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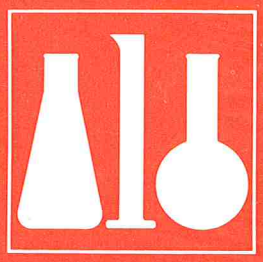

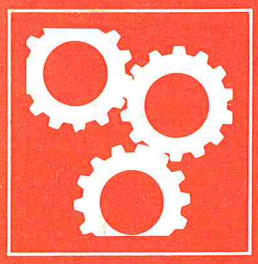

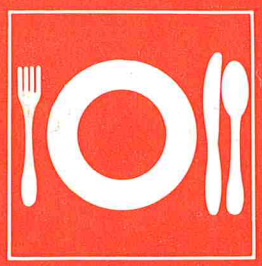

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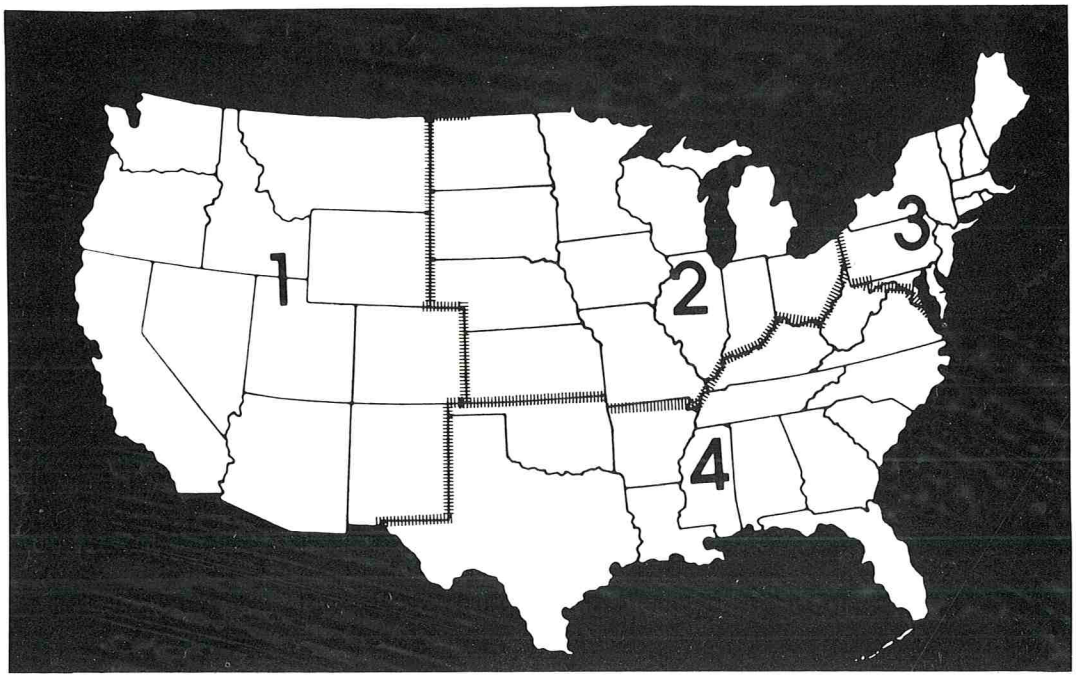

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Description and Microbiology of Home-Canned Tomatoes and Tomato Juice

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ABSTRACT

Home canners completed questionnaires and submitted 378 jars of tomatoes and 382 jars of tomato juice for analysis. Nearly 50% of the containers were non-canning jars, and 25 jars had held food items such as instant tea or coffee or jelly. A few closures were reused from grocery stock; the remainder were the two-part rim and lid or zinc cap and ring. The headspace in 14% of the jars was below 16/32 inch and in 17% between 31/32 and 64/32 inch. Serum layers in 70% of the jars of tomato juice ranged from 20 to 64% of the volume. Eleven percent of the samples were processed with the method of hot fill and seal only, 66% were processed in boiling water, and 23% in the pressure canner. Vacuums of 13 to 15 inches of mercury were most frequently recorded in jars processed with any of the three methods. The pH ranged from 3.5 to 4.7. The average titratable acidity as citric acid, of tomatoes was 0.444% and of tomato juice 0.471%. Microorganisms were recovered from 33% of the jars of tomatoes and from 15% of the tomato juice; however, the percentage of recovery decreased during the period of analysis, suggesting autosterilization. The microorganisms recovered included three fermentative yeasts, one *Rhodotorula,* three filamentous fungi, 15 *Bacillus licheniformis,* and the remainder were *Klebsiella pneumoniae.* It is concluded that directions given to home canners are adequate for home preservation of tomatoes and tomato juice, and that most canners engage in good canning practices.

The fact that thousands of jars of tomatoes and tomato juice are canned and consumed annually without incident suggests effectiveness of the canning process, but descriptions of the microbiology of the canned products are meager. Recipes for canning of these items are readily available, and they appear to be quite uniform. Fields et al. (*7*) studied home-canned tomatoes and green beans in Missouri in 1975. Among their observations were the prominent use of nonstandard canning jars and the recovery of microorganisms, chiefly *Bacillus* spp., from 40% or 280 jars of tomatoes. Tischer and Esselen (*16*) received 17 jars of tomatoes and two of tomato juice during their study of home canning in 1945. Thirteen jars of tomatoes either had no vacuum or had a positive pressure and 10 jars were characterized by a malodorous condition. Both jars of tomato juice were spoiled.

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Home-canned tomatoes and tomato juice packed during 1976 in Tennessee were studied to obtain descriptive processing and microbiological information. Questionnaires accompanying the samples provided information on the source, degree of ripeness, and condition of the tomatoes, lapse of time between harvesting and canning, and the method of processing. Laboratory observations were made on the types of jars and closures, amount of serum in the jars of tomato juice, and headspace of the containers, vacuum, pH, titratable acidity, and presence of viable microorganisms which were capable of growth in an acidified substrate.

MATERIALS AND METHODS

Samples

Samples of tomatoes and tomato juice were gathered through cooperation of the Health and Nutrition Department of the University of Tennessee Agricultural Extension Service. Home canners in the various counties of Tennessee were asked to donate one jar of tomatoes or tomato juice for analysis which was representative of the normal processing procedure used. Each home canner was also asked to complete a questionnaire to provide information regarding the source of the tomatoes, estimated maturity at the time of use, lapse in time between harvesting and canning, processing procedure used, and processing time.

Samples were transported to Knoxville and stored in the laboratory at 22 C. Samples were analyzed in the order of receipt; those originating in the western portion of the state were analyzed first, and in the eastern portion last. The period of analysis extended from early October, 1976, through, March, 1977.

At the time of analysis the rings of two-part closures were removed. The headspace was measured with a canner's gauge. The percent serum in tomato juice was determined by measuring the height of the serum layer and the total height of the contents of the jar. Brands and types of containers and closures were recorded.

The jars were then inverted several times to mix the contents and immersed in inverted position in an iodine disinfectant for at least 1 min. The partial vacuum was measured with an American Can Co. vacuum gauge. The lids or other closures were then removed and portion of the contents were removed for microbiological analysis. The pH and titratable acidity were determined on the remainder of the contents.

pH and titratable acidity

The pH was measured with a Corning Research Model 12 pH meter. Portions or entire contents of jars of tomatoes were homogenized with a

blender. Duplicate 10-g portions of blended tomatoes or tomato juice were diluted with 40 ml of distilled water and titrated to pH 8.1 with standardized NaOH solution. The acidity was calculated as percent citric acid.

Microbiology

Ten to 12 g of mixtures of tomatoes and fluid or tomato juice were transferred with a sterilized spoon to tubes containing 1 ml each of 10% polypeptone and 10% dextrose which had been sterilized at 121 C for 15 min. One loopful of material from tubes with outgrowth after 2 to 3 days of incubation at 32 C was streaked on nutrient-glucose agar. Colonies from these outgrowths were selected to nutrient and nutrient-glucose agar slants. The bacteria were identified according to *Bergey's Manual* (*3*) and Edwards and Ewing (*5*).

One hundred sequential samples of tomatoes and tomato juice were cultured in Lactobacilli MRS broth containing 0.02% sodium azide for detection of lactic acid bacteria. The tubes were incubated 2 to 3 days at 32 C.

Studies of Klebsiella

Portions of pooled tomato juice were adjusted to pH 4.0 through 4.6 at 0.1-pH increments, tubed in 6-ml quantities and sterilized at 121 C for 15 min. The tubes were inoculated with 0.1 ml of 24-h-old broth cultures of 10 randomly selected strains of *Klebsiella pneumoniae* which were obtained from tomatoes and tomato juice. Tubes of juice similarly prepared were inoculated with serial dilutions of 24-h-old broth cultures which were also serially plated to determine the bacterial populations. The contents of all tubes were mixed after inoculation with a vortex agitator and incubated at 37 C for 48 h.

Strains of *K. pneumoniae* were grown in 2 ml of nutrient broth for 20 h at 37 C. The tubes were removed from the incubator to a water bath adjusted to 60 C and, after an allowance of 1 min for come-up temperature, tubes were removed at intervals through 30 min and cooled promptly in water. One-half ml of the contents was transferred to a nutrient or nutrient-lactose broth. These tubes were incubated 2 days at 37 C.

RESULTS

Data in this paper are taken from observations and studies of 378 jars of tomatoes and 382 jars of tomato juice. Five jars of tomatoes and 5 jars of juice were spoiled on receipt, and these are considered separately.

Tomatoes

Information obtained from the questionnaires revealed that 97% of the tomatoes were grown by the home canner (Table 1). The home canners estimated that 93% of the tomatoes were fully ripe at the time of the use, 3% were somewhat green and 4% were overripe. Bruised or bruised and cracked tomatoes were used by 18% of the home canners. Eighty percent of the tomatoes were canned either on the day of harvest or on the following day, with several canners reporting a lapse of as many as 5 days between harvesting and canning.

TABLE 1. *Reported quality of tomatoes used for home canning of tomatoes and tomato juice (from 726 questionnaires).*

Grown by the home canner	97%
Processed during:	
Day of harvest	43%
Following day	37%
Two to five days after harvest	20%
Estimated ripeness:	
Green-ripe	3%
Fully-ripe	93%
Overripe	4%
Tomatoes used were:	
Sound	82%
Some bruised	14%
Bruised and cracked	4%

Jars and closures

Standard canning jars were used by 52% of the home canners which agrees with the 54% by Fields et al. (*7*). Twenty-five nonstandard jars were identified as having held dried coffee or tea, salted nuts, olives, or jelly. The partial vacuum in the 25 jars was similar to that recorded for standard canning jars. The remainder of the jars were clear glass, straight sided pint and quart jars. Although the thread size of these jars may differ slightly from that of the standard canning jar, the home makers were able to acquire tight seals and high vacuums. The home canners reported failure to seal in a total of 47 jars among the lots from which the samples were drawn. The failures occurred among lots which were represented by both standard and nonstandard jars. Four of the 10 spoiled samples were canned in nonstandard jars.

One jar was closed with a zinc cap and rubber ring. Thirteen closures were the embossed caps of salad dressing and similar jars. The partial vacuum of 10 of these jars was between 13 and 19 inches of mercury. All other closures were the two-part rim and single-use lid.

Vacuum and methods of processing

Nearly 95% of the jars had a vacuum of 10 or more inches of mercury (Table 2). The percentage of distribution of the vacuum values was approximately equal between each of the three methods of heat processing. Vacuum below 7 inches of mercury was measured in 2.5% of the jars, distributed among each of the three methods of processing. Ten percent of the jars were processed by the method of hot fill and sealing without further heating, 66% were heated after filling and sealing in boiling water for 5 to 30 min, and 24% were processed under pressure of 5 to 15 lb. for 5 to 15 min (Table 2).

Table 2. *Partial vacuum ranges achieved with each method of heat processing of home-canned tomatoes and tomato juice.*

Inches of vacuum	Number	Method of heat processing		
		Hot fill and seal	Boiling water	Pressure
0	2	0	2	0
1 - 3	6	2	3	1
4 - 6	9	0	8	1
7 - 9	20	3	13	4
10 - 12	89	9	66	14
13 - 15	203	29	126	48
16 - 18	165	18	116	31
19 - 21	123	7	80	36
22 - 24	69	0	44	25
25 - 26	7	0	2	5
Number	693	68	460	165

Serum

The serum depth in the jars of tomato juice ranged from 0 to 62%. The serum levels in 70% of the jars was in excess of 20%, suggesting the probable preparation of juice by the cold-break process during which pectic enzymes are active (*4, 15*).

Headspace

The headspace of 14% of the jars ranged from 3 to 15/32 inch, of 69% between 16 and 30/32 inch, and of 17% between 31 and 64/32 inch. A headspace of 1/2 inch

is recommended for the home canning of tomatoes and tomato juice. No statistical correlation was found between low headspace and low vacuum values. Tomatoes and juice of samples with large headspace and low vacuum values exhibited darkening at the surface as storage progressed. Microorganisms were obtained from 23% of the jars with 30/32 inch or less headspace, and from 40% of the jars with more than 30/32 inch headspace.

pH and titratable acidity

The pH and titratable acidities are recorded in Table 3. A few home canners indicated that citric acid was added. Addition of acid may account for the low pH and high titratable acidity of some samples. Both pH and titratable acidity appear to reflect the normal range for fresh tomatoes (*13*). The questionnaire did not include a question regarding the cultivar of tomatoes used by the home canners. At least five of the cultivars which produce fruit with high pH values (*13*), Beefmaster hybrid, Big Early Hybrid, Big Girl, Garden State, and Burpee VF Hybrid, are available to home gardeners of the state either as plants obtainable on local markets or as seeds. Sapers et al. (*13*) estimated that the probability of pH at or above 4.8 for these varieties is in the range of 0.000 to 0.004, and at pH 4.6 or above is in the range of 0.053 to 0.242.

TABLE 3. *pH and titratable acidity of 366 containers home-canned tomatoes and 357 containers home-canned tomato juice.*

pH	Product	Number	Titratable acidity, percent	
			Average	Range
3.5	Tomatoes	1	1.08	
3.7	Juice	1	2.20	
4.0	Tomatoes	6	0.531	0.455 - 0.702
	Juice	22	0.534	0.387- 0.765
4.1	Tomatoes	30	0.477	0.314 - 0.642
	Juice	57	0.454	0.290 - 0.754
4.2	Tomatoes	81	0.454	0.335 - 0.598
	Juice	92	0.509	0.322 - 0.740
4.3	Tomatoes	81	0.442	0.380 - 0.710
	Juice	90	0.448	0.237 - 0.660
4.4	Tomatoes	97	0.425	0.251 - 0.710
	Juice	64	0.444	0.237 - 0.660
4.5	Tomatoes	50	0.399	0.285 - 0.609
	Juice	30	0.481	0.309 - 0.805
4.6	Tomatoes	9	0.395	0.275 - 0.578
	Juice	1	0.424	
4.7	Tomatoes	7	0.429	0.270 - 0.535

None of the samples of tomatoes or juice included in this study had a pH 4.8 or above. Fields et al. (*7*), Powers (*12*), and Sapers et al. (*13*) have reviewed and discussed the relationship of botulism associated with acid canned foods. Results represented later suggest that pH of tomatoes may be less a factor in the growth of *Clostridium botulinum* in tomatoes and juice than are other factors in the home canning process.

If the titratable acidities associated with pH values below 4.0 are omitted, the average titratable acidity of the tomatoes was 0.444% acid, and of the juice, 0.471% acid. The higher value of titrable acidity of the juice, coupled with the high percent serum in many samples, suggests that either the cold-break process was used or that tomatoes used for preparation of juice were insufficiently or slowly heated. When the cold-break process is used, the pH is lower and the titratable acidity is higher than when the hot-break process is used (*15*). The settling of tomato particles in the container, however, may enable a rise in pH in the upper portion of the container (*14*).

Spoiled samples

Five jars of tomatoes and five of tomato juice were spoiled on receipt. No microorganisms were recovered from three jars. *Bacillus subtilis, Klebsiella pneumoniae,* and *Aureobasidium* were recovered in single instances. *Bacillus licheniformis* was obtained from four jars.

Microbiology

Microorganisms were obtained from 123 (33%) of the tomatoes and from 57 (15%) of the tomato juice. They were recovered in the range of 18% to 33% from jars with all levels of vacuum, with the lowest percent recovery from containers with vacuum values of 13 to 15 inches of mercury.

The highest percentage of recovery was obtained from samples canned in West Tennessee. The lowest percentage recovery was obtained from samples canned in East Tennessee which were analyzed 20 or more weeks after canning. The results suggest the occurrence of autosterilization which has been observed to occur in sugar (*8*) and canned vegetables (*11*).

No lactic acid bacteria were detected. A gassy, malodorous fermentation was noted in 158 tubes of the primary culture medium. Sixty-eight of these were subcultured and subsequently the bacteria were identified as being *K. pneumoniae.* In addition, three fermentative yeasts, one *Rhodotorula*, two *Pencillium* sp., and 15 *B. licheniformis* were also obtained. Fields et al. obtained chiefly *Bacillus* spp. from tomatoes during their study; however, they cultured small volumes of fluid on neutral agar. This study involved the addition of large quantities of tomatoes and serum or juice to a medium to detect those microorganisms which might grow under acidic conditions. Pelliculate outgrowths of bacilli were frequently obtained when tomatoes or juice were cultured in medium which was alkalinized to bring the pH to 6.5 after the inocula of tomatoes or juice were added.

Studies with K. pneumoniae

Five of 10 strains of *K. pneumoniae* introduced as broth cultured inocula grew in tomato juice at pH 4.1, and all strains grew in tomato juice adjusted to pH 4.2 or higher. None grew in juice adjusted to pH 4.0. In all instances of growth, the pH was lowered slightly or by no more than 0.2 unit.

Growth occurred in tubes of juice of pH 4.2 which were inoculated with 37 to 137 cells as determined by the plate count. Three of 10 strains grew when tomato juice was inoculated with the next dilution of inocula estimated to contain 9 to 14 cells, and no growth occurred in tubes with estimated inocula of 4 to 13 cells.

Four of 68 strains of *K. pneumoniae* survived heating to 60 C for 30 min, and 23 survived heating to 20 but not

30 min, as determined by outgrowths of transfers of heated cells to nutrient-lactose broth.

DISCUSSION

Although many jars of home-canned vegetables studies by Tischer and Esselen (*16*) were spoiled, they concluded that directions for home canning were adequate for proper preservation (*6*). Results of the present study of home-canned tomatoes and tomato juice lead to the same conclusion.

Use of noncommercial canning jars is not recommended, but home canners do use them, apparently successfully. Despite the difference in thread size, home canners achieved effective seals with such jars. The reported failures to seal occurred with both commercial and noncommercial jars. Reuse of closures is a highly questionable practice, because of the possible failure of the sealing compound on repeated use. As determined by the degree of vacuum attained, any one of the three methods of heat-processing, whether by hot fill and sealing without further heating, processing in boiling water, or processing under pressure, leads to satisfactory results.

The results indicate an apparent lack of attention to published instructions on the part of some home canners. This is probably an expected observation when one considers the many individuals who are engaged in home canning. Lack of attention is indicated by reuse of closures, low vacuum in some containers, overfilling, under-filling, and recovery of heat-sensitive yeasts and molds. Simple canning directions do not convey the consequences of failure to follow recommended procedures. Constructively, those in contact with the canning public may find it advisable to emphasize the reasons for faithful adherence to recommended procedures and the consequences pursuant to the failure to follow sound practices.

K. pneumoniae is widespread in the environment and it has been isolated from many vegetables (*2*). It has been isolated consistently from fresh tomatoes in this laboratory, and this may account for its prevalence in the canned tomatoes and juice.

Viable cells in canned tomatoes and juice without apparent growth may possibly be due to the survival of extremely small numbers of cells, as suggested by the experimental work with quantitated inocula. They were not detected in this study. The cells may have suffered thermal damage which necessitated the presence of oxygen or supplementary nutrition for the initiation of growth. The need for oxygen is suspected, because in many instances of recovery during analysis and later during studies of juice at adjusted pH levels, the initial outgrowth was strongly pelliculate, with fermentation not apparent until 1 to 3 days later. Since foods containing viable, nonproliferating bacteria are known to undergo autosterilization, this phenomenon appears to have occurred in tomatoes and juice during storage.

Recovery of yeasts and filamentous fungi must be interpreted with a great degree of concern. Since they are readily destroyed by heat, a liberty with respect to maintaining adequate temperature during the canning practice appears to have occurred. Yeasts were isolated from a container with 12 inches of mercury, thus post-sterilizing contamination may not be the sole factor involved. At least two instances of botulism have occurred during which yeasts were isolated. A yeast-like organism was associated with a single instance of botulism (*1*). Meyer et al. (*9*) demonstrated growth of *C. botulinum* when it was introduced into pear juice at pH 3.66 with a lactobacillus and a yeast, but *C. botulinum* did not grow when introduced alone into pear juice at pH 6.0.

REFERENCES

1. Anonymous. 1974. Botulism - Idaho, Utah. MMWR 23 (27):241-242.
2. Browth, C., and R. J. Seidler. 1973. Potential pathogens in the environment: *Klebsiella pneumoniae*, a taxonomic and ecological enigma. Appl. Microbiol. 25:900-904.
3. Buchanan, R. E., and N. E. Gibbons. (eds.). 1974. Bergey's manual of determinative bacteriology. 8th ed. Williams and Wilkins Co., Baltimore.
4. Cruess, W. V. 1958. Commercial fruit and vegetable products. 4th ed. McGraw-Hill, New York.
5. Edwards, P. R., and W. H. Ewing. 1972. Identification of Enterobacteriaceae. 3rd ed. Burgess Publishing Co., Minneapolis, Minn.
6. Esselen, W. B., and R. G. Tischer. 1945. Home canning. II. Determinations of process times of home-canned food. Food Res. 10:215-226.
7. Fields, M. L., A. F. Zamora, and M. Bradsher. 1977. Microbiological analysis of home-canned tomatoes and green beans. J. Food Sci. 42:931-934.
8. Hall, H. H. 1939. Survival of thermophilic food-spoilage organisms in store white beet sugar. Food Res. 4:259-267.
9. Meyer, K. F., and J. B. Gunnison. 1929. Botulism due to home canned Bartlet pears. J. Infect. Dis. 45:135-147.
10. Official methods of analysis of the Association of Official Analytical chemists. 1975. W. Horwitz, ed. 12th ed. A. O. A. C., Washington, D.C.
11. Pearce, W. E., and E. Wheaton. 1952. Auto-sterilization of thermophilic spores in canned foods. Food Res. 17:487-494.
12. Powers, J. J. 1976. Effect of acidification of canned tomatoes on quality and shelf life. Crit. Rev. Food Sci. Nutr. 7:371-396.
13. Sapers, G. M., J. G. Phillips, and A. K. Stoner. 1977. Tomato acidity and the safety of home canned tomatoes. Hort. Sci. 12:204-208.
14. Schoenemann, D. R., A. Lopez, and F. W. Cooler. 1974. pH and acidic stability during storage of acidified and nonacidified canned tomatoes. J. Food Sci. 39:257-259.
15. Stadtman, F. H., J. E. Buhlert, and G. L. Marsh. 1977. Titratable acidity of tomato juice as affected by break procedures. J. Food Sci. 42:370-382.
16. Tischer, R. G., and W. B. Esselen. 1945. Home canning. I. Survey of bacteriological and other factors responsible for spoilage of home canned foods. Food Res. 10:197-213.

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Microbiological Quality of Commercial Frozen Minced Fish Blocks

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ABSTRACT

Two hundred and eight commercial blocks of imported frozen minced fish examined for aerobic plate count, fecal coliforms, and coagulase-positive staphylococci were found to be in compliance with the proposed standards of the International Commission on the Microbiological Specifications for Foods. There was no significant correlation between aerobic plate count (35 C) and certain indicator organisms (coliforms, fecal streptococci, and coagulase-positive staphylococci).

The recent decline in stocks of the traditional oceanic species of fish, coupled with an increased world demand for animal protein, has led to implementation of methods and devices for increasing the efficiency of utilization of marine food resources, particularly through employment of mechanical meat deboning machines (*11*). Fish processed in this manner is referred to as minced fish and is imported into the United States in the form of frozen rectangular blocks usually 16.5 or 18.5 lb. (7.5 or 8.4 kg) for further processing into fish sticks, portions, and other products. The nature of the mincing process affords ample opportunity for increased microbial content in the finished product unless good manufacturing practices (GMP) are strictly adhered to. Thorough washing of the headed and gutted fish before mincing and frequent washing of all equipment during the operation are essential. In addition, the increased surface area created and disruptance of cellular integrity provide favorable conditions for microbial growth unless proper temperature control and minimal holding time before freezing are maintained. Blackwood (*3*) compared microbiological data of minced fish produced from the same raw material in two different plants, the first operating under good GMP, the second somewhat lax in this practice. The average plate count of samples from the first plant was 67,000/g compared to 1.5 million/g for the second plant. Fecal coliform counts were also lower in product from the first plant.

Another important factor which deserves serious consideration is the initial quality of the fish. Cann and Taylor (*6*) reported that for producing a mince of acceptable microbial content, fresh fish iced for only up to 4 days could be used. Also, mince prepared from frames (skeletal structure remaining after filleting) was of lesser bacteriological quality compared to mince prepared from fillets or trimmings. Babbitt et al. (*2*) similarly reported that mince recovered from frames was higher in total plate count than mince prepared from whole fish (headed and gutted) which, in turn, was slightly higher than counts from intact fillets.

There is some concern that the mincing process may become a salvage operation for fish on the verge of spoilage. In recent years, the U.S. Food and Drug Administration has expressed its intention to establish microbial standards for various processed foods including frozen seafoods. Since there are relatively few published data concerning the microbial quality of minced fish used by commercial processors in this country, this study was initiated to obtain such information for a data pool that might serve as a guideline upon which to establish potential future standards and, also, to evaluate the microbiological quality of commercial minced fish blocks on the basis of the standards proposed by the International Commission on Microbiological Specifications for Foods (ICMSF) (*10*).

