medium (Difco) was, however, enough to sustain an Eh_7 of –100 mv for more than 80 h.

KCW medium was equilibrated with mixtures of O_2 and prepurified N_2 to adjust the Eh_7 to values suitable for testing growth and sporulation of *C. perfringens.* In some instances, 0.1% sodium thioglycolate or 0.005 *M* K_3Fe $(CN)_6$ was added to adjust the potential under anaerobic conditions. Table 1 lists equilibrated Eh_7 values for media tested. Nitrogen was used in the system as a carrier or diluent for air and as a means of obtaining constant and adequate mixing in the system.

TABLE 1. *Eh_7 values of media equilibrated with N_2 or mixtures of N_2 and air*

Media	Air flow[a] in ml/min/liter	Eh_7 in mv
KCW	0	0
KCW	3.6	+250
KCW	7.0	+275
KCW	20.0	+300
KCW	32.0	+320
KCW	36.4	+350
KCW (0.1% Na thioglycolate)	0	–109
KCW (0.005 *M* K_3Fe $(CN)_6$)	0	+358
Ellner's	0	– 20
Fluid thioglycolate (Difco)	0	–100

[a] N_2 was added to the gas mixture to bring the total amount of gas passing through the system to 87 ml/min/l. 0 indicates that pure nitrogen was used.

Patterns of growth, sporulation, Eh_7, and pH under anaerobic conditions for the three strains of *C. perfringens* are compared in Fig. 2. All strains attained levels of 10^8 cells/ml after 9 h of incubation; the number of cells present thereafter either remained steady or decreased slightly for the remainder of the 72-h incubation period. The Eh_7 pattern was similar for all strains. The initial fall coincided with the log phase of growth; Burrows (*5*) also observed this phenomenon. The Eh_7 dropped to nearly –450 mv and then rose slightly to –400 mv. The ability to produced spores varied with the strain. Strain S-45 formed 5×10^5 spores/ml; strain 1, 1×10^2 spores/ml; and strain 15, 9×10^6 spores/ml. The pH pattern also varied with strain. The pH of strains 1 and 15 fell to about 5.65 within 6-8 h followed by a slow rise to pH 5.85. The pH of S-45 slowly rose 0.15 pH unit, then fell slowly, and did not reach its minimum value of 6.0 until 66 h had elapsed.

Table 2 illustrates results obtained when small amounts of air were added to the nitrogen bubbled through the medium. The pH is not mentioned in Table 2 because it followed the characteristic pattern of each strain. When the Eh_7 was raised to +350 mv (36.4 ml air/min/liter), the bacteria were unable to grow; and the pH remained constant at its initial value.

A higher limiting Eh_7 was found in this work than in many previous studies (Table 3). *C. perfringens* could grow at an initial Eh_7 of +320 mv but not at +350 mv; strain 15, however, had a long lag at +320 mv and, in one instance, did not grow at all. The variations in limiting Eh values noted in Table 3 can be attributed, in part, to differences in media; other factors, such as strains of *C.*

TABLE 2. *Effect of O_2 tension and inital Eh_7 upon cell and spore numbers and minimum and final Eh_7 for three strains of* C. perfringens

Initial Eh_7 (mv)	Air flow[a] (ml/min/liter)	Maximum cells/ml	Spores/ml (72 h)	Cells/ml (72 h)	Minimum Eh_7 (mv)	Eh_7 (mv, 72 h)
Strain S-45						
0	0	5.4×10^8	4.5×10^5	1.6×10^8	−420	−375
+250	3.6	8.4×10^7	7.0×10^4	3.4×10^6	−420	−225
+300	20.0	2.2×10^7	1.7×10^5	1.0×10^4	−395	+150
+320	32.0	3.4×10^7	2.3×10^5	5.5×10^6	−415	+50
+350	36.4	no growth	0	0	—	—
Strain 1						
0	0	4.2×10^8	1.3×10^2	2.2×10^7	−445	−360
+250[b]	3.6	6.0×10^8	0	2.8×10^3	−430	+100
+250[c]	3.6	7.0×10^6	1.5×10^5	4.5×10^6	−425	+30
+275	7.0	1.8×10^8	2.4×10^4	3.2×10^6	−460	+75
+300	20.0	7.0×10^7	3.0×10^5	8.0×10^6	−440	+100
+320	32.0	8.2×10^6	3.1×10^4	1.5×10^6	−440	−50
+350	36.4	no growth	0	0	—	—
Strain 15						
0	0	1.3×10^8	8.9×10^6	2.2×10^7	−450	−380
+250	3.6	1.2×10^7	3.0×10^7	1.3×10^7	−450	0
+275	7.0	9.5×10^7	6.4×10^6	6.0×10^6	−440	+100
+320[d]	32.0	1.8×10^7	5.2×10^6	4.4×10^6	−440	+100
+350	36.4	no growth	0	0	—	—

[a] N_2 in the gas mixture was adjusted to provide a total flow of 87 ml/min/liter to provide constant agitation.
[b] Initial inoculum of 10^7 cells/ml.
[c] Initial inoculum of 7×10^4 cells/ml.
[d] One inoculated vessel did not grow or sporulate at all; values reported here are for the duplicate vessel that sustained growth.

TABLE 3. *Upper limits of Eh for growth of* Clostridium perfringens

Eh (mv)[a]	Initial pH	Medium	Reference
+230 to +250 (+169 to +189)	6.0	Cooked meat broth	Barnes and Ingram (*3*)
+160 (+123)	6.4	Glucose, peptone meat extract	Hanke and Bailey (*7*)
+90 (+90)	7.0	Glucose, peptone meat extract	Hanke and Bailey (*7*)
0 to +30 (+49 to +79)	7.8	Glucose, peptone meat extract	Hanke and Bailey (*7*)
+165 (+141)	6.6	Glucose nutrient broth	Hanke and Katz (*8*)
−125 (−125)	7.0	Peptone-vitamin C broth	Kligler and Guggenheim (*10*)
+194 to +238 (+194 to +238)	7.0	Tryptone, beef extract, yeast extract, glucose	Mead (*13*)
+200 to +250 (+237 to +287)	7.6	Peptone-agar broth	Reed and Orr (*14*)

[a] Numbers in parentheses are the calculated Eh_7.

perfringens used and means of measuring Eh, can influence the values obtained. Allyn and Baldwin (*1*) found that the limiting Eh for growth depended upon factors characteristic of the growth medium. A more suitable ion-balance or more favorable nitrogen source, for example, could facilitate growth at unfavorable oxidation-reduction potentials. In addition, repeated subculturing to obtain healthy log phase bacteria in this study could have made them capable of growth under more adverse conditions than is normally possible. Another explanation for the high limiting Eh_7 could be the comparatively large inoculum of approximately 10^4 cells/ml used. Barnes and Ingram (*3*) were unable to obtain growth of *C. perfringens* at high Eh levels unless a large inoculum of 10^6 cells/ml was used. Reed and 'Orr (*14*) explained that growth at higher Eh levels was possible with a large inoculum because a localized negative drift was formed in close proximity to the cells.

Except for strain 1, the number of spores formed remained at the level characteristic of each strain when aeration was increased until, at 36.4 ml air/min/liter (initial Eh_7 of +350 mv), the inoculum could not grow, and no spores were formed (Table 2). Strain 1 originally sporulated erratically under anaerobic conditions, usually forming 10^2 to 10^3 spores/ml, but occasionally forming no spores at all. A year later, with aeration greater spore numbers of 10^4–10^5 spores/ml were obtained. After completion of the Eh_7 experiments, strain 1 was again grown anaerobically and consistently formed approximately 5×10^4 spores/ml. Evidently, the sporulating capacity of strain 1, isolated from food at the beginning of the Eh experiments, had somehow changed upon storage in the laboratory. Only production of spores seemed affected; the pH, Eh_7, and growth patterns remained the same throughout. The motility nitrate test and lecithinase and hydrogen sulfide

production remained as when the strain was first isolated.

All strains were able to form spores at an aeration rate of 32 ml air/min/liter (initial Eh_7 of +320 mv), but not at 36.4 ml air/min/liter (initial Eh_7 of +350 mv). If the bacteria could grow at all, they sporulated, producing numbers of spores characteristic for each strain. The cells did not form spores until the Eh_7 reached its minimum of –420 to –450 mv; this indicates that highly reducing conditions may be necessary for sporulation or perhaps that formation of some "sporogenic" compound as a result of growth is necessary for sporulation to commence.

Maximum cell populations achieved decreased slightly as a result of aeration (Table 2). For all strains, the maximum cell numbers at 36.4 ml air/min/liter (initial Eh_7 of +320 mv) were about 10 times lower than under anaerobic conditions (initial Eh_7 of 0). In general, cells died off more rapidly as aeration was increased. Only 10^6 cells/ml were present after 72 h at 32 ml air/min/liter (initial Eh_7 of +320 mv) compared with 10^7 or 10^8 cells/ml under anaerobic conditions (initial Eh_7 of 0 mv).

C. perfringens reduced the Eh_7 to approximately –420 mv (strain S–45) or –440 mv (strains 1 and 15) regardless of aeration rates as long as the inoculum could grow. However, at increased aeration rates the Eh_7 rose 300 to 500 mv within 72 h rather than remaining near –400 mv as under anaerobic conditions (Table 2). These strains could establish intense reducing conditions during the log phase, but as the cells became less active during the stationary phase, aeration oxidized the medium's constitutents faster than the bacteria's metabolic activities could reduce them.

Addition of 0.1% Na thioglycolate to KCW medium reduced the equilibrated Eh_7 from 0 to – 109 mv under anaerobic conditions. Maximum cell numbers and cells/ml at 72 h were essentially the same as in KCW medium, except for strains-45. The addition of 0.1% Na thioglycolate was slightly toxic to S-45, reducing the cell numbers one log cycle. Sporulation, pH, and Eh_7 patterns were essentially the same in KCW medium without reducing agent or with 0.1% Na thioglycolate added.

To separate the effects of oxygen from redox potential, 0.005 *M* $K_3Fe(CN)_6$ was added to the medium to raise the Eh_7 to +358 mv, the level at which *C. perfringens* would not grow when air was the sole poising agent. Only strain 1 was able to grow under these conditions. Strain S-45 died off within 4 h and strain 15 survived 18 h; the same survival times were obtained when air was the poising agent. It seems that oxygen tension is a more efficient inhibitor of growth than Eh_7 poised with 0.005 *M* $K_3Fe(CN)_6$ for at least one strain of *C. perfringens*. Knaysi and Dutky (*11*) also obtained growth of a *Clostridium* at higher Eh when potassium ferricyanide was used to poise the medium than when oxygen was the sole poising agent. They found that a butanol-producing *Clostridium* would grow at +335 mv maintained by potassium ferricyanide in the absence of oxygen, but that an oxygen concentration corresponding to a potential of about +300 mv inhibited growth.

Further work in the area of oxidation-reduction potentials and their relation to bacterial growth might be directed toward correlation of effects of redox potential and aeration by measuring both the redox potential and the dissolved oxygen concentration in growing cultures. Another area of interest would be maintenance of a constant Eh_7 during growth of *C. perfringens* by aeration or addition of other oxidizing agents to determine the limiting Eh_7 for sporulation; maintenance of a constant Eh is not easily achieved.

In summary, media varied in their poising capacity and, as a result, affected the oxidation-reduction potentials observed in bacterial cultures. Maximal cell populations were reached nearly coincidentally with minimal Eh values. The limiting Eh_7 for growth of the three strains of *C. perfringens* was +350 mv; at this Eh_7, the cultures slowly died without affecting Eh or pH. Increased aeration caused a reduction in the maximum number of cells produced, hastened their lysis, and raised the final Eh_7 of the system. Sporulation occurred at numbers typical for the individual strains as long as growth occurred.

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The First Annual Summary of Food-borne Disease in Canada

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ABSTRACT

Data on food-borne disease that occurred in 1973 were collected from all parts of Canada. A total of 378 incidents, comprising 343 outbreaks and 35 single cases and involving 3347 ill persons, was recorded. In incidents where the etiology was known, microorganisms were the main causative agents. *Staphylococcus aureus* was responsible for more incidents (*33*) and more cases (*606*) than any other agent. Other incidents were caused by *Salmonella* (*14*), *Clostridium perfringens* (*7*), and *Clostridium botulinum* (*5*). Illness from parasites, plants, and chemicals occurred less frequently. Over 40% of the incidents were associated with meat and poultry; other products playing a significant role were baked goods, vegetables and fruits, Chinese foods, and sandwiches. Mishandling of food in food-service establishments was the cause of over a third of the incidents. Seasonal variation in the frequency of illness was small, but more incidents were reported from the west and center of the country than from the east.

The status of food-borne disease in Canada is generally deduced from annual summary reports from the United States. This practice is questionable, since even for the United States the data are incomplete and not representative of the whole country. Comparable Canadian data are limited at present. There is no official reporting system for food-borne illness to a federal department in Canada, except for numbers of persons ill from salmonellosis and botulism, which are notifiable in all provinces, and from staphylococcal food poisoning which is notifiable in all provinces except Ontario; these are printed weekly by Statistics Canada. The widely-read *Epidemiological Bulletin,* produced by the Health Protection Branch, and its recent successor the *Canada Diseases Weekly Report* contain articles relating to food-borne outbreaks, often with extensive detail. However, information from both these sources is not sufficiently complete to evaluate satisfactorily the status of food-borne illness.

As a first step towards a national system that collects and disseminates data on food-borne disease in Canada information on food-poisoning incidents with respect to causative agents, case numbers and food vehicles has been collected for 1973 from health and environmental agencies across Canada.

With a few exceptions, the format of this report is similar to that of the *Annual Summaries of Foodborne and Waterborne Disease Outbreaks* produced by the Center for Disease Control, Atlanta. Even though the definitions employed are not exactly the same, a direct comparison can be made between the reports of the two countries. A more detailed summary report is available from the author.

DEFINITIONS

The following definitions are used in this summary.

Food: any substance for human consumption, excluding drinking water.

Outbreak: an incident in which two or more persons experience a similar illness after ingestion of a common vehicle (food) and epidemiological analysis implicates the vehicle as the source of the illness.

Case: a person who has been ill as a result of consuming food shown to be contaminated on the basis of epidemiological evidence or laboratory analysis.

Single case: one case, as far as can be ascertained, unrelated to other cases in respect to vehicle consumed.

Incident: an outbreak or a single case.

Laboratory confirmed incident: with the specific etiological agent determined by laboratory analysis.

These definitions differ somewhat from those stated in the C.D.C. *Annual Summaries of Foodborne Disease Outbreaks;* in this report, (*a*) outbreaks invariably consist of two or more ill persons and, (*b*) single cases become a distinct category. It was felt that there was not sufficient justification for single cases of botulism or chemical food poisoning being included in the category of outbreak, as in C.D.C. *Annual Summaries.* Yet this report would be incomplete if many of the single cases were ignored. It was, therefore, decided to list the outbreaks and single cases separately, where this was appropriate.