MATERIALS AND METHODS

Two hundred and eight frozen minced fish blocks weighing either 16.5 or 18.5 lb. were obtained from several commercial seafood processors during the period March 1975 through September 1976. The various fish species involved included cod (*Gadus morhua*) 98 blocks, Alaska pollock (*Theragra chalcogramma*) 65 blocks, pollock (*Pollachius virens*) 26 blocks, haddock (*Melanogrammus aeglefinus*) nine blocks, hake (*Urophycis tenuis* or *Merluccius bilinearis*) four blocks, ling cod (*Molva molva*) three blocks, and ocean catfish (*Anarhichas lupus*) three blocks. The source of the Alaska pollock was the North Pacific ocean, whereas all other species were from the North Atlantic ocean. The Alaska pollock blocks originated from Japan. The remainder were produced in Canada, Denmark, Greenland, Iceland, Norway, or Poland.

Microbiological tests were conducted in accordance with the procedures outlined in the *Bacteriological Analytical Manual* (*8*) of the

FDA except where noted. Each block was sampled in duplicate. The counts of both samples were averaged for reporting the block count. A general description of the test procedures follows.

Aerobic plate count (APC)

A 50-g sample was obtained from the frozen block using an electric drill with a sterile 1-¼ inch (32 mm) high-speed bit. This was blended with 450 ml of phosphate buffer; and from this primary dilution, further decimal dilutions were prepared. Four pour plates were made from each appropriate dilution using the TPE agar (Standard Methods agar reinforced with 0.5% Bacto-peptone and 0.5% NaCl) recommended by Lee and Pfeifer (12). Two plates were incubated at 21 ± 1 C and two at 35 ± 0.3 C, and colony counts were made after 5 days or 2 days, respectively.

Coliforms

Coliform count was estimated by a 3-tube (lauryl sulfate tryptose broth (LST) MPN procedure with dilutions ranging from 10^{-1} to 10^{-4} and with confirmation in brilliant green lactose bile 2% broth (BGLB). A loopful from gas-positive LST tubes was inoculated into EC broth fermentation tubes and incubated for 48 h in a water bath maintained at 45.5 ± 0.05 C to determine fecal coliforms. A loopful from gas-positive EC tubes was streaked on Levine's EMB agar, and typical *E. coli* colonies were subjected to the IMViC test for confirmation.

Fecal streptococci

These types were enumerated by a direct plate count on KF streptococcus agar.

Coagulase-positive [CP] staphylococci

This count was estimated by employing a 3-tube (trypticase soy broth with 10% NaCl) MPN procedure followed by streaking on Vogel-Johnson agar and subsequent testing of typical colonies for coagulase activity.

Salmonella

A 25-g sample was blended with 225 ml of lactose broth; and following incubation, aliquots were subcultured in selenite cystine broth and tetrathionate broth. After further incubation, a loopful from each broth was streaked onto brilliant green agar, and suspect colonies were tested for biochemical activity on triple sugar iron agar, lysine iron agar, urea broth, phenol red dulcitol broth, malonate broth, and for IMViC pattern.

Analysis of data

Linear regression analysis was done on the data using a Wang[a] 600 Series Advanced Programming Calculator.

RESULTS AND DISCUSSION

The cumulative percent frequency of aerobic plate count (21 C) for the minced blocks plotted according to species is shown in Fig. 1. This type of graph can be useful since it enables one to estimate the percent of samples complying with a specified count or conversely the maximum count for a specified percent of samples (or producers). The Alaska pollock blocks had the lowest plate count with numbers ranging from 10 to 100,000/g; whereas, blocks made from cod-frame mince were highest in plate count with a range of about 30,000 to 3,000,000/g. The general low count of the Alaska pollock blocks could be rationalized on the basis of high-quality fish or, possibly, the blocks were old. The production date of these blocks was not known; however, it is acknowledged that during continued storage at commercial freezer temperatures (approx. -18 to -21 C), there is a gradual die-off of vegetative microorganisms (*15*). A more likely explanation is that a reduction in bacterial numbers occurred as a result of the several cold water washings which the mince normally received during the manufacture of "surimi" blocks (*1*). The mince produced in Canada or European countries is usually not washed.

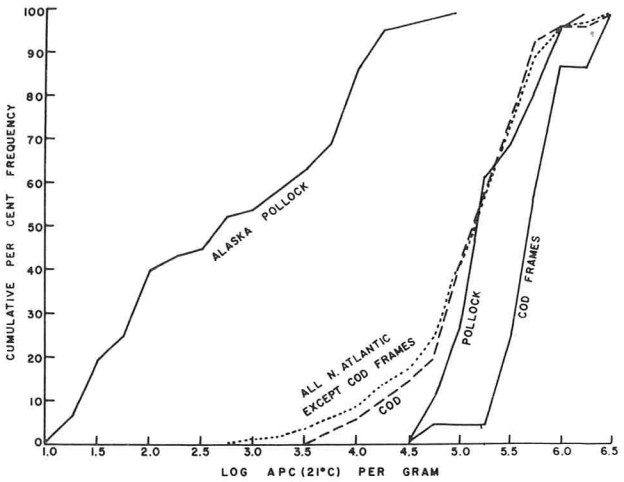

Figure 1. *Cumulative percent frequency of APC [21 C] for various species minced fish blocks.*

The finding of a higher plate count for minced blocks made from frames compared to the other minced blocks (made from fillets, V-cuts, or trim) substantiates the reports of other investigators (*2,6*). Bond (*4*) states that the quality of mince produced from frames is highly dependent on adequate refrigeration and sanitary and expeditious handling. The higher bacterial count usually encountered in frame mince may be a reflection of the difficulty of maintaining these GMP's.

The ICMSF has proposed microbial limits for standard plate count (25 C) for frozen minced fish based on a 3-class sampling plan as follows (*10*): Sample 5 units (n = 5) and accept the lot if no plate count exceeds 10^6/g (m = 10^6) or if not more than 3 plate counts (c = 3) fall between 10^6 - 10^7/g (m - M), providing none of the counts exceed 10^7/g (M = 10^7). Applying these criteria, it can be concluded from probability tables (*10*) that all the different specied blocks examined in this study, each species constituting an individual lot, would be regarded as acceptable (p = 1) since none of the plate counts exceeded 10^7/g (percent defective = 0), and less than 13% for any lot (cod frames) fell between 10^6 to 10^7/g (percent marginal = 13). Crabb and Griffiths (*7*) sampled 115 blocks of frozen minced fish in international trade and similarly reported all to perform satisfactorily. Ninety-two percent of the plate counts were less than 10^6/g and 7% fell within the range 10^6 to 10^7/g.

It should be pointed out that the plate counts obtained in the present study would probably be higher than those obtained following the ICMSF procedure since the latter method employs standard methods agar which was found to yield a lower count than the TPE agar used in this study. The reason for using TPE agar was that a preliminary study, in which four different media were evaluated for APC using five different species of frozen fish, had indicated maximum counts at incubation temperatures of either 21 C or 35 C with TPE agar. The other three media compared were (*a*) standard methods

[a]*Mention of trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.*

agar, (*b*) standard methods agar + 0.5% NaCl, and (*c*) Eugon agar + 0.5% yeast extract.

Since the initial microbial flora of the fish species from which these minced blocks were made is composed principally of psychrotrophs, the APC at 21 C is usually an indication of the microbial quality of the raw material used for mincing (*13*). On the other hand, the APC at 35 C can serve as an indicator of sanitary conditions or human contamination during processing or handling (*15*). It has also been demonstrated that the marine psychrophiles normally associated with fish either fail to grow or grow poorly at 35 C (*12*). The mesophilic plate count at 35 C or 21 C should be comparable. Consequently, a high APC at 35 C and a low ratio of APC_{21}/APC_{35} could serve as an index of poor GMP. In this investigation, the plate counts at 35 C were generally found to be less than the corresponding counts at 21 C. Log APC at 35 C was plotted as a function of the log APC at 21 C. Regression analysis was done separately on the Alaska pollock data and the combined North Atlantic fish data since two slightly different trends of the plotted data were apparent visually. For the Alaska pollock, the equation of the regression line was:

$$Y = 0.87\,X + 0.06$$

The correlation coefficient (r) was 0.96, and the standard error of estimate ($S_{y.x}$) was 0.26. For the combined North Atlantic fish samples, the equation for the regression line was:

$$Y = 1.00\,X - 0.48$$
$$\text{with } r = 0.90 \text{ and } S_{y.x} = 0.33$$

In general, the plate count at 21 C for all samples was 2 to 3 times greater than the count at 35 C. There were no instances in which the APC (35 C) was high (>10^6/g) and the ratio of APC (21 C)/APC (35 C) equal to approx. 1. This would tend to indicate that overall the true mesophilic count was not excessive and that the blocks were produced under acceptable hygienic conditions.

Thirty-six blocks out of the 208 total were positive for coliforms. The cumulative percent frequency of coliforms for the various blocks arranged according to fish species is presented in Table 1. The coliform density was generally low, most blocks (95%) not exceeding a count of 15/g. In 58 instances in which a presumptive positive coliform count was obtained with LST tubes, four proved to be false positives, but this only occurred when the presumptive count was low (4/g). In two instances, the confirmed count was slightly less than the presumptive count; but in all other instances, both counts were equivalent. In a microbiological examination of frozen fish and seafoods, Hall et al. (*9*) reported that 80% of the positive specimens in LST broth tubes confirmed as positive in BGLB. Thus, for monitoring coliform counts of fish blocks for routine quality control purposes, a 3-tube LST broth MPN procedure would probably be satisfactory. However, this would not apply to batter/breaded seafoods which may contain fermentable sugars other than lactose.

Fecal coliforms were only detected in four blocks, three cod and one cod frame. Three of the blocks had an average count of 4/g or less; and for the other block (cod), the average count was 7/g. The ICMSF (*10*) proposed fecal coliform limits for minced fish blocks is based on a 3-class sampling plan with the following criteria: n = 5, c = 3, m = 4, M = 400. Since none of the fecal coliform counts exceeded M and only one count fell between m and M, the blocks examined in this study would be readily acceptable by this proposed standard.

The frequency of *E. coli*-positive blocks was very low. This organism was only detected in one cod block and one cod frame block. In both instances, the MPN count was 4/g.

Use of the coliform count as an index of pollution in frozen foods has been criticized because of the susceptibility of this group of microorganisms to freezing injury resulting in a gradual disappearance in their numbers in frozen foods during continued storage. The fecal streptococcus count remains more stable in this situation, and it has been proposed to replace the coliform count as a pollution indicator for frozen foods (*5,16*). However, Varga and Anderson (*17*) concluded that presence of enterococci in fish fillets originated from improperly sanitized working surfaces and that their numbers should be interpreted to reflect plant sanitation rather than fecal contamination. Table 2 presents the cumulative percent frequency of fecal streptococci in the various frozen minced blocks. The Alaska pollock blocks were least contaminated with about 95% of the blocks having a count not exceeding 25/g; whereas, for this same percentage of cod blocks, the count ranged up to 4000/g. The ICMSF does not include the enterococcus count in

TABLE 1. *Cumulative percent frequency of coliforms in frozen minced blocks made from various species of fish.*

	Species				
Coliforms/g	Alaska pollock	Cod	Cod frames	Pollock	All N. Atlantic
0-3	100	83.8	70.8	92.3	84.6
3.1-6		86.5	83.3	100	89.5
6.1-9		93.2	83.3		93.0
9.1-15		94.6	100		96.5
15.1-25		98.6			98.6
25.1-43		100			100

its recommended microbiological limits for fish. Shewan (*14*) has tabulated some suggested bacteriological standards for fish and fishery products, and the value cited for the enterococcus count in 1000/g. All the Alaska pollock blocks and about 90% of the combined North Atlantic species blocks examined in this study met this suggested standard.

An attempt was made to correlate the fecal streptococcus count with the coliform count. This could not be done with Alaska pollock blocks since all were negative (<3/g) for coliforms. For the North Atlantic species blocks, the incidence of positive coliforms was 34/140 and for positive fecal streptococci, it was 98/140. There were 71 instances in which coliforms were not detected but fecal streptococci were, and only five instances in which the reverse situation prevailed. These results could be indicative of a higher survival rate of the fecal streptococci during frozen storage. Regression analysis done on the data plotted as fecal streptococcus count as a function of coliform count indicated no correlation ($r = -0.018$).

Coagulase-positive (C.P.) staphylococci were detected in 40 of the 208 blocks. The maximum count did not exceed 24/g. The Alaska pollock blocks had the lowest level of contamination; however, 95% of the North Atlantic species blocks did not exceed a level of 10/g. The cumulative percent frequency of C.P. staphylococci is shown in Table 3. The ICMSF (*10*) does have a proposed limit for C.P. staphylococci in minced fish blocks based on a 3-class sampling plan, and the parameters are: $n = 5$, $c = 3$, $m = 10^3$/g, and $M = 2 \times 10^3$/g. This requirement was easily met by the blocks sampled in this survey since no block had a count that exceeded m.

Salmonella was not detected in a 50-g sample taken from any of the blocks. It may be argued that in non-liquid foods, salmonellae contaminations are usually localized and that the small sample taken in this study could easily miss the contaminated areas; however, absence of salmonella in all the sub-samples taken indicates that the level of contamination in the blocks would at least be low, if at all. There are no proposed limits for Salmonella in fish blocks since the FDA regards the presence of this pathogen in foods as adulteration.

For marine fish taken from temperate waters, the APC at 35 C, the coliform counts, fecal streptococcus count, and C.P. staphylococci are usually associated with improper sanitation or human contamination. It was considered of interest to determine whether any correlation existed between the APC at 35 C and any of the other three indicator counts. It was not possible to involve the coliform counts in any regression analysis since most of that data consisted of a negative (<3/g) count. The C.P. staphylococcus count was also predominantly negative (<3/g); but for 37 samples of North Atlantic fish which were C.P. staphylococcus positive, there was no correlation ($r = 0.09$) with the APC (35 C). One hundred and twenty-eight minced blocks of North Atlantic fish were positive for fecal streptococci. Regression analysis of log fecal streptococcus count as a function of log APC (35 C) indicated a correlation coefficient of 0.47 which, although indicative of some correlation, cannot be considered significant.

If the 208 blocks examined in this survey were regarded as a single lot, it would be considered acceptable in microbiological quality in accordance with the proposed ICMSF standards since none of the counts

TABLE 2. *Cumulative percent frequency of fecal streptococci in frozen minced blocks made from various species of fish.*

Fecal strept./g	Species				
	Alaska pollock	Cod	Cod frames	Pollock	All N. Atlantic
0-10	92.3	29.7	45.8	19.2	32.2
11-25	96.9	37.8	54.1	26.9	41.3
26-50	98.4	48.6	58.3	38.4	49.7
51-100	100	51.3	62.5	49.9	55.9
101-250		66.1	70.8	73.0	70.6
251-500		85.0	79.1	73.0	83.9
501-1000		90.4	83.2	84.5	89.5
1001-2000		91.8	91.5	96.0	93.7
2001-4000		94.5	95.6	96.0	95.8
4001-8000		95.9	100	100	97.9
8001-16,000		95.9			97.9
16,001-32,000		98.6			99.3
32,001-64,000		100			100

TABLE 3. *Cumulative percent frequency of coagulase positive staphylococci in frozen minced blocks made from various species of fish.*

C.P. Staph./g	Species				
	Alaska pollock	Cod	Cod frames	Pollock	All N. Atlantic
0-3	96.9	83.8	79.2	88.5	84.6
3.1-6	98.5	90.5	87.5	96.2	92.3
6.1-10	98.5	94.6	91.7	96.2	95.1
10.1-16	100	98.6	100	100	99.3
16.1-24		100			100

exceeded the maximum value (M) for APC, fecal coliforms, and C.P. staphylococci; and only an insignificant number, if any, fell within the conditional range (m-M). Crabb and Griffiths (7) sampled 115 blocks of frozen minced fish in international trade for microbiological quality, and they similarly reported generally satisfactory results. Only one block did not comply with the proposed ICMSF standards and that was because the plate count exceeded 10^7/g.

From the results obtained in this survey and others thus far reported, it does not appear that frozen minced fish blocks in commerce present any public health hazard, and most blocks produced should meet the requirements of the proposed ICMSF microbiological standards.

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REFERENCES

1. Amano, K. 1971. Inspection and quality of assessment of Japanese comminuted fish products. pp. 119-124. In R. Kreuzer (ed.) Fish inspection and quality control. Fishing News (Books) Ltd., London, England.
2. Babbitt, J. K., D. L. Crawford, and D. K. Law. 1974. Quality and utilization of minced deboned fish muscle. pp. 32-43. In R. E. Martin (ed.) Second technical seminar on mechanical recovery and utilization of fish flesh. National Fisheries Institute, Washington, D.C.
3. Blackwood, C. M. 1974. Utilization of mechanically separated fish flesh - Canadian experience. pp. 325-329. In R. Kreuzer (ed.) Fishery products. Fishing News (Books) Ltd., London, England.
4. Bond, R. M. 1975. Background paper on minced fish. FAO fisheries circular No. C332. Food and Agriculture Organization of the United Nations, Rome 24p.
5. Burton, M. O. 1949. Comparison of coliform and enterococcus organisms as indices of pollution in frozen foods. Food Res. 14:434-438.
6. Cann, D. C., and L. Y. Taylor. 1976. The bacteriology of minced fish prepared and stored under experimental conditions. Conference on the production and utilizations of mechanically recovered fish flesh (minced fish). April 1976. Torry Research Station, Aberdeen, Scotland.
7. Crabb, W. E., and D. J. Griffiths. 1976. Bacteriological status of mechanically recovered flesh from the cod. Conference on the production and utilization of mechanically recovered fish flesh (minced fish). April 1976. Torry Research Station, Aberdeen, Scotland.
8. Food and Drug Administration. 1976. Bacteriological analytical manual for foods. Food and Drug Administration, Bureau of Foods Division of Microbiology, Washington, D.C. 340 p.
9. Hall, H. E., D. F. Brown, and K. H. Lewis. 1967. Examination of market foods for coliform organisms. Appl. Microbiol. 15: 1062-1069.
10. International Commission on Microbiological Specifications for Foods. 1974. Microorganisms in foods, Vol. II. Univ. of Toronto Press, Toronto, Canada. 213p.
11. King, F. J., and J. H. Carver. 1970. How to use nearly all the ocean's food. Com. Fish. Rev. 32(12):12-21.
12. Lee, J. S., and D. K. Pfeifer. 1974. Influences of recovery media and incubation temperatures on the types of microorganisms isolated from seafoods. J. Milk Food Technol. 37:553-556.
13. Liston, J., and J. R. Matches. 1976. Fish, crustaceans, and precooked seafoods. pp. 507-521. In M. L. Speck (ed.) Compendium of methods for the microbiological examination of foods. American Public Health Association, 1015 Eighteenth Street, N.W., Washington, D.C.
14. Shewan, J. M. 1970. Bacteriological standards for fish and fishery products. Chem. Ind. Feb. 7, 1970: 193-199.
15. Speck, M. L., and B. Ray. 1977. Effects of freezing and storage on microorganisms in frozen foods: A review. J. Food Prot. 40:333-336.
16. Thatcher, F. S., and D. S. Clark (ed.) 1968. Microorganisms in foods: Their significance and methods of enumeration. Univ. of Toronto Press. Toronto, Canada. 234p.
17. Varga, S., and G. W. Anderson. 1968. Significance of coliforms and enterococci in fish products. Appl. Microbiol. 16:193-196.

Annual Report of BISSC Committee

This committee has had one meeting with the Baking Industry Sanitation Standards Committee (BISSC) since our last report. The usual Fall BISSC meeting was not held in 1977. In place of the Fall BISSC meeting the Baking Exposition was held in Atlantic City, N.J. Our committee was very active during the Exposition in Atlantic City where our members helped man the BISSC booth and covered the Exposition discussing with the exhibitors, the sanitary construction of equipment and the BISSC standards.

The 61st meeting of BISSC was held in Chicago March 2-5, 1978. This meeting was a very active one with our committee members serving as public health and sanitation consultants to industry task committees engaged in the formulation of seven (7) new standards which included oven loaders, unloaders, pan stackers and unstackers, chocolate melting and refining equipment, grinders, food choppers and pulverizers, laminators, extruding and cutting machines, tray loaders, pattern formers and continuous mix equipment.

My recent appointment to the BISSC Board of the Office of Certification provides the IAMFES with representation and imput to that function of BISSC. The Fall 1978 BISSC Meeting will be held in San Francisco, California September 14 to 16.

At present there are BISSC standards for thirty-three (33) categories of Baking Equipment. Seventy (70) companies have been given authorization certificates for equipment manufactured in compliance with these standards. BISSC information booklets as well as all BISSC standards are available to sanitarians. Copies may be obtained by writing to Mr. Ray Walter, Executive Secretary, BISSC, 521 Fifth Ave., New York, N.Y. 10017.

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Food Poisoning Potential of Pathogens Inoculated onto Bologna in Sandwiches[1]

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ABSTRACT

Bologna sandwiches inoculated separately with low levels (100 to 1000 per g) of specific pathogens at time of sandwich preparation to simulate conditions that might occur in home or food service preparation, were stored at 4, 21 and 30 C for 0, 4, 8 and 25 h and monitored for growth of pathogens. All pathogens, except *Clostridium perfringens*, were capable of significant growth after more than 8 h of incubation at 30 C, but not at 4 or 21 C. Significant growth at 21 C only occurred with *Staphylococcus aureus* after 25 h of incubation. *C. perfringens* failed to grow on bologna in all sandwiches. All other pathogens, except *S. aureus*, failed to grow on bologna with low pH (pH <6.1). Growth of *S. aureus*, was retarded on bologna at pH 5.5, and inhibited at pH 5.1. Only gram negative pathogens (enteropathogenic *Escherichia coli* and *Salmonella typhimurium*) were adversely affected by increased bacterial competition. Results indicated that bologna in sandwiches under these experimental conditions would only become a potential vehicle for food poisoning under almost unrealistic conditions of handling and storage.

Storage abuse and improper handling are major factors involved in food poisoning outbreaks in North America (*2, 3*). Sandwiches are products which could be exposed to considerable abuse before consumption. Despite this potential for food poisoning from sandwiches, there are few reported cases of sandwich-borne food poisonings. This study was undertaken to investigate the food poisoning potential of sandwiches. However, to limit the scope of the study, bologna was chosen as the only sandwich filling.

Bologna supports growth of microorganisms, but may not be an ideal medium for bacterial growth. A variety of inhibitory and selective agents present in bologna can affect survival of specific microorganisms. The most important inhibitory agents include: nitrite, pH, salt concentration, competition, oxygen and carbon dioxide partial pressures and storage temperature. The combination of factors in cooked, cured meats are reported to select gram positive, salt tolerant microorganisms, such as *Bacillus, Micrococcus, Sarcina, Lactobacillus* and *Microbacterium* spp. (*1, 10, 11, 12, 16, 18, 19, 25, 26, 29*). On this basis, it may be expected that potential food poisoning organisms such as *Bacillus cereus* and *Staphylococcus aureus* can develop in cooked, cured meats.

An earlier study (*20*) indicated that bologna could be obtained in the retail marketplace with either a high pH (near pH 6.5) or a low pH (below pH 5.5), and that pH change was not a reliable function of product age, since certain manufacturers' product had a high pH with high microbial counts after 30 days shelf-life. This study was designed to evaluate the effects of differences in competition with other bacteria (age of product), pH and storage temperature on growth of several selected pathogens.

MATERIALS AND METHODS

Freshly sliced, but not packaged, bologna was obtained directly from two manufacturers, selected for differences in product pH at the end of the product's shelf-life. The product was returned to the laboratory and vacuum packaged in aluminum-nylon-polypropylene pouches (Cryovac Division, Grace Chemicals, Mississauga, Canada) under 26 lb/sq. in. vacuum. Packaged bologna was stored at 4 C for 2 and 30 days, representing "new" (low competition) and "old" (high competition) bologna, respectively.

Sandwiches were prepared by placing one slice of bologna between two slices of enriched, white bread, which had been spread with one teaspoonful of soft margarine (Parkay). The bread with margarine was equilibrated to 21 C before bologna was added. Triplicate samples of the three bologna types (high pH, low competition; high pH, high competition; low pH, high competition) were evaluated for their ability to support growth of selected pathogens: *S. aureus* S-6, *Salmonella typhimurium* (ATCC 13311), enteropathogenic *Escherichia coli* 0124 DM, *B. cereus* B4AC and *Clostridium perfringens* 8239-H. (All cultures, except *S. typhimurium* 13311, were obtained from Dr. A. Hauschild, Health Protection Branch, Health and Welfare Canada, Ottawa, Canada).

At the time of sandwich preparation the bologna slice was inoculated with low levels (100 to 1,000 per g) of test organisms, by spreading 0.05 ml of appropriately diluted overnight culture on one side of each slice, using a sterile, glass hockey stick. Inoculated sandwiches were placed in plastic bags (Zip Lock Seal, Dow Chemicals) and incubated at 4, 21 and 30 C for 0, 4, 8 and 25 h.

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Following incubation, counts of test organisms were determined on the following media: *S. aureus* on Difco Baird-Parker medium, incubated 36 C for 48 h; *S. typhimurium* on Difco Brilliant-Green agar, incubated 36 C for 24 h; *E. coli* on Difco Violet Red Bile agar, overlayered and incubated 36 C for 24 h; *B. cereus* on Mossel et al. (*17*) Mannitol, Egg-Yolk, Phenol Red, Polymyxin (MYP) agar, incubated 30 C for 20-24 h, but in the case of heavily contaminated plates for 40 h; *C. perfringens* on egg-yolk free Tryptose-Sulfite-Cycloserine (TSC) agar, incubated anaerobically (using BBL "Gas-Paks" to obtain a H_2/CO_2 atmosphere) 36 C for 24 h.

Counts were confirmed as follows: *S. aureus* using Difco coagulase plasma; *S. typhimurium* using MacConkey agar, TSI slants, and serological screening with Salmonella O Antiserum Poly A-I and Group B factors 1, 4, 5 and 12; *E. coli* using MacConkey agar and gas production in EC medium at 45.5 ± 0.05 C; *B. cereus* using gram stain and catalase production; and *C. perfringens* using gram stain, nitrate reduction and motility. Confirmation was also inferred from the absence of growth of typical organisms from uninoculated control sandwiches.