THE DATA

Reports of food-borne incidents originate from many sources (consumers, physicians, institutions, food industries, etc.), and are usually investigated by federal, provincial, or local health inspectors and reported to federal and provincial epidemiologists. Special requests for reports of food-borne illnesses occurring in 1973 were made to the Health Protection Branch, to provincial epidemiologists and, for more detailed information, to provincial laboratories, and municipal and regional

TABLE 1. *Numbers of food-borne incidents and cases in 1973*

Etiology	Outbreaks	Single Cases	Incidents		Cases	
			Total	Percentage	Total	Percentage
Microbiological	57	12	69	18.3	1516	45.3
Parasitic	3	2	5	1.3	65	1.9
Plant	2	—	2	0.5	4	0.1
Chemical	6	7	13	3.4	29	0.9
Unknown	275	14	289	76.5	1733	51.8
Total	343	35	378	100.0	3347	100.0

TABLE 2. *Number of persons ill in food-borne incidents by specific cause in 1973*

Etiology	Number of outbreaks	Number of cases in outbreaks	Number of cases/outbreak		Single cases
			Range	Median	
MICROBIOLOGICAL					
Staphylococcus aureus	27	600	2-131	4	6
Salmonella	13	334	2-150	10	1
Clostridium perfringens	7	535	2-325	23	—
Clostridium botulinum	2	5	2-3	2.5	3
Bacillus species	3	19	2-15	2	—
Streptococci	2	4	2	2	—
Mold	3	7	2-3	2	1
Yeast	—	—	—	—	1
Subtotal	57	1504	2-325	4	12
PARASITIC					
Trichinella	3	63	2-53	4	2
PLANT					
Mushroom toxin	1	2	NA*	2	—
Alkaloid	1	2	NA	2	—
CHEMICAL					
Metals	4	14	2-5	3.5	—
Cleaning and disinfecting solutions	—	—	—	—	6
Other chemicals	2	8	3-5	4	1
Subtotal	6	22	2-5	3.5	7
UNKNOWN	275	1719	2-175	3	14
TOTAL	343	3312	2-325	3	35

* NA = not applicable

health authorities. Only information on acute disease was received; chronic food-borne illnesses, resulting from consumption of mycotoxins, pesticides, etc., remain unreported. Once obtained, the information was edited, and the following tables were prepared.

Table 1 shows that 378 food-borne incidents, comprising 343 outbreaks and 35 single cases, occurred in 1973, and 3347 persons were reported ill. Unfortunately, only 89 (23.5%) of the incidents involving 1614 (48.2%) of the cases were laboratory confirmed. Of these, microbiological agents were the most frequent cause of illness. The large number of incidents with unknown etiology indicates that many investigations were not sufficiently thorough.

Table 2 lists incidents caused by specific agents, including the range of cases and the median number of cases per outbreak. The agent responsible for most outbreaks (27) and cases (600) with a median of four cases per outbreak was *Staphylococcus aureus.* Although *Salmonella* ranked second in number of outbreaks (*13*), the number of cases (334) was fewer than for *Clostridium perfringens* (535). The median number of cases for both these organisms was relatively high, 10 for *Salmonella* and 23 for *C. perfringens. Bacillus* species (including *Bacillus cereus*), streptococci (*Streptococcus pyogenes* and *Streptococcus mitis*), and yeasts and molds also caused illness. There were five incidents of trichinosis involving game meat and pork products. Plant products responsible for illness were toxin from *Amanita* mushrooms, and solanine from potatoes; some chemicals were tin, chlorine, caustic wash, gasoline, and a pesticide. The ranges and medians for outbreaks involving plants and chemicals were low.

In all, there were five reported deaths from consumption of food. Three Eskimos died in separate incidents when they ate game meat contaminated with *Clostridium botulinum* type E toxin. One man was found dead with evidence of staphylococcal enterotoxemia, and another died from eating bear meat containing *Trichinella.*

Foods associated with illnesses are shown in Table 3. Meat and poultry together account for over 40% of the incidents. Bakery products, vegetables and fruits, Chinese foods and sandwiches also played a significant role in food-borne illness. Eggs and dairy products accounted for less than 4%.

Table 4 shows the place of mishandling. Food-service facilities were responsible for over a third of all incidents

TABLE 3. *Foods associated with food-borne incidents and cases*

Food	Incidents		Cases	
	Number	Percentage	Number	Percentage
Meat	118	31.2	1313	39.2
beef	24	6.4	405	12.1
ham and pork	19	5.0	391	11.7
hamburger	21	5.5	94	2.8
sausages	26	6.9	66	1.9
game meat	7	1.9	62	1.9
other or unspecified	21	5.5	295	8.8
Fish	10	2.6	27	0.8
Shellfish	12	3.2	129	3.9
Poultry	41	10.8	811	24.2
chicken	25	6.6	105	3.1
turkey	10	2.6	595	17.8
other or unspecified	6	1.6	111	3.3
Eggs	3	0.8	25	0.7
Dairy foods	9	2.4	99	3.0
Bakery products	29	7.7	131	3.9
bread	4	1.0	22	0.7
pizzas	6	1.6	18	0.5
cakes	7	1.9	35	1.1
pies and puddings	5	1.3	38	1.1
other baked products	7	1.9	18	0.5
Confectionery	3	0.8	27	0.8
Vegetables and fruits	34	9.0	116	3.5
raw vegetables and fruits	7	1.9	16	0.5
processed low acid vegetables	4	1.0	36	1.1
processed acid vegetables and fruits	17	4.5	46	1.4
other vegetables and fruits	6	1.6	18	0.5
Chinese foods	23	6.1	104	3.1
Salads	9	2.4	31	0.9
Sandwiches	18	4.8	173	5.2
Beverages	13	3.4	27	0.8
soft drinks	10	2.6	18	0.5
other drinks	3	0.8	9	0.3
Multiple vehicles	1	0.3	6	0.2
Other foods	8	2.1	36	1.1
Unknown	47	12.4	292	8.7
Total	378	100.0	3347	100.0

(34.1%). Mishandling in the home (19.1%), in the processing plant (11.4%), and in the retail store (10.0%) was also significant.

The incidents caused by mishandling of foods in food-processing establishments are summarized in Table 5. The processor was responsible for 140 ill persons in 43 separate incidents. Six of these incidents and 25 cases involved microbiological agents compared with 10 incidents and 20 cases associated with chemical agents. The incidents involving botulism in marinated mushrooms and *Salmonella eastbourne* in chocolate received widespread publicity because of the commercial distribution of the contaminated products. The chemical agents were either metals dissolved in acid juices or cleaning and disinfecting chemicals remaining in soft drink bottles.

TABLE 4 *Place where food mishandled in food-borne incidents in 1973*

Establishment	Number of outbreaks	Number or single cases	Total	Total percentage
Food-service establishments	127	2	129	34.1
Homes	62	10	72	19.1
Food-processing establishments	32	11	43	11.4
Retail food establishments	31	7	38	10.0
Wholesale suppliers	4	—	4	1.1
Unknown	87	5	92	24.3
Total	343	35	378	100.0

In general, incidents were distributed equally throughout the year, except for a decline in January and February and peaks in June and November. The regional distribution in food-borne incidents across Canada is shown in Table 6. Ontario had the greatest number of incidents which accounted for 52.4% of the total. However, per 100,000 of a population the rate of food-borne illness in British Columbia (3.2) was higher than that in Ontario (2.5). Whether the high rate experienced in the sparsely-populated Northwest Territories (7.9) is significant will depend on a comparison of data in subsequent years; three incidents were recorded for 1973.

EXAMPLES OF OUTBREAKS

The following are examples, in summary form, of outbreaks which occurred in 1973 in Canada and are not

TABLE 5. *Incidents of food-borne illness caused by mishandling of food in food-processing establishments in 1973*

Etiology	Vehicle	Number of Incidents	Number of Cases
Clostridium botulinum type B	Marinated mushrooms	1	1
Salmonella eastbourne[a]	Chocolate	1	17
Salmonella weltevreden[a]	Prob. pepper	1	1
Staphylococcu aureus	Canned crab meat	1	1
Mold	Canned tomato juice	1	2
Mold	Rye bread	1	3
Trichinella spiralis	Ham	1	4
Tin	Canned tomato juice	3	12
Zinc	Galvanized steel drinking canteen	1	2
Chlorine	Bottled soft drink	1	1
Caustic wash	Bottled soft drink	5	5
Unknown	Bakery products	7	30
Unknown	Acid canned and bottled products	7	19
Unknown	Bottled drinks	4	11
Unknown	Low acid canned products	3	7
Unknown	Cheese curds	1	11
Unknown	Chocolate	1	7
Unknown	Liver	1	2
Unknown	Wieners	1	2
Unknown	Popcorn	1	2
Total		43	140

[a]Other cases of this outbreak also occurred in 1974.

published elsewhere. Not all of the examples chosen were necessarily the best documented. Some of them illustrate incomplete epidemiological or laboratory analysis, which, in some circumstances were beyond the control of the investigating team. These indicate some of the problems involved in collecting data on an outbreak and partially explain why so many incidents were of unknown etiology.

Botulism from commercially marinated mushrooms

On July 4, a woman in Montreal ate 4-5 marinated mushrooms, commercially bottled and imported from the United States. She did not eat more because she did not like the taste and put the bottle in the refrigerator. One day later she felt tired and had a headache, and in the following few days developed symptoms typical of botulism—double vision, difficulty in breathing and swallowing, and a dry mouth. She was admitted to a hospital on July 6 where she received trivalent (A, B, and E) botulinum antiserum and a tracheotomy was performed. She gradually recovered and was released from the hospital on July 24. The contents of the refrigerated bottle were found to contain *C. botulinum* type B spores and toxin with a titer of 625 mouse LD_{50} per g. If it is assumed that the ill woman ate 20 g of mushrooms, her intake was 12,500 LD_{50}. As a result of this finding, all bottles in Canada (8656) and in the United States were recalled. Subsequent analysis of all the 8658 bottles showed that a further 10 bottles contained *C. botulinum* type B toxin with LD_{50} values ranging from 10 to 100,000/g. Many other of the bottles contained contents with a high pH (>4.75) and had butyric odors. It was concluded that the contents of many of the bottles were not acidified sufficiently during processing.

Hospital outbreak involving egg salad sandwiches

In October, patients and staff members of an Ontario hospital ate a lunch consisting of egg salad sandwiches, tomato juice and canned peaches. About 2 h after consumption of the lunch the first reports were received of patients suffering from nausea and vomiting. A total of 125 persons was reported ill. Most of these started to recover by the evening. Laboratory analysis of the egg salad sandwiches showed a count of $>3 \times 10^6$ *Staphylococcus aureus*/g, and this strain produced enterotoxin type C_2. The previous day the eggs had been prepared up to, and including, the dicing stage. Dicing was normally done just before making the sandwiches, but there was to be a reduction of electrical power and all stages in the preparation requiring equipment were carried out the day before. The diced eggs were stored overnight in a large container in the refrigerator and mixed with mayonnaise the next morning. Preparation of the sandwiches was complete at 9:45 A.M. and they were

TABLE 6. *Regional distribution of food-borne incidents in 1973*

Province or Territory	Outbreaks and single cases: Number of outbreaks	Number of single cases	Total	Total percentage	No. per 100,000 population[a]
British Columbia	69	4	73	19.3	3.2
Alberta	5	2	7	1.9	0.4
Saskatchewan	11	2	13	3.4	1.4
Manitoba	9	3	12	3.2	1.2
Ontario	183	15	198	52.4	2.5
Quebec	52	4	56	14.8	0.9
New Brunswick	5	4	9	2.4	1.9
Nova Scotia	2	—	2	0.5	0.3
Prince Edward Island	—	—	—	0.0	0.0
Newfoundland	1	1	2	0.5	0.4
Northwest Territories	3	—	3	0.8	7.9
Yukon	—	—	—	0.0	0.0
More than one province or territory	3	—	3	0.8	NA[b]
Canada	343	35	378	100.0	1.7

[a]Based on 1973 estimates by the Census Division, Statistics Canada
[b]NA: Not applicable

sent to three cafeterias located in different parts of the hospital for dispensing to the wards. Refrigeration of the diced eggs was slow overnight because of the large volume of product, and no refrigeration took place from 8:00 A.M. till serving at noon. The cause of the outbreak was staphylococcal enterotoxin, likely produced during the preparation and distribution steps.

Salmonellosis from a banquet

At a banquet catered by a church group near Windsor, Ontario on June 16, 376 participants were served cold sliced turkey, cold sliced ham, meat balls, several salads, strawberries, short cake and beverages. Twelve to 44 h later, 100-200 persons became ill with nausea, vomiting, cramps, diarrhea, headaches and fever which lasted for 2-10 days. Three persons were hospitalized. *Salmonella saint-paul* was isolated from all the stool samples submitted by ill persons (23). No food was available for testing, and there was no way of tracing most of those attending. However, interview of 15 families suggested turkey as the most likely vehicle. Since refrigeration was grossly inadequate and ambient temperatures on the days of preparation and serving were very high, the conditions were suitable for multiplication of the *Salmonella.*

Zinc poisoning from a drinking canteen

In August, two teenage boys in British Columbia went for a hike and carried with them a cloth-covered metal canteen filled with orange flavored crystals dissolved in water. One and a half hours after the canteen was filled, the boys each drank a cupful of the beverage. One half hour later, violent cramps, bloating, and vomiting seized the boys, and one was hospitalized. The father of one observed fizzing when he put a few drops of the beverage onto the metal of the canteen. Subsequent analysis of another canteen indicated that the boys each had consumed the equivalent of 950 mg of zinc sulfate; very little cadmium or tin was found. Whereas the canteen was adequate for containing water, the acid beverage reacted with the galvanized steel and dissolved the zinc. The distributor of the imported product labelled the canteens with a caution against using them for beverages other than water.

Illness from raisin bread

In June, a woman in Saskatchewan bought seven loaves of raisin bread at a special price, froze the bread, and thawed a loaf when required. Two persons ate a few slices and became ill with nausea, vomiting, sore throat, hot flashes, and dizziness less than half an hour later. Analysis of one of the loaves showed that the bread was soggy in parts and contained 5% ethyl alcohol, 0.2% ethyl acetate, and a small amount of acetic acid. According to the woman, other persons were ill from bread bought from the same supermarket, but this was not found out until a year later. It is probable that the bread became damp and allowed yeast fermentation. It is known that ethyl acetate is an irritant that can cause a sore throat and also has a narcotic action. No microbiological analysis, however, was carried out.