RESULTS AND DISCUSSION

Microflora, pH and moisture content of the bologna samples were measured. New (low competition), high pH product had total counts ranging from 1.2×10^2 to 4.3×10^4 organisms per g, pH range 6.4 to 6.7 and moisture content 58.0 to 59.4%. Old (high competition), high pH product had total counts above 1×10^6 organisms per g, pH range 6.3 to 6.7 and moisture content 55.8 to 57.3%. Old, low pH product had total count above 1×10^6 organisms per g, pH range 5.1 to 5.5 and moisture content 53.5 to 55.2%. An additional old sample with pH 6.1 was analyzed. Salt content was approximately 2.4% (data supplied by manufacturers) and levels of other inhibitors, with the possible exception of nitrite, were controlled. Nitrite levels in old bologna were probably 15 - 20% less than in new product, as a result of storage depletion (*24*); however, breakdown products of nitrite might also be involved in microbial inhibition (*13, 21, 22*).

Growth of test organisms on bologna in sandwiches is shown in Fig. 1 - 5. Growth is expressed in terms of relative population change by plotting $\log_{10}$ of the ratio of the number of pathogens recovered after incubation to the number inoculated. The most striking observation

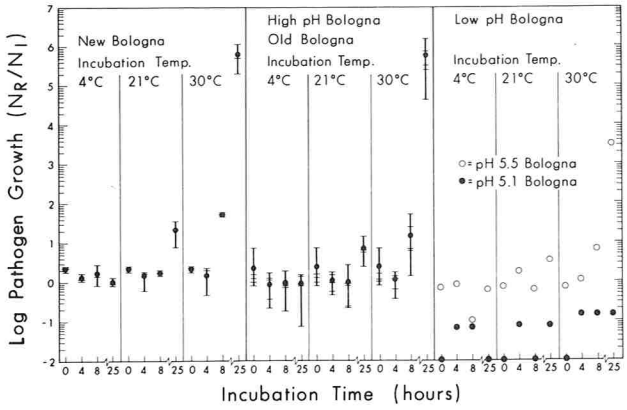

Figure 1. *Influence of time and temperature of incubation, age and pH of bologna on growth of* S. aureus. N_R *number of pathogens recovered after incubation;*
N_I *number of pathogens inoculated;*
• *mean of log pathogen growth; except for low pH, as explained above;*
- *individual observations of log pathogen growth and range.*

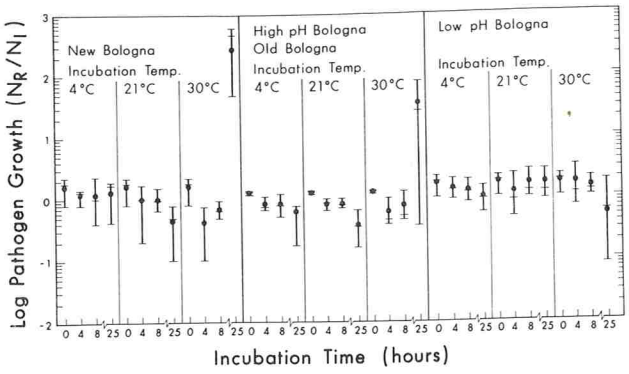

Figure 2. *Influence of time and temperature of incubation, age and pH of bologna on growth of* S. typhimurium.
N_R *number of pathogens recovered after incubation;*
N_I *number of pathogens inoculated;*
• *mean of log pathogen growth;*
- *individual observations of log pathogen growth and range.*

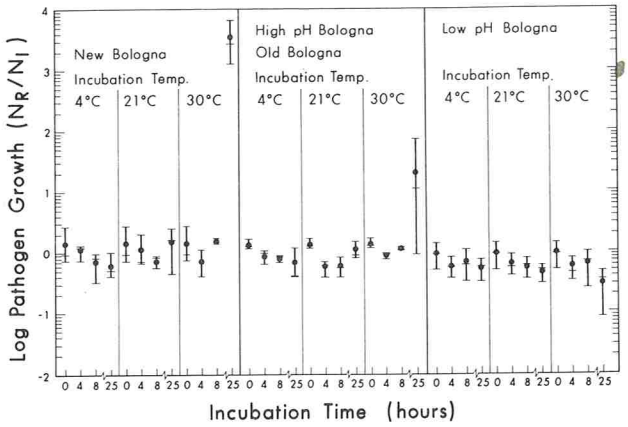

Figure 3. *Influence of time and temperature of incubation, age and pH of bologna on growth of* E. coli. (*See Fig. 2 for explanation of symbols*).

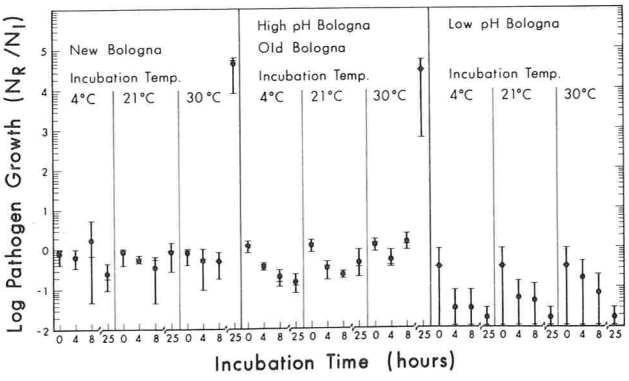

Figure 4. *Influence of time and temperature of incubation, age and pH of bologna on growth of* B. cereus. (*See Fig. 2 for explanation of symbols*).

was that significant growth only occurred after more than 8 h of incubation at 30 C. All test pathogens, except *C. perfringens*, grew on high pH bologna (new and old) after 25 h of incubation at 30 C. *C. perfringens* generally failed to grow, and declined in numbers after exposure to more abusive storage conditions, especially in low pH product (Fig. 5).

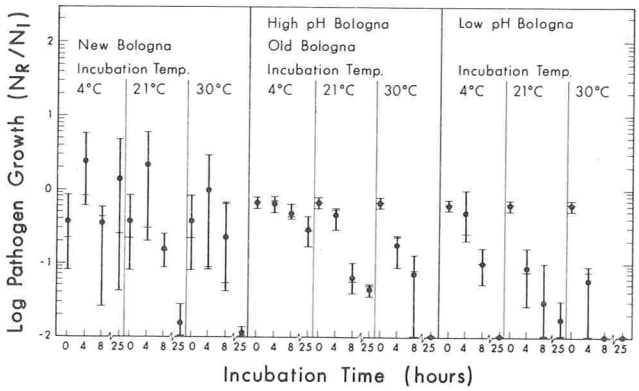

Figure 5. *Influence of time and temperature of incubation, age and pH of bologna on growth of* C. perfringens. *(See Fig. 2 for explanation of symbols).*

Only after 25 h of incubation at 30 C had *S. aureus* and *B. cereus* grown to potentially hazardous levels on new and old, high pH bologna. Enteropathogenic *E. coli* and possibly *S. typhimurium* increased to potentially hazardous levels only on new, high pH bologna. Greater growth of *S. aureus* and *B. cereus* could probably be attributed to their greater tolerance to salt (*28*) and other curing agents (*11*).

Under conditions less abusive than incubation at 30 C, *S. aureus* was the only pathogen that grew (Fig. 1). Growth was less than 100-fold population increase, and only occurred after 25 h of incubation at 21 C. As such, a food poisoning risk would only occur if high counts of *S. aureus* (10^4 to 10^5 organisms per g) were present in the food at time of sandwich preparation. No growth of pathogens in sandwiches occurred at 4 C, as would be expected for mesophilic organisms. A prolonged lag phase was reported for *S. aureus* (*6, 14, 28*) and *Salmonella* spp. (*28*) after exposure to levels of inhibitors found in cured meats, confirming the fact that no growth occurred within 4 h in any samples.

Effects of pH may also be interpreted from Fig. 1 - 5. In high pH bologna, incubated at 30 C, all inoculated pathogens grew, except *C. perfringens*. In low pH bologna, however, growth of pathogens was not only prevented, but in the case of *S. aureus* and *B. cereus*, in addition to *C. perfringens*, death occurred. This loss of viability was independent of incubation temperature for both *S. aureus* and *B. cereus.* The low initial inoculation of pathogens made death rates difficult to record. Counts rapidly became unreliable, decreasing to less than 30 colonies per plate. This probably accounted for the large differences between replicates, in samples where the test organisms were dying.

The effect of competitive inhibition can also be interpreted from Fig. 1-5. However these effects, indicated by results for different age (new and old) product, were less apparent than effects of pH, storage time of sandwiches or incubation temperature. All pathogens, except *C. perfringens*, grew on both age types of high pH bologna after 25 h of incubation at 30 C. *E. coli* (Fig. 3) and possibly *S. typhimurium* (Fig. 2) were the only test organisms affected by competition (age). Relative increases of *E. coli* were 1,200- to 6,300-fold and for *S. typhimurium* were 50- to 640- fold on new bologna, compared to 1- to 74-fold and 0.5- to 80-fold relative increases on old bologna, respectively. These results support the observations of Heiszler et al. (*11*) that gram negative bacteria could not compete with the natural flora of cooked, cured meats.

The lack of increased inhibition of *S. aureus* and *B. cereus* in older product was surprising because both organisms are reportedly inhibited by the normal saprophytic flora of most foods (*4, 5, 7, 8, 9, 15, 23, 27*). Lactic acid bacteria and group D streptococci are even more antagonistic to *S. aureus* and *B. cereus* (*4, 5, 8, 9*), but some of the test product had lower lactic and group D *Streptococcus* counts than expected. Competitive inhibition may occur even when low bacterial populations are present (*4*), however, such an eventuality was not indicated in, nor could it be determined from this study.

Subjective evaluation of the bologna in the sandwiches (i.e. cursory evaluation of appearance and odor) was made after incubation. Gross changes were not observed in any new product samples, even after 25 h incubation at 30 C. This was in accordance with normal expectations that, most food poisoning bacteria grow in foods without visible signs of deterioration. In contrast, however, deterioration of bologna could be detected before significant pathogen growth occurred in old product samples. Typical forms of deterioration were off-odors and greening.

CONCLUSIONS

These studies indicated that bologna in sandwiches contaminated with low levels of bacterial pathogens at time of preparation was only likely to be a vehicle of food poisoning under almost unrealistic conditions. Only product contaminated with *S. aureus*, *B. cereus*, enteropathogenic *E. coli* or salmonellae exposed to highly abusive storage (i.e. more than 8 h at 30 C), with low initial bacterial counts and high intial pH had food poisoning potential. Reasonable handling of sandwiches, especially with refrigerated storage, should give a large margin of safety for such products contaminated with low levels of typical food poisoning pathogens at time of sandwich making.

Although pH and age (microbial competition) are reported and interpreted as the factors affecting the ability of pathogens to grow, other factors might also be related. Older product was less likely to support pathogen growth than new product, even under abusive conditions, and detectable spoilage occurred on old product before, or at the same time as pathogen growth. This study indicated that restrictions on the total microbial load of a vacuum packaged, sliced luncheon meat such as bologna might be totally unrealistic, since the older, high competition product appeared even safer for consumer handling than newer, low competition product.

REFERENCES

1. Alm, F., I. Ericksen, and N. Molin. 1961. The effect of vacuum packaging on some sliced processed meat products as judged by organoleptic and bacteriologic analysis. Food Technol. 15:199-203.
2. American Meat Institute Foundation. 1960. The science of meat and meat products. W. H. Freeman and Company. San Francisco.
3. Center for Disease Control. 1974. Foodborne and waterborne disease outbreaks - Annual summary 1973. U.S. Dept. Health, Education and Welfare/Public Health Service. Atlanta.
4. Dack, G. M., and G. Lippitz. 1962. Fate of staphylococci and enteric microorganisms introduced into slurry of frozen pot pies. Appl. Microbiol. 10:472-479.
5. Daly, C., M. LaChance, W. E. Sandine, and P. R. Elliker. 1973. Control of *Staphylococcus aureus* in sausage by starter cultures and chemical acidulation. J. Food Sci. 38:426-430.
6. Dempster, J. F. 1976. Pathogenic microorganisms in cured meats. Food Manuf. 51(1):41-46.
7. Eddy, B. P., and M. Ingram. 1962. The occurrence and growth of staphylococci on packed bacon, with special reference to *Staphylococcus aureus*. J. Appl. Bacteriol. 25:237-247.
8. Gilliland, S. E., and M. L. Speck. 1972. Interactions of food starter cultures and food-borne pathogens: Lactic streptococci versus staphylococci and salmonella. J. Milk Food Technol. 35:307-310.
9. Goepfert, J. M., W. M. Spira, and H. U. Kim. 1972. *Bacillus cereus*: Food poisoning organism. A review. J. Milk Food Technol. 35:213-227.
10. Graham, P. P., and T. N. Blumer. 1971. Bacterial flora of prefrozen dry-cured ham at three processing time periods and its relationship to quality. J. Milk Food Technol. 34:586-592.
11. Heiszler, M. G., A. A. Kraft, C. R. Rey, and R. E. Rust. 1972. Effect of time and temperature of smoking on microorganisms in frankfurters. J. Food Sci. 37:845-849.
12. Holtzapffel, D., and D. A. A. Mossel. 1968. The survival of pathogenic bacteria in, and the microbial spoilage of, salads, containing meat, fish and vegetable. J. Milk Food Technol. 3:223-239.
13. Ingram, M. 1974. The microbiological effects of nitrite; discussion. pp. 63-75. In B. Krol, and B. J. Tinbergen (eds.) Proceedings of the International Symposium on Nitrite in Meat Products. Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands.
14. Julseth, R. M., and R. H. Deibel. 1974. Microbial profile of selected spices and herbs at import. J. Milk Food Technol. 37:414-419.
15. Minor, T. E., and E. H. Marth. 1971. *Staphylococcus aureus* and staphylococcal food intoxications. A review. I. The staphylococci: Characteristics, isolation and behavior in artificial media. J. Milk Food Technol. 34:557-564.
16. Mol, J. H. H., J. E. A. Hietbrink, H. W. M. Mollen, and J. von Tinteren. 1971. Observations on the microflora of vacuum packed sliced cooked meat products. J. Appl. Bacteriol. 34:377-397.
17. Mossel, D. A. A., M. J. Koopman, and E. Jongerius. 1967. Enumeration of *Bacillus cereus* in foods. Appl. Microbiol. 15:650-653.
18. Nickerson, J. T., and A. J. Sinskey. 1972. Microbiology of foods and food processing. American Elsevier Publishing Co. New York.
19. Palumbo, S. A., C. N. Huhtanen, and J. L. Smith. 1974. Microbiology of the frankfurter process: Salmonella and natural aerobic flora. Appl. Microbiol. 27:724-732.
20. Paradis, D. C., and M. E. Stiles. 1978. A study of microbial quality of vacuum packaged, sliced bologna. J. Food Prot. 41:811-815.
21. Riha, W. E., and M. Solberg. 1975. *Clostridium perfringens* inhibition by sodium nitrite as a function of pH, inoculum size and heat. J. Food Sci. 40:439-442.
22. Riha, W. E., and M. Solberg. 1975. *Clostridium perfringens* growth in nitrite containing defined medium sterilized by heat or filtration. J. Food Sci. 40:443-445.
23. Scheusner, D. L., and L. G. Harmon. 1973. Growth and enterotoxin production by various strains of *Staphylococcus aureus* in selected foods. J. Food Sci. 38:474-476.
24. Sebranek, J. G., R. G. Cassens, and W. G. Hockstra. 1974. Fate of added nitrite. pp. 139-148. In B. Krol, and B. J. Tinbergen (eds.) Proceedings of the International Symposium on Nitrite in Meat Products. Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands.
25. Smith, J. L., S. A. Palumbo, J. C. Kissinger, and C. N. Huhtanen. 1975. Survival of *Salmonella dublin* and *Salmonella typhimurium* in Lebanon bologna. J. Milk Food Technol. 38:150-154.
26. Steinke, P. K. W., and E. M. Foster. 1951. Effect of temperature of storage on microbial changes in liver sausage and bologna. Food Res. 16:372-376.
27. Tatini, S. A. 1973. Influence of food environments on growth of *Staphylococcus aureus* and production of various enterotoxins. J. Milk Food Technol. 36:559-563.
28. Troller, J. A. 1973. The water relations of food-borne bacterial pathogens. A review. J. Milk Food Technol. 36:276-288.
29. Warnecke, M. O., H. W. Ockerman, H. H. Weiser, and V. R. Cahill. 1966. Quality of processed comminuted meat as affected by microbial flora of the raw constituents. Food Technol. 20:686-688.

Report of the Journal Management Committee 1978

The *Journal* Management Committee met on August 14, 1978 and discussed the actions taken on previous recommendations as well as new ideas for the improvement of the *Journal*. The Committee decided to reaffirm a few of its previous recommendations to the Executive Board and to make new recommendations for the Board's consideration as follows:

1. Although there has been improvement in the amount of material published that is of direct interest to the practicing sanitarian, further progress would be desirable. To this end, the Committee recommends that the Assistant Managing Editor of the *Journal* review material in other publications to identify and obtain appropriate articles for reprinting in the *Journal*.
2. The Committee recommends that the Instructions to Authors material be expanded by adding a section specifically for the writing of articles for the practicing sanitarian.
3. The Committee recommends that the Assistant Managing Editor proceed with the plan to appoint a committee of knowlegable sanitarians and a committee of dairy fieldman whose responsibilities would be to develop lists of subjects of current concern that should be developed and published in the *Journal* as well as to identify people who could prepare these articles.
4. The Committee recommends that the Assistant Managing Editor be charged with the review of the Federal Register and similar publications to obtain information of interest to the membership and to prepare appropriate articles on this for the *Journal*. Similarly, the Morbidity and Mortality Reports of the Center for Disease Control should be reviewed for information of interest to the membership and this information published in the *Journal*.
5. The Committee recommends that the possibility be investigated of advertisements for the *Journal* being placed in other journals on a reciprocal basis.
6. The Committee recommends that the listing of articles titles and authors on the title page of the *Journal* be set up on a 2 column format.
7. The Committee recommends that the source of the material printed as filler in the main section of the *Journal* and in the News and Events section be identified at the end of each article.

R. B. Read, Jr., Ph.D.
Chairman

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Storage Stability and Bacteriological Profile of Refrigerated Ground Beef from Electrically-Stimulated Hot-Boned Carcasses

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ABSTRACT

An incubation temperature of 22 C was found adequate for estimating the psychrotrophic bacterial population of ground beef from electrically-stimulated carcasses. The aerobic plate count of the product was as low as $8.0 - 9.0 \times 10^3$/g. Electrical stimulation of beef carcasses prolonged the lag phase of the psychrotrophic bacterial population by 2 days but enhanced the growth rate during the logarithmic phase of growth (12.2 and 9.9 generations formed in the electrically-stimulated and control samples respectively). The shelf life of the ground beef from electrically-stimulated carcasses was prolonged by 3 days as compared to the control (4-5 vs. 7-8 days respectively). Nonpigmented *Pseudomonas* predominated in the flora at spoilage of ground beef from electrically-stimulated carcasses and the control. *Clostridium perfringens, Salmonella* and coliform organisms were not detected, but *Staphylococcus aureus* was found at a level as low as 10 cells/g.

The demand for ground beef is increasing every year in the U.S. The present consumption is 18.2 kg/capita and a 25% increase is expected (*1*).

The bacteriological quality of raw ground beef is of concern to all segments of the industry. Reduced shelf life and discoloration of the product due to bacterial growth are often encountered. Goepfert (*8*) reported that 34% of the 955 samples of ground beef analyzed had an Aerobic Plate Count (APC) of 5×10^6/g. Duitschaever (*6*) showed that more than 50% of 108 ground beef samples had an APC in the range of 5×10^7/g. The source of meat for ground beef and its holding time may affect the bacteriological quality of the product and its storage stability. Proposed bacteriological standards for ground beef in some states set a maximum range of 1.0×10^6 to 5.0×10^6 bacteria/g (*8*). The United Kingdom has proposed a level of 10^6/g (*9*) while in Canada the maximum limit is 10^7/g (*18*). It is well established that most psychrotrophic meat-spoilage bacteria are of the gram-negative type (*3,16*). The nonpigmented *Pseudomonas* organisms predominate in the meat flora at spoilage (*2,11*).

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Although several studies dealing with the storage stability, and flora at spoilage of ground beef have been completed, there is relatively little, if any, information on the effect of beef prefabrication procedures on the bacteriological quality and storage stability of the ground product. The purpose of this work was to study the effect of electrical stimulation of beef carcasses on storage stability of refrigerated ground beef, its bacteriological quality, and spoilage flora.

MATERIALS AND METHODS

Electrical stimulation

The electrical stimulation (a square wave pulse of 300 V, 400 cpm with a duration of 0.5 msec and a current of 1.6 to 1.8 amp) of beef sides (commercial Angus and Hereford steers) started at 30 min post mortem and continued for 15 min. Both sides, stimulated and control (non-stimulated), were held at 16 C during the stimulation and up to 1.5-h post mortem.

Ground beef

At 1.5-h post mortem, portions of the chuck were hot-boned, on-the-rail, from both the electrically stimulated and control sides. The meat was ground and frozen (-25 C) until used.

Storage

Twelve portions of ground beef, each of 250 g, were packed using a polystyrene foam tray wrapped with polyvinyl chloride and stored at 5 ± 1 C.

Bacterial counts

A 10-g sample of ground beef was removed every 22 - 24 h and blended, for 2 min, with 90 ml of 0.1% peptone (Difco) water. Further dilutions were obtained using the same diluent. The plates for the Aerobic Plate Count (APC) were prepared by spreading appropriate dilutions of ground beef samples on prepoured Plate Count Agar (PCA) (Difco) and incubated at 22 C for 48 h. The same procedure and medium were used for the Psychrotrophic Bacterial Count (PBC). The plates were incubated at 7 C for 10 days. The results of each treatment are arithmetic averages of six determinations.

Number of generations

The number of generations (G) was calculated according to the following formula: $G = 3.3 \log b/B$ in which b = number of bacteria at the end of a given time period, and B = Initial number of bacteria.

Shelf life determination

The meat samples were organoleptically evaluated for "off odors" by a three-people panel. A sample evolving "off odors" was considered spoiled.

Pathogenic and indicator organisms

Clostridium perfringens, Salmonella, Staphylococcus aureus and the coliform organisms were detected and counted according to the methods of the *Bacteriological Analytical Manual for Foods.* (7).

Identification of spoilage organisms

Thirty colonies were randomly selected from PCA plates of each of the electrically-stimulated and control samples. Each colony was purified and transferred to a Nutrient Agar (Difco) slant (incubated at 22 C) which served as the stock culture.

The following tests were conducted for the taxonomical classification: gram stain, motility, catalase, benzidine (5), cytochrome oxidase (*14*), oxidation-fermentation (glucose) (*12*), pigment production (*13*), reducing compounds from gluconate (*10*), starch hydrolysis (7), and hydrogen sulfide production (*22*). The isolates were broadly classified in a scheme based upon the work of Shewan (*19*) and Shewan et al. (*20*). All tests were conducted at 22 C.

Statistical analysis

The correlation and the linear regression between the APC and the PBC were calculated. Results of the APC of the ground beef sample from the electrically stimulated (ES) carcass and its control were subjected to the new multiple range test (*21*).

RESULTS AND DISCUSSION

The linear regression between the APC and the PBC of the electrically-stimulated (ES) samples (n = 6) is shown in Fig. 1. The equation of the regression line was $Y = 0.78 + 0.85\ X$ with a correlation coefficient of 0.96, significant at $P < 0.01$. Figure 2 shows the linear regression between the APC and the PBC of the control (non electrically-stimulated) samples (n = 6). The equation of the regression line was $Y = -0.3 + 1.06\ X$ with a correlation coefficient of 0.99, significant at $P < 0.01$. These results show that one can get an accurate estimation of the psychrotrophic bacterial population of ground beef from ES carcasses by using an incubation temperature of 22 C. This elevated temperature (22 C) shortens the incubation time from 10 days (7 C) to 48 h. Since the psychrotrophic bacterial population of ground beef is responsible for spoilage (2), it is also responsible for the storage stability of the refrigerated product. It is important to the industry, for quality control purposes, to have rapid means for estimation of the psychrotrophic bacterial population to take adequate measures concerning processing and marketing of the meat product.

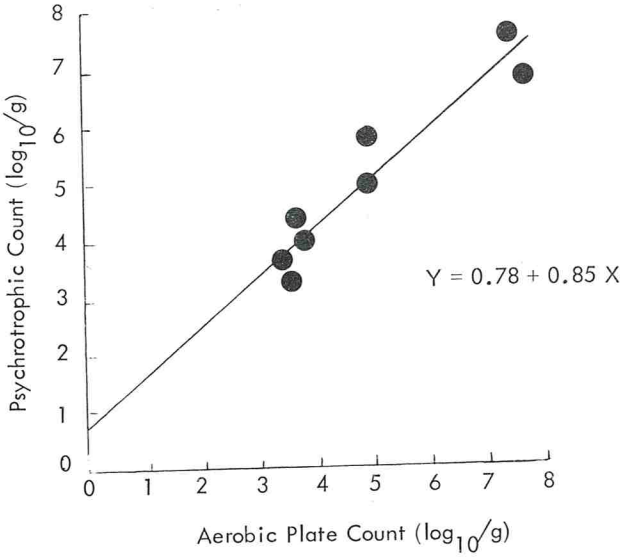

Figure 1. *The linear regression between the Aerobic Plate Count and Psychrotrophic Bacterial Count of ground beef from electrically stimulated carcasses.*

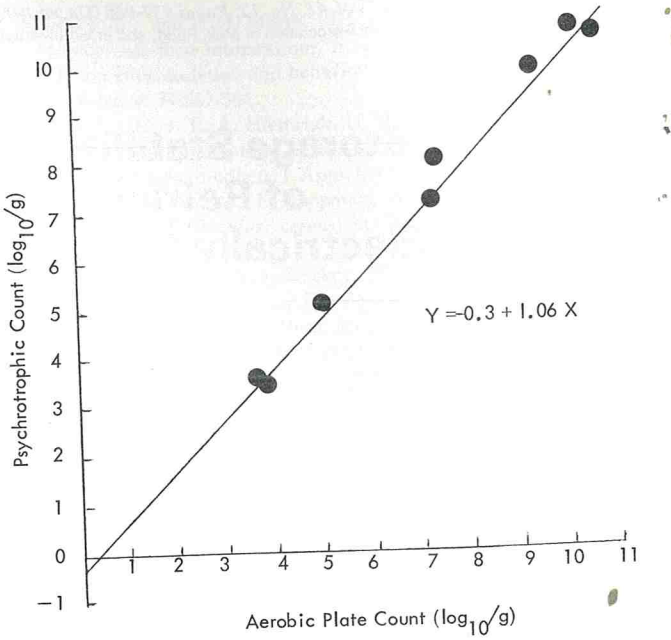

Figure 2. *The linear regression between the Aerobic Plate Count and Psychrotrophic Bacterial Count of ground beef from non-stimulated carcasses.*

A 22-C incubation temperature was used for monitoring the APC of the electrically-stimulated and control samples of ground beef stored at 5 C (Fig. 3). The initial bacterial count of ground beef from ES carcasses or the control was as low as 8.0 - 9.0 × 10^3/g. This level was lower by 100 to 1000-fold than currently proposed bacteriological standards for ground beef (*8,9,18*). As can be seen (Fig. 3), the bacterial population of the samples from ES carcasses had a lag phase of 3 days as compared to 1 day for the control bacterial population. It is probable that the electrical stimulation may have impaired the bacterial cell metabolism (*17*) and causing an extended lag phase. A significant difference ($P < 0.05$)

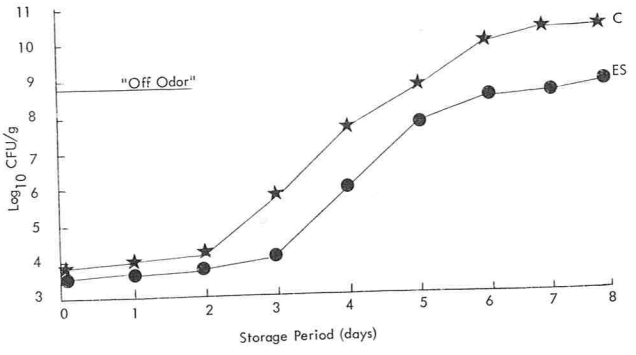

Figure 3. *The growth of the endogenous flora of ground beef from electrically stimulated carcasses stored at 5 C. (C = control; ES = electrically stimulated; CFU = colony forming unit).*

was found among the APC of the samples from ES carcasses and the control on the third, fifth, sixth, and seventh days of storage. Between the third and fifth day of storage, the bacterial population formed 12.2 generations in samples from ES carcasses while only 9.9 in the control.