Gastroenteritis from canned corn

In November, two adults and their children (3 and 5) in New Brunswick ate some corn from a newly opened can along with fried fish for an evening meal. They noticed an off-taste, but the "bad" odor was not at first attributed to the corn. Later, after realizing that he had eaten some "bad" corn, the father made himself vomit and experienced no other symptoms. The mother developed stomach pains 45 min after the meal, followed by vomiting and diarrhea which lasted for about 4½ h. Because of the mother's symptoms, the children were taken to a local clinic where vomiting was induced; no symptoms were noticed thereafter. The meal history for the previous 24 h revealed no other suspect foods. The suspected can containing the remainder of the contents was sent refrigerated to a laboratory. A foul smell was noticed by the analyst, and the dominant organisms found were *Clostridium butyricum* and *Streptococcus faecalis.* The aerobic and anaerobic colony counts were 85,000/g and 125,000/g, respectively. A leak in the side seam produced during manufacture was detected in the can. No other reports of illness were received concerning the same lot of this product. The organism actually responsible for the illness was not determined.

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Local Health Agencies: (*a*) Ontario: Algoma Health Unit; Brant County Health Unit; Durham Region Health Unit; Etobicoke Borough Health Department; Grey-Owen Sound County Health Unit; Haldimand-Norfolk Health Unit; Haliburton-Kawartha-Pine Ridge District Health Unit; Halton County Health Unit; Hamilton-Wentworth Health Unit; Hastings and Prince Edward Counties Health Unit; Huron County Health Unit; Kingston-Frontenac and Lennox and Addington Health Unit; Leeds, Grenville, and Lanark District Health Unit; Muskoka-Parry Sound Health Unit; Niagara Regional Area Health Unit; North Bay and District Health Unit; Northwestern Health Unit; North York Borough Health Department; Ottawa-Carleton Regional Area Health Unit; Oxford Health Unit; Peel County Health Unit; Perth District Health Unit; Peterborough County-City Health Unit; Porcupine Health Unit; Renfrew County and District Health Unit; St Lawrence and Ottawa Valleys Health Unit; Scarborough Borough Health Department; Simcoe County District Health Unit; Sudbury and District Health Unit; Thunder Bay District Health Unit; Timiskaming Health Unit; Toronto City Health Department; Waterloo Regional Health Unit; Wellington-Dufferin-Guelph Health Unit; Metro Windsor-Essex County Health Unit; York Borough Health Department; and York Regional Health Unit. (*b*) Québec: Montréal Urban Community. (*c*) Hospital: St Paul's Hospital (Grey Nuns') of

Saskatoon, Saskatchewan. (*d*) University: Department of Chemistry and Chemical Engineering, University of Saskatoon, Saskatchewan.

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New Milk Product was Begun in OSU Laboratory

Sweet, palatable acidophilus milk, being accepted across the nation as a new dairy product with potential beneficial qualities, got its start at Oregon State University 17 years ago.

In 1959, three Agricultural Experiment Station scientists at OSU—microbiologists Dennis E. Duggan, A. W. Anderson and Paul R. Elliker—published their work on producing a frozen concentrate of the bacteria used in preparing a palatable acidophilus milk. Their research results, including how to prepare the better tasting product, have been available to the public without charge since.

Today, Elliker is still head of the Department of Microbiology at OSU. Anderson also is a professor in the department. Duggan is a member of the Department of Biology at Florida State University, Gainesville.

Milks containing live lactobacilli, with nutritional attributes rivaling those of yogurt, have long been a favorite in many countries. For years, they have been available in soft drink machines in Japan. But they have never gained wide acceptance in the United States until recently because of their acid flavor.

Now being marketed in several cities under different names, sweet, palatable acidophilus milk—fresh, homogenized and pasteurized milk with a strain of *Lactobacillus acidophilus* (LBA) culture added—provides a beneficial type of bacteria for the intestinal tract.

The beneficial effect of lactobacillus-containing drinks, consumed in other parts of the world for centuries, is believed to have validity, said Floyd Bodyflet, OSU food scientist. However, he and the other OSU workers emphasize that some claims for beneficial effects of LBA and other cultured milks still require additional research for substantiation.

Acidophilus milk has been suggested in the U.S. for years to relieve certain intestinal disturbances and some undesirable effects of prolonged antibiotic therapy. As the three OSU scientists pointed out in 1959, in some instances the use of antibiotics over an extended period has been shown to disturb the normal balance of desirable intestinal tract bacteria.

A unique feature of the OSU study involved development of strains of LBA resistant to antibiotics commonly used 17 years ago.

Their report in the August 1959 volume of Food Technology, Journal of the Institute of Food Technologists, also noted reports of other investigators that LBA could be implanted in the intestine and by some competitive means, perhaps acid production, inhibited some undesirable activities until normal balance was restored.

The OSU team not only worked out a method to prepare frozen concentrates of LBA but also developed a supplemented whey medium to support growth and recovery of LBA cells by centrifugation.

The concentrate of LBA developed by the OSU researchers probably represented the first successful prototype of frozen culture concentrates of lactic acid and related bacteria now extensively used throughout the world for dairy and other food products.

The OSU investigations showed that not only did the concentrates have a high survival rate of LBA but also could be stored frozen for months and then could be added, when convenient, to milk for consumption.

The sweet acidophilus milk, they found, was as palatable as fresh milk. Freshly thawed acidophilus concentrate could be added to fresh, cold, pasteurized milk and stored for extended periods in the refrigerator with no change in acidity of milk and no significant loss in desirable organisms.

"The entire procedure of production and preservation of this concentrate required certain specialized equipment but the techniques involved could be adapted easily to commercial production," said the report.

"Sufficient concentrate to last for several months could be made and frozen in one day, thereby eliminating the need for daily preparation of small quantities. The concentrate could be stored in an ice cream freezing room or a deep freeze."

A number of companies in the U.S. and abroad now sell concentrates of LBA and related lactobacilli produced basically by the procedures reported in 1959 from OSU. These concentrates are suitable for preparing sweet, palatable acidophilus milk at a cost of a fraction of a cent per half gallon of milk for the added LBA bacteria.

Other lactobacillus investigations are being followed today by OSU microbiologists W. E. Sandine, K. S. Muralidhara, G. G. Sheggeby and Elliker who have worked with animal scientist David C. England on the effect of feeding lactobacilli on intestinal colonization of young pigs.

Their present study, soon to be published, shows that a lactobacillus organism (other than LBA) can colonize in the intestines of pigs. The strain of lactobacillus used also may colonize the human intestine since the intestinal tract of pigs has similarities to that of humans.

Actually, the strain of lactobacillus used in the study on young pigs has been reported by other workers to implant and grow in the human intestine. Testing of human intestinal tissue, taken by biopsy from individuals fed the lactobacillus, would provide needed proof, the scientists point out.

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Thermal Stability of Enterotoxins in Food

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ABSTRACT

Biological and serological properties of staphylococcal enterotoxins exhibit remarkable thermal stability. The slope of the thermal destruction curve (z value) for enterotoxin A or D is 27-28 C and it is 32 C for enterotoxin B regardless of the initial concentration of enterotoxin, the heating medium, or the assay system used to detect the enterotoxin. Thermal stability of enterotoxins is influenced by the nature of the food, pH, presence of NaCl, etc., and the type of enterotoxin; enterotoxin A is relatively more stable to heat at pH 6.0 (or higher) than at pH 4.5-5.5. Enterotoxin D is relatively more stable at pH 4.5-5.5 than at pH 6.0 or greater. Heat treatments commonly used in food processing, that is pasteurization (72 C for 15 sec) or ultrahigh temperature heating (290 F for 9 sec) in a "Spiratherm" Unit of Fluid whole milk, or smoking and heating of cured sausage to 70-100 C, or heating of Cheddar cheese to 70-90 C, are not effective for complete destruction (to levels below detection by the microslide serological assay) of enterotoxin A or D when present initially at levels normally expected to be found in foods (0.5-1.0 μg per 100 ml or g). Enterotoxin A or B (30 μg/ml) heated at 100 C for 25 min in saline, containing 2% gelatin and 0.3% proteose peptone and at pH 7.0, retained biological activity toward humans with a suggestion of increased biological activity after heat treatment, especially with enterotoxin A. The length of time required at a given temperature for complete destruction of enterotoxin in a given food system depends upon the sensitivity of the detection system used and the amount of food sample used for recovery of the enterotoxin. Treatment of heated enterotoxin A or D with urea and its removal by dialysis before serological assay, increased the recovery of enterotoxin by almost 4-fold as compared to controls which were simply heated and were not treated with urea. Enterotoxin A or D present initially at 1 μg/ml in whole milk (using 2 ml for recovery of enterotoxin with urea treatment following heating and assay by the micro slide method of Casman and Bennett) was found to withstand heating of milk at 121.1 C for 15 min. In the absence of urea treatment of heated toxins in milk, both toxins were found to be inactivated (to less than 0.02 μg/ml(within 15 min at 121.1C; between 13.8-14.8 min for toxin A and 12.8-13.8 min for toxin D.

Biological activity of staphylococcal enterotoxins exhibit rather remarkable thermal stability. This fact was recognized early by the studies of Jordan et al. and Bergdoll et al. (*3, 10*). Their data showed that enterotoxins produced in veal-infusion broth by growth of enterotoxigenic staphylococci at 37 C for 2 days, survived boiling (100 C) for 30 min. When humans were fed heated toxin with pasteurized milk, an emetic response was produced. In subsequent studies with monkeys (using intravenous injection) these authors demonstrated that enterotoxin is extremely heat resistant, but that its activity decreased with prolonged heating. Enterotoxin heated at 100 C for 30 min at pH 6 and 7.5 showed a 95% inactivation when fed to monkeys (3). These studies also pointed out that oral feeding to monkeys is not as sensitive as injecting the toxin into cats or monkeys. Epidemiological investigations in natural outbreaks of staphylococcal food poisoning provide more direct evidence of the remarkable thermal stability of the biological activity of enterotoxins when tested with humans. Several people who consumed enterotoxins from boiled goat's milk used in coffee, showed typical food poisoning symptoms. In this case the unboiled goat's milk yielded viable staphylococci. The boiled milk, although free of staphylococci, caused vomitting and diarrhea when a portion of it was fed to a monkey (*6*). In another outbreak, 16 of the 30 people who consumed thoroughly cooked sausage having no viable staphylococci became ill. Also, spray dried nonfat dry milk with no viable staphylococci caused typical staphylococcal food poisoning symptoms in humans in outbreaks that occurred in England and in Puerto Rico (*1, 2*).

Studies of the quantitative thermal stability (or destruction) of enterotoxins had to await development of practical, reliable, and reproducible serological assays as well as the availability of somewhat purified enterotoxins. These developments came about during the mid 1960's. Since then several investigators (*4, 5, 7-9, 11-16*) have studied the thermal inactivation kinetics of enterotoxins A and B and the influence on thermal inactivation of such factors as concentration of toxin, pH, presence of NaCl, and the heating medium itself. Of the four enterotoxins A, B, C, and D, enterotoxins A and D are the ones more commonly found in food poisoning outbreaks.

KINETIC TERMS USED IN EXPRESSING THERMAL STABILITY

Thermal stability or resistance of enterotoxins can be expressed in kinetic terms such as activation energy; standard enthalpy or entropy; or they may be expressed as D, z, or F values. The latter three values are commonly used in thermal processing of foods.

D value is the time (in minutes) necessary, at a given temperature and under specified conditions of heating, to reduce the activity (concentration) of enterotoxin by 90%. F value is the time necessary (in minutes) at a given temperature and under specified conditions of heating, to destroy a specified amount of enterotoxin. While the D value is independent of enterotoxin concentration, the F value is dependent on concentration. Both D and F values

are dependent on heating temperature and the heating system (substrate, pH, presence of salt or sugar, etc.). A plot of the log of the D or F value against heating temperature is referred to as the thermal death time or inactivation curve and the slope of this curve is called the z value. The z value is the temperature increase or decrease necessary to decrease or increase, respectively, the time necessary to bring about a 10-fold change in enterotoxin activity. The z value is independent of both the concentration of enterotoxin and the heating substrate.

The F value is dependent on the concentration of heated enterotoxin and on the sensitivity of the assay or detection system employed. The F value increases with an increase in concentration of enterotoxin and it also increases with an increase in the sensitivity of the detection system employed.

THERMAL STABILITY OF ENTEROTOXINS IN BUFFER SYSTEMS

Table 1 summarizes the published data on thermal inactivation of staphylococcal enterotoxins heated in buffer solutions at 212 F (100 C) or higher. Enterotoxin A, which is produced in relatively smaller concentrations than B, is the most common type encountered in food poisoning outbreaks. The z value for enterotoxin B is higher than that of enterotoxin A indicating that the former is somewhat more resistant to heat than the latter. As might be anticipated, the F value for enterotoxin A was larger with the more sensitive detection system, i.e., cat vs. monkey, 11 vs. 8 min. Read and Bradshaw (*12*) showed that there was a good correlation between results from the cat assay and the serological assay in detecting heated enterotoxin B. In general, the z value for enterotoxin A or B determined with bioassay or serological assay was about the same, i.e. 46-50 F for toxin A and 52-58 F for toxin B.

The initial concentration of enterotoxin A or B used in these studies was rather large in comparison to what might be expected in foods. Moreover, the heat treatments were so great as to be employed only in canned sterilized foods.

Table 2 shows data on thermal inactivation of enterotoxins A, B, C, and D heated at temperatures of 100 C or less and enterotoxin concentrations of 1 μg or less for toxins A and D. These data indicate that there was some reactivation of enterotoxin B (*7*) upon storage. On the other hand data of Soo et al. (*15*) show that enterotoxin A requires treatment with chemical denaturants for reactivation to occur. In the absence of

TABLE 1. *Thermal inactivation characteristics of staphylococcal enterotoxins*

Source of information	Type of enterotoxin	Assay system used	Sensitivity or amount injected or fed	Initial toxin conc. (μg/ml)	Heating medium	Heating Temp. (°F)	F_{250} (min)	z value (°F)
					Dialyzed growth material dissolved in			
Denny et al. (*4*)	A	Monkey feeding	47.25 μg	7	Distilled water	212-250	8	46
	A	Cat; intravenous injection	2.3 μg	7	Distilled water	212-250	11	48
Read and Bradshaw (*12*)	B	Serological; double diffusion tube	0.7 μg/ml	30	0.04 *M* veronal buffer, pH 7.2	204-259	19-16.4	55-58
		Cat; intravenous injection	2.0 μg	30	0.04 *M* veronal buffer, ph 7.2	240	32.5	—
Hilker et al. (*8*)	A	Serological; single diffusion tube	1 μg/ml	21	0.04 *M* veronal buffer, pH 7.2	212-250	22	50
Stinson and Troller (*16*)	B	Serological; single diffusion tube	1 μg/ml	10-100	0.04 *M* veronal buffer, pH 7.2	250-320 320	62 2.8	52 —

TABLE 2. *Thermal inactivation of serological activity of staphylococcal enterotoxins at temperatures of 100 C or less*

Source of information	Type of enterotoxin	Heating		Serological activity of enterotoxins		
		Temp. (°C)	Time (min)	Before heating (μg/ml)	After heating (%)	After storage or other treatment (%)
Jamlang et al. (*9*)	B	70	15	100	10	35
		100	15	100	60	—[a]
Fung et al. (*7*)	B	80	60	100	22	42
		100	60	100	15	20
	C	80	60	100	50	85
		100	60	100	35	60
Soo et al. (*15*)	A	70	15	5	0.2	6.4
		90	15	5	0.4	4.8
		70	15	1	0.1	—
		90	15	1	1.0	—
	D	70	7.5	5	3	95[b]
		90	7.5	5	6	95
		70	7.5	0.5	8	—
		90	7.5	0.5	24	—

[a]—Not tested.
[b]Toxin was heated in the presence of 6 *M* urea and pH 5.

these, specific reactivation (including storage of enterotoxin B following heating) one would conclude that inactivation was faster at the lower heating temperatures. For example, toxin B heated at 70 C for 15 min showed a recovery of 10% as compared to 60% recovery when heated at 100 C. When heated at 70 C and then reheated to 100 C for 6 min, there was an increased recovery of toxin; 10% vs. 35%. This increase upon reheating was observed by Jamlang et al. (*9*) both serologically and biologically by intravenous injection in dogs.

EFFECT OF pH OF BUFFER ON THERMAL STABILITY OF ENTEROTOXINS

The pH of the heating medium affects the rate of inactivation of a given enterotoxin. As can be seen in Fig. 1, enterotoxin A was inactivated faster at pH values ≤ 5.5 than at pH 6.5 or higher. Likewise, enterotoxin A was found to survive 7 min at 250 F when the pH was 7.8 as compared to its complete inactivation in less than 1 min when the pH was 5.3 (Data of Denny et al. presented at an ASM-Seminar on staphylococcal enterotoxins, Miami Beach, Florida, 1973). On the other hand, enterotoxin D, Fig. 2, was inactivated faster at pH 6.5 than at pH ≤ 5.5.

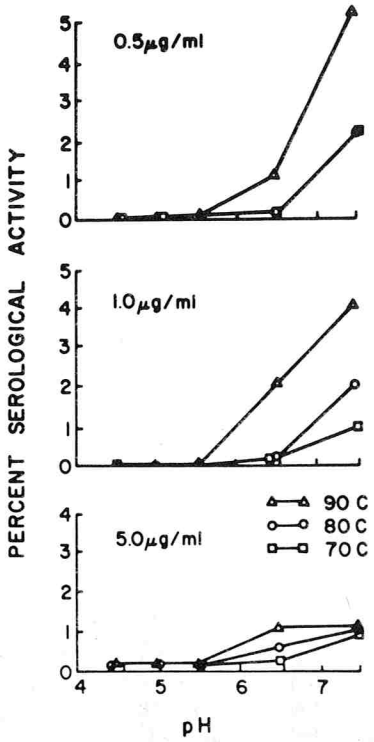

Figure 1. *Effect of pH on thermal inactivation of enterotoxin A heated in sodium acetate or sodium phosphate buffer (0.01 ionic strength) for 5 min.*

THERMAL STABILITY OF ENTEROTOXINS IN FOODS

Thermal stability of enterotoxins varies with the heating system or the food system. Enterotoxins are more thermostable in crude form than they are in the purified form.

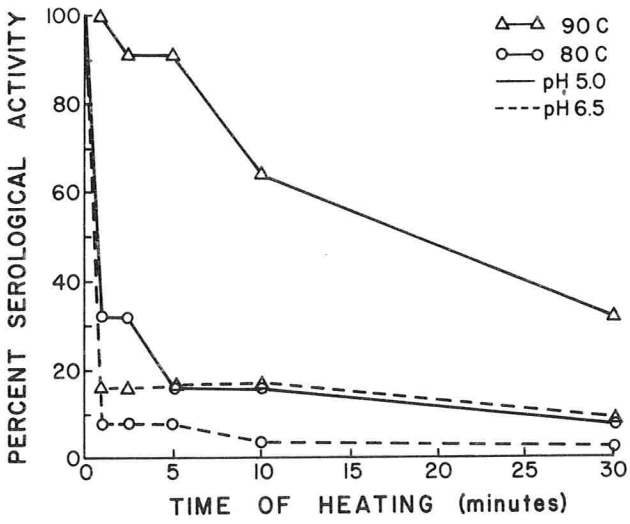

Figure 2. *Effect of pH on thermal inactivation of enterotoxin D heated in sodium acetate or sodium phosphate buffer (0.01 ionic strength) for 5 min.*

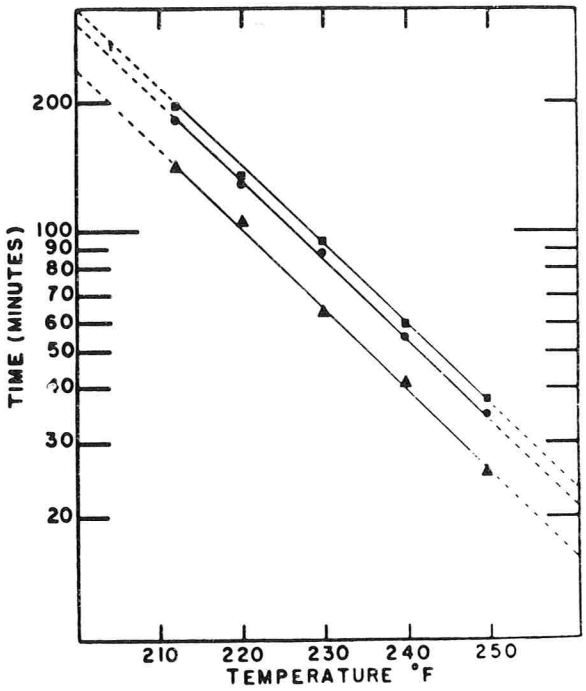

Figure 3. *Heat inactivation curves of enterotoxin A in beef bouillon. Enterotoxin concentrations: ■ 60 µg/ml; • 20 µg/ml; ▲ 5 µg/ml. Symbols represent the longest heating period at each temperature giving a positive serological reaction. Denny et al. (5).*

Figure 3 shows thermal inactivation data of enterotoxin A sufficient to reduce serological activity to <1 µg/ml in beef bouillon of pH 6.2. The z value, or the slope of the thermal inactivation curve, was between 49-52 F. With 5µg/ml initial concentration, the F_{250} value was 27 min and this value was five times greater

than that found with phosphate buffer of pH 7.2 that is 5 min vs. 27 min. Table 3 shows data on thermal inactivation of enterotoxin A when present initially at 1 μg/ml concentration. The F value at each temperature was found to be greater by serological test than by the monkey assay, in this instance 45 vs. 40, 28 vs. 22, and 16 vs. 13 min. Figure 4 shows data of Soo et al. (*14*) on

TABLE 3. *Thermal stability of Staphylococcal enterotoxin A (1 μg/ml) in beef bouillon at pH 6.2*[a]

Temperature of heating (°C)	End points (minutes) of inactivation			
	Serological assay[b]		Monkey feeding[c]	
	Positive	Negative	Positive	Negative
110	40	45	35	40
115	26	28	20	22
121.1	15	16	12	13

[a]Data of Denney, C.B., National Canners Association, Washington, D.C. Presented in an ASM seminar on staphylococcal enterotoxins, annual meeting of the American Society for Microbiology, Miami Beach, Florida, May 6-11, 1973.
[b]Oudin-gel diffusion procedure.
[c]Monkeys were fed a dosage equivalent to about 47 μg (estimated serologically) of unheated enterotoxin.

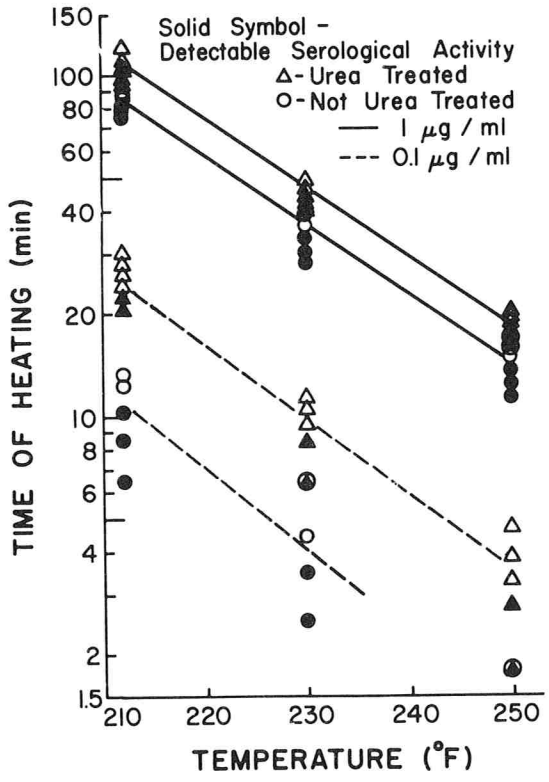

Figure 4. *Thermal inactivation curves of enterotoxin A in whole milk of pH 6.6.*

thermal inactivation of enterotoxin A in whole milk. The slope of the inactivation curve was 49 F which is in excellent agreement with that found in tests with beef bouillon by Denny et al. (*5*). The F_{250} for 1 μg/ml was 18 min in milk as compared to 16 min in beef bouillon, using a serological assay. With 0.1 μg/ml of milk, the F_{250} was found to be 3.8 min. Read and Bradshaw (*11*) reported a z value of 46.6 F for enterotoxin B in whole milk and an F_{250} value of 18.4 min for 30 μg/ml to be reduced to <0.5 μg/ml. Figure 5 shows data of Soo et al. (*14*) on thermal inactivation of enterotoxin D in whole milk. The z value was 52 F and the F_{250} value with 1

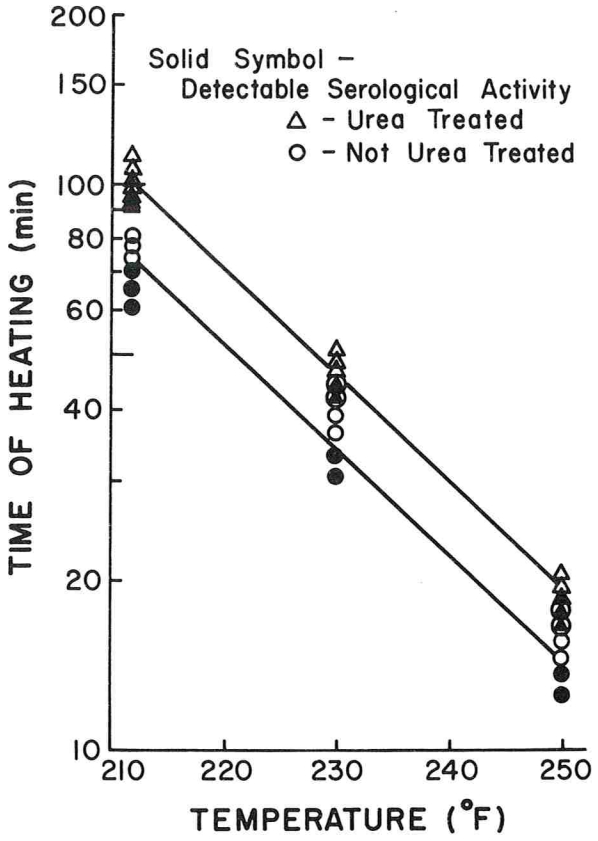

Figure 5. *Thermal inactivation curves of enterotoxin D in whole milk of pH 6.6.*

μg/ml was 19.8 min. These data indicate that if 1 μg/ml of enterotoxin A or D is present in whole milk (as based on serological tests and urea treatment before assay), these toxins would not be inactivated by heating at 250 F for 15 min.

EFFECTS OF PROCESSING OF MILK PRODUCTS ON THERMAL STABILITY OF ENTEROTOXINS

Data in Table 4 show thermal inactivation of enterotoxins A and D by pasteurization of milk. A substantial amount of enterotoxin A or D survived the pasteurization heat treatment. The likelihood of finding enterotoxin in pasteurized or heated milk and dry milk would depend upon the sensitivity of the assay system used, the amount of sample used for assay, and the concentration of enterotoxin produced in milk by *S. aureus* before heat treatment. Table 5 shows data on recovery of enterotoxin from nonfat dry milk (NFDM). Enterotoxin was produced in fluid pasteurized skim milk by growth of *S. aureus* (196E; 18 h at 37 C). This milk

TABLE 4. *Thermal inactivation of staphylococcal enterotoxins A and D in milk, skimmilk, cream, evaporated milk, and reconstituted nonfat dry milk [NFDM] at 72 C for 15 sec*[a]

Type of sample	Percent serolgical activity remaining[b]			
	Enterotoxin A		Enterotoxin D	
	Without urea	With urea	Without urea	With urea
Whole milk	36	71	30	67
Skimmilk	56	71	30	67
Cream	24	45	15	30
Evaporated milk	56	72	NT[c]	NT
Reconstituted NFDM	45	75	NT	NT

[a]40-50 sec to reach 72 C.
[b]1 μg/ml initial concentration; based on the recovery of added enterotoxins A and D to milk, skimmilk, evaporated milk, reconstituted NFDM (90%), and cream (70%).
[c]Not tested.

was heated at 77-82 C for 15 min to destroy viable *S. aureus,* then was condensed to 35-40% solids concentration in a vacuum pan, and finally was spray dried. Drying conditions for this study included 380 F inlet and 175 F outlet air temperature and 12-15 min drying time. Toxin was assayed by intravenous injection of cats. As can be seen from data in this table, enterotoxin was detectable biologically in NFDM. The enterotoxin was stable to processing treatments of NFDM when present at those levels expected to be found. Unfortunately, at the time this study was conducted, the serological assay was unavailable to quantify the enterotoxin concentration. However, examination of this toxic product after storage for over 10 years at < 4 C, using the serological microslide assay and using 100 g for the assay, showed the presence of enterotoxin A at a level of approximately 1 μg/100 g.

TABLE 5. *Thermal stability of enterotoxin produced by* Staphylococcus aureus *(196 E) during manufacture of nonfat dry milk, as determined by cat assay*[a]

Dose injected[b]	No. cats injected	No. cats ill
4	2	2
3	5	4
2	4	1
1	4	2
0.5	4	1

[a]Data of Elmer George, Ph.D Thesis, University of Minnesota (1964).
[b]Grams equivalent of NFDM; filter sterilized supernatant from rennet-treated recombined NFDM was injected intravenously.

Table 6 shows data on thermal inactivation of enterotoxin A and D in whole milk heated at 290 F for 9 sec in a "Spiratherm" unit. Even at the lowest initial concentration of enterotoxin (0.005 μg/ml) both toxins survived this heat treatment. The data also show that one needs to examine a large enough sample (100 ml) to demonstrate survival of the toxin.

EFFECTS OF SMOKING AND COOKING OF SAUSAGE ON THERMAL STABILITY OF ENTEROTOXINS

Table 7 shows data on thermal inactivation of enterotoxins A and D in smoked and cooked sausage.