The shelf life (i.e. time to "off odor") of a refrigerated product in general and of a meat product in particular is determined among other reasons by bacterial growth. Any processing or preservation procedure that will induce a long lag phase and will slow the growth rate of the bacterial population will also extend the shelf life of the product. "Off odors" were detected after 4-5 days in the control samples but after 7-8 days in the samples from ES carcasses with a corresponding bacterial count of 8×10^8/g ground beef. Electrical stimulation extended the shelf life of the ground beef by 3 days. The rapid growth of the bacterial population of the electrically-stimulated samples did not compensate for the 3-day lag phase (Fig. 3) resulting in a lower population level than that of the control samples (up to the eighth day of storage). This low population level did not impair the organoleptic properties of the product from ES carcasses while the control samples spoiled after 4-5 days.

At spoilage, 30 colonies were picked from PCA plates of each of the ES samples and the control. Most colonies (22-23 isolates) from both samples had the properties described in Table 1. These properties are characteristic of the nonpigmented *Pseudomonas* group II (*20*) or *Pseudomonas fragi* (*3*). Two isolates from each of the ES carcasses and the control were gram negative, nonmotile, nonpigmented but had an oxidative reaction in Hugh-Leifson medium. These four isolates were designated *Moraxella*-oxidative in accordance with the description of Thornley (*22*). A gram positive, catalase-positive, benzidine- and hydrogen sulfide-negative coccobacillus organism was isolated from the control sample; it was identical to a culture of *Microbacterium thermosphactum* (*4,15*). These results show that electrical stimulation did not affect the nature of the spoilage flora of ground beef. The nonpigmented *Pseudomonas* predominated in the flora at spoilage.

C. perfringens, Salmonella and coliform organisms were not detected in the samples from either ES carcasses or the control. *S. aureus* was detected at a level as low as 10 cells/g of ground beef. This level coincides with proposed bacteriological standards (*8,9,18*). These results point out one of the advantages of electrical stimulation and on-the-rail hot-boning in reducing sanitation problems especially preventing bacterial recontamination of the product from surfaces as with on-the-table boning.

The study showed that both the samples from ES carcasses and the control were free of pathogens except *S. aureus.* The sample from ES carcasses had a prolonged shelf life as compared with the control with no unique problem concerning the nature of the spoilage flora.

TABLE 1. *Properties common to most[a] of the microoganisms isolated from spoiled ground beef from electrically stimulated and non-stimulated carcasses.*

Character	Result
Gram reaction	-
Morphology	Short rods
Motility	+
Oxidase	+
Oxidation/fermentation test (glucose)	Oxidative
Pigmentation	-
Reducing compounds from gluconate	+
Starch hydrolysis	-
Growth at 4 C	+
Growth at 41 C	-

[a]Number of isolates 55 (23 and 22 isolates from ground beef from ES carcasses and the control respectively).

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REFERENCES

1. Anonymous, 1977. Per capita ground beef consumption. Meat Plant Magazine, Newsletter edition 38 (12).
2. Ayres, J. C. 1960. The relationship of organisms of the genus *Pseudomonas* to the spoilage of meat, poultry and eggs. J. Appl. Bacteriol. 23:471-486.
3. Davidson, C. M., M. J. Dowdell, and R. G. Board. 1973. Properties of gram negative aerobes isolated from meats. J. Food Sci. 38:303-305.
4. Davidson, C. M., P. Mobbs, and J. M. Stubbs. 1968. Some morphological and physiological properties of *Microbacterium thermosphactum*. J. Appl. Bacteriol. 31:551-559.
5. Deibel, R. H., and J. B. Evans. 1960. Modified benzidine test for the detection of cytochrome containing respiratory systems in microorganisms. J. Bacteriol. 79:356-360.
6. Duitschaever, C. L., D. H. Bullock, and D. R. Arnott. 1977. Bacteriological evaluation of retail ground beef, frozen beef patties and cooked hamburger. J. Food Prot. 40:378-381.
7. Food and Drug Administration. 1976. Bacteriological analytical manual for foods. Association of Official Analytical Chemists, Washington D.C.
8. Geopfert, J. M. 1976. The aerobic plate count, coliform and *Escherichia coli* content of raw ground beef at the retail level. J. Milk Food Technol. 39:175-178.
9. Green, S. I. 1976. Microbiological aspects of quality control. Food Man. p. 55 & 71, July, 1976.
10. Haynes, W. C. 1951. *Pseudomonas aeruginosa* - its characterization and identification. J. Gen. Microbiol. 5:939-950.
11. Herbert, R. A. 1975. Microbial ecology of fish spoilage. Proc. Biochem. December 1975:9-11.
12. Hugh, R., and E. Leifson. 1954. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various Gram negative bacteria. J. Bacteriol. 66:24-26.
13. King, E. O., M. K. Ward, and D. E. Raney. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 54:301-308.
14. Kovacs, W. 1957. Identification of *Pseudomonas pyocyanea* by the oxidase reaction. Nature (London) 178:703.
15. McLean, R. A., and W. L. Sulzbacher. 1953. *Microbacterium thermosphactum* spec. nov. a nonheat resistant bacterium from fresh pork sausage. J. Bacteriol. 65:428-433.
16. Mossel, D. A. A. 1968. Microbiological qualities. J. Food Technol. 3:401-421.

17. Munnert, H., and D. Gradman. 1976. Voltage dependent potassium fluxes and the significance of action potentials in Acetabularia. Biochim. Biophys. Acta 443:443-450.
18. Pivnick, H., J. E. Erdman, D. Collins-Thompson, and G. Roberts. 1975. Microbiological standards for meat in Canada. J. Milk Food Technol. 38:638.
19. Shewan, J. M. 1963. The differentiation of certain bacteria frequently encountered in marine environments. pp. 499-521. In: C. H. Oppenheimer (ed.) Marine microbiology. Charles C. Thomas, Springfield, Il.
20. Shewan, J. M., G. Hobbs, and W. Hodgkiss. 1960. A determinative scheme for the identification of certain genera of gram-negative bacteria, with special reference to the *Pseudomonadaceae*. J. Appl. Bacteriol. 23:379-390.
21. Steel, R. G. D. 1960. Principles and procedures of statistics. McGraw-Hill Book Company, New York, N.Y.
22. Thornley, M. J. 1967. A taxonomic study of *Acinetobacter* and related genera. J. Gen. Microbiol. 49:211-257.

International Dairy Federation Representative Report 1978

Harold Wainess

The International Dairy Federation is a technical organization with membership from 31 countries throughout the world. It was established in 1903 and has its General Secretariat in Brussels, Belgium. As yet the U.S.A. has not officially joined the organization although a concentrated effort is underway to bring the U.S.A. into the ranks.

In each of the member countries, a National Committee has been created to represent as many national dairy interests as possible, including milk production and processing, engineering, technology, sanitation, economics, education, research and involved governmental groups.

Expenses are derived from membership fees paid by each National Committee who also appoint delegates to the IDF Annual Meetings and to their working groups of experts (technical committees). Many of these committees are similar to those established by the IAMFES and work on many of the same technical subjects.

Each year an annual session is held at which most of the groups of Experts report on their activities during the year. When the results merit these reports are published as Standards, recommended procedures, technical guides, scientific reports, topical monographs, research reports, etc. To date, 184 Documents and Standards of interest to dairy technologists have been published.

Seminars and symposia on current subjects are held whenever the need arises and every four years the IDF sponsors the International Dairy Congress, which took place this year in Paris in conjunction with the annual meetings.

There are 95 Groups of Experts who hold meetings between successive annual sessions. A considerable amount of the work done is by correspondence and a number of U.S.A. dairy technologists have been invited to contribute to the deliberations of these groups.

IDF has close contacts with other international bodies, including FAO, AOAC, ISO, ADSA and IAMFES.

Officials are elected for a normal four-year term and the current IDF president is Mr. K. G. Savage of Canada and the president of the Commission of Studies, Dr. H. Kay of Germany. In addition they have a permanent secretary general and staff, Mr. P. F. J. Staal in Brussels.

Some ideas of the scope of their work and its similarities to IAMFES can be gleaned from the following selection of the 91 topics discussed during the 1978 Annual Meeting in Paris.

- Quality of milk as a basis for payment
- Bacteriological quality of raw milk
- Standard method for somatic cell counting in milk
- Aseptic collection of milk samples—Isolation and identification of mastitogenic organisms — recommended procedures
- Chemical residues
- Heavy metals
- Lipolysis
- Code of practice for the hygienic production of milk
- Machine milking
- Standardization of refrigerated farm milk tanks
- Code of practice for the design and construction of milk collection tankers
- Recombination of milk and milk products — technology and engineering aspects
- Methods for improving the quality of heat treated milk
- Technical aspects of packaging milk and milk products
- General code of hygienic practice for the dairy industry
- Automated cleaning and disinfection of milk processing plants
- Control of water and waste waters in the dairy industry
- Membrane processes and resulting products
- Symbols used by dairy equipment manufacturers
- Prevention of noise
- Air pollution problems
- Energy use and conservation
- Sensory evaluation of milk and milk products
- Hygienic requirements in standards of identity
- Corrosion in dairying
- Water content of milk and milk products
- Pesticide residues
- Heavy metals and other elements in dairy products
- Protein content of milk
- Instrumental methods
- Behaviour of pathogens in cheese
- Flavour in milk and milk products
- Heat resistant proteinases in milk
- Effect of heat treatment on the physico-chemical properties of milk
- Cold storage of bulk milk and technological problems

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Comparison of Bacterial Species Isolated from Ground Beef, Textured Soy Protein and Ground Beef Extended with Textured Soy Protein [1,2]

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ABSTRACT

A survey of the bacterial species of public health importance which could be isolated from ground beef (GB), textured soy protein (TSP) and ground beef extended with TSP (SGB) after 3 and 10 days of storage at 4 C was conducted. *Escherichia coli* was the most frequent gram-negative isolate from GB and SGB. Few gram-negative organisms were found in TSP. *Clostridium perfringens* was the most frequent gram-positive isolate from GB and SGB while *Bacillus* sp. was isolated most frequently from TSP. *Salmonella enteriditis* ser. *worthington* was isolated from GB and TSP. These products contained a wide variety of microorganisms, some of which might result in a food-associated infection or intoxication. However, if properly handled and cooked before consumption, these products should present few public health hazards.

Ground beef extended with soy protein has increased in popularity in recent years, especially in mass-feeding institutional foodservice systems. Use of soy protein for meat extension in the Class A government subsidized school lunch program was approved in 1971 by the U.S. Department of Agriculture. Since then, increased costs of red meat items have made meat products extended with soy protein economically attractive to hospitals and other institutional foodservices.

Foodborne disease outbreaks are often associated with mass-feeding systems such as those found in cafeterias, restaurants and hospitals (*15-17*). A review (*18*) of epidemiological data pertaining to ground beef indicated that this product was involved in 3.6% of the identified foodborne disease outbreaks from 1967 through 1973. Data pertaining to the place where these ground beef items were mishandled were available in 27 of the 65 outbreaks. Mishandling in foodservice establishments was responsible for 67% of the outbreaks, while mishandling in private homes was implicated in 30% of the outbreaks and mishandling in the food processing industry accounted for 3% of the outbreaks.

Since ground beef and ground beef extended with soy protein are frequently used in institutional foodservices, the bacterial species which could cause or have been associated with foodborne illness or infection were investigated. Additionally, investigations were done to determine if these organisms were present after 7 days of storage at 4 C.

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MATERIALS AND METHODS

Samples

Duplicate units from 31 production lots of ground beef (GB), textured soy protein (TSP), and the corresponding lots manufactured into ground beef extended with TSP (SGB) were obtained from a production facility in the San Francisco Bay area. Units were held at 4 ± 1 C and analyzed after 3 and 10 days of storage from the date of production.

Sample preparation

A 25-g portion of each unit was weighed into a sterile one-liter blender. Following addition of 225 ml of sterile phosphate buffered water, the sample was blended at high speed for 3 min.

Isolation and identification of aerobic bacteria

Gram-negative and gram-positive organisms were isolated and identified by use of the methods described by Guthertz et al. (6). No attempt was made to quantitate the numbers of each species isolated. Results are reported as the percent of samples positive for each bacterial species isolated.

Isolation of Clostridium perfringens

C. perfringens was isolated and identified by using the procedures of Fruin et al. (*4*).

RESULTS

The aerobic gram-negative organisms isolated from each product after 3 and 10 days of storage are presented in Table 1. The most frequent isolates from the 3-day GB, in order of occurrence, were *Escherichia coli, Enterobacter cloacae, Citrobacter freundii, Acinetobacter calcoaceticus* var. *anitratum, Klebsiella pneumoniae, Aeromonas hydrophilia, Proteus vulgaris,* and *Enterobacter hafniae. Arizona hinshawii* (*Salmonella arizonae*) was isolated from one sample. After the 10-day

TABLE 1. *Gram-negative organisms*[a] *isolated from ground beef, textured soy protein (TSP) and TSP extended ground beef.*

Organisms	Ground beef		Textured soy protein		TSP extended ground beef	
	3 Day	10 Day	3 Day	10 Day	3 Day	10 Day
	P[b]	P	P	P	P	P
Acinetobacter calcoaceticus var. *anitratum*	35.5	33.3	9.7	3.3	41.9	46.7
Achromobacter xylosoxidans	-	3.3	-	-	-	-
Aeromonas hydrophilia	19.4	10.0	6.5	6.7	6.5	10.0
Alcaligenes sp.	3.2	3.3	-	-	9.7	-
Arizona hinshawii	3.2	-	-	-	3.2	-
Chromobacterium typhiflavum	-	3.3	-	3.3	-	-
Citrobacter diversus	-	3.3	-	-	-	-
Citrobacter freundii	58.1	43.3	9.7	10.0	32.3	36.7
Citrobacter sp.	3.2	-	-	-	-	-
Enterobacter aerogenes	3.2	-	-	-	-	-
Enterobacter agglomerans	-	-	3.2	3.3	3.2	-
Enterobacter cloacae	64.5	40.0	6.5	3.3	48.4	30.0
Enterobacter hafniae	12.9	66.7	6.5	16.7	12.9	63.3
Escherichia coli	83.9	86.7	9.7	10.0	100.0	93.3
Klebsiella pneumoniae	22.6	13.3	-	3.3	51.6	36.7
Pasteurella multocida	-	-	-	-	3.2	-
Proteus mirabilis	6.5	3.3	-	3.3	-	-
Proteus morganii	3.2	3.3	-	-	-	-
Proteus vulgaris	16.1	3.3	3.2	-	3.2	10.0
Pseudomonas aeruginosa	6.5	-	-	3.3	-	-
Pseudomonas fluorescens	9.7	3.3	6.5	6.7	6.5	3.3
Pseudomonas fluorescens grp.	3.2	6.7	-	13.3	6.5	6.7
Pseudomonas maltophilia	-	-	3.2	-	-	-
Pseudomonas putida	3.2	3.3	-	-	3.2	-
Pseudomonas sp.	9.7	3.3	3.2	3.3	29.0	16.7
Salmonella enteriditis ser. *worthington*	-	3.3	3.2	3.3	-	-
Serratia liquefaciens	6.5	33.3	6.5	16.7	6.5	20.0
Serratia marcescens	-	-	-	3.3	-	-
Yersinia enterocolitica	-	-	-	3.3	-	-

[a]Genus and species names are from Analytical Profile Index, Analytab Products, Inc., Plainview, NY.
[b]P = Percent of samples positive.

storage at 4 C, *E. coli* was the most frequently isolated gram-negative organism followed by *E. hafniae, C. freundii, E. cloacae, A. calcoaceticus* var. *anitratum, Serratia liquefaciens, K. pneumoniae* and *A. hydrophilia. Salmonella enteriditis* ser. *worthington* was isolated from one sample of the 10-day GB.

The 3-day TSP contained fewer gram-negative organisms than the GB. Isolates occurring most frequently included *E. coli, C. freundii,* and *A. calcoaceticus* var. *anitratum* all of which occurred in less than 10% of the samples. *S. enteriditis* ser. *worthington* was isolated from one sample of TSP after 3 days of storage. Gram-negative organisms were isolated more frequently from the 10-day TSP samples. *E. hafniae* and *S. liquefaciens* were isolated most often followed by *Pseudomonas fluorescens* grp., *E. coli,* and *C. freundii. S. enteriditis* ser. *worthington* was isolated from one sample (same lots as the 3-day isolate). One isolate of *Yersinia enterocolitica* was found in the 10-day TSP.

Fewer types of organisms were isolated from SGB than GB. After the 3-day storage, *E. coli* was the most frequent gram-negative organism isolated followed by *K. pneumoniae, E. cloacae, A. calcoaceticus* var. *anitratum, C. freundii, Pseudomonas* sp., and *E. hafniae. A. hinshawii* (*S. arizonae*) was isolated from one sample. After 10 days of refrigeration, there were even fewer species of organisms found; however, substantial increases in the occurrence of some species were observed. *E. coli* remained the most frequent isolate with *E. hafniae, A. calcoaceticus* var. *anitratum, K. pneumoniae, C. freundii, E. cloacae, S. liquefaciens, Pseudomonas* sp., *A. hydrophilia* and *P. vulgaris* following in order of occurrence.

Gram-positive organisms of public health importance isolated from GB, TSP and SGB are shown in Table 2. The most frequent isolates from GB at 3 days of storage were *Clostridium perfringens, Streptococcus faecalis* var. *liquefaciens, Staphylococcus epidermidis, Staphylococcus aureus,* and *Bacillus cereus.* After 10 days of storage at 4 C, there was an overall reduction in the number of gram-positive isolates. *C. perfringens* remained the most frequently isolated organism with 73% of the samples positive. The incidence of *S. aureus* and *S. faecalis* increased while the incidence of most other isolates decreased.

The 3-day TSP produced few gram-positive isolates with *Bacillus* sp., *B. cereus* and *C. perfringens* being isolated most frequently. Following the 10-day storage period the percent of samples positive for *C. perfringens, B. cereus,* and *S. faecalis* var. *liquefaciens* were reduced. The percent recovery of all other gram-positive organisms was increased.

The SGB after 3 days of storage contained the largest variety of gram-positive isolates and in most instances

TABLE 2. *Gram-positive organisms isolated from ground beef, textured soy protein (TSP) and TSP extended ground beef.*

Organisms	Ground beef		Textured soy protein		TSP extended ground beef	
	3 Day	10 Day	3 Day	10 Day	3 Day	10 Day
	P[a]	P	P	P	P	P
Bacillus cereus	45.2	-	54.8	10.0	54.8	3.3
Bacillus sp.	45.2	43.3	67.7	73.3	45.2	53.3
Clostridium perfringens	96.8	73.3	25.8	20.0	96.8	90.0
Corynebacterium sp.	3.2	3.3	-	3.3	-	-
Diphtheroids	35.5	36.7	16.1	20.0	54.8	40.0
Erysipelothrix sp.	-	6.7	-	-	-	3.3
Micrococcus sp.	41.9	50.0	12.9	30.0	61.3	50.0
Staphylococcus aureus	48.4	30.0	9.7	10.0	51.6	40.0
Staphylococcus epidermidis	58.8	56.7	12.9	26.7	58.1	53.3
Streptococcus anginosus	-	-	-	3.3	3.2	6.7
Streptococcus avium	-	-	-	-	3.2	-
Streptococcus casseliflavus	-	-	-	-	-	3.3
Streptococcus cremoris	-	-	-	3.3	-	3.3
Streptococcus durans	9.7	3.3	-	-	3.2	-
Streptococcus faecalis	35.5	36.7	6.5	3.3	38.7	23.3
Streptococcus faecalis var. *liquefaciens*	64.5	43.3	19.4	13.3	54.8	56.7
Streptococcus faecium	6.5	-	6.5	3.3	3.2	20.0
Streptococcus lactis	-	-	-	3.3	3.2	3.3
Streptococcus sanguis	9.7	3.3	3.2	10.0	16.1	6.7
Streptococcus sp.	35.5	23.3	-	10.0	29.0	36.7

[a]P - Percent of samples positive.

the highest number of positive samples for all products tested. *C. perfringens* remained as the most frequently isolated organism (96.8% positive). *Micrococcus* sp., *S. epidermidis, B. cereus,* diphtheroids, *S. faecalis* var. *liquefaciens,* and *S. aureus* were all present in at least 50% of the samples. In the 10-day SGB the previous pattern of isolates was found with few exceptions. However, *B. cereus*, diphtheroids and the *S. aureus* isolations were notably reduced. Several *Streptococcus* sp. and *Bacillus* sp. showed a marked increase in occurrence.

DISCUSSION

A review of the literature revealed only one report where specific aerobic organisms from ground beef and ground beef extended with soy protein were identified (*2*). *Serratia* and *Enterobacter* were the predominant gram-negative genera reported. Gram-positive organisms were not identified.

In the present study, *E. coli* was the isolate found most frequently in the GB and SGB at the 3- and 10-day sampling periods. The 3- and 10-day TSP samples contained few gram-negative organisms with *E. coli, E. hafniae* and *S. liquefaciens* occurring most frequently. There was no indication that addition of soy protein to the ground beef had any stimulatory effect upon any one organism or group of organisms. However, it must be noted that this cannot be directly shown from these data because all organisms present in each sample were not identified, only the morphologically different organisms were examined. Overall the SGB contained fewer species of organisms than the GB. This observation could be due to the extension of the product with TSP. The TSP contained few species of gram-negative organisms; many samples yielded no isolates. Therefore, 20% extension by weight of GB with TSP would result in a product with a reduced microbial load. Finding fewer species of gram-negative organisms present in the SGB after 10 days of storage at 4 C suggests that either the product was not a favorable growth medium or that some bacterial species were overgrown by other organisms more adaptable to psychrotrophic growth conditions. Additionally, methods used to detect the bacterial species present would preclude recovery of injured organisms.

The gram-positive isolates presented a somewhat different pattern than the gram-negative isolates. Overall, the SGB contained a larger variety of gram-positive organisms than either of its two components. *C. perfringens* was found most often in the beef products, while *Bacillus* sp. was the most frequent isolate from the TSP. The incidence of *C. perfringens* from ground meat products in this study is similar to those previously reported (*3, 8*). Studies by Schroder and Busta (*12,13*) and Kokoczka and Stevenson (*7*) have indicated that soy protein extension of ground beef products has variable effects on growth of *C. perfringens*. Further studies (*5, 14, 20*) of the characteristics of *C. perfringens* have shown that this organism has limited ability to survive refrigerated storage. Results of this study agree with other reports that survival of *C. perfringens* is reduced with refrigerated storage. However, a reduction of 23.5% in the frequency of positive samples was found in GB while only a 6.8% reduction was shown in SGB after 10 days of refrigerated storage. Additionally, a reduction of 5.8% was noted in the TSP after the same storage period. Apparently, addition of soy protein provides some cryoprotection for *C. perfringens*. If this is true, food handlers should be aware of the increased potential of these products to cause food poisoning.

Normal healthy individuals are resistant to subinfectious doses of most bacteria. Therefore, ingestion of cooked or partially cooked ground beef items, with their

concomitant microflora, constitutes minimum risk for most of this population. However, if this food item is used in institutional foodservice systems, especially hospitals and convalescent homes, the potential for food associated infection is increased. Cross-contamination of other food items, particularly those served uncooked, could lead to outbreaks of foodborne infection. In hospitals and convalescent homes, cross-contamination of other food items through mishandling could have critical or even fatal consequences. Persons entering health care facilities are usually debilitated which results in a lowered response to bacterial insult. Therefore, foods containing organisms not commonly associated with foodborne illness can become vehicles capable of transmitting infectious agents.

In addition to isolation of documented food poisoning organisms, numerous organisms capable of causing opportunistic infection were recovered (Tables 1 and 2). A number of these organisms have been identified as the etiologic agent in infections primarily from compromised individuals.