TABLE 6. *Thermal inactivation of staphylococcal enterotoxins A and D in milk at 143.3 C for 9.12 sec*

Type of enterotoxin	Concentration (μg/ml)	Amount of Milk tested (ml)	Percent serological activity remaining[a]	
			Without urea treatment	Treated with (6*M*) urea after heating
A	0.5	2	8[b]	8
A	0.05	2	UD[b]	UD
A	0.05	20	4	4
A	0.005	2	UD	UD
A	0.005	20	UD	UD
A	0.005	100	4	4
D	0.05	2	UD	UD
D	0.05	100	10	10
D	0.005	2	UD	UD
D	0.005	100	16	16

[a]Based on recovery of enterotoxin added and recovered from the same but unheated milk; 40% for 100 ml and 90% for 2 or 20 ml of milk tested.
[b]Undetectable (less than 0.01 μg).

Both toxins survived cooking at 100 C, but to demonstrate the survival of toxin it was necessary to use urea treatment in the first step of extraction.

TABLE 7. *Thermal inactivation of staphylococcal enterotoxins A and D in cooked sausage*[a]

Type of entero-toxin	Amount of enterotoxin (ug) accounted for in the lyophilized extract from 100 g meat					
	Amount added	Amount recovered from the unheated control	After smoking and cooking to internal temperature of 71.2 C		Boiling to internal temp of 100 C after smoking and cooking[c]	
			Without urea	With urea	Without urea	With urea
A	1.0	0.4	0.16	0.16	UD[b]	0.02
D	1.0	0.4	0.4	0.4	0.02	0.02

[a]Samples containing enterotoxins were smoked for 2 h and cooked to 71.2 C before heating at 100 C.
[b]Undetectable (less than 0.02 μg).
[c]9 min to reach 100 C and 7 min to cool down to 48 C.

THERMAL STABILITY OF ENTEROTOXINS AS MEASURED BY HUMAN FEEDING

As noted, thermal inactivation of enterotoxins has been measured biologically, using monkeys or cats for the assay, as well as serologically. Monkeys require 5-10 μg of oral feeding to show an emetic response, while, cats, injected intravenously, show a response at a dose level of about 0.25-0.5 μg per cat. Since monkeys require larger amounts of enterotoxin to create a response than do humans, and since intravenous injection into cats is not the normal route of enterotoxins intake, one needs to determine the thermal stability of enterotoxins by feeding them to humans to evaluate the safety of a given thermal process. Such a study has been conducted by Dangerfield and data are shown in Tables 8 and 9. Enterotoxin A or B was heated at 100 C in saline containing 2% gelatin and 0.3% proteose peptone at pH 7.0. The appropriate dilution of heated or unheated enterotoxin was mixed in milk and was ingested by humans following breakfast. With increase in length of heating there was a decrease in the number of persons becoming ill. There was also a slight decrease in the

serological activity that is, 50% reduction after 25 min of heating and no decrease until after 8 min at 100 C (Table 8). Even though there was no decrease in the serological activity or in the number of persons becoming ill with toxin heated for 1.6 min, persons ingesting heated toxin were more severely ill than those ingesting the same dose of unheated toxin. In fact, three of six persons required parenteral replacement of fluid and electrolytes. Fever occurred in 10 out of the 16 ill individuals and one person showed an asymptomatic response. This might suggest an increase in the biological activity of toxin after heating. The results for enterotoxin A were more dramatic and are shown in Table 9. All persons who consumed enterotoxin A heated at 100 C for 25 min became ill and all showed more severe clinical symptoms than those receiving a similar dose of unheated toxin. One of six developed rapid onset of severe generalized abdominal cramping pain associated with marked vomitting and diarrhea. The symptoms persisted 12 h. When smaller doses of heated toxins were fed to humans, one person at each dose of 0.005 and 0.01 μg/kg showed mild symptoms which were compatible with staphylococcal intoxication.

These results suggest that though there would appear to be a decrease in the serological activity of heated enterotoxins, there is some potentiation or increase in the biological activity after heat treatment.

TABLE 8. *Thermal stability of staphylococcal enterotoxin B (30 μg/ml) in 0.85% saline, 2% gelatin, and 0.3% proteose-peptone*[a]

Minutes heated[b] at 100 C	Enterotoxin[c] (μg/ml)	Results of human feeding: Dose fed (μg/kg)	No. fed	No. ill
0	10	0.4	4	4
1.6	10	0.4	6	6
8.0	10	0.4	6	5
15.0	5-10	0.4	6	3
25.0	5	0.4	6	2

[a]Data of Dangerfield, H.C., U.S. Army Med. Res. Inst. Infectious diseases, Fort Detrick, Md; Presented in an ASM seminar on staphylococcal enterotoxins annual meeting of the American Society for Microbiology, Miami Beach, Florida, May 6-11, 1973 and Bennett, R.W., FDA-Food and Drug Administration, Washington, D.C. and unpublished data (1973).
[b]Does not include 4-4.5 min heating time to bring to 100 C.
[c]Microslide assay of Casman and Bennett.

TABLE 9. *Thermal stability of staphylococcal enterotoxin A (30 μg/ml) in 0.85% saline, 2% gelatin, and 0.3% proteose-peptone*[a]

Minutes heated at 100 C	Enterotoxin (μg/ml)	Results of human feeding: Dose fed (μg/kg)	No. fed	No. ill
0	10	0.4	3	3
0	10	0.2	8	6
25	5-10	0.4	6	6
25	5-10	0.05	6	1
25	5-10	0.01	6	1[d]
25	5-10	0.005	6	1[d]

[abc]See footnotes in Table 8.
[d]Persons showing vomitting and/or diarrhea were considered ill. These individuals showed milk symptoms (nausea and anorexia) compatible with intoxication.

RECOVERY OF SEROLOGICAL ACTIVITY OF HEATED ENTEROTOXIN AND ITS RELATIONSHIP TO BIOLOGICAL ACTIVITY

Our work indicates that heated enterotoxin A or D cannot be measured as well serologically as can the unheated toxin. We have also been able to show some enhancement in detectability (by 3- to 4-fold) by urea treatment of the heated toxin and removal of the urea before assay. This suggests that the serological sites are unavailable after heating and that urea reestablishes these masked sites, possibly by some refolding of partially denatured enterotoxin back to the native state thus making the serological sites available for the reaction. An attempt has also been made (Tatini et al. unpublished data) to determine if urea treatment which enhances the recovery of heat-altered toxin also results in an enhanced recovery of biological activity of enterotoxin A in cats and monkeys. Some of the preliminary results are shown in Table 10. Enterotoxin A (100 μg/ml, in phosphate buffer of pH 6.5) was placed in 2-ml volumes in screw cap test tubes and was heated in a water bath to 70 C and cooled immediately. The heated and unheated toxins were lyophilized and used for the assay. Urea was removed by dialysis before lyophilizing. Though fewer cats were tested for heated than unheated at each dose level of toxin, it would appear that ED_{50} for cats might be around 0.25-0.5 μg for unheated toxin, 0.125 to 0.25 μg for heated, and 0.03-0.06 μg for heated toxin treated with urea. If this is true, then there are 2-4-fold more emetic doses for the cat in the heated toxin than in the unheated toxin.

In the monkey, also, heated toxin at a serologically measured activity of 5 μg appeared to be more toxic than the corresponding unheated toxin; none of six with unheated, and two of four with heated toxin showed a positive emetic response. These results, and especially

TABLE 10. *Relationship between serological and biological activity of heated and unheated staphylococcal enterotoxin A*

Heat treatment	Serological assay	Cat assay: Dose[a]	Cat assay: No. ill/No. injected	Monkey assay: Dose[a]	Monkey assay: No. ill/No. fed
	(μg/ml)	(μg)		(μg)	
Unheated	100	0.50	7/10	6	0/6
		0.25	3/8	12	1/6
		0.125	1/7		
		0.06	1/6		
		0.03	0/3		
Heated to reach 70 C	5-6	0.50	2/2		
		0.25	2/2	5.5	2/4
		0.125	1/2		
		0.06	1/2		
		0.03	0/3		
Heated to reach 70 C and treated with urea[b]	21-24	0.50	4/6		
		0.25	2/3		
		0.125	2/2	13	1/2
		0.06	1/1		
		0.03	1/3		

[a]Based on microslide-serological method of Casman and Bennett.
[b]Urea was removed by dialysis before lyophilizing for serological assay.

those using cats as test animals, are in agreement with results on humans presented earlier; that is an increase in biological activity of heated toxins as compared to unheated toxin seems to occur. The consequences of these findings are rather obvious, that staphylococcal growth and production of enterotoxin must be prevented in foods because thermal processing cannot be relied upon to inactivate the toxins. At the same time there is reason to believe that there may even be an increase in the toxicity of enterotoxin A in thermally processed foods. It is also apparent that appropriate methodology should be developed to measure heat-altered toxins in foods.

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ABSTRACT

Processing of milk into dry milk, cheese, and butter was an important part of Biblical man's survival in the desert wasteland. Meat processing was limited, and little of it was likely consumed during the 40 years of wandering of the Israelites. The Bible does mention processing of quail which were salted and dried. Locusts were a common food item for traveling Israelites and were prepared and consumed whenever found. This was done by boiling them in salt water, then baking, drying, or smoking before consuming. Cereal grain formed an important part of the Hebrews' diet. It was threshed and ground with a hand mill and then made into bread. Another method of processing was by using the mortar and pestle to make flour for bread. Manna seems to have been processed in a manner similar to that used for cereal grains. It was the sole source of food for many years while the Israelites traveled in the desert. Vegetables were not processed and are only briefly mentioned, whereas spices are referred to throughout the Old Testament. Because of the availability of natural vegetation and the difficulties of storing processed foods, extensive processing methods were not developed. Many of the processing techniques used by Biblical man are the same as those used by present-day Bedouin Arabs.

The Old Testament of the Bible is an excellent source of information about food processing methods used in one of the most highly civilized parts of the ancient world. Only the most explicitly explained processing techniques, those for milk, meat, and cereal grain products, are discussed in this paper. A nomadic lifestyle dictated the use of foods that were easily transported, such as meat and milk in the form of goats and other herd animals; easily cultivated, such as wheat and corn; or easily found in the wild, such as locust and manna. These foods were either eaten immediately or processed to minimize susceptibility to microbial spoilage.

PROCESSING OF MILK

Milk was a main staple in the diet of the Hebrews and others and is discussed at some length in the Old Testament. As Doughty (*4*) points out, milk is an important part of the diets of nomadic Bedouin tribes today. They produce a type of dry milk called *mereesy*."Mereesy is the buttermilk of the flock, dried by boiling to a hard shard and resembles chalk." It will stay in this dry chalk-like form for a year or more. To rehydrate it for consumption, it is rubbed between the hands and then mixed with water. For a special delicacy, mereesy is ground into a flour and mixed with sugar which is rehydrated into another type of beverage (*4*). Although there is no mention in the Bible of the wandering Hebrews using a milk product of this type, it was probably processed and consumed frequently. When milk consumption is mentioned in the Bible, it is generally either as fresh milk or buttermilk. Abraham gave some fresh milk to travelers (Gen. 18.8). Deuteromony again mentions milk, "the butter of kine and milk of sheep" (Deut. 32.14). Doughty (*4*) explains that the butter of kine is the butter of cows and that this and the milk of sheep are also common among the Arabs. In Judges 4:19 it is stated that Jael opened a bottle (which is a skin) of milk to give Sisera a drink. This is a clue that the Hebrews perhaps carried buttermilk in goat skin pouches, as do the present day Bedouins.

Buttermilk was produced by the Bedouin Arabs as they traveled by rocking a skin full of sour milk on their knees (*4*). Again, this is not specifically stated in the Bible, but it is a simple process which is almost a necessity for a traveler who cannot preserve goods such as raw milk.

Butter making, however, is discussed in the Bible. Proverbs 30:33 (*5*) mentions, "Surely the churning of milk bringeth forth butter." Butter is considered by Doughty to have been a luxury item (*4*). It is a little more complicated process than that for manufacture of buttermilk. Because of the hot climate, butter was melted and clarified and stored in leather bottles in a semi-liquid state.

There are problems in interpretations concerning butter because butter and curdled milk are the same word in Hebrew. Thus, the meaning must be taken from the context as curds were also a common feature of the Hebrew diet. The problem with meaning can be illustrated with the passage from Judges (5:24-25). This passage also shows somewhat the status of butter and curds as a luxury item. "Blessed above women shall Jael the wife of Heber the Kenite be, blessed shall she be above women in the tent. He asked for water, and she gave him milk; she brought forth butter in a lordly dish." It is possible that this must mean curds and not butter as is stated.

Job 10:10 states: "Hast thou not poured me out as milk, and curdled me like cheese?" It is not known if this

[1]*Biblical quotations in this paper are from King James version.*

is the fault of the terminology or if the process of cheese making did indeed come that much later. There is no mention of how the cheese was made, but during that time it was probably made by a primitive method of of drying and curdling milk. In I Samuel 17:18, Jesse, the father of David, sent the officer under whose orders his sons were serving a present of 10 cheeses. This would seem to indicate that cheese was also somewhat a luxury item.

MEAT PROCESSING

Meat is another equally important element in the diet of the nomad. Dairy cattle and oxen were eaten by the Hebrews. Unfortunately, there is no reference to the processing of the meat. Goat and lamb were also eaten by the Hebrews, but again there is no mention of the process of preservation used if there was one.

It is felt that the Israelites did not eat meat during the 40 years they were traveling in the desert searching for the Promised Land. When they left Egypt after 40 years they arrived in Jordan with an extensive stock of cattle, sheep, and goats. Numbers 31:32-34 tells of the booty they took just from the Midianites as spoil: "six hundred and seventy-five thousand sheep, and seventy-two thousand cattle and sixty-one thousand asses." With this large number of animals it would seem that there would be enough meat to eat. But in fact, after they made the required sacrifices to their god, and considering that the company was several million strong and had not been able to get meat in Egypt, the herds would not have lasted until they reached their destination. Some Biblical scholars have estimated that the meat would have lasted them one month at most if eaten rather than sacrificed as directed (*7*). The only way it would have been possible for them to transport their herds to the Promised Land was to use them only for the required sacrifices. This is probably the reason that there is no specific reference to the preservation of meat in this portion of the Bible.

At one point God did providentially send them a flock of quail and the method of preserving them is explained in Numbers 11:31-33. "And there went forth a wind from the Lord, and brought quails from the sea, and let them fall by the camp, as it were, a day's journey on this side, and as it were two cubits high upon the face of the earth. And the people stood up all that day, and all that night and all the next day, and they gathered the quail.....and they spread them all abroad for themselves round about the camp. And while the flesh was yet between their teeth, ere it was chewed (before it was consumed) the wrath of the Lord was kindled against the people.....because there they buried the people that lusted (who craved meat)." It has been stated that quail of this type has a fatty flesh that is excellent to taste, but consumption of large quantities can cause digestive problems (*2*). It was necessary to dry and salt cure the meat. This passage indicates that the quail were placed around the camp to sun dry and that those "who had the craving" and ate the raw birds in large quantities died. A. G. von Veen states that it is possible that they died from an acute poisoning. Authors such as Pliny the Elder, Lucretius, and Galen mention that quail can be poisonous to man after the birds have eaten plants such as *Helleborus* and *Conicum maculatum* (hemlock). Von Veen states that Sargent found that hemlock seed is harmless to quail, even in high dosage and in prolonged feeding test, but very toxic when fed to dogs. The symptoms of poisoning were the same as those described in human beings. This then is a logical cause of death due to eating the uncured birds (*8*).

This does illustrate that the Hebrews must have known how to dry their meat by splitting the carcass, salting, and drying in the sun to make a stable food product. This type of processing may well be the technique used by the Hebrew for all types of meat. It would certainly give them a more stable food with a longer keeping time.

INSECTS

Locusts do not sound particularly appetizing to modern palates, yet God told the Israelites in Leviticus 11:22, "Of them you may eat: the locust according to its kind, the bald locust according to it kind, the cricket according to its kind, and the grasshopper according to its kind." The locust is said by Biblical authorities to be thrown alive into boiling salt water, and its head, wings and legs pulled off after a few minutes of cooking. After this they were either roasted, baked or dried and smoked (*7*). Doughty says that the Bedouins prepare their locusts in much the same manner. The locusts after processing smell something like rancid fish oil. They are stored in leather sacks if not eaten immediately (*4*). Much later, John the Baptist ate locusts as part of his regular diet (Matt. 3:4). It is not known how frequently locusts were eaten by the Hebrews. They likely did not have enough time to process the locusts while traveling. The locusts were probably consumed immediately and not kept as reserve food supply.

PROCESSING OF CEREALS

Cereal grains were an important part of the Hebrew diet. Grains were mainly processed into flour and bread. The first threshing of wheat appears in the Bible in Judges 6:11 where Gideon is beating out wheat in the wine press. Wheat flour was given religious significance in Exodus 29:2 when the Israelites receive their orders on what they should eat: "And unleavened bread, and cakes unleavened tempered with oil, and wafers unleavened anointed with oil; of wheaten flour shalt thou make them." From this text one can see that the flour must have been made by some type of milling process. Such a process for cereal grain is described in Numbers 11:8, "the people went about and gathered it and ground it in mills or beat it in mortars." The mortar is the oldest method for processing cereals known to man. The milk used is described as a hand-mill comprised of two circular stones fitting one inside the other (*2*). In Deuteronomy 24:6, Moses told the Israelites that the millstones, or even the upper millstone, was not to be taken as a pledge (payment for debt) because flour had to be made daily for

bread.

Corn was likely processed in a similar manner. Corn in biblical terms means any cereal grain, i.e., wheat, barley, vetch, etc. These cereal grains were staples from which the Hebrew and Arab nomads made some of their basic food items. Grain processing is touched upon in Deuteronomy 25:4, "Thou shall not muzzle the ox when he treadeth out the corn." Grain was tread on by oxen to separate the kernels from the rest of the plant. Corn obtained in this manner was used to make bread. The flour milled from grain other than wheat was called bread whereas flour made from wheat was called fine bread.

Lentils are believed to be the first cultivated crop of man. They are mentioned in the Bible in Genesis 25:34 when Jacob gave Esau bread and pottage of lentils. Pottage is the Hebrew word for soup. Lentils are legumes with small, edible seeds. The plants are easily grown and when dried can be stored for long periods. Ezekiel 4:9 gives a Biblical recipe for making bread, "Take thou also unto thee wheat, and barley and beans and lentils and millet and fitches and put them in one vessel and make thee bread thereof." The lentils were probably milled much like wheat and other grains for breadmaking (*6*). The passage from Ezekiel hints at many domesticated grain and vegetable products available at that time.

MANNA

The most widely known Biblical food is manna, which is described in Numbers 11:7, "Manna was as coriander seed, and the color thereof as the color of bdellium." The coriander seed is a small seed about a quarter of an inch in diameter, and bdellium is a yellowish color. The next verse in this passage explains that the manna was milled, baked, and made into cakes much the same way as cereal grains. Manna is said to taste like a wafer made with honey. At the present time manna is gathered in small quantities by Bedouins of northern Arabia (*2*). It is processed after cleaning then cooled in water. It can be also stored for some time in leather skins. Bodenheimer (*1*) hypothesizes that manna is a material secreted by an insect which feeds on the sap of the tamarisk tree. He agrees with the Biblical report concerning manna and states that the location where manna was found by the Israelites is approximately the same as the area where it is found today. The Bible gives the time as the second month after the exodus, late May and early June, which is the same time as it is found in those areas today. Other characteristics of the Biblical manna, its shape, size, disappearance at midday, taste like honey are consistent with a description of the present tamarisk manna.

However, when one examines the story of Biblical manna more closely, it becomes evident that it is impossible that the two are the same food. Tamarisk manna cannot be the miracle food on which the Hebrews existed for 40 years as they wandered through the desert. It is found in the Sinai only during the months of June and July. The manna which the Hebrews ate was received every morning for 40 years. It also fed the millions of Israelites and comprised the major staple in their diet. Today manna is not found every year, and when there is a good year, it can be found in few kilo quantities. The Biblical manna could not be saved for the next day yet tamarisk manna can be stored for several days. Tamarisk manna cannot be pounded with a mortar and ground in a mill as Numbers 11:8 because it is too soft. It is generally agreed that tamarisk manna cannot be made into a flour (*7*). If these two foods are the same, it is indeed a rare coincidence - perhaps even a miracle.

PROCESSING OF VEGETABLES

A brief mention of vegetables is made in Numbers 11:5 where the congregation complained to Moses, "We remember the fish, which we did eat in Egypt freely; the cucumbers, and the melons, and the leeks, and the onions, and the garlick." This indicates that there was some extremely sophisticated farming occurring in rich lands such as the banks of the Nile river in Egypt (*6*), but that the stark conditions of the desert did not allow for a larger variety of foods other than the ones mentioned above.The stock of these vegetables they took with them on their exodus must have been consumed shortly after leaving Egypt, and it is unlikely that these could have been cultivated as they traveled.

In conclusion, those foods which were consumed by the Israelite were only those foods which he had the technical capability to process. A nomad for 40 years, he likely took little interest in processing many foods and generally consumed natural vegetation encountered in the desert as he traveled. Many of the processing techniques used were the same as those practiced by some of today's primitive societies.

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Health, the Environment, and the Urban Community

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ABSTRACT

The health of a community must be viewed from a three-dimensional perspective: (*a*) personal health, (*b*) environmental health, and (*c*) social conditions related to health. Not only are the physical, chemical, and biological components of the environment significant determinants of community health, but the problems of poverty, substandard housing, stressful occupational environments, and unemployment are also integrated constituents of health and well-being. Accordingly, health service programs cannot be compartmentalized; they must reflect the three-dimensions of health and they must be designed to achieve the goals and objectives necessary to improve community health.

In the Eleventh Bronfman Lecture before the Ninety-Ninth Annual Meeting of the American Public Health Association the late Herman Hilleboe, (5) reminded us that "health is not primarily an end in itself but rather a means of attaining human well-being within the constraints of the physical, biological, and socio-cultural environment in which man finds himself."

Accordingly, comprehensive health policies and plans are part of the social, the economic and the political developments of a community or a nation.

DIMENSIONS OF HEALTH

From this broad perspective we must view health in three dimensions: (*a*) personal health, (*b*) environmental health, and (*c*) social conditions related to health. For example, to ignore the problems of poverty, substandard housing, stressful occupational environments on the one hand, and unemployment on the other is to assure an inefficient use of most of the investment of scarce health resources.

To be sure, disease and disability may be viewed as a lack of an adequate adjustment of man to factors of the environment. The significant feature is that the social, physical, and biological components of the environment function as an integrated unit—one acting upon the other—so it is difficult to compartmentalize them intelligently, although we do it constantly to the community, to man and to his mind.

However, the traditional environmental objectives of clean air and water and preservation of national parks and wildernesses have not always been the central concern of all Americans, especially those who live in urban communities. They focus instead upon more immediate economic and social interests. For many of these residents the overwhelming concerns are their residential environment, including neighborhood sanitation, housing, high crime rates, uncollected garbage and trash, lead-based paint poisoning and rat infestations, and their work environment with excessive physical and chemical stresses.

Available employment of the type for which low-income adults might qualify is generally not available in the areas in which they live because central cities have increasingly become the white-collar employment centers while the suburbs have become the job employment areas for new blue-collar workers. This is ironical in view of the fact that low-paid blue-collar workers live in the central cities while the white-collar workers are increasingly living in the suburbs. Traveling to and from work becomes increasingly difficult for both, and it has generated problems in the environmental fields of transportation, noise, air and land resources management.

Within this setting, it would be trite to rehearse the morbidity and mortality statistics which show the personal health status of urban area residents. At the first nationwide conference on the health status of the Negro, held during the centennial observance of Howard University (1967), the conferees agreed that the unskilled and semiskilled workers have the most serious health problems and receive the least adequate health care. The great majority of the workers in these two occupational groups have health problems which stem mainly from this fact. The health services received by them tend to be provided by two sources: (*a*) charity wards and clinics and (*b*) private care by overworked and harried general practitioners; and this continues to be a major concern of those policy makers who are sensitive to health needs of urban dwellers. As a result there is encouragingly widespread acceptance of the need to accelerate improvements in the environmental health services and in comprehensive health care programs.

Only five years ago the Task Force on Medicaid and Related Programs warned that among other things: (*a*) health must remain high on the scale of social, political, and economic priorities; and (*b*) health care is too often delivered at the time and place and in a way more convenient to the provider than to the consumer. The day is past, reported the task force, when doctors and hospital administrators and trustees and their associates may rely only on their judgement on how to best

distribute all the skills and resources at their disposal to what they see as the greatest advantage for the people they think they are serving.

But before that task force and even since its report to the then Secretary of Health, Education and Welfare, Elliot Richardson, there has been a broad spectrum of developments which were designed to remove deficiencies in health services and environmental management activities. It is instructive to sketch, in fairly broad strokes, some of these developments.

Titles 18 and 19 of the Social Security Act, more popularly called Medicare and Medicaid, were considered important medical care financing programs for the elderly and the poor. The Comprehensive Health Planning and Public Health Service Amendments of 1966 (*1*) called for new breeds of comprehensive health planners who were expected to prepare long-range as well as short-term new and innovative comprehensive health plans. One of the more interesting debates in Congress was how heavily the government should promote formation of Health Maintenance Organizations (HMOs) which are founded on the theory that it is better to try to keep people well than to wait for them to develop illnesses before treating them. The Heart Disease, Cancer, and Stroke Amendments of 1965 commanded a total national commitment to search for a cure for cancer. A similar mandate was called for in the National Heart, Blood Vessel, Lung and Blood Act of 1972. The introduction of Workmen's Compensation Laws, the Child Labor Laws, the establishment of a bureau of Industrial Hygiene in the Public Health Service, the Walsh-Healy Act, and the Coal Mine Health and Safety Act, culminated in the Occupational Health and Safety Act of 1970 which declared the intent of Congress "to assure so far as possible to every working man and woman in the nation safe and healthful working conditions and to preserve our human resources."

The Demonstration Cities and Metropolitan Development Act (P.L. 89-745) was designed as an improvement over earlier slum clearance and urban renewal programs. It attempted, among other goals, to encourage local coordination of federal grants. The "reward" for local consolidation of separate federal programs was the Model Cities Supplemental Grant. This grant was intended to improve the federal "delivery system" of aid to urban areas but it could not do so without matching coordination at the federal level of the program linkage it was supposed to bring about at the local level. Also, the federal air quality program was to change dramatically when the Clean Air Amendments of 1970 became law.

Finally, after more than a decade of debate and discussion, the 92nd Congress enacted a general revenue sharing proposal. The State and Local Fiscal Assistance Act of 1972 authorized $30.2 billion in permanent appropriations for general revenue sharing with state and local governments and the District of Columbia.

Obviously, in cataloging these developments, we have merely scratched the surface. Nevertheless, these selected examples indicate, in part, that there is a broad legislative (and in a broader sense, political) foundation upon which to make improvements in preventive medicine, in community health services, and in environmental conditions in urban communities. But in the context of this topic, it is essential to examine in general some of the issues which continue to require a substantial input of resources.

THE RESIDENTIAL ENVIRONMENT

No paper, no discussion or argument on health and the urban dweller can proceed very far without considering the quality of the residential environment. After more than two generations of agitation and amid the greatest physical abundance of material wealth in world history, the nation still has more than six million substandard dwelling units and almost four million more which are overcrowded (although physically sound).

The National Commission on Urban Problems (*2*) reported that "hundreds of thousands of people live in jurisdictions which do not have housing codes to establish minimum standards of health, safety and welfare in all existing housing. Where they do exist, a serious difficulty in enforcing exists. . . ."

The U.S. Council on Environmental Quality (*4*) in its report to Congress, describes the problem as follows: "Deteriorating on the inside, litter strewn on the outside, block after block of decay provides a depressing setting for life. Buildings designed in earlier days have been subdivided into numerous crowded living units, with little provision for storage areas, common spaces or refuse collection systems."

In March, 1972 Secretary George Romney of the U.S. Department of Housing and Urban Development told the Economic Club of Detroit that the programs of his department cannot solve inner city housing problems, and that the housing subsidy program has gone wrong. Other housing and urban field specialists support this admission. While public housing and its related programs for moderate income groups have been of some slight help in meeting the housing needs of the poor, urban renewal has been essentially irrelevant to the housing needs of low income groups. Data from studies of urban renewal and relocation in Springfield, Massachusetts (*7*) show that 40% of all families relocated because of urban renewal found it necessary to move again within 4 years. Moreover, more than one third of those moving a second time had to move for precisely the same reason they moved in the first place—substandard housing.

We are not suggesting that this country has made no progress in improving the quality of the residential environment. As a matter of fact, a phenomenal record has been made over the past two decades in building housing and upgrading the residential environment for the middle and affluent classes, mainly at the edge of the

inner city and in the suburbs. The efforts of private enterprise account for most of the improvement, but government policy has provided significant incentives and help through mortgage guarantees, secondary credit facilities, and federal income tax deductions for interest payments and local property taxes.

In this connection both Congress and the Administration have displayed their dissatisfaction with escalating housing subsidy costs, fraudulent housing practices, problems of locating low income housing, and the continuing abandonment of central city housing.

It is expected that the Housing and Community Development Act, signed into law in 1974, will deal more effectively with the interdependent nature of housing and the residential environment, and move closer to the goal of "a decent home and a better neighborhood for every American."