A. hydrophilia, C. freundii, E. aerogenes, E. cloacae, K. pneumoniae, P. mirabilis and *S. marcescens* have been documented as the causative organisms in urinary tract infections (*9,19*). Additionally, *A. hydrophilia* has been reported as the etiologic agent in severe gastroenteritis, superficial wound infections and bacteremia (*9,11*). *A. calcoaceticus* var. *anitratum, Alcaligenes* sp., *E. agglomerans* and *C. freundii* have been reported in patients with septicemia (*19*). Bacterial endocarditis resulting from *S. marcescens* and *P. aeruginosa* has been documented (*19*). *K. pneumoniae* is a frequent agent of nosocomial infection, and has been demonstrated in respiratory infections and cases of secondary septicemia (*19*). *P. aeruginosa* has been associated with burn wound infections as well as eye and wound infections, pneumonitis, superficial skin infections and severe enterocolitis in newborns (*19*). *Y. enterocolitica,* an organism which grows well under psychrotrophic conditions, has been shown to cause severe enterocolitis (*1*). In a recent report, *Chromobacterium typhiflavum* was isolated in a case of bacteremia (*10*). Although many opportunistic infections have been reported in the literature, it must be noted that ground beef has not been implicated as the source of this type of infection in physiologically stressed individuals.

The presence of these organisms in raw meat items does not indicate that the products are unsafe for consumption. However, it does indicate that food handlers and microbiologists must be aware of the potential these products have for cross-contaminating work surfaces, equipment, workers, and other foods which are served uncooked.

REFERENCES

1. Bisset, M. J. 1976. *Yersinia enterocolitica* isolates from humans in California, 1968-1975. J. Clin. Microbiol. 4:137-144.
2. Craven, S. E., and A. J. Mercuri. 1977. Total aerobic and coliform counts in beef-soy and chicken-soy during refrigerated storage. J. Food Prot. 40:112-115.
3. Emswiler, B. S., C. J. Pierson, and A. W. Kotula. 1976. Bacteriological quality and shelf life of ground beef. Appl. Environ. Microbiol. 31:826-830.
4. Fruin, J. T., J. F. Foster, and L. S. Guthertz. 1978. Comparison of recovery methods for *Clostridium perfringens* from selected foods. J. Food Quality 1:135-140.
5. Fruin, J. T. 1974. Estimations of populations of *Clostridium perfringens* in a meat medium held at low temperatures. Ph.D. thesis, Purdue University, West Lafayette, IN.
6. Guthertz, L. S., J. T. Fruin, D. Spicer, and J. L. Fowler. 1976. Microbiology of fresh comminuted turkey meat. J. Milk Food Technol. 39:823-829.
7. Kokoczka, P. J., and K. E. Stevenson. 1976. Effect of cottonseed and soy products on the growth of *Clostridium perfringens*. J. Food Sci. 41:1360-1362.
8. Ladiges, W. C., J. F. Foster, and W. M. Ganz. 1974. Incidence and viability of *Clostridium perfringens* in ground beef. J. Milk Food Technol. 37:622-633.
9. McCracken, A. W., and R. Barkley. 1972. Isolation of *Aeromonas* species from clinical sources. J. Clin. Pathol. 25:970-975.
10. Pien, F. D. 1977. Group VE-2 (*Chromobacterium typhiflavum*) bacteremia. J. Clin. Microbiol. 6:435-436.
11. Rosner, R. 1964. *Aeromonas hydrophilia* as the etiologic agent in a case of severe gastroenteritis. Am. J. Clin. Pathol. 42:402-404.
12. Schroder, D. J., and F. F. Busta. 1971. Growth of *C. perfringens* in a meat loaf with and without added soybean protein. J. Milk Food Technol. 34:215-217.
13. Schroder, D. J., and F. F. Busta. 1973. Effect of synthetic meat components on growth of *C. perfringens*. J. Milk Food Technol. 36:189-193.
14. Strong, D. H., and N. M. Ripp. 1967. Effect of cookery and holding on hams and turkey rolls contaminated with *Clostridium perfringens*. Appl. Microbiol. 15:1172-1177.
15. U.S. Department of Health, Education and Welfare, Public Health Service. Foodborne and Waterborne Disease Outbreaks. Annual Summary 1976.
16. U.S. Department of Health, Education and Welfare, Public Health Service. Foodborne and Waterborne Disease Outbreaks. Annual Summary 1975.
17. U.S. Department of Health, Education and Welfare, Public Health Service. Foodborne and Waterborne Disease Outbreaks. Annual Summary 1974.
18. U.S. Department of Health, Education and Welfare, Public Health Service. 1975. Current trends - microbiologic standards for raw ground beef, cold cuts and frankfurters. Morbidity and Mortality 24:229-230.
19. von Graevenitz, A. 1977. The role of opportunistic bacteria in human disease. Ann. Rev. Microbiol. 31:447-471.
20. Woodburn, M., and C. H. Kim. 1966. Survival of *Clostridium perfringens* during baking and holding of turkey stuffing. Appl. Microbiol. 14:914-920.

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Significance of Abuse Chemical Contamination of Returnable Dairy Containers: Hazard Assessment

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ABSTRACT

Certain pesticide residues reabsorbed into milk (or water) contents when stored in LEXAN® resin and polyethylene plastic returnable milk containers that had been exposed to diluted pesticide products, washed, and subsequently filled. The significance of these laboratory test results is comprehensively discussed by attempting to characterize the nature of exposure to pesticide residues from a washed returnable dairy container, as opposed to exposure to milk containing pesticide residues of non-container origin. Laboratory findings are compared to actual public use experience with returnable plastic dairy containers. A hazard assessment is presented, which includes a comparison of quantities of pesticide residues found extracting with existing Federal milk tolerance standards, food tolerance standards, unavoidable contaminant food additive regulations, World Health Organization and Safe Drinking Water Committee acceptable daily intake values. The probability of purchase of milk that is contaminated with pesticide residues, where the washed returnable dairy container is the source, is presented.

Two previous publications by Gasaway (*7,8*) reported results of exposure of dilute pesticide products in returnable dairy containers under controlled conditions. Storage of dilute pesticide products in LEXAN resin containers resulted in approximately a 30% incompatibility factor, while high density polyethylene (HDPE) plastic and glass showed good compatibility. In-line contaminant detector testing of washed containers found compatible during pesticide product storage studies revealed that the device did not always function as a fail-safe device. Organoleptic testing was shown to be more effective in isolating containers that had off-odors than the in-line contaminant detector. Milk or water extraction studies with pesticide-exposed and washed returnable dairy containers resulted in isolating those products which are responsible for pesticide extraction.

The purposes of this publication are to: (*a*) report results of rewashing experiments of returnable dairy containers previously exposed to pesticides, (*b*) characterize the nature of exposure to pesticide residues from a washed returnable dairy container, (*c*) report the public use experience of the HDPE plastic returnable container in Canada and the LEXAN resin containers at Garden State Farms from the standpoint of abuse chemical exposure, (*d*) assess the hazard of exposure to pesticide residues from a washed returnable dairy container, and (*e*) report the results of three independent assessments of risk from exposure to pesticide residues from a washed dairy container by a medically qualified toxicologist, a statistician and the Pittsburgh Poison Control Center, Pittsburgh, Pennsylvania.

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MATERIALS AND METHODS

Rewashing study

After exposure to selected pesticides, washing, and extraction analyses conducted at the Wisconsin Alumni Research Foundation Institute (WARF), those LEXAN resin containers found positive for extracted pesticides (Table 1) were returned to the Garden State Farms dairy plant for a second bottle washing with PBS detergent (Pennwalt Chemical Company). Following the second washing, extraction studies were repeated at WARF Institute, Inc. (Table 2). For one product, Ortho-Klor 74, a third washing and extraction scheme was undertaken (Table 3). Following the third wash and extraction, the Ortho-Klor 74 container was rinsed to remove milk residues with two volumes of cold tap water, and a section of the side wall of the container (0.73 g) was removed for chlordane analysis. The LEXAN resin sample was dissolved in methylene chloride (150 ml). Separation of the dissolved LEXAN resin from chlordane was accomplished using a Florisil column. The chlordane was eluted from the column by the three washings with 5% by volume diethyl ether in petroleum ether (200 ml). The three ether elutions were evaporated to 2 ml. Analysis was accomplished by direct injection using a Hewlett Packard 5710A GC equipped with a linear EC detector. The column was 1.8 m × 4.0-mm glass packed with 3% OVI on 80/100 mesh Gas Chrome Q. The injector, column and detector temperatures were 250, 190 and 300 C, respectively.

RESULTS AND DISCUSSION

Rewashing study

The purpose of conducting repetitive washing studies of LEXAN resin containers previously exposed to dilute

pesticides was to determine if one washing satisfactorily removed all residues. For those LEXAN resin containers exposed to 3 days of dilute pesticides, seven product cases resulted in residue extraction after washing and milk, or water, extraction (Table 1). For those LEXAN resin containers exposed to 10 days of dilute pesticides, six product cases resulted in residue extraction after washing and milk or water extraction (Table 1.)

After a second bottle washing, using PBS detergent, pesticide extraction was noted for two treated containers (Ortho-Klor 74 and 3-D Weedone) for both periods of storage (3 and 10 days exposure) (Table 2). For Ortho-Klor 74, pesticide extraction was confirmed after a third washing operation for the container (Table 3). To obtain a total chlordane analysis of the container after three commercial washings, a sample of the container wall was removed and analyzed. The total chlordane content of the sample was related to the entire container weight. While the analysis did not confirm the presence of chlordane (< 0.77 μg), it was assumed that the detection level amount was present. Chlordane (0.77 μg), if present, would result in 0.0004 ppm in milk.

Assessment of pesticide residue exposure from returnable containers

The very nature of exposure to pesticide residues from washed dairy containers is different than other types of exposures to chemicals. From an acute standpoint, exposure to pesticides may have negative implications during manufacture, transportation, application and storage. An acute accident with pesticides is typically a singular instance, although there may be specific pesticides implicated in more than one accident. The quantities involved and/or the toxicity of these economic poisons usually lead to a medical emergency. Should container misuse occur, the possible extraction of pesticide residues into milk contents is generally a singular episode, however, the concentrations of pesticide ingredients involved are typically near or below the allowable chronic levels of exposure.

There have been many studies summarized by Hayes (*9*) directed at characterizing the insipient nature of chronic pesticide exposure. The quantities of pesticides are small, and result from application to livestock and foodstuffs or result from environmental spraying that leads to contamination of air, water and food. The exposure to pesticides from a washed dairy container is not a chronic problem because that would imply repeated dosing, but the levels of exposure from the washed dairy container are of the type found in chronic situations.

TABLE 1. *Milk (water) extraction test results for Lexan resin containers (ppm) - A summary.*[c]

Product and manufacturer		Pesticide exposure time			
		3 Days		10 Days	
		Milk extraction time		Milk extraction time	
Ingredients (%)	M/W[a]	3 Days	10 Days	3 Days	10 Days
Ammate-X, Bonide					
Ammonium sulfamate (95)	W	0.4	1.1	< 0.2	< 0.2
3-D Weedone, Amchem					
Butoxyethanol ester of 2,4-D(17.7)	M	0.44	0.48	0.59	0.48
Butoxyethanol ester of Silvex (8.3)	M	0.10	0.14	0.31	0.10
Dicamba (1.5)	M	< 0.05	< 0.05	< 0.05	< 0.05
Ortho-Klor 74, Ortho					
Chlordane (74)	M	0.55 (AVG)[b]	1.30 (AVG)	0.66 (AVG)	2.06 (AVG)
6E Oil Spray, Pratt					
Petroleum Oil (96)	W	< 2.0	< 2.0	< 2.0	4.0
Ethion (2)	W	< 0.0015	< 0.0015	< 0.0015	< 0.0015
Rose/flower dust/spray, Pratt					
Carbaryl (3)	M	< 0.05	< 0.05	< 0.05	< 0.05
Malathion (4)	M	< 0.01	< 0.01	< 0.01	< 0.01
Folpet (5)	M	0.017	0.091	0.013	0.015
Kelthane (1.5)	M	0.047	0.044	< 0.01	0.02
Imidan 12.5WP, Pratt					
Phosmet (12.5)	M	< 0.01	0.11	0.13	0.09
Multi-purpose garden dust or spray, Science					
Methoxychlor (5)	M	0.042	0.072	0.025	0.121
Malathion (5)	M	< 0.01	< 0.01	< 0.01	< 0.01
Captan (5)	M	< 0.005	< 0.005	< 0.005	0.009
Methoxychlor 50 WP, Science					
Methoxychlor (50)	M	< 0.005	0.19	< 0.005	< 0.005
Totals					
Product cases showing extraction		7		6	
Product cases not showing extraction (not listed above)		44		44	
Total number of ingredients found extracting		9		9	
Chemically different ingredients found extracting		8		9	

[a] = "M" for milk and "W" for water used as extraction medium.
[b] = Average of several chlordane analyses.
[c] = Pesticide ingredients found extracting after first commercial wash.

TABLE 2. *Milk (water) extraction test results for Lexan resin containers (ppm) - second commercial wash.*

Product and manufacturer		Pesticide exposure time: 3 Days	Pesticide exposure time: 10 Days
		Extraction time	
Ingredients (%)	M/W[a]	10 Days	10 Days
Ammate-X, Bonide			
Ammonium sulfamate (95)	W	< 0.2	-[b]
3-D Weedone, Amchem			
Butoxyethanol ester of 2,4-D (17.7)	M	0.72	0.68
Butoxyethanol ester of Silvex (8.3)	M	0.36	0.08
Dicamba (1.5)	-	-	-
Ortho-Klor 74, Ortho			
Chlordane (74)	M	0.015	0.013
6E Oil Spray, Pratt			
Petroleum Oil (96)	W	-	< 2.0
Ethion (2)	-	-	-
Rose/Flower Dust/Spray, Pratt			
Carbaryl (3)	M	-	-
Malathion (4)	M	-	-
Folpet (5)	M	< 0.005	< 0.005
Kelthane (1.5)	M	< 0.01	< 0.01
Imidan 12.5WP, Pratt			
Phosmet (12.5)	M	< 0.01	< 0.01
Multi-Purpose Garden Dust or Spray, Science			
Methoxychlor (5)	M	< 0.005	< 0.005
Malathion (5)	-	-	-
Captan (5)	M	-	< 0.005
Methoxychlor 50 WP, Science			
Methoxychlor (50)	M	< 0.005	-
Totals			
Product cases showing extraction After second wash		2	2
Product cases not showing extraction After second wash		5	4
Total number of ingredients found extracting After second wash		3	3
Chemically different ingredients found Extracting After second wash		3	3

[a] ="M" for milk and "W" for water used as extraction medium.
[b] = Not tested for extraction in second wash scheme because not found to extract in first wash scheme.

TABLE 3. *Milk extraction test results for Lexan resin containers (ppm) - third commercial wash.*

Product and manufacturer		Pesticide exposure time: 3 Days	Pesticide exposure time: 10 Days
		Extraction time	
Ingredients (%)	M[a]	10 Days	10 Days
Ortho-Klor 74, Ortho			
Chlordane (74)	M	0.0125	0.010
Totals			
Product cases showing extraction After third wash		1	1
Product cases not showing extraction After third wash[b]		0	0
Total number of ingredients found extracting After third wash		1	1
Chemically different ingredients found Extracting after third wash		1	1

[a] ="M" for milk used as extraction medium.
[b] =3-D Weedone, Amchem, showed positive milk extraction after second wash, but was not tested further.

Hence, exposure to pesticide residues, if misuse of a container takes place, must be described as a singular episode at chronic levels in the case of the washed returnable dairy container. While illnesses have been reported from consumption of fluid dairy production returnable containers (*2,13*), there has not been a confirmed case of illness where a returnable container was shown to be the source of illness. As regards pesticides specifically, the most significant group of chemicals toxicologically, there are no known instances where pesticide residues have been found in fluid dairy products where the container is the source.

It is interesting to explore the instances of pesticide poisonings from containers in general, even though the author's interest is principally the washed container. Hayes (*10*) reviewed category E865 of the *Vital Statistics of the United States,* which reported for 1968 all accidental poisonings by pesticides, fertilizers and plant foods. In that year there were 87 deaths reported, a reduction from 111 deaths in 1961 and 152 deaths in 1956. He reported for 1968 that

> "At least 12 deaths involved pesticides stored in inappropriate, unlabeled containers, mainly soft drink or liquor bottles. Three involved "empty" containers that had been discarded carelessly and later found by children. One 70-year-old man was killed when he drank diazinon from an old whiskey bottle in a bar where he worked, which illustrates that improper placement of pesticides often is associated with other safety violations. Some deaths in children involved errors of other children or adults. A 5-year-old boy not only got milk from a refrigerator for his 2-year-old sister, but added to it some insecticide from a bottle left on the kitchen windowsill. A mother mistook a mixture of parathion, diazinon and chlordane for coffee and gave it to her child with milk. An uncle gave his niece what he supposed was soda pop; when the child started to choke, the man remembered that it might be weed killer borrowed from a friend and tasted the mixture. Both died of acute arsenic poisoning. An amateur exterminator dusted a home with parathion. A 1-year-old boy ingested the poison after exploring a hole in the wall near his crib and died."

The purpose of citing these unfortunate reports is one of validating the most commonly reported route of poisoning from a container, and that is consumption of pesticides directly, which is not the instance of a washed container.

By 1975 the total deaths attributed to accidental poisoning by pesticides, fertilizers and plant foods had been reduced to 30 (*11*). The incompatibility of LEXAN resin with pesticide formulations is quite positive as regards the safe use of the returnable container. It says that, if anything, perhaps fewer long-term storage cases would take place due to the material's inability to contain the product, as contrasted with glass and HDPE plastic containers which have been shown to have good compatibility with stored chemicals.

Use of the LEXAN resin container around the home would not be expected to increase the incidence of container use for pesticide mixing and dilution. All types of containers have been available around the home for years. More single trip containers are available to consumers today than ever before; however, the deaths associated with pesticide poisonings have declined, while reporting systems have improved. If anything, the use of returnable containers for fluid dairy products reduces the absolute number of containers that may be found around the home. The reason for reduction in the number of containers is that the container is returnable

and not disposable. For the LEXAN resin container, which achieves 100 trips on the average from dairy to consumer, the number of containers is reduced by 99 containers, relative to single service containers. In addition, the container's return is encouraged via deposits. When a consumer pays a deposit, there is added incentive to protect the bottle from abuse, enabling its return for deposit value.

By this reasoning the use of the LEXAN resin container for packaging fluid dairy products will decrease the risk of pesticide poisonings by reducing the number and availability of empty disposable dairy containers around the home.

Experience with the HDPE plastic returnable container in Canada

Canada's experience with dairy packaging has been different than the United States. Glass returnable containers were largely replaced in the mid 1960's in Canada as soon as the returnable HDPE plastic container became available. Canada did not have a developed glass industry, so the move to domestic production of blow-molded returnable HDPE plastic containers was a natural complement to an expanding paper container industry.

In the 1960's the decision was made by the Canadian government not to require the use of the in-line contaminant detector with the returnable HDPE plastic container. In the Province of Ontario (*18*), HDPE plastic returnable containers have had as high as 30% market share. In September, 1977, 4.1MM HDPE plastic returnable containers were shipped within the Province of Ontario. During that month, the Minister of Health received 50 complaints from consumers, which represents a frequency of 0.0012% based on container shipments. Of these complaints, 99% were for foreign objects, and 1% was for hydrocarbon contamination. Based on container shipments, hydrocarbon complaints showed a frequency of 0.000012%. On an annualized basis, the hydrocarbon complaints would number six complaints for 49.2MM container shipments. This frequency of complaints was reported as average, and represents a frequency rate without the in-line contaminant detector.

There is still no consideration being given to employing an in-line contaminant detector system in Canada. Furthermore, the opinion has been expressed that the use of a LEXAN resin container shows promise of reducing the foreign object complaint rate by 100% because of the clear nature of the container, and there is the likelihood that incompatibility of chemicals with the container will result in a net reduction of hydrocarbon type complaints.

Sales experience with the LEXAN resin container.

While there have been consumer complaints associated with the use of the HDPE plastic multiuse container (*2,13*), there have not been LEXAN resin container complaints received by Garden State Farms, which has used the LEXAN resin container most extensively. Since complete float conversion from glass returnable containers to LEXAN resin containers, 21.3MM LEXAN resin containers have been distributed. These shipments have been made over a 3-year period, and do not reflect shipments made during the mixed glass/LEXAN resin container float period. This no-complaint record has been established without the use of the in-line contaminant detector as well, based on a waiver authorized by the Department of Health, State of New Jersey, for purposes of evaluation and the United States Public Health Service.

The actual experience in the dairy with the returnable LEXAN resin container must be compared with results obtained from laboratory experiments. The specific consumer record of returnable dairy containers is positive as regards the health and safety record.

Hazard assessment for LEXAN resin returnable dairy containers

The field experience with the LEXAN resin container has been satisfactory with respect to no reported or encountered consumer complaints for chemical contamination or milk off-flavors. A hazard assessment is indicated.

According to Hayes (*9*), hazard has a specific definition for the purposes of hazard assessment.

Hazard = Toxicity × Dosage × Probability of Dosing

Hazard assessment must take into account all three variables. To differentiate between toxicity and hazard, toxicity is the inherent capacity of a chemical to cause harm, while hazard is the risk that under any particular set of circumstances harm will occur. A report of quantities of pesticides found to extract from returnable dairy containers that have been abused purposely in the laboratory, that does not also include a discussion of toxicity and probability of dosing, has only marginal application. Use of incomplete dosage information by Dean (*6*) to conclude that plastic returnable dairy containers "could lead to some serious health problems" suggests emotionalism and should be discounted, since dosage is not equivalent to hazard (*3*) according to the National Science Foundation.

Published milk tolerance action levels, other food tolerance ranges and unavoidable contaminant food additive regulations (*19*) provide bench marks for comparison with the respective amounts of pesticide residues found extracting from the washed LEXAN resin containers (Table 4). While these same guidelines would be appropriate for hazard assessment of the HDPE plastic returnable container following pesticide storage and washing, that assessment was not pursued by this author. The LEXAN resin extraction results are directly compared (Fig. 1) with these aforementioned values, using the highest concentration of pesticide ingredients found during extraction testing (Tables 1-3). For Captan, Phaltan, Ammonium Sulfamate, Imidan, Petroleum Oil and Kelthane there are no reported milk tolerance values for comparison. For these chemicals the maximum

TABLE 4. *Published tolerances, regulations and acceptable daily intake values.*

Pesticide Ingredient	Milk tolerance (ppm)	Range of food tolerances (ppm)	Unavoidable contaminant, Food additive regulations (ppm)	World Health Organization, Acceptable Daily Intake (mg/kg/day)	Safe Drinking Water Committee, Acceptable Daily Intake (mg/kg/day)
Captan	None	2-100	50	0.125	0.05
Phaltan (Folpet)	None	15-50	None	0.16	0.16
Ammonium sulfamate	None	5	None	None	Yes
2,4-D	0.1	0.05-1000	0.1 and 5.0	0.3	0.0125
Silvex	0.05	0.05-0.1	0.5	0.06	0.00075
Kelthane	None	0.1-30	45	0.025	None
Chlordane	0.01	0.01-0.03	None	0.001	None
Imidan (Phosmet)	None	0.1-40	None	None	None
Methoxychlor	1.25	1-100	None	0.1	0.1
Petroleum oil	None	None	None	None	None

amount found extracting is less than food tolerance values for non-dairy foods, where published, and less than unavoidable contaminant food additive regulations, where published. While it might be argued that in these cases, should there be a milk tolerance value published, that the level of that value would or should be less than for other foods based on the fact that milk is consumed to a greater extent, the list of other foods represents some widely consumed commodities. Hence, as a guide, the food tolerance values can serve as pertinent comparisons.

Milk tolerance values have been published for 2,4-D, Silvex and Methoxychlor, and there is a pending tolerance for Chlordane. The maximum amount of Methoxychlor found extracting is less than the published milk tolerance value. In the Silvex, 2,4-D and Chlordane instances, the published or pending milk tolerance values are exceeded.

Acceptable daily intake values have been established for many pesticide ingredients by the World Health Organization and by the Safe Drinking Water Committee. The acceptable daily intake (ADI) of a chemical is that amount which may be consumed on a daily basis an entire lifetime without appreciable risk to health (*20*). The ADI is a chronic dose value, determined after first considering the no-effect level of a chemical during animal feeding studies. That no-effect level is then divided by an uncertainty factor, typically 100 or 1,000, to determine the amount considered acceptable for daily consumption. Hence, the ADI has a built-in safety factor. This author has suggested that the exposure to pesticide residues from a washed dairy container is a singular instance at the chronic quantity levels, hence comparison of extraction results with ADI's is of interest (Fig. 2). Neither organization has published ADI levels for Ammonium Sulfamate, Imidan or Petroleum Oil. Either one organization or the other has published values for the remaining pesticide ingredients found extracting from the washed LEXAN resin container.

To compare published ADI values with amounts of pesticide residues found extracting requires conversion of units, from ppm's to mg/gallon. Implicit in a comparison of this type is the selection of a typical body weight. The author chose 70 kg, a value widely used and accepted by various governmental agencies. A further assumption was made that the 70-kg adult human would consume 1 gal. of milk at one sitting, an amount 15 times greater than the average daily consumption of milk according to the Milk Industry Foundation. The reason for selecting such a large quantity of milk for singular consumption is that 1 gal. of milk would be the maximum quantity in a single container that could be purchased in the retail trade today by the consumer. In addition, because of the large float volumes of bottles involved in a dairy operation, a consumer would not be able to purchase a second container during the container's lifetime that had been exposed to the same pesticide chemical, as determined from probability analysis.

Using the assumptions outlined above it may be shown that the amounts of pesticide chemicals, Captan, Phaltan, Methoxychlor and Kelthane that would be contained in 1 gal. of milk, are less than the published ADI values of the World Health Organization and the Safe Drinking Water Committee. The amounts of Chlordane that would be contained in 1 gal. of milk, should it be consumed entirely at one sitting by the adult human, would exceed the World Health Organization ADI following the first container washing. For the second and subsequent container washing, the values would not be exceeded. The amounts of 2,4-D and Silvex that would be consumed by the adult human from drinking 1 gal. of milk at a single sitting would be less than the World Health Organization ADI and the Dow Chemical Company ADI published values, respectively. For both 2,4-D and Silvex, however, the Safe Drinking Water Committee ADI would be exceeded.