NATURAL RESOURCES MANAGEMENT

Air pollution control, which is beset with many difficulties, including confrontation and criticism, has made significant progress, but a massive effort is still needed to meet the national ambient air quality standards. From the outset the Environmental Protection Agency (EPA), charged with implementing the Clean Air Act of 1970, faced adversaries within the Government as well as groups outside the federal establishment. The Department of Commerce, the Bureau of Mines of the Department of Interior, and the now defunct Office of Science and Technology opposed any vigorous pollution control for various reasons.

Last September the major industrial leaders asked the Government to hold off imposition of any new environmental standards, warning that antipollution and safety devices use up money that business could invest to increase output and thus hold down inflation. In addition, the "energy crisis" which prompted the enactment of the Energy Supply and Environmental Coordination Act of 1974 (P.L. 93-319) required a readjustment of the strategies for achieving cleaner air. No one can say for certain what effects these readjustments will have on personal health, despite the recent predictions of the number of deaths resulting from the physiological stresses of high concentrations of pollutants in the ambient air.

It is encouraging to note that P.L. 93-319 authorized funds and directed the National Institute of Environmental Health Sciences to determine health effects of emissions of sulfur oxides into the air, resulting from any conversions to burning coal in efforts to conserve scarce fuels.

'Perhaps de Nevers (*3*) best describes the intricacies of implementing the Clean Air Act of 1970. Cataloging the political, social and economic ramifications of the Act, he said "the fact that difficulties have arisen shows only that it is hard to write a far reaching law that does not require study and amplification."

In dealing with air pollution everyone is acting on the basis of inadequate information. The relation between dose and response is simply not known for the major pollutants. As more information emerges about the true effects of air pollution, however, a more refined and cost-effective scheme for regulating pollution is likely to be developed.

Inextricably tied to the questions of air and water resources management is the issue of land use. Throughout our history our greatest resource has been our land—forests and plains, mountains and marshlands, rivers and lakes. Today throughout the nation there is a critical need for more effective land use and better control over the use of land and the living systems that depend on it.

Nevertheless, something happened to the Land Use Planning Act on its way to passage in the House of Representatives. It was defeated by a vote of 211-204. Notwithstanding the *Wall Street Journal's* editorial (June 14, 1974) which proclaimed that the House acted with considerable wisdom and that the defeat of the Act was no great loss, land use decisions are still an important determinant of environmental quality. Although planning and control of land use are largely the responsibility of local government, the impacts often reach statewide, regionwide or nationwide significance. Purely local regulations, such as zoning laws, often frustrate environmental management goals and objectives. Thus, it is hoped that the House defeat was merely a temporary setback for national land use legislation and that Congress will review the need for appropriate forward action to control land use planning and future urban and rural growth.

CHRONIC DISEASES AND OCCUPATIONAL HEALTH

In contrast to the earlier recommendation of the Task Force on Medicaid and Related Programs, the priorities in the prevention and control of chronic diseases may have shifted. In fact, there is some indication that these diseases, which are a major cause of impaired activity, are not as vigorously researched as they should be.

This view was expressed by the U.S. Senate Committee on Appropriations for the Department of Health, Education and Welfare. In its 1974 appropriations report the Committee pointed out that: "Although diseases of the heart, lung, and blood vessels are responsible for many more deaths than cancer and are a major cause of impaired activity, the budget estimate for the National Heart and Lung Institute is only $303.3 million—or half of what is proposed for cancer. Moreover, this amount represents a decrease of $20 million below the 1974 operating (obligation) level of $329,511,000 which includes the availability of $43 million in funds previously impounded. In view of the current rate of inflation, this amount will clearly not permit the Institute to carry out its new and broader mandate under the National Heart, Blood Vessel, Lung and Blood Act of 1972."

Further, the Committee disagrees with the apparent policy of gradually eroding support for biomedical research on such important and tragic health problems as kidney failure, dental caries, diabetes, infectious diseases, neurological and muscular disabilities, mental retardation and a host of other diseases and disabilities.

Because traditional problems are often a heritage for government, as well as some segments of the private sector, occupational health and safety has all too often been overlooked by those responsible for the total health service system. Yet on any working day in any community the productivity of its labor forces is directly affected by the conditions of the workers' environment.

As chairman of the American Public Health Association's Task Force on Occupational Health and Safety, I am keenly aware of some progress that has been made in eliminating visible occupational diseases in the work environment by appropriate use of the tools and techniques of medicine, engineering, toxicology, and chemistry.

I am also aware of the continuing gaps and deficiencies in our efforts to monitor the workplace and prevent unnecessary physical and chemical stresses on the worker, wherever he may perform his daily assignments.

As Kerr (*6*) states: "There is just now a realization that pollution of the workplace is partially responsible for the degradation and exploitation of workers. In addition, many of the same pollutants have a deleterious impact on the surrounding community. . . ." Over the past year we have read and heard of the mounting issues of occupational health and safety.

The debate and discussions of these issues have at times been furious and have left the blue-collar worker, organized labor, and many others wondering whether health and safety in the work place will ultimately prevail over purely political goals and objectives.

Here are a few examples: (*a*) The *Washington Post,* July 16, 1974: "The Labor Department delayed or toned down its implementation of worker health and safety standards in 1972 as 'a sales point' to attract corporate donations. . . ." (*b*) The *Washington Post,* July 23, 1974: "Recent published interviews with two Labor Department officials indicated that Labor's Occupational Safety and Health Administration found HEW reports politically embarrassing since the HEW recommendations are often more stringent than the standards the Labor Department wants to adopt." (*c*) The *Washington Post,* August 22, 1974: "Dr. Irving J. Selikoff, a cancer researcher, cited (before a Senate Commerce Subcommittee) three recent studies which suggest that vinyl chloride used in thousands of plastic products, may be not only a cancer causing-agent but mutagenic—creating mutations." (*d*) The *Washington Post,* September 21, 1974: "Citing a potential cancer threat to an estimated 1.5 million U.S. workers from inorganic aresenic, union officials yesterday formally petitioned the federal Occupational Health and Safety Administration to implement emergency measures virtually eliminating worker exposure to the chemical."

It would be productive if we could report here that the above charges have had more than desultory interest of the entire health profession. But unfortunately such a report cannot be made.

In fact, it is difficult to understand why we have been so slow in developing a personal health and environmental control service system in which prevention, diagnosis, treatment, and rehabilitation of occupational illnesses and injuries are thoroughly coordinated and effectively integrated with a comprehensive family health care program.

It is encouraging to note that labor is no longer waiting for public health officials to develop an epidemiological basis for the control of occupational hazards. An increasing number of labor unions are contracting with employers and medical centers to develop surveillance systems to detect diseases attributed to the occupational environment.

The question, then, is no longer whether government should have the total responsibility of developing health service systems. The real question is that of determining the most effective way of combining and applying resources at the various levels of government and in the private sector to ensure prevention and control of occupationally-related diseases and injuries. From an ecological perspective, we would urge that some version of national health insurance, if and when it finally emerges, provides an appropriate catalyst for moving toward this desirable goal.

CONCLUSIONS

The kind of "broad brush" inventory attempted in the preceding discussion is not a new exercise for professional health workers in urban America. In literally hundreds of professional meetings each year we have reexamined our responsibilities, analyzed our progress and plotted our future course. Underlying the deliberations at these annual assemblies has been a strong sense of our stewardship for the health protection of our nation, a realization that as public health workers, environmentalists, behavioral scientists, and politicians in the broadest sense we have both the privilege and the duty of serving all people.

In the end, however, our most difficult task may not be to devise better structures or critical mechanisms for health service programs, but to involve our community, and our state, federal, and local leaders in developing a clearer understanding of the three dimensions of health, in setting goals and priorities and in designing a strategy for achieving these goals.

To do so, we still need, as Senator Edmund Muskie has stated, "an informed electorate, a concerned electorate, and an active electorate (who) are able to see the relationships among health and urban development, housing, land use, education, manpower training, equal opportunity, capital investment, taxation, and the

fulfillment of the individual and society." As in the case of the good physician who treats the whole man, we need more than ever to treat the whole society—its *environment,* its *health,* and its *community institutions,* be they political, social, or economic.

ACKNOWLEDGMENT

Presented at the Annual Meeting of the American Public Health Association, New Orleans, Louisiana, October 24, 1974.

REFERENCES

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1976 Star Spangled Meeting Presents LIFE

1976 Star Spangled Meeting presents for its main entertainment at the Wednesday, August 11, 1976, Banquet and Dinner Dance a dynamo of entertainment, LIFE.

LIFE, a relatively young show group, has rapidly gained a reputation as the top new musical sensation from coast to coast. This group of four women and seven men—a combination of the most accomplished young entertainers from both this country and Canada—has earned overwhelming approval from audience after audience.

Vibrantly costumed and dynamically choreographed, their shows cover every musical style imaginable, including contemporary tunes, country, old standards, 50s rock'n roll, spirituals, ballads, Broadway favorites and patriotic songs. The group skillfully provides their own musical accompaniment to create a total continuity for every performance.

LIFE's credits include successful engagements at the Sahara (main room) in Las Vegas, the Nugget (main room) in Reno, the Fontainebleau in Miami, the Conrad Hilton in Chicago, the Waldorf Astoria in New York, the Four Seasons in Toronto, Canada and the Princess Hotel in Bermuda. LIFE has performed across the country for convention shows, industrial trade shows, supper clubs, fairs, colleges and resorts.

Among the entertainers who have been proud to appear with LIFE are such familiar names as Tennessee Ernie Ford, Tony Bennett, Bob Hope, Lena Horne, Jim Nabors, Bobby Vinton, Anita Bryant, Loretta Lynn, and the Smothers Brothers.

More than performing in the conventional manner, this spirited group of musicians animates each musical arrangement of every production in their own style. In every exhilarating performance, LIFE infuses their audiences with the excitement that only total professionalism can create, and the audiences invariably respond with unprecedented standing ovations.

Dynamic in music, movement and mood, LIFE is the first and last word in total entertainment.

To help us celebrate our bicentennial, be sure you and your wife plan to attend the 1976 Star Spangled Meeting Banquet and Dinner Dance. Don't miss LIFE.

News and Events

DFISA Presents Awards

Gordon A. Houran, immediate past president of Dairy and Food Industries Supply Association, was selected as the 1976 recipient of the DFISA Honorary Plaque, highest award for service made by the association. Houran, who is vice president-administration, DeLaval Separator Co., was recognized for his contributions over the years to DFISA, the 400-member-company association of equipment and supplier manufacturers.

Among Houran's major contributions was chairmanship of DFISA's important technical committee from 1962 to 1972, of the Exposition committee in 1972, and service on the board of directors since 1965. He is only the 20th DFISA member selected for the award. Presentation was made by William Gardner, Ladish Co., Tri-Clover div., chairman of the awards committee, at DFISA's 57th Annual Meeting on April 7, 1976.

In other ceremonies, three members were recipients of the first DFISA Achievement Awards, a new class of honors. Jack Bronson, DeLaval Separator Co., was recognized for "devoted service as a member and chairman of the marketing committee during which he directed conversion of the Dairy Processor Market Guide from a manual to a computerized compilation" and for his other contributions to the DFISA marketing programs.

Darl Evans, Babson Brothers Co., Oak Brook, Ill., was recognized for his long and dedicated membership on and chairmanship of the DFISA farm tanks task committee and his leadership in developing the important 1960 3-A sanitary standard for farm tanks at a time when the public health need for it was acute.

Walter Laun, Cherry-Burrell Corp., Cedar Rapids, Iowa, was honored for his "extraordinary" service to the 3-A sanitary standards program as co-chairman of the technical committee for more than 10 years, chairman of task committees which brought pending 3-A sanitary standards to successful conclusion and leadership in helping advance the new E-3-A standards for the egg products industry.

Food Mycology Short Course

A five day short course on food mycology will be held at the University of Minnesota, St. Paul Campus in the Food Science and Nutrition Building on September 13-17, 1976.

The purpose of the course is to inform quality assurance and other interested personnel in the food industry about the identification, isolation and significance of fungi found in food products. The course will consist of lecture and laboratory exercises concentrating on the above items. Identification of microscopic counting methods and agar plate identification techniques will be stressed in the laboratory. Specific problem foodstuffs will be analyzed for the presence of mold. The lectures and discussion periods will concentrate on the listed topics as well as the significance of molds in food products. For further information contact The Office of Special Programs, University of Minnesota, St. Paul, MN 55108. (612-373-0725)

Fiber in Diet Report Available

The proceedings of the tenth annual symposium sponsored by the Western New York Section of the Institute of Food Technologists, the Institute of Food Science, Cornell University and the New York State Cooperative Extension are represented in Special Report Number 21, entitled "The Role of Fiber in the Diet." Requests for copies should be addressed to: D. L. Downing, Dept. of Food Science and Technology, New York State Agricultural Experiment Station, Geneva, NY 14456.

News and Events

University of Minnesota Press Publishes Consumers' Guide to Processed Foods

A book which provides authentic, factual information about food processing, additives, nutrition, and other important and often controversial aspects of today's consumer foods will be published by the University of Minnesota Press on June 1. The book is *Processed Foods and the Consumer: Additives, Labeling, Standards, and Nutrition* by Vernal S. Packard, Jr., professor and extension specialist in food science and nutrition at the University of Minnesota (clothbound, $12.00, paperbound, $5.95).

Professor Packard offers an overview of the food industry, focusing on many of the major issues now being raised about food safety, ecology and the environment, and mankind's ability to feed itself. The book deals with regulations governing food and food processors; relates supermarket foods to the latest nutritional recommendations of the Food and Nutrition Board, National Academy of Sciences/National Research Council; provides a basis for understanding food labels and the use of label information in making purchases of food and vitamin supplements; and brings together the health-related issues of food additives and nutrition.

The book will be equally useful as a guide for consumers, as a reference manual for those in food industries and regulatory and health agencies, and as a text for course use in classes in food science or technology, nutrition, dietetics, institutional food management, public health, and related courses.

As the author points out and the book's content demonstrates, a maze of food ingredients, regulations, and standards confront government agencies and citizen in the development of a national food policy, a policy that must consider the needs of human health and nutrition not only in this country but abroad. The book presents the details involved in this comprehensive problem and discusses them in a logical sequence which reflects today's scientific consensus.

There are separate chapters on food definitions and standards, food names, additives, flavor, color, preservatives, emulsifiers and stabilizers, toxic metals in food, antinutritional factors in "natural" foods, natural/organic foods, nutrients, nutrient sources, and label information, food and food supplement labels, and nutritional quality guidelines.

Questions concerning the use and safety of food additives are important in the discussion. The author says that much of the difficulty people encounter in reaching a balanced view of the benefits and hazards of food additives stems from a failure to understand the meaning of terms. He explains that in food technology additives are called either "intentional" or "incidental," terms which the layman might understand better if the words "ingredients" and "contaminants" were substituted, respectively. Professor Packard makes it clear that not all additives are potentially harmful and also emphasizes that "harmlessness" is an impossible condition in connection with food consumption.

In the chapter on food color the author traces the controversy over Red No. 2, the dye which the Federal Food and Drug Administration banned in January because of concern that the dye might be a weak cancer-causing agent. He explains that further hearings on the matter are scheduled and the status of the dye may yet be changed again.