The three pesticide chemicals in violation of published milk tolerance values or exceeding ADI values are Chlordane, 2,4-D and Silvex. This is not to say that each time one of these pesticide chemicals is found in a pesticide product, and container misuse takes place, if it does, that extraction exceeding these values will result. There were three products in this study that contained Chlordane as an ingredient. Two products did not result in milk extraction and one did, Ortho-Klor 74. Six pesticide products studied contained 2,4-D as an

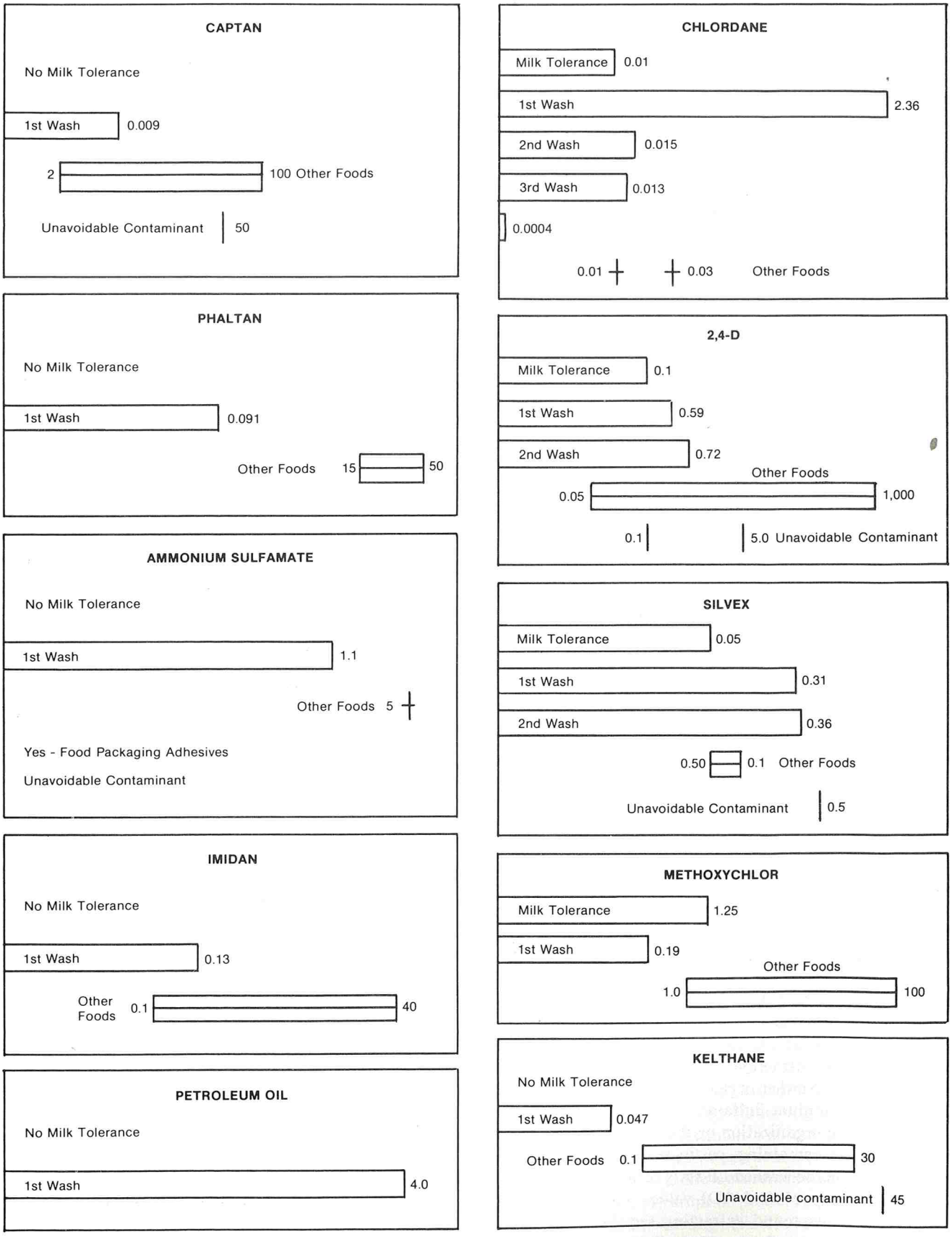

Figure 1. *Comparison of $\log_{10}$ quantity of pesticide ingredients found extracting with milk tolerance and other foods tolerance values and unavoidable contaminant food additive regulation values (ppm).*

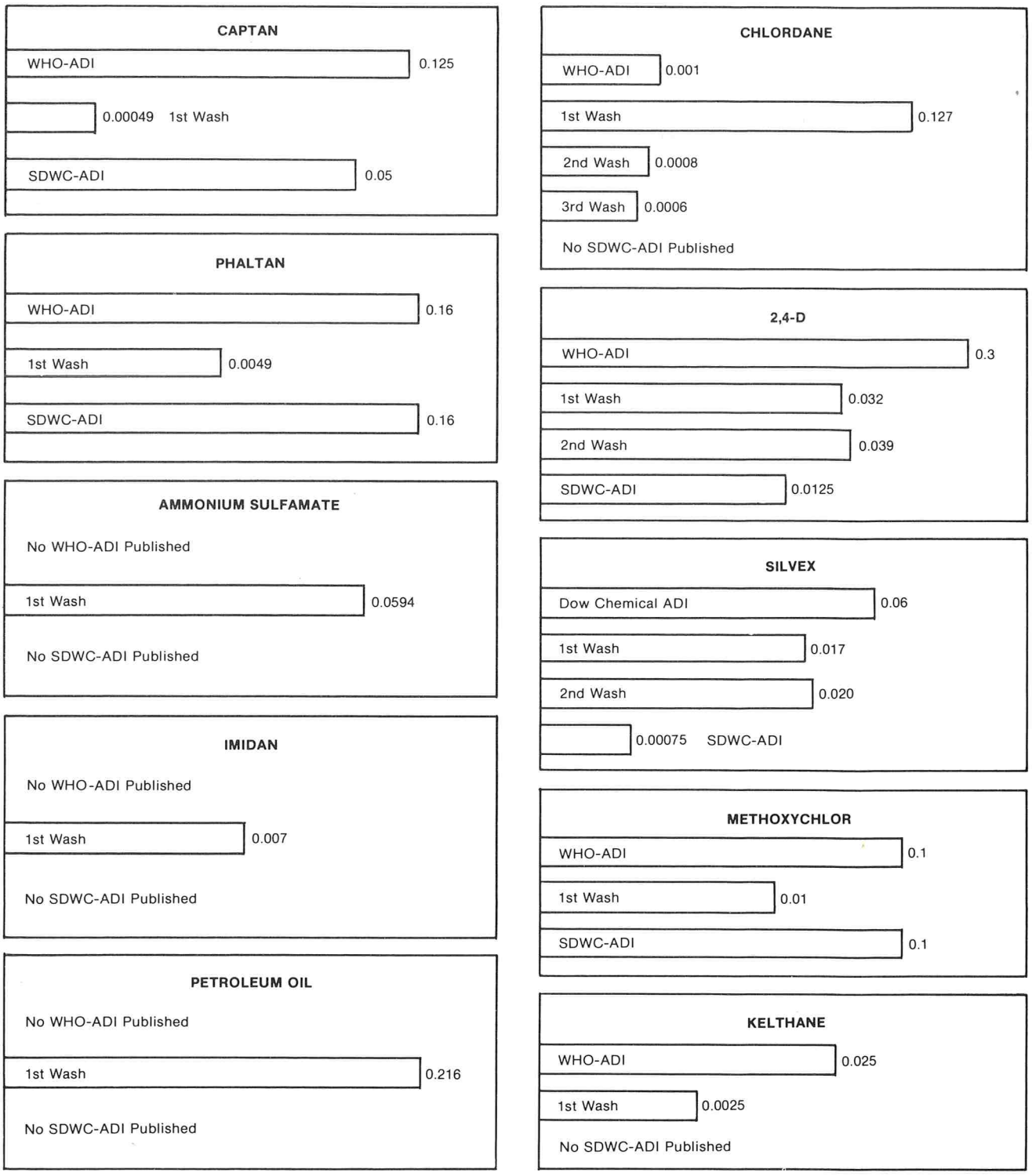

Figure 2. *Comparison of $\log_{10}$ quantity of pesticide ingredients found extracting with World Health Organization and Safe Drinking Water Committee Acceptable Daily Intake Values for Lexan resin (mg/kg).*

ingredient. One resulted in positive milk extraction, 3-D Weedone, one product was not compatible with the LEXAN resin container, and four products showed no milk extraction. Three pesticide products contained Silvex as a pesticide ingredient. Two showed no extraction, and one did, 3-D Weedone. For those pesticide ingredients found extracting at levels that result in values less than the published ADI's, there are also instances where a singular product containing the ingredient is implicated, while other pesticide products containing the ingredient are either incompatible with LEXAN resin or were shown not to extract by Gasaway

(*7*). It may be concluded for the three ingredients, Chlordane, 2,4-D and Silvex, that a specific product would have to be stored for extraction to be confirmed.

In September, 1976, Chlordane lost approval for many intended uses and for incorporation in pesticide products designed for consumer application. Existing packaged stocks were allowed to be sold. While there may be existing stocks of Ortho-Klor 74, and like products containing 74% technical Chlordane, in homes in this country, it is the author's opinion that the probability of dairy container misuse with this chemical has declined and will continue to do so. An analogous situation is consumer use of arsenicals. Consumer poisoning by arsenicals dropped to zero when this pesticide ingredient was restricted to selected uses. In addition, the only reported poisonings by Chlordane have involved consumption directly of concentrate or stored dilute solutions (*1,4,5,14*).

Both Silvex and 2,4-D extraction occurred from the LEXAN resin container exposed to 3-D Weedone. In retrospect, reviewing the specifics related to 3-D Weedone testing, one finds that after 3 days of storage the container showed no instances of incompatibility. After 10 days of storage of 3-D Weedone, however, a slight degree of clouding was noted compared to a control container. The degree of clouding was not sufficient to conclude that the container should be judged incompatible, because the untrained eye might not detect the degree of clouding. This observation does validate nonetheless the power of incompatibility inherent in the LEXAN resin container, a built-in safety valve not available for HDPE plastic or glass containers.

Independent consultant assessment - Pittsburgh Poison Control Center

The General Electric Company requested information on poisonings that had taken place within the population base served by the Pittsburgh Poison Control Center during 1975 and 1976 where the pesticides had been transferred from the original container in which the product was purchased. This particular sub-population of all reported poisonings is of interest, since it implicates a container, which could conceivably be a milk container. The selection of the Pittsburgh, Pennsylvania, center relates to a specific computer program developed by Moriarty and Barton (*17*), which details the container type in recorded poisonings. The Pittsburgh center has been the only center to date in the National Poison Center Network that has used a computer program of this type. Of the 1,505 total pesticide poisonings reported to the center, 49 cases involved access to pesticides in a container other than the original container, for 1975 and 1976. These 49 cases represent 0.1% of all reported poisonings at this center. The Pittsburgh Poison Control Center (*16*) reports cases where poisonings took place from exposure to stored pesticide formulations *only*. A specific case involving a returnable type of container was conspicuously absent, and this supports the notion of reducing poisonings by reducing the number of single-trip throw-away containers available for consumer use.

Independent consultant assessment - toxicology

Lyman (*15*) assessed the hazard (Toxicity × Dosage) of the 10 pesticide ingredients found extracting from the washed LEXAN resin returnable dairy container. He compared the quantities found with reported ADI values and 10 × ADI values, since he felt that the second value was more representative for single dose assessment. He chose the 70-kg adult human and the 3-kg newborn human, and assumed total intake of 2 liters and 16 oz., respectively. He concluded that the amount of 2,4-D that would be consumed in either case would be acceptable. The Safe Drinking Water Committee ADI for Silvex is much lower than the Dow Chemical Company reported value. Silvex consumption from a washed LEXAN resin container would be acceptable for the adult but not for the infant using the Safe Drinking Water Committee ADI and the 10-fold safety factor. Using the Dow Chemical Company ADI, however, both the adult and infant would fall within acceptable ranges. Consumption of chlordane, at the concentration found to extract into milk, would not be considered acceptable by the aforementioned guidelines, for either the adult or child. Before the hazard level may be characterized, the above conclusion has to be compared with the probability of incurred container abuse.

Independent consultant assessment - statistics

Whiston (*21*) completed a probability analysis of pesticide extraction into fluid milk and subsequent ingestion after improper storage for the LEXAN resin container case. Some key points reported by him are as follows:

(*a*) Direct ingestion of poisonous materials as stored is the sole recorded mode of poisoning, and elimination of single-trip containers may be the best available next step in the campaign against accidental poisonings.

(*b*) A commercially washed container has not been shown to result in poisoning.

(*c*) Organoleptic or sensory analysis is consistent, and more sensitive than any other known and practical method, including in-line contaminant detection and laboratory extracting studies.

(*d*) Virtually every container of milk ever packaged is subjected to human sensory perception and analysis before consumption.

(*e*) Serious contamination of milk products in LEXAN resin containers will be a small fraction of the observed contamination in HDPE plastic returnable containers.

(*f*) The probability of contaminated milk being purchased once in a person's lifetime is 0.0000012 for the HDPE plastic returnable container.

(*g*) The probability of contaminated milk being purchased once in a person's lifetime is 0.00000005 for the LEXAN resin returnable container, or 1 chance in 20,000,000. That is 4.16% of the HDPE plastic contaminated milk consumption probability.

(*h*) What makes the chances so remote is the 100 average trips that a single LEXAN resin container makes to the consumer, thus implying that the misuse must take place on the first trip, for after that the probability becomes smaller.

CONCLUSIONS

This publication, and the two previous publications by Gasaway (*7,8*) have addressed the significance of pesticide abuse chemical contamination of returnable dairy containers. The incompatibility mechanism of LEXAN resin has been shown to be more effective in culling misused containers, should pesticide abuse take place, than the in-line contaminant detector for HDPE plastic returnable containers. The in-line contaminant detector has not been shown to be effective in culling any LEXAN resin containers previously exposed to pesticides, because of the inherent and preemptory response of incompatibility. Organoleptic or sensory testing has been shown to be the best method of detecting misused containers, and is probably the best consumer method for determining the suitability of packaged dairy products for consumption. The reports of "chemically" contaminated milk through use of the HDPE plastic returnable container have been few. Field experience with the LEXAN resin container has not resulted in "chemically" contaminated milk complaints. Based on experiments conducted at the Wisconsin Alumni Research Foundation Institute, Inc., three pesticide ingredients have been shown to extract at levels that would exceed acceptable daily intake values - Chlordane, 2,4-D and Silvex. The over-all probability of consumption of milk containing one of these chemicals is the product of 0.00000005 times the probability of storage of either Ortho-Klor 74 or 3-D Weedone. There is no method available to know what the latter probability is, because the number of containers sold of these two products is not known, much less how many remain in the marketplace or are in storage in households. It is known that Chlordane is no longer being incorporated in consumer-oriented pesticide products for wide distribution. It is the author's opinion that there is little hazard associated with the use of the LEXAN resin returnable container for packaging and distributing fluid dairy products. Based on the various market basket studies conducted by the Food and Drug Administration, there seems to be ample evidence to suggest that pesticide exposure to the consumer is more likely from consuming a balanced diet than being exposed to pesticides via container misuse (*9*). The author's no-hazard opinion is consistent with that expressed by Kennedy (*12*) of the Food and Drug Administration, which states in part, relative to chemical contamination of returnable plastic dairy containers: "The number of identifiable incidents of this type have been so few, and the level of contamination which could result from such occurrences is so low that these occurrences, in our opinion, do not constitute a public health hazard...".

REFERENCES

1. Aldrich, F. D., and J. H. Holmes. 1969. Acute chlordane intoxication in a child. Arch. Environ. Health. 19:129-132.
2. Bodyfelt, F. W., M. E. Morgan, R. A. Scanlan, and D. D. Bills. 1976. A critical study of the multiuse polyethylene plastic milk container system. J. Milk Food Technol. 39:481-485.
3. Chemicals and Health - Report of the panel on chemicals and health of the president's science advisory committee. 1973. Science and Technology Policy Office, National Science Foundation.
4. Curley, A., and L. K. Garrettson. 1969. Acute chlordane poisoning. Arch. Environ. Health. 18:211-215.
5. Dadey, J. L., and A. G. Krammer, 1953. Chlordane intoxication. J. Am. Med. Assoc. 153:723-5.
6. Dean, M. P. 1977. Correspondence with Mr. Donald Kennedy, Commissioner, Food and Drug Administration, July 26.
7. Gasaway, J. J. 1978. Significance of abuse chemical contamination of returnable dairy containers: organoleptic and extraction studies. J. Food Prot. 41:863-877.
8. Gasaway, J. M. 1978. Significance of abuse chemical contamination of returnable dairy containers: pesticide storage and detector evaluation. J. Food Prot. 41:851-862.
9. Hayes, W. J., Jr. 1975. Toxicology of pesticides. The Williams & Wilkins Company, Baltimore.
10. Hayes, W. J., Jr. 1976. Mortality in 1969 from pesticides, including aerosols. Arch. Environ. Health. 31:61-72.
11. In Consultation. 1977. The perils of pesticides. Med. W. News. 10:46-48.
12. Kennedy, D. 1977. Correspondence with Mr. M. P. Dean, University of Wisconsin.
13. Landsberg, J. D., F. W. Bodyfelt, and M. E. Morgan. 1977. Retention of chemical contaminants by glass, polyethylene, and polycarbonate multiuse milk containers. J. Food Prot. 40:772-777.
14. Lensky, P. and L. E. Evans. 1952. Human poisoning by chlordane. J. Am. Med. Assoc. 149:1394-5.
15. Lyman, F. L. 1977. Pesticide hazards from contaminated LEXAN® milk bottles. Consultant report to the General Electric Company.
16. Moriarty, R. W. 1977. Consultant report on hazard assessment to the General Electric Company.
17. Moriarty, R. W., and F. B. Barton, 1977. Computerization of patient data at poison control centers. Bull. De Med. Leg., Urg. Med. Cent. Anti-Poisons. 20:282-291.
18. Personal interview with Minister of Health, Province of Ontario, Canada. 1977.
19. Pesticide Chemical News Guide. 1977. Edited by R. E. Duggan and P. D. Duggan. Food Chemical News, Inc., Washington, D.C.
20. Pesticide Study Series - 11. Laws and institution mechanisms controlling the release of pesticides into the environment. Office of Water Programs, U.S. Environmental Protection Agency.
21. Whiston, W. B. 1978. Pesticide migration - estimation of the risk exposure for milk in returnable LEXAN® containers. Consultant report to the General Electric Company.

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Occurrence of Aflatoxin in Hypoallergenic Milk Substitutes

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ABSTRACT

Aflatoxin B_1 was detected, and its identity confirmed, in hypoallergenic milk substitutes composed, among other things, of the following ingredients susceptible to possible aflatoxin contamination: soya protein isolate, soy and coconut oils, cornstarch, and corn syrup. Except for one determination all findings were under 1 ng of aflatoxin/ml of formula at drinking concentration. Manufacturers' reserve samples of soya protein isolate, the ingredient thought to be the most likely source of the aflatoxin, were found to be uncontaminated. Reserve samples of other ingredients were not examined. Because hypoallergenic formula may be the major nutrient of an individual with a metabolic defect at infancy, a vulnerable stage of life, available control measures should be used to avoid aflatoxin-contaminated ingredients.

The association of mycotoxins with human and animal disease generally has been based on symptoms occurring shortly after ingestion. Such mycotoxicoses in animals have been well documented; few such situations have been reported for man. The extent of the mycotoxin hazard has been reviewed (*6*), but data in regard to man are limited.

In one study of a possible relation between dietary aflatoxin levels and primary liver cancer in Thai populations the investigators noted a relation between aflatoxin levels and the incidence of Reye's syndrome (*7*), an often fatal pediatric disease. The correlation included a finding of aflatoxin B_1 in the tissues of the stricken children. We have been pursuing this observation with Reye's syndrome patients at the University of Mississippi Medical Center. Because of this interest and because the young of the species studied have been the most sensitive to the acute effects of aflatoxin (*12*), major dietary components of some of the patients were examined. This included a hypoallergenic milk substitute being used at the Medical Center.

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EXPERIMENTAL PROCEDURES

Sample preparation and distribution

A measured volume of liquid formula was freeze-dried in tared beakers; the beakers were then reweighed, the difference (ca 2.5 g) representing the freeze-dried weight of the formula. The first four samples were divided into equal lots; one lot of each formula was analyzed at the University of Mississippi Medical Center (UMMC), the other at the Bureau of Foods laboratories of the Food and Drug Administration (FDA). Subsequent samples were analyzed only at UMMC.

UMMC method

Freeze-dried samples were extracted using a method (*1*) of the Association of Official Analytical Chemists (AOAC), modified as follows to accommodate the small sample size. Lyophilizate, 20 3-mm diameter glass beads and 35 ml of acetone-water (7:3, v/v) were shaken together in a 125-ml Erlenmeyer flask at 300 rpm for 30 min. The content of each flask was vacuum filtered and the residue washed with acetone-water (10 ml). The filtrate was quantitatively transferred to a 100-ml beaker, evaporated to about 5 ml on a steam bath, and 2 ml of lead acetate solution (200 g $Pb(OAC)_2 \cdot 3H_2O$ +3 ml HOAc, diluted to 1 liter) mixed in by swirling, followed by 2 ml of saturated sodium sulfate (ACS grade) solution. The mixture was transferred to a 13-ml centrifuge tube in a 13.5-cm radius rotor and operated 20 min at 2500 rpm. The supernatant fluid was decanted into a 30-ml separatory funnel, and defatted with two 10-ml portions of hexane. The aflatoxins then were extracted with three 10-ml aliquots of chloroform. Pooled chloroform extracts were dehydrated over anhydrous granular sodium sulfate, evaporated to 1 ml and quantitatively transferred to a small test tube (10 × 75 mm). The extract was evaporated to near dryness with heat and finally to dryness under vacuum. The dried residue was dissolved in 50 or 100 µl of the HPLC elution solvent for analysis.

Liquid samples were extracted by the following modification: Liquid formula (20 ml) was mixed with lead acetate solution (2 ml), followed by saturated sodium sulfate solution (2 ml). The mixture was transferred to a 30 ml centrifuge tube in a 13.5-cm radius rotor and operated at 4000 rpm for 30 min. The supernatant fluid was transferred to a 60-ml separatory funnel, defatted with two 20-ml portions of hexane and then extracted with two 20-ml portions of chloroform. The pooled chloroform extracts were prepared for HPLC in the same manner as above for the freeze-dried samples.

Extracts were analyzed for aflatoxins B_1, B_2, G_1 and G_2 using a Waters Associates (Milford, MA 01757) high-pressure liquid chromatograph. Sample components were separated on a µPorasil column (Waters Associates, Inc.) and detected by UV absorbance at 365 nm at sensitivities of 0.005-0.01 absorbance units full scale. The aflatoxins were identified by retention time and quantitated by peak height or peak area determined by digital electronic integration. The mobile

phase consisted of H_2O-saturated chloroform:cyclohexane:acetonitrile:2-propanol (75:22.5:3:2, v/v/v/v) (*5*). The solvent flow rate was 2 ml/min, at a pressure of 1400 psi.

FDA method

Weighed samples of freeze-dried formula (0.94-1.63 g) were extracted using the procedure of Trucksess et al. (*11*) modified to accommodate the small sample size. The freeze-dried material was mixed with 30 ml of water and 14 ml of saturated sodium chloride, followed by 100 ml of acetone. The mixture was defecated with 10 ml of lead acetate solution and 75 ml of water, stirred and allowed to stand; 5 min later diatomaceous earth was added, and the mixture filtered. The filtrate was defatted in a separatory funnel with petroleum ether (b.p. 30-60 C), and aflatoxins were extracted into chloroform. The chloroform extract was passed through a butt tube column containing ca. 10 g of granular silica gel over ca. 2 cm of anhydrous sodium sulfate. The filtrate was collected in a clean 300-ml Erlenmeyer flask. The aqueous layer remaining in the separatory funnel was reextracted with acetone:chloroform (1:1, v/v), the chloroform layer was passed through the same butt tube, and the column washed with 20 ml of acetone:chloroform (1:9, v/v). All chloroform eluants were combined in the same Erlenmeyer flask. The adsorbed aflatoxins were eluted from the column with chloroform:acetone (9:1, v/v). The combined eluants were evaporated in a steam bath to near dryness under a stream of nitrogen. The dried extract was then dissolved in 100 μl of chloroform for thin layer chromatography (TLC). To detect both aflatoxins B_1 and M_1 on the same plate, the two-dimensional TLC developing solvents were modified as follows: first direction, anhydrous ethyl ether:methanol:water (96:3:1, v/v/v); second direction; chloroform:acetone:isopropanol (85:10:5, v/v/v). Confirmation of the identity of aflatoxin B_1 was accomplished by overspotting origin spots of positive samples with trifluoroacetic acid to form the hemiacetal derivative, aflatoxin B_{2a}, followed by two-dimensional TLC using the following developing solvents: first direction, anhydrous ethyl ether:methanol:water (96:3:1, v/v/v); second direction, chloroform:acetone:isopropanol (85:12.5:2.5, v/v/v). Quantitation was by densitometry. A portion of the extract, after conversion of aflatoxin B_1 to B_{2a} for fluorescence enhancement (*10*), was analyzed also by HPLC using a reverse phase column and water:methanol:acetonitrile (60:25:15, v/v/v) as the elution solvent.

RESULTS AND DISCUSSION

Aflatoxin B_1 was found in 3 of 4 samples taken from Medical Center stock, all of the same brand but bearing different production codes (Table 1). The original finding was confirmed by a second laboratory, including a chemical confirmation of the aflatoxin B_1 identity. The quantitative disparity observed is to be expected when measurements are made, as these were, with relatively small samples and close to the detection limits of the methods. Following this observation, 12 samples were picked up on the open market, eight of the same brand as used at the hospital, three of another brand and one of a third brand, all bearing different production codes. Aflatoxin was found in two of the open market samples - one of the original brand tested, and the other the single sample of the third brand.

The composition of the hypoallergenic milk substitutes (*3*) is given in Table 2. Of the components listed, possible sources of aflatoxin contamination are the soy protein isolate, coconut and soy oils, corn syrup, and cornstarch. Because of the usual source of the corn used and the distribution of any aflatoxin that might have been present into refinery products other than starch (*13*), the possibility of finding aflatoxin in cornstarch or corn syrup (made by hydrolysis of starch) is remote. Although not specified, the coconut and soy oils used must be refined to avoid undesirable flavors and odors. The usual refining process effectively removes any aflatoxins that might have been present from contaminated oilseed (*2,4*). Aflatoxin contamination of soybeans is not usually encountered (*8*), but if by chance aflatoxin-contaminated beans had been used, aflatoxin would have been concentrated in the soy protein isolate (*9*).