In an appendix chapter entitled "Cyclamates: A Chronicle of Confusion" the author traces the sequence of events which led to the banning of cyclamates by the FDA. Other appendixes cover standards of identity for foods, categories of additive functions as used in the regulation of food ingredients, 101 varieties of food additives, food categories used in the regulation of food ingredients, a listing of some natural and artificial flavoring compounds, some commonly used chemical preservatives, a glossary of terms, and a bibliography.

Vernal S. Packard, Jr. is a regular contributor of research articles to the *Journal of Milk and Food Technology* and has also contributed to *Food Scope* and *Dairy Plant Fieldman.*

Boosinger Appointed Director of Dairy Division

Jay B. Boosinger, 36, was appointed director of the Dairy Division, Florida Department of Agriculture and Consumer Services, effective February 12, 1976.

Boosinger, a graduate of Manatee County High School in Bradenton and the University of Florida with a bachelor's degree in dairy manufacturing, has been with the Department of Agriculture since 1964. He was a dairy specialist from 1964 to 1967. He was named sanitarian of the year for 1972-73 by the Florida Association of Milk, Food and Environmental Sanitarians, and presently is president-elect of the organization.

Boosinger also is vice chairman of the National Conference on Interstate Milk Shippers and serves as chairman of one of three NCIMS governing councils. He also has been president of the Southern States Dairy Division of the National Association of State Departments of Agriculture and past chairman of the Florida Milk Quality Council. Boosinger has been a member of IAMFES for eleven years.

News and Events

Food Scientist Kenneth Weckel Retires

A hard working University of Wisconsin-Madison scientist who has worked to improve the quality of American foods for nearly half a century is retiring this June. He is Kenneth G. Weckel.

A symposium of "Food Regulations: Present and Future" held at UW-Madison on May 6 honored Weckel's contributions to U.S. food technology. At a recognition dinner held that evening at the Wisconsin Center, Glenn S. Pound, dean and director of the College of Agriculture and Life Sciences, Harold E. Calbert, chairman of the Department of Food Science, and other colleagues recalled Weckel's contributions. They presented the scientist with leather bound volumes containing all of his professional publications.

Weckel was born in Canton, Ohio in 1905. He studied at the University of Wisconsin-Madison where he earned his Ph.D. in Dairy Science in 1935. His richly productive career has shown no sign of slowing down ever since he began helping to eliminate rickets as a public health hazard in the 1930's.

"I was a student under Professor Harry Steenbock then," he recalls. "Of course Steenbock discovered that when sterol in milk is exposed to ultraviolet light, it converts to vitamin D. My job was to adapt the Steenbock process of vitamin D fortification for use by the milk processing industry." In 1938 Weckel received the Borden Award from the American Dairy Science Association for his efforts.

His colleagues also credit Weckel as the originator of a broad food technology curriculum at UW-Madison.

The food scientist has attacked nearly every conceivable food processing or quality problem from candied cranberries to smoked chub. He is an authority in the fields of dairy products, fruit and vegetable processing, confectionary technology, nutrition-vitamin technology and regulatory practices and procedures. He is especially respected for his knowledge and contributions in the area of food sanitation.

Weckel belongs to a score of technical and professional organizations and has received citations from such diverse groups as the Wisconsin Society of Professional Engineers, the National Confectioners Association and the International Association of Milk, Food and Environmental Sanitarians, Incorporated.

Japan Science and Technology Award

Katsuto Okada, a senior managing director of Morinaga Milk Industry Company and president of the Morinaga subsidiary M. O. Engineering Company, was recently awarded the Science Technology Medal of the Science and Technology Agency of the Japanese government. The award was in recognition of Okada's outstanding contributions to the development of a spray drying plant for processing milk into powder form. The development of the spray drying plant was completed in 1966 by Morinaga. It has been patented in 17 countries and is in operation in many locations, including Japan, Norway, New Zealand and West Germany. The plant, basically a vertical type concurrent spray dryer, overcomes many troubes associated with the conventional spray dryers and also provides the product cooling function within the plant.

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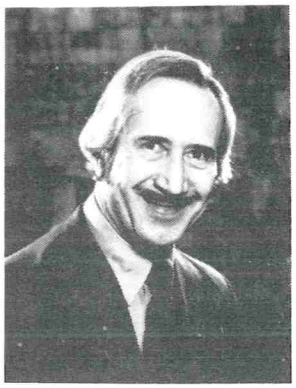
National Association of Dairy Fieldmen to Affiliate With IAMFES

Officers of the National Association of Dairy Fieldmen are (clockwise from top left) William E. Stallard, First Vice President; Clifford Mack, Second Vice President; Charles Montgomery and Sidney H. Beale, President. Secretary-Treasurer Art Johnson is not pictured.

Recognizing the need for maintaining a professional organization, the board of directors and advisors of the National Association of Dairy Fieldmen have recommended that the NADF become an affiliate association of the International Association of Milk, Food and Environmental Sanitarians, Inc. This will aid in publishing articles of interest to the professional fieldman. Reports of the association's activities will be carried in the Journal of Milk and Food Technology.

Plans have been made to devote one session of the program at the 1976 IAMFES Annual Meeting to dairy fieldmen. The special session will be held on August 11, 1976 at the Arlington Park Hilton, Arlington Heights, Illinois. Registration blanks are available in this issue of the Journal. On August 12 a special program is being held by the National Mastitis Council that should be of value to all dairy fieldmen.

How Long Are We Going to Live?



Robert M. Brown

On a sunny day in 1776 you could stand on a colonial hill, fill your lungs with the purest of air and behold the sharply etched landscape for miles in all directions. From the surface of a country lake or pool you could see the shimmering movements of game fish in the clear water below. Everywhere in young America the outside environment was pristine . . . while the *inside* environment was lethal. Everyone and everything was menaced by the presence of unseen and unsuspected micro-organisms. Mothers and babies died in childbirth. Epidemics raged through homes, barracks and ships quarters while infections in wounds were commonly fatal.

A fortunate few would attain great old age but the life expectancy of an American boy or girl born in 1776 was only 35 years.

With the passing of two centuries, the quality of our external and internal environments has had some reversals. Our air and water have become contaminated. Much of our land has been despoiled, and treasured creatures in the chain of nature have been brought to extinction.

At the same time, the humanly controlled *inside environment* has dramatically improved with advancements in medicine, public health services, sanitation, water supply, waste disposal and other health-related technologies. All, of course, have flourished in a rain of brilliant inventions and discoveries during the same two hundred years.

Human control of the inside environment has helped to build a protective barrier against disease and infections in homes, schools, hospitals, stores, restaurants, workshops and meeting places so that the span of life has doubled. The life expectancy of an American boy or girl born in 1976 is now more than seventy years.

This one hundred percent increase in life expectancy (assisted and shared by people of other advanced nations) must stand as one of the great milestones in human history.

When we think of the saving of human lives we are likely to evoke images of masked surgeons during tense hours of brain surgery, of jet planes rushing cargoes of serum to disaster victims, or the pattern of heartbeats moving across monitor screens at an intensive care station.

The fact is that environmental controls may be adding more years to your life than medical science. Your chances of living to age seventy are vigilantly guarded by sanitary engineers and water engineers in your community as well as dedicated members of your public health department. Your long, healthy future is a matter of real concern to the manufacturer of equipment that protects the food you eat in a public restaurant. This is equally true of the designer of the filter that guards the water in your swimming pool or the maker of plastic pipes that bring drinking water to your home. And the EPA's successful efforts in cleaning our air and water may prove to be your personal salvation.

Some scientists believe that we should someday be able to live active and productive lives up to age 100. If we do expand our lives in this direction it will probably come from further advances in environmental quality made possible by all sorts of people in public, private and professional services.

President

NSF

NSF — the National Sanitation Foundation — is an independent non profit, non governmental organization dedicated to environmental quality. It fulfills its mission through research, education and the development of standards for health related products and services.

Headquarters and laboratories are in the NSF Building, Ann Arbor, Michigan.

Affiliate Annual Meetings

California—October 18-20, 1976, Airport Marina Hotel, Burlingame (San Francisco Bay Area).
Connecticut—January 1976.
Florida—March 16-18, 1976 Langford Hotel, Winter-Park.
Illinois—May 24, 1976, Blue Moon Restaurant, Elgin.
Indiana—October 7-9, 1975, Holiday Inn, Merrillville.
Iowa—October 27, 1976, Scheman Continuing Education Bldg., Ames.
Kansas—October 1-3, 1975, Holiday Inn, Manhattan.
Kentucky—February 22-23, 1977, Stouffer's Inn, Louisville.
Michigan—March 1976.
Mississippi—April 15-16, 1976, Holiday Inn North, Jackson.
Missouri—April 5-7, 1976, Ramada Inn, Columbia.
New York—September 17-19, 1975, Granit Hotel, Kerhonkson.
Ontario—Eastern, November 1976, Kemptville.
Oregon—November 17, 1975, Oregon Department of Agriculture & Kings Table of International Restaurant, Salem.
South Dakota—May 11-14, 1976, Holiday Inn, Aberdeen.
Virginia—March 8-10, 1976, Donaldson, Brown Center, Blacksburg.
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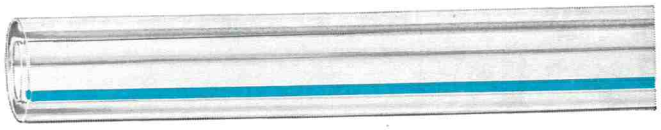
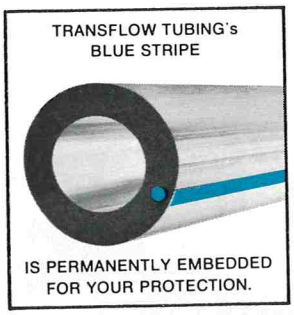
Always insist on the Blue Stripe®...



It tells you it's genuine Norton Transflow® M-34R milk tubing

It tells you it meets the tough FDA and 3-A Standards

Putting the Blue Stripe on Transflow milk tubing is one of the most important things we do, because the embedded Blue Stripe — Norton's Registered Trade Mark — is your assurance that you're getting genuine Transflow M-34R tubing.



Transflow tubing, you see, is "something special." It's the first tubing ever developed especially to handle raw milk. It is made under the most advanced quality assurance procedures using only the purest raw materials. And, of course, it meets the toughest FDA and 3-A* requirements.

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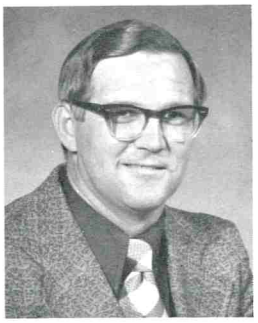
Dairy authorities speak out on better cow milking



Dr. Robert D. Appleman
Professor of Animal Science
University of Minnesota

Automatic take-off milking units: They can save and protect.

There are two primary reasons why an investment in more mechanized milking is being considered by many dairymen. One is to reduce labor. The other is to improve udder health and maintain production of high quality milk.

LABOR TAKES A BIG BITE

Labor accounts for 15 to 30 percent of all costs in a dairy operation. About 55 percent of this labor is expended in the milking operation. In general, the total labor cost to produce 100 pounds of milk in a herd averaging 12,000 pounds per cow annually when labor* is valued at $3.00 per hour approaches $2.50 per cwt. in 30-cow herds; $2.10 in 50-cow herds; $1.68 in 100-cow herds; $1.13 in 250-cow herds; and $.91 in 500-cow herds**.

With an investment to modernize milking parlors, including unit take-off, it is not unusual to substantially lower the labor costs of producing milk.

Many of the milking chores are repetitious and result in drudgery. According to our studies, 5 to 10 percent of the milker's chore time is spent removing the milking unit. On top of that, the typical milker spends from 12 to 30 percent of his time machine-stripping cows.

THE OPERATOR IS A BUSY MAN

Proper stimulation of cows in a milking parlor is important to good milk letdown. Recent New Zealand work shows there is a loss of up to 1,000 pounds of milk per cow yearly when cows are not properly stimulated. In many barns the milker cannot effectively handle as many milking units as today's economy demands. Frequently, washing and stimulation time is limited to less than 15 seconds per cow because the milker is too "busy" with machine stripping or handling other units. The result is slow milking combined with considerable overmilking. Automatic unit take-off should improve this situation. Addition of automated prep stalls will help even more, provided they function properly.

SOME RESEARCH RESULTS

Research studies comparing automatic take-off and conventional milking units involving 550 cows in a Louisiana herd resulted in these conclusions:

1. Automatic unit take-off significantly reduced the number of quarters infected with mastitis.
2. Automatic unit take-off reduced udder irritation as evidenced by lower CMT scores.
3. The men operating the automatic take-off units reduced their walking distance in the parlor by more than 25%.

Dr. Nelson Philpot, leader of this study, says one should not expect miracles. Automatic take-off units do not make a poor operator better. They do, however, allow a conscientious operator to do an even better job on more cows.

According to dairymen using this equipment, proper maintenance and proper operation of equipment is even more important with non-automated systems. The ability, cooperative attitude and location of your local serviceman should become a primary factor in deciding whether to install this more sophisticated and expensive equipment.

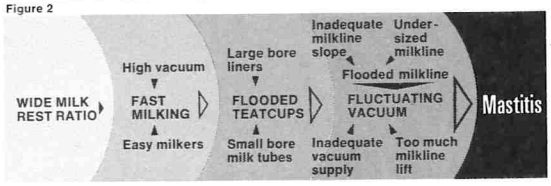
MASTITIS PREVENTION NOT SIMPLE

Dairymen should not necessarily expect a reduction in the number of cows requiring treatment for clinical mastitis (gargot). In a marginal system, more cases may result because the significance of a single variable is not the same in every milking system or in every situation.

Frank Smith, California milking system specialist, illustrates this point well. He indicates that too many researchers and educators have attempted to over-simplify the cause of mastitis. In turn, they have over-simplified its prevention. *The concept of a direct, independent relationship shown in Figure 1 is incorrect.* Figure 2 arranges these same variables in a manner which is sequential, additive, and interdependent.

Figure 1

Figure 2



As mentioned earlier, installing automatic unit take-off may allow one to milk cows faster and reduce overmilking. However, if such a change resulted in flooded milk lines and fluctuating vacuum, the incidence of mastitis might increase rather than decrease. Providing all other deficiencies in the system were corrected, automatic take-off would prove highly beneficial.

AUTOMATIC TAKE-OFFS A COMMON SIGHT?

Where cost of this mechanization is not excessive and such installations prove to be reasonably trouble-free over time, I'm sure that automatic take-off units will become an increasingly more common sight on dairy farms.

*For our purposes, the labor figures include all dairy chore labor, feeding labor, and the raising of offspring. Field labor isn't included.

**In 250-cow and 500-cow herds, we assume the existence of a parlor and a free-stall barn with mechanized feeding and waste handling.

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