There are only three commercial sources of soy protein isolate. Each manufacturer was alerted to a possible problem. Two of the manufacturers sent us portions of their reserve samples, 30 from one and 23 from another. The third manufacturer, using in-house facilities and a method that we provided, analyzed 40 of his reserve samples. The method would have detected an aflatoxin B_1 level of 0.5 ng/g. Since the soy protein component is approximately 2% of the final formula weight, this detection level translates to 0.01 ng/ml of the formula, more than adequate to have detected in the protein the quantities found in the formula. No aflatoxin was detected in any of the samples tested by us or by the manufacturer. We did not attempt retrospective sampling of the other components susceptible to possible aflatoxin contamination.

There is no basis for judging whether the levels of aflatoxin B_1 encountered in these formulas present a risk of harm. However, since the formula can constitute the major nutrient of an individual with a metabolic defect, at the most vulnerable stage of life, there is reason to be ultraconservative in judgement. Controls for preventing incorporation of aflatoxin-contaminated ingredients are easily applied and an adequate supply of ingredients with no detectable aflatoxin (<0.5 ng/g) should be available.

TABLE 1. *Aflatoxin B_1 (ng/ml[a]) analyses of hypoallergenic milk substitute samples with detectable aflatoxins.*

Sample Source	Analyzing laboratory	Determinative step	Sample number 1	3	4
Medical Center	UMMC	HPLC	1.19[b]	0.18	0.08
	FDA	HPLC	0.23[c]	0.32[d]	0.12[e]
	FDA	TLC	0.25	0.62	0.20
			5	12	
Open market	UMMC	HPLC	0.9	0.14	

[a]At drinking dilution.
Other aflatoxins detected (ng/ml):
[b]B_2, 1.16; G_1, 3.79; G_2, 6.24.
[c]B_2, 0.11.
[d]B_2, 0.05.
[e]B_2, 0.11.

TABLE 2. *Listed components of hypoallergenic milk substitutes.*

Brand A	Brand B	Brand C
Water	Water	Water
Soy protein isolate	Soy protein isolate	Soy protein isolate
Sucrose	Sucrose	Sucrose
Salts & vitamins	Salts & vitamins	Salts & vitamins
Coconut & soy oils	Soy oil	Soy oil
Corn syrup	Corn syrup solids	
Modified cornstarch		

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REFERENCES

1. Association of Official Analytical Chemists. 1975. Official methods of analysis, Chapter 26, revised March 1975, Sections 26.A09-26.A16. Washington, D.C. 20044.
2. Dwarakanath, C. T., V. Sreenivasamurthy, and H. A. B. Parpia. 1969. Aflatoxin in Indian peanut oil. J. Food Sci. Technol. (Mysore) 6:107.
3. Medical Economics Co. 1977. Physicians desk reference. Oradell, N.J.
4. Parker, W. A., and D. Melnich. 1966. Absence of aflatoxin from refined vegetable oils. J. Am. Oil Chem. Soc. 43:635.
5. Pons, W. A., Jr. 1976. Resolution of aflatoxins B_1, B_2, G_1, and G_2 by high pressure liquid chromatography. J. Assoc. Off. Anal. Chem. 59:101.
6. Rodricks, J. V., C. W. Hesseltine, and M. A. Mehlman (eds.). 1977. Proc. conf. mycotoxins in human and animal health, Pathotox Publishers Inc., Park Forest South, Ill.
7. Shank, R. C., C. H. Bourgeois, N. Keschamuras, and P. Chandavimol. 1971. Aflatoxins in autopsy specimens from Thai children with an acute disease of unknown aetiology. Food Cosmet. Toxicol. 9:501.
8. Stoloff, L. 1976. Incidence, distribution, and disposition of products containing aflatoxins. Proc. Am. Phytopathol. Soc. 3:156.
9. Stoloff, L., M. Trucksess, and W. Martinez. 1976. The fate of aflatoxins in the preparation of protein concentrates and isolates from contaminated peanut and cottonseed flours. J. Food Sci. 41:1251.
10. Thorpe, C. W., and L. Stoloff. 1975. Determination of aflatoxins by HPLC using a fluorescence detector; fluorescence enhancement by hemiacetal formation. Abstract No. 58 in 89th Ann. Mtg. Assoc. Off. Anal. Chem. (Assoc. Off. Anal. Chem., Washington, DC 20044).
11. Trucksess, M. W., L. Stoloff, W. A. Pons, Jr., A. F. Cucullu, L. S. Lee, and A. O. Franz, Jr. 1977. Thin layer chromatographic determination of aflatoxin B_1 in eggs. J. Assoc. Off. Anal. Chem. 60:795.
12. Wogan, G. N. 1968. Aflatoxin risks and control measures. Fed. Proc. 27:932.
13. Yahl, K. R., S. A. Watson, R. J. Smith, and R. Barbolok. 1971. Laboratory wet-milling of corn containing high levels of aflatoxin and a survey of commercial wet-milling products. Cereal Chem. 48:385.

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Microbiology of the Soda Cracker Process

I. Isolation and Identification of Microflora

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ABSTRACT

A survey of the soda cracker manufacturing process revealed that starter cultures were not used and that fermentation for the process relied primarily on chance contamination. Over 200 isolates from sponge and dough samples, obtained from two commercial plants, were screened. Microorganisms responsible for fermentation of commercial soda cracker sponge and dough were isolated and identified. Besides Bakers' yeast (*Saccharomyces cerevisiae*), which is part of the standard formulation, three species of lactic bacteria were found to have prominent roles in the fermentation process. *Lactobacillus plantarum* was found to be the dominant species. Two other species found in significant numbers were *Lactobacillus delbrueckii* and *Lactobacillus leichmannii*.

Soda crackers have been made since 1840 and their manufacture has grown into a giant industry which now includes crackers of various forms and flavor. It is difficult to comprehend that the soda cracker industry still relies on chance contamination for fermentation of sponge and dough. A few microbiological and chemical studies have been published, notably by Micka (*7,8,9*). Chemical and biochemical changes during fermentation of sponge and dough have been studied but the microorganisms responsible for the process have never been isolated or identified (*1,3,4,12,13,14*).

Following our successful studies on the lactic fermentation of San Francisco sourdough French bread (*5,10*) and the subsequent development of pure culture technology (*11*), these techniques have been directed toward the complex lactic fermentations in soda cracker sponge and dough. It was the goal of these studies to develop pure culture fermentation to eliminate batch variations and quality control problems that have continuously plagued the industry.

MATERIALS AND METHODS

Isolation of bacteria

Sponge and dough samples from two commercial soda cracker plants were studied. Samples packed in insulated boxes, with frozen refrigerant (canned gel), were air-freighted and received for analysis within 24 h of shipment. Temperature of the samples, on arrival, varied from 3 to 6 C. For isolation and enumeration of the microorganisms, 11 g of sample was aseptically blended for 90 sec, at a reduced speed with 99 ml of sterile 0.1% aqueous peptone in a sterilized 8-oz. Osterizer jar[1]. After further serial dilution, also in sterile 0.1% peptone, samples were plated on selected agar media by the spread plate technique and incubated for 2 days at 30 C. Preliminary counts were made using tomato juice agar (Difco Laboratories, Detroit, Michigan), APT agar (Baltimore Biological Laboratories, Cockeysville, Maryland), Mycophil agar (BBL), Wort agar (BBL), Malt agar (Difco), PCA agar (Difco), L-agar (BBL), MRS agar (Difco) and SDB agar (*5*). MRS agar and tomato juice agar were found to be the only suitable media. MRS agar was selected for visual ease in colony counting and reproducibility of the media. Samples from the two commercial plants were received once a month, for six consecutive months, to study the possibility of changing microflora.

Identification of microorganisms

Single colonies from spread plates were picked and carried on MRS slants. Several hundred isolates were collected for screening. The yeasts were eliminated from the screening process since the standard formula includes 5×10^6 Bakers' yeasts (*Saccharomyces cerevisiae*) per gram of sponge. Contaminating yeasts would be few, if any.

The MRS medium, in a modified form (*6*), was used for biochemical studies. It was made up as follows: peptone, 10.0 g; yeast extract, 5.0 g; Tween-80, 1.0 g; ammonium citrate, 2.0 g; sodium acetate, 5.0 g; magnesium sulfate, 0.1 g; manganese sulfate, 0.05 g; and disodium phosphate, 2.0 g per liter of distilled water. The pH of the basal medium was adjusted to 6.5 with HCl. Bromthymol blue (0.1 g/l) was added to give a dense green color. Carbohydrate solutions (6%) were made and then sterilized by passing through a 0.20-micron filter membrane. Fermentations were conducted in 20 mm × 125 mm screw capped culture tubes with a 10 mm × 44 mm inverted culture tube insert for gas collection. Culture tubes, with inserts and 8 ml of basal media, were sterilized at 15 psi steam for 15 min. After cooling, 4 ml of sterile carbohydrate solution was added.

Inoculum was prepared by suspending the growth of 24-h-old MRS slant in 5 ml of sterile distilled water. Tubes were inoculated with 0.1 ml of the suspension and incubated at 30 C.

Metabolic data were recorded at 24 and 48 h. *Bergey's Manual of Determinative Bacteriology* (*2*) was used for determining species of lactic bacteria isolates.

RESULTS AND DISCUSSION

Soda cracker sponge samples had total counts of 4 to 49×10^7 per gram (yeast counts were 1 to 15×10^7 and bacteria counts 1 to 41×10^7). Soda cracker dough samples had total counts of 9 to 90×10^7 per gram (yeast counts were 1 to 12×10^7 and bacteria counts 9 to 80 ×

[1]*Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.*

TABLE 1. *Microbial counts*[1] *of soda cracker sponge and doughs (commercial).*

Source	Sponge			Dough		
	Total Count	Yeast	Bacteria	Total Count	Yeast	Bacteria
A	49	8	41	44	9	35
A	13	10	3	90	10	80
A	7	6	1	60	5	55
A	5	1	4	10	1	9
A	15	1	14	9	1	8
B	23	5	18	12	2	10
B	28	2	26	20	9	11
B	40	15	25	60	12	48
B	4	2	2	26	4	22
B	30	2	28	30	5	25

[1]Microbial counts for all figures are 1×10^7.

10^7). Table 1 summarizes the microbial counts over a period of 5 months. The data show a 10-fold variation in counts. However, this spread did not have any noticeable effect on quality and production of soda crackers. The yeast and lactic bacteria ratio and numbers were quite similar to those found in our studies on San Francisco sour dough French bread fermentation (*5,10*).

A total of 183 bacterial isolates were examined during this study. They were all found to be gram-positive, catalase-negative and non-motile rods. Carbohydrate reactions, shown in Table 2, when compared with descriptive data in *Bergey's Manual*, indicated that the dominant species found was *Lactobacillus plantarum*. This was true for both of the two commercial soda cracker sponge and dough systems studied. Two other species found in most samples, in substantial numbers, were *Lactobacillus delbrueckii* and *Lactobacillus leichmannii*. Lactic bacteria found in smaller numbers were *Lactobacillus brevis, Lactobacillus casei* and *Lactobacillus fermentum*. Table 3 shows the distribution of bacteria found in the studies.

TABLE 2. *Carbohydrate reactions of isolates*[1].

Carbohydrate	Isolate No. 9	Isolate No. 24	Isolate No. 160
Amygdalin	+	+	–
Arabinose	+	–	–
Cellobiose	+	+	–
Fructose	+	+	+
Galactose	+	–	±
Glucose (acid)	+	+	+
Glucose (gas)	–	–	–
Lactose	+	+	–
Maltose	+	+	+
Mannitol	+	–	–
Mannose	+	+	+
Melezitose	+	–	–
Melibiose	+	–	–
Raffinose	+	–	–
Rhamnose	–	–	–
Rhibose	+	–	–
Salicin	+	+	–
Sorbitol	+	–	–
Sucrose	+	+	+
Trehalose	+	+	–
Turanose	+	–	–
Xylose	+	–	–
Esculin	+	+	–

[1]Isolate No. 9 = *L. plantarum*; No. 24 = *L. leichmannii*; No. 160 = *L. delbrueckii.*

TABLE 3. *Species distribution of lactic bacteria found in soda cracker sponge and doughs.*

Species	% of Isolates
Lactobacillus plantarum	47
Lactobacillus delbrueckii	16
Lactobacillus leichmannii	13
Lactobacillus brevis	5
Lactobacillus casei	4
Lactobacillus fermentum	3
Other lactic bacteria	12

Further research will be conducted to develop stabilized concentrated cultures of *L. plantarum, L. delbrueckii* and *L. leichmannii* for possible use in pure culture fermentation of soda cracker sponge and dough.

REFERENCES

1. Bohn, R. T., and R. J. Martz. 1926. A rapid method for the colorimetric determination of hydrogen-ion concentration of crackers. Cereal Chem. 3:183-187.
2. Buchanan, R. E., and N. E. Gibbons. 1974. Bergey's manual of determinative bacteriology, 8th ed. The Williams and Wilkins Co., Baltimore.
3. Johnson, A. H., and C. H. Bailey. 1924. A physico-chemical study of cracker dough fermentation. Cereal Chem. 1:327- 410.
4. Johnson, A. H. 1925. Identification and estimation of the organic acids produced during bread dough and cracker dough fermentation. Cereal Chem. 2:345-364.
5. Kline, L., and T. F. Sugihara. 1971. Microorganisms of the San Francisco sour dough bread process. II. Isolation and characterization of undescribed bacterial species responsible for the souring activity. Appl. Microbiol. 21:459-465.
6. Man, J. C. de, M. Rogosa, and M. E. Sharpe. 1960. A medium for the cultivation of lactobacilli. J. Appl. Bacteriol. 23:130-135.
7. Micka, J. 1941. Soda requirements of cracker flours. Cereal Chem. 18:483-497.
8. Micka, J. 1948. Lactic fermentation and trace iron effect on cracker quality. Food Ind. 20(1):102-107, 234.
9. Micka, J. 1955. Bacterial aspects of soda cracker fermentation. Cereal Chem. 32:125-131.
10. Sugihara, T. F., L. Kline, and M. W. Miller. 1971. Microorganisms of the San Francisco sour dough bread process. I. Yeasts responsible for the leavening action. Appl. Microbiol. 21:456-458.
11. Sugihara, T. F., and L. Kline. 1975. Further studies on a growth medium for *Lactobacillus sanfrancisco.* J. Milk Food Technol. 38:667-672.
12. Triebold, H. O., and C. H. Bailey. 1932. A chemical study of rancidity. I. Autoxidation of shortenings and crackers. Cereal Chem. 9:50-64.

13. Triebold, H. O., and C. H. Bailey. 1932. A chemical study of rancidity. II. Factors influencing the keeping quality of shortenings and crackers. Cereal Chem. 9:91-103.

14. Vaufel, E. A., and W. Hanks. 1944. pH variations in soda crackers and their control with diammonium phosphate. Cereal Chem. 21:16-27.

Report of the Committee on Applied Laboratory Methods — International Association of Milk, Food, and Environmental Sanitarians, 1976-1978.

This committee, during the past 2 years, has provided in-depth assistance and consultation to the Association and others in the following ways:

1. Conducted collaborative and/or comparative studies on new or modified methods, which have been published in the Association journal. Most of these studies have provided necessary information on methods to Chapter Chairpersons and the Intersociety Council of the American Public Health Association (APHA) in the development of the 14th edition of *Standard Methods for the Examination of Dairy Products* (SMEDP).
2. Provided consultation on abnormal milk testing procedures to The National Mestitis Council (NMC) and the NMC Research Committee.
3. Provided consultation on microbiological methods and abnormal milk screening tests to the National Conference of Interstate Milk Shippers (NCIMS) Laboratory and Abnormal Milk Committees.
4. Provided assistance and consultation on Abnormal Milk Confirmation Procedures to the responsible AOAC Associate Referee and his committee.
5. Continued to stress the importance of variation of analyst data because criteria have not been developed for certification of microbiological media, reagents, materials, and instrumentation in all laboratory disciplines.
6. Provided liaison to the Association Farm Methods Committee and to laboratory committees of Regional Associations of Food and Drug Officials. Offered the assistance of this committee to Regional Food and Drug Officials Associations.
7. Assisted in the development of Drinking Water Laboratory Certification Regulations of the Environmental Protection Agency.
8. Participated as Chapter members in the development of two APHA texts recently published: *Compendium of Methods for the Microbiological Examination of Foods and Quality Assurance Practices for Health Laboratories.*

As of January 1, 1978, the Chairpersons for three Subcommittees of this Committee had started on their new assignments. Mr. Charles Huhtanen had transferred to Chairperson of the Subcommittee Laboratory on Methods for the Examination of Foods, Dr. Michael Wehr accepted the position of Chairperson of the Subcommittee on Laboratory Methods for the Examination of Milk and Milk Products, and Mr. Ernest Shipe, Jr. became Chairperson of the Subcommittee on Methods for the Examination of Water and other Environmental Samples.

A. Richard Brazis, Ph.D.
Chairperson, Applied Laboratory Method Committee

Subcommittee on Laboratory Methods for Examination of Milk and Milk Products

This subcommittee has continued to be very active in the affairs of the Association. Changes in administrative responsibilities of the two former Co-chairpersons forced their request for reassignment of a new Subcommittee Chairperson in 1977-78; Dr. Mike Wehr of the State Department of Agriculture in Oregon has accepted Chairpersonship of this subcommittee provided he also does not receive new responsibilites, which unfortunately for the Association appear to be headed his way. The activities of this Subcommittee for 1977 were shown in the 1977 report of the Committee, which has not been published. The changes in leadership in this subcommittee have resulted in no action on new studies.

H. Michael Wehr, Ph.D., Chairperson

Members: William Arledge, Guenther Blankenagel, Ph.D., Edwin H. Connell, Earl W. Cook, Ph.D., Vernon R. Cupps, Roy Ginn, Clair Gothard, James J. Jezeski, Ph.D., Oliver W. Kaufmann, Ph.D., Wesley N. Kelly, William S. LaGrange, Ph.D., H. E. Randolph, Ph. D., James A. Rolloff, Edmond Sing, Ph.D., Maurice Weber, Ph.D., Kenneth W. Whaley

Subcommittee on Laboratory Methods for the Examination of Foods

The members of this subcommittee have been reviewing microbiological methods published in AOAC and APHA laboratory manuals to determine projects worthy of consideration for subcommittee comparative and collaborative studies.

These include: Reports of microbial growth on acidified media for enumeration of yeast and/or mold contaminants has resulted in publications specifying the need of one or more antibiotics in media to suppress bacterial growth, and the influence of water activity (A_w) on microbial growth, suggesting the need for preenrichment media prior to examination and detection of pathogens and food spoilage microorganisms. The Chairperson has authored a chapter on potentially pathogenic microorganisms and parasites of meat which will be published in a handbook of The American Meat Science Association in 1979.

C. H. Huhtanen, Chairperson

Members: Harold Bengsch, J. J. Jezeski, Ph.D., W. S. LaGrange, Ph.D., R. T. Marshall, Ph.D., Don Pursch, E. L. Sing, Ph.D., H. Michael Wehr, Ph.D.

Subcommittee on Laboratory Methods for the Examination of Water and Other Environmental Samples

This subcommittee has had difficulty getting off the ground during the past two years. There are critical questions which should be resolved concerning presumptive test media for coliforms in water supplies, influence of transit time on recovery of coliforms, on-site methods for rapid enumeration of coliforms in rural water supplies, variability of coliform growth in different lots of brilliant green bile lactose broth, sterilization of media containing carbohydrate, importance of magnesium sulfate in dilution water, chemical quality of laboratory water and last, but not least, sanitary significance of enterobacteriaceae in water supplies.

Ernest L. Shipe, Jr., Chairperson

Members: Berry Gay, Jr., Arnold Salinger, Kenneth Whaley

Journal of Food Protection Vol. 41, No. 12, Pages 980-982 (December, 1978)

Microbiology of the Soda Cracker Process

II. Pure Culture Fermentation Studies

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ABSTRACT

Stable, pure-culture, frozen concentrates of *Lactobacillus plantarum, Lactobacillus delbrueckii* and *Lactobacillus leichmannii* were developed for use in the fermentation of soda cracker sponge and dough. Sponge fermentation time was reduced from the conventional 18 h to 4 h. Dough fermentation time was also reduced from 4 h to 2 h. The conventional 24-h soda cracker process could be reduced to 8 h by the use of pure-culture technology.

Pure-culture fermentation for soda cracker production which would improve sanitation and quality control, as well as substantially reduce process time, has been developed on a laboratory scale. Three of the bacterial isolates from commercial soda cracker sponge and dough, *Lactobacillus plantarum, Lactobacillus delbrueckii,* and *Lactobacillus leichmannii* were grown in large quantities (*4*). The cells were separated from the growth medium by centrifugation, suspended in a stabilizing menstruum (*1*), frozen and stored at -100 C, and used as stock cultures for our fermentation studies. Storage stability studies were conducted concurrently.

MATERIALS AND METHODS

Preparation of stabilized pure cultures

The bacterial isolates were propagated in Fernbach flasks containing 1 liter of MRS broth (Difco Laboratories, Detroit, Michigan)[1]. They were inoculated (10%) with a 24-h-old broth culture. Incubation was conducted in a New Brunswick Rotary Shaker Incubator with agitation at 150 rpm and a temperature of 30 C. After 24 h, cultures were cooled to 8 C and cells were then separated by means of a Sharples Super Centrifuge. The cells were resuspended in a stabilizing menstruum consisting of 60% of an aqueous solution made up of 8% non-fat milk solids, 2% mono-sodium glutamate and 0.5% Tween 80; and 40% glycerol. The harvested cells were mixed with the stabilizing menstruum in a ratio of 1:4. The mixture was homogenized with a Kraft Non-Aerating Stirrer (Model S-25). Stabilized cell concentrates were stored in screw-capped polyethylene bottles which were frozen and held at -100 C (Liquid nitrogen atmosphere).

A "rapid" activity test

A "rapid" activity test was devised to check starter cultures. The cultures were tested at the start of storage and then periodically during storage. It was patterned after the liquid sponge phase of the proposed pure-culture technique for production of soda crackers. A 10% flour and water suspension (59 g of flour and 590 ml of distilled water) was inoculated with 10 ml of starter culture (thawed rapidly in a 30 C bath) to be tested. The mixture was poured into a sterile 2-liter Erlenmeyer flask and then placed in a Rotary Shaker Incubator (150 rpm) at 30 C. At 0, 2, and 4 h the pH of the test mixture was measured and total counts were determined by plating on MRS agar. A typical "active" starter culture would give a pH curve as shown on Figure 1.

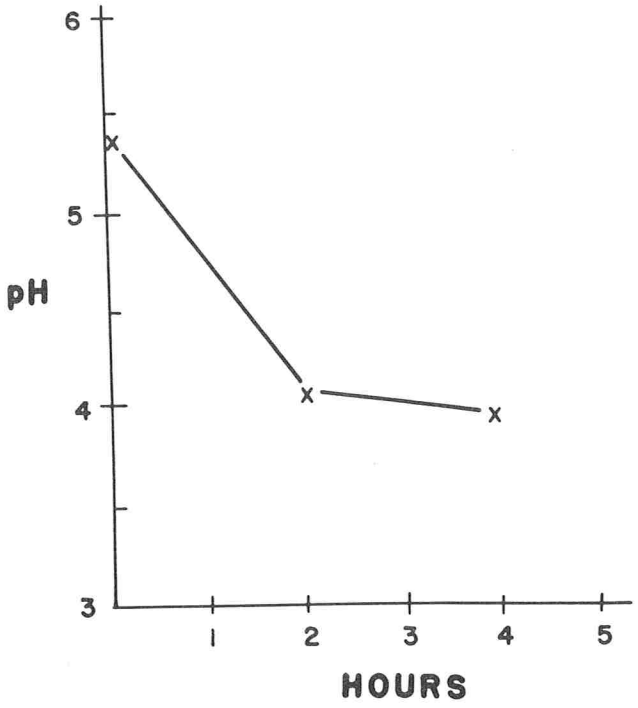

Figure 1. *Curve showing typical "activity" of a* Lactobacillus *isolate.*

Stability studies

Stored (-100 C) stabilized culture concentrates were tested monthly over a period of 16 months. "Activity" tests as well as total counts were determined, as described in the previous section.

Laboratory-scale soda crackers

To test experimental cultures, laboratory-scale soda cracker formulation and procedures were developed. A modified soda cracker formulation used in the fermentation studies is shown in Table 1. Conventional soda cracker sponge is fermented for 18 to 20 h at 30 C and the dough for 4 h at 32 C. The "control" soda crackers were made

[1]*Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.*

TABLE 1. *Formulation for soda crackers on a laboratory-scale.*

Ingredient	Grams
Sponge	
Flour (9% protein)	1350
Shortening	284
Yeast (compressed)	4.5
Water	590
Mixed in Hobart Mixer, Model 200A at speed 1 for 1 ½ min.	
Dough	
Flour	909
Malt Syrup (diastatic)	23
Salt	27
Soda	15 (varies with pH)
All of the above sponge	
Mixed in Hobart Mixer, Model 200A at speed 1 for 2 min.	

according to this schedule. However, for ease in handling and economics of pure culture use, a "liquid-sponge" step was developed for our studies. A 10% flour and water slurry was inoculated with the test culture concentrate (10%). The mixture was incubated at 30 C in a New Brunswick Rotary Shaker Incubator (150 rpm) for 4 h. The "active" inoculum was then used to inoculate a conventional sponge (formulation was modified to allow all of the water to be from the liquid-sponge).

A major problem was adaptation of existing equipment for machining or forming of the soda cracker dough. After testing various available equipment, the Acme Rol-Sheeter, Model 8 (D. R. McClain and Son, Pico Rivera, California) was found to be suitable for this procedure. The final dough was first passed through the Acme Rol-Sheeter with the rollers set at half-open (this was repeated three times). The clearance was then set at the first mark. The dough was passed through once, folded to give a lamination of six layers, then passed through again to produce the final dough sheet. The sheet was trimmed to size (6 inches × 12 inches), perforated into eight squares and docked using a Singer Tracing Wheel (The Singer Company, New York, N.Y.). The dough was baked for 160 sec. at 550 F in a National Reel Oven (National Mfg. Co., Lincoln, Nebraska).

RESULTS

Culture concentrates with viable counts of 10 to 17 × 10^{10} were prepared, frozen and stored at -100 C. All three isolates studied, *L. plantarum* (Isolate No. 9), *L. leichmannii* (Isolate No. 24) and *L. delbrueckii* (Isolate No. 160), showed excellent frozen stability properties (Table 2). Viable counts for Isolate No. 9 remained constant for at least 10 months and the other two isolates showed some drop but maintained sufficient numbers for production of a suitable product. The "rapid" activity tests indicated little or no loss in acid producing abilities.

The "liquid-sponge" step, necessary for ease of handling and economics of pure culture use, was found to be very stable. It could be cooled to 13 C and used after as long as 48 h without any loss of activity. This unusual stability would make it possible to prepare large quantities of "liquid-sponge" for up to 3 days of production. It would, of course, require an investment in temperature-controlled sanitary tanks but the saving in labor would certainly offset this cost in a short time.

TABLE 2. *Viable cell counts and "activity" of frozen concentrates after 10 months storage at -100 C.*

Isolate No.	Counts/g		"Activity" test	
	"0" Time	10 Months	pH at Start	pH at 4 h
9	10×10^{10}	8×10^{10}	5.30	3.75
24	17×10^{10}	8×10^{10}	5.30	3.95
160	15×10^{10}	8×10^{10}	5.30	3.95

Use of "liquid-sponge" to inoculate the conventional sponge gave a starting lactic bacteria count of approximately 1×10^{8}. The usual "lag" phase in the sponge fermentation was eliminated. Sponge fermentation was completed in 4 h, determined by drop in pH (conventional sponge fermentation takes 18 to 20 h). Dough fermentation continued at a rapid pace and was completed in 2 h (conventional dough fermentation takes 4 h). Allowing time for mixing, forming and baking, it is conceivable to schedule an 8-h production run using pure culture technology. A comparison of the two fermentation schedules is shown in Table 3. The shorter fermentation times, when using pure cultures, are due to the greater microbial population and the growth-stage of the microorganisms. This is shown in Table 4.

TABLE 3. *Comparison of conventional and pure culture (proposed) fermentation for soda cracker production.*

Process	Fermentation time (h)	
	Conventional	Pure culture
Liquid-sponge	0	4
Sponge	18	4
Dough	4	2
Total time	22	10[1]

[1]Total fermentation time can be reduced to 6 h by preparing liquid-sponge 24-48 h before use and storing at 13 C.

TABLE 4. *Comparison of microbial counts in conventional and pure culture fermentations.*

Time (h)	Total Bacterial Count	
	Conventional	Pure Culture (Isolate No. 9)
	Sponge	
0	24,500	50,000,000
4	25,000	130,000,000
10	1,500,000	—
15	25,000,000	—
20	75,000,000	—
	Dough	
0	33,000,000	110,000,000
2	—	120,000,000
4	22,000,000	—

Single-culture and mixed-culture fermentations were compared. Single-culture fermentations were made with *L. plantarum, L. delbrueckii* and *L. leichmannii*. Mixed culture fermentations were made with *L. plantarum* combined with either *L. delbrueckii* or *L. leichmannii*. Starting with identical microbial counts, fermentation rates for both single and mixed cultures were found to be approximately the same. However, organoleptic tests indicated a slight flavor difference among the various cultures. This is probably due to the differences in organic acid composition and the levels of diacetyl

present in the final product. Organic acid and diacetyl production, by lactic bacteria, varies according to species and growth conditions (*3*).

DISCUSSION

Today's soda cracker fermentation, which relies primarily on chance contamination for an inoculum, can be made more efficient by using pure-culture technology. Fermentation schedules would be much shorter and predictable. Quality control would be greatly improved since delayed fermentations and off-fermentations would be eliminated. It would also be possible to control the flavor of the soda cracker by using different lactic bacteria and different combinations of lactic bacteria in the starter culture. Levels of organic acids as well as diacetyl can be controlled. All in all, pure culture fermentation would provide a uniform, flavorful and wholesome product made efficiently (*2*).

Our studies, reported here, have been conducted on a laboratory-scale and we realize that production problems may occur. But, the principle of pure-culture fermentation has been shown to be adaptable to soda cracker production.

REFERENCES

1. Gilliland, S. E., and M. L. Speck. 1974. Frozen concentrated cultures of lactic starter bacteria. A review. J. Milk Food Technol. 37:107-111.
2. Ng, H. 1972. Factors affecting organic acid production by sourdough (San Francisco) bacteria. Appl. Microbiol. 23:1153-1159.
3. Sugihara, T. F. 1977. Non-traditional fermentations in the production of baked goods. Bakers' Digest 51(5):76, 78, 80, 142.
4. Sugihara, T. F. 1978. Microbiology of the soda cracker process. I. Isolation and identification of microflora. J. Food Prot. 41:981-983.

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Immunotoxicology of Foodborne Substances: An Overview

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ABSTRACT

The current status of research concerning immunotoxicology of foodborne substances is discussed. Several food additives and bacterial toxins interact directly with cells involved in the immune response and could interfere with natural immunoregulation in the gut. Specialized antibodies produced locally in the gastrointestinal tract help facilitate enzymatic degradation of the substances with which they react by retaining them along the mucous lining; this process is known as immune exclusion. Interference with local production of intestinal antibodies could lead to loss of local immunity and immune exclusion of large, potentially immunogenic proteins. Consequences of chemically induced loss of immune exclusion could include increase in incidence of autoimmune diseases, gastrointestinal allergies, toxigenic diarrheas, and pathogenic invasion through the gut wall. The need for more research is apparent.

The thrust of current research in food toxicology has, for the most part, been directed at methods for detecting chemical mutagens and/or carcinogens. In vitro methods, various cell culture methods, and tests done with *Drosophila* and rats have been used to screen for chemicals that are potentially mutagenic and possibly carcinogenic for man (*8*). This area of research is extremely important and has yielded much useful information. Many mutagenic or carcinogenic changes (transformations) in mammals are dealt with effectively by the body's own natural defense mechanism, the immune system. This extremely complex system is responsible for the well-being of the human animal in many ways, including (*a*) rejection and destruction of both living and nonliving foreign material, (*b*) neutralization of pathogens such as viruses, mycoplasma, bacteria and their toxins, and other parasites, and (*c*) recognition and destruction of cells that have undergone transformation to a malignant state. In a healthy individual, the immune system carries out its protective functions with remarkable efficiency. When the individual's immune system is compromised by congenital defects, infection, or nutritional factors, a multitude of serious disorders can result (reviewed in *1,2,3*). The purpose of this article is to provide an overview of past and current work concerning the possible immunologic consequences of ingesting foodborne immunosuppressive chemicals or chemicals capable of altering immune regulation. Food additives, some natural constituents, and bacterial toxins will be discussed; pesticide residues have been reviewed elsewhere (*30*).

THE IMMUNE SYSTEM

Figure 1 illustrates some of the cells and interactions involved in the various types of immune responses. The cells from which immunocompetent cells are derived are termed "stem cells." These migrate from the bone marrow and, under the influence of various organs, become lymphocytes in one or the other of the two major compartments of the immune system. Stem cells influenced by the thymus become the T-lymphocytes. These can be further divided into subpopulations, some of which act as (*a*) killer cells that are specifically cytotoxic to other cells (foreign or transformed) to which they are sensitized, (*b*) helper cells that are required for an antibody response to many antigens, and (*c*) suppressor cells that can inhibit formation of antibody, a normal negative control mechanism, via soluble mediator(*s*). The other major compartment is made up of the B-lymphocytes. These arise from stem cells that come under the influence of the so-called bursa equivalent (from studies in chickens). In man, the bursa equivalent is believed to be fetal liver and spleen, and bone marrow. B-cells have surface antibodies specific for antigen and are the precursors to antibody-forming cells (AFC). When triggered by their appropriate antigen, and possibly after interaction with the regulatory T-cells, B-cells mature into plasma cells that produce antibody directed against the stimulating antigen. Not shown in Fig. 1, but of great importance to immune mechanisms, are the monocytes and macrophages. Besides their duties as scavenger cells and processors of antigens, regulatory functions have recently been ascribed to macrophages (*9,10*). The interactions among the various cells are not

yet fully understood, but they are known to involve many soluble products (lymphokines), the list of which is rapidly expanding (reviewed in *31*).

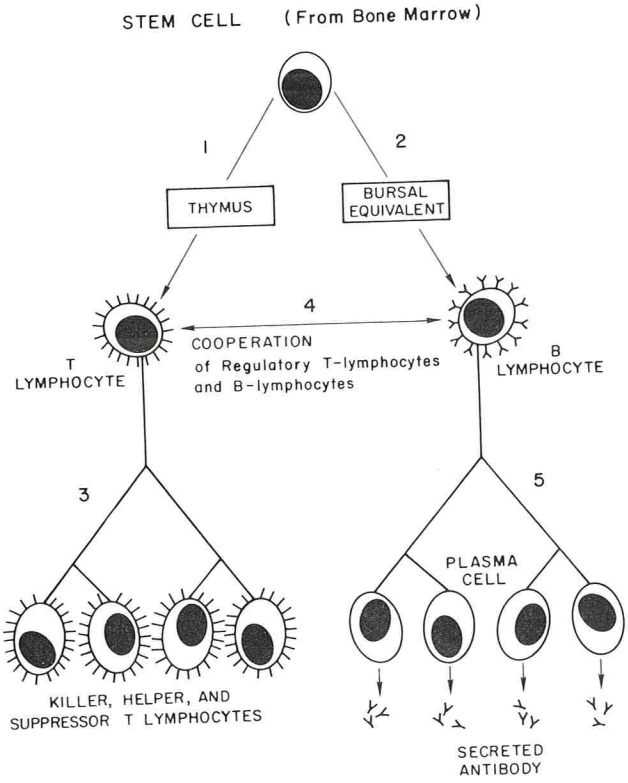


Figure 1. *Bone marrow stem cell influenced by the thymus (1) becomes a T-lymphocyte, and influenced by the "bursa equivalent" (2) becomes a B-lymphocyte; these are two distinct populations of small lymphocytes. Exposure of either T- or B-lymphocytes to appropriate stimulus (antigen or mitogen) causes proliferation. Stimulated T-lymphocytes (3) become one of several subpopulations of mature T-lymphocytes, which act as regulatory cells or killer cells. B-lymphocytes, when stimulated (5), become antibody-secreting plasma cells. For most protein antigens, cooperation (4) of T-lymphocytes and B-lymphocytes is necessary for B-lymphocytes to become antibody-secreting plasma cells. The regulatory influence (4) of T-lymphocytes, either help or suppression, ultimately affects the B-lymphocyte's ability to become an antibody-secreting plasma cell.*

FOOD ADDITIVES AND CONSTITUENTS

Certain chemicals, food additives, and/or constituents were recently shown to be capable of directly inhibiting lymphoid cells from carrying out their functions in an in vitro system (*4,5,7*). The system used to evaluate the immunosuppressive potential of such compounds was the Mishell-Dutton (M-D) system (*20*), which quantitates AFC. Although the M-D system is an in vitro test, cellular interactions between B-lymphocytes, T-lymphocytes, and accessory mononuclear cells that are required for in vivo antibody response are also required for in vitro responses. The fact that the M-D system is well characterized is advantageous, as many ways are available to dissect the system and determine the particular cell type (or cell product) affected by a chemical introduced into the system.

The M-D system used dissociated mouse spleen cells as a source of lymphocytes and mononuclear cells (Fig. 2). After the cells are dissociated and washed, cultures of 1-1.5 × 10^7 lymphocytes are cultured in 1-ml volumes. Antigens used in the system vary from those that require help from T-lymphocytes (most protein antigens) to those that require no T-lymphocyte help (lipopolysaccharide, hapten-conjugated Ficoll, pneumococcal polysaccharide, etc.). Cultures are maintained in a controlled environment for 4 to 5 days and fed daily. On the day of harvest, cells are removed from the culture and washed. AFC to sheep erythrocytes, the most commonly used antigen, are quantitated by antibody-complement lysis of sheep erythrocytes contained in a matrix composed of either agarose and buffer or a special liquid-filled chamber. Released antibody reacts with the target erythrocyte and, with complement, lyses antibody-coated cells, resulting in a zone of lysis, or plaque, around the AFC (Fig. 3). Visualization of the response to antigens other than erythrocytes is made possible by coupling (or sensitizing) the antigen or haptenic determinant to the erythrocyte to make a lyseable target for antibody and complement. Usually the primary antibody response (IgM production)

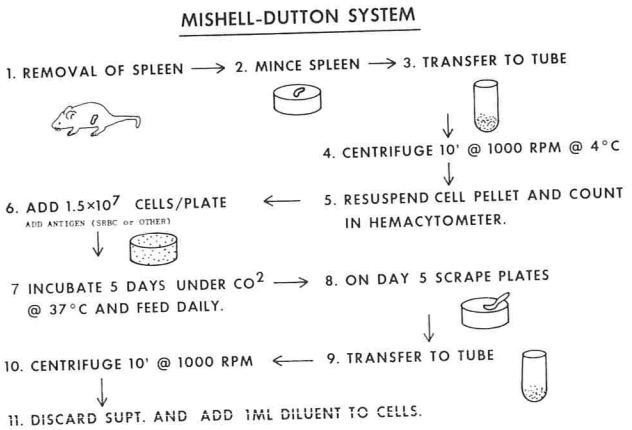


Figure 2. *Spleenic lymphocytes are stimulated by antigen in vitro to become antibody-secreting plasma cells. Besides T- and B-lymphocytes, macrophages are required for the immune response to many antigens in vitro. Since much is known about the Mishell-Dutton system, the target cell type of many toxicants can be ascertained.*

is studied, but the system can be adapted to visualize antibody responses of the IgG and IgA types as well (*23*). Cultures can be sensitized with antigen in vitro, or animals can be immunized in vivo by a variety of routes and the plaque-forming cell (PFC) response determined in vitro. This gives the system flexibility.

Utilizing the M-D system, we determined that several phenolic food additives were immunosuppressive, but that this effect was not due to overt toxicity. Furthermore, the M-D system was specific as to which chemicals could inhibit the AFC response; slight changes in reactive groups or group positions on the phenol ring caused significant variations in biological activity (Table 1) (*4,5,7*). One surprising result was that few compounds had an effect on the AFC response.

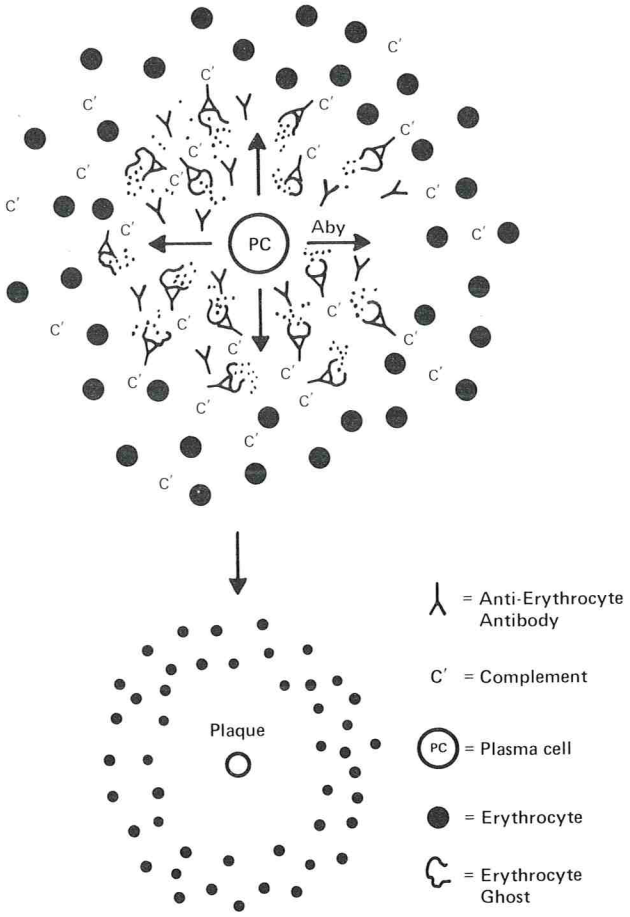


Figure 3. *Direct plaque formation by IgM-secreting plasma cells stimulated in vitro. The reaction of antigen (erythrocytes or antigen-coupled erythrocytes) with antibody (IgM) secreted by plasma cells in the presence of complement causes hemolysis. The clear zone around the plasma cell is a plaque.*

The results obtained with gallic acid, a metabolite of the food additives propyl gallate and tannic acid and a natural constituent of some foods, are particularly interesting and exemplify the ability to ascertain the affected cell type using the M-D system. The ability of gallic acid to suppress the AFC response to sheep erythrocytes was shown to be reversed by 2-mercaptoethanol, a compound capable of substituting for some macrophage functions (*11,18*). Further work showed that gallic acid blocked functions of T-lymphocytes that were dependent on the presence of functional macrophages (*5*); both helper T-cell function and mitogen-induced suppressor T-cell function (*6*) were shown to be macrophage-dependent and therefore susceptible to gallic acid-induced suppression. Detailed studies on the other compounds found to be immunosuppressive in vitro remain to be done to ascertain the affected cell type for each compound.

Another food additive, carrageenan, has been shown to be immunosuppressive (*28*); like gallic acid, carrageenan's target cell is the macrophage (*28*). Carrageenan has also been shown to facilitate the growth of some tumors by blocking the tumor-destroying capability of macrophages (*19*). Carrageenan is not absorbed through the gut under normal circumstances and would therefore not be as potentially detrimental as chemicals that are easily absorbed and capable of direct interactions with immunocompetent cells in the lamina propria of the gut wall.

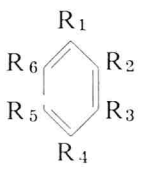
Calcium cyclamate was not directly immunosuppressive in the M-D test. This result is contrary to in vivo feeding studies involving rabbits. In those tests, rabbits fed diets containing 5% calcium cyclamate for 150 days demonstrated reduced antibody responses to antigenic challenge (*14*). It was not demonstrated whether this reduction was due to direct effects on immunocompetent cells, an indirect effect of involvement of another organ, or hormonal imbalance.

Spiers et al. (*27*) have recently proposed an elaborate model system for evaluating the effect of potentially immunosuppressive compounds in mice. This system employs a vaccine consisting of DPT (diphtheria, tetanus toxoid, pertussis) with S3 pneumococcal polysaccharide added; this mixture permits assessment of the effect on both T-lymphocyte-dependent and T-lymphocyte-independent antibody responses. The effect of an established immunosuppressant, cyclophosphamide, was studied in this system (*27*). Time of administration of either the antigen cocktail or immunosuppressant could be varied, giving the system great flexibility. This predictive tool should prove to be valuable for determining the immunosuppressive potential of various compounds.

BACTERIAL TOXINS

Bacterial toxins have also been shown to disrupt normal immunoregulatory mechanisms. Staphylococcal enterotoxins A and B, for example, can exert an immunosuppressive effect on antibody formation in vitro (*25*). This effect is thought to be mediated by soluble T-lymphocyte products (lymphokines), which, once produced, can spread from the toxin-affected T-cell to exert their suppressive effect on nearby cells. These studies were carried out using mouse cells in vitro. Enterotoxin A in amounts as small as 10^{-6} μg/ml has been recently shown to activate human peripheral lymphocytes to divide and to produce large amounts of immune interferon, a lymphokine (personal communication, M. L. Langford, G. J. Stanton, H. M. Johnson, U. of Texas Medical Branch, Galveston, Texas. 1978). Sub-emetic doses of these toxins could have a significant effect on the normal immunoregulatory processes in the gastrointestinal tract. Induction of suppressor T-lymphocytes, a normal immunoregulatory process, can be reversed by cyclic adenosine monophosphate (cyclic-AMP) or its inducers in vitro (*17*). Toxins such as cholera toxin and possibly those from other gram-negative organisms are potent inducers of cyclic-AMP activity (*12*). The effects of toxin-induced disruption of immunoregulation require in vivo confirmation. Although effects on the immune response would be more difficult to assess

Table 1. The effects[a] of some common phenolic food additives or food additive metabolites on the Mishell-Dutton system.

Compound	Functional groups						90% PFCSD μg/culture	50% VRD μg/culture
	R_1	R_2	R_3	R_4	R_5	R_6		
p-Hydroxybenzoic acid	OH	H	H	COOH	H	H	>200	>200
Methyl paraben	OH	H	H	$COOCH_3$	H	H	25	>200
Propyl paraben	OH	H	H	$COO(CH_2)_2CH_3$	H	H	50	50
Vanillin	OH	OCH_3	H	CHO	H	H	stimulatory	>200
Vanillic acid	OH	OCH_3	H	COOH	H	H	stimulatory	>200
Butylated hydroxyanisole 2-isomer	OH	$C(CH_3)_3$	H	OCH_3	H	H	50	>200
Butylated hydroxyanisole 3-isomer	OH	H	$C(CH_3)_3$	OCH_3	H	H	25	>200
Butylated hydroxytoluene	OH	$C(CH_3)_3$	H	CH_3	H	$C(CH_3)_3$	50	≃150
Gallic acid	OH	OH	H	COOH	H	OH	1–2	>200
Propyl gallate	OH	OH	H	$COO(CH_2)_2CH_3$	H	OH	5	50
Pyrogallol	OH	OH	H	H	H	OH	5	5

[a]Suppression of the in vitro antibody response to sheep erythrocytes is presented as the dose of test compound (μg/culture) which resulted in ≥ 90% suppression of the PFC response (90% PFCSD). Toxicity was accessed by trypan blue dye exclusion and is presented as the dose of test compound (μg/culture) which resulted in a 50% viability reduction (50% VRD) at the end of the 5 day culture period. Control anti-SRBC PFC responses ranged from 10,000 to 25,000 PFC/culture and were background-corrected before the mean was calculated. All compounds were added to cultures at the time of antigen addition.

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than overt symptoms of intoxication (vomiting or diarrhea), the potential hazards posed are great and will be discussed later.

PRINCIPLE OF IMMUNE EXCLUSION

The major antibody in all secretions along the mucous membranes is a specialized antibody, secretory IgA(sIgA), which is a dimeric antibody composed of two 7s IgA molecules coupled by a joining chain (J chain) and finally coupled to a secretory component (SC). The final structure, sIgA, is released into the mucus covering the intestinal villi, the glycocalyx. This specialized antibody is resistant to enzymatic degradation by intestinal enzymes. In a series of experiments, Walker and his associates have demonstrated a novel role for sIgA. Intact proteins were thought to be able to traverse the intestinal barrier in newborns but not in adults. Warshaw et al. (*38*) showed that immunogenic quantities of bovine serum albumin could traverse the intestine of adult rats. Up to 2% of the administered protein entered the intestinal lymphatics, and subsequently, the general circulation in an unaltered form. Using an everted gut sac technique, Walker et al. (*36,37*) proposed that intestinal antibody complexes with undigested proteins and retains them in the mucous lining, thus increasing their chances for degradation by intestinal proteases. Walker et al. (*35*) had previously shown that oral immunization decreased uptake of the immunizing antigen, but not of unrelated antigens; this result suggested that local immunity interfered with protein uptake in a specific manner. Thus intestinal antibodies may play a role in exclusion of intestinal antigens from either dietary or bacterial sources (detailed in Fig. 4). In a recent review of intestinal antibodies, Walker and Isselbacher (*34*) pointed out that the mucous barrier of the intestine, with its secretory antibodies, has become known as "antiseptic paint" to describe its function. This function is most pronounced in premature and newborn infants whose sIgA AFC are not yet functional. As pointed out by Walker and Isselbacher (*34*), allergy-prone infants showed an increased incidence of skin sensitivity to a variety of allergens when exposed before sIgA appearance as compared with infants exposed after sIgA appearance. The earlier that cow's milk was introduced to infants, the higher the circulating antibody level to milk proteins (*32*).

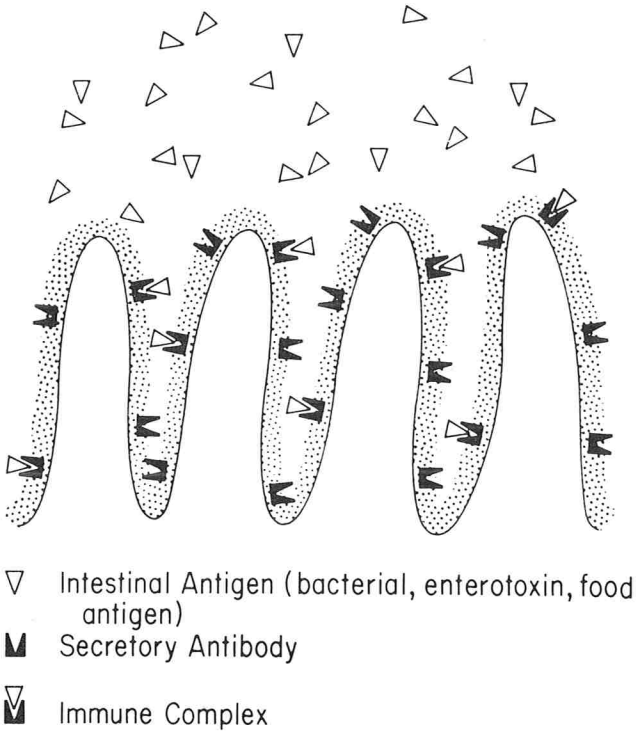

Figure 4. *Secretory antibodies (IgA) are produced in the lamina propria of the intestinal lining and are transported across the epithelium to the mucous lining of the gut, the glycocalyx. The antibodies, after reacting with their corresponding antigens, may facilitate enzymatic breakdown of the antigen, neutralize toxic substances or viruses, or prevent colonization of bacteria along the gut wall by preventing attachment. Reprinted, by permission, from The New England Journal of Medicine (297:767, 1977).*

LOCAL IMMUNITY AND CONSEQUENCES OF LOSS OF LOCAL IMMUNITY

Much evidence exists to show that the IgA-producing plasma cells in the lamina propria of the intestinal tract (and along all mucosal surfaces) are capable of interacting with absorbed antigens and responding locally (reviewed in *29*). Much of the original evidence for this phenomenon came from studies of poliovirus-induced antibody (*21*). Data collected to date suggest that the regulatory events controlling the local immune response are likewise expressed locally (*39*). Recent studies have shown that foodborne chemicals, both additives and natural constituents, are capable of direct interaction with cells required for the in vitro immune response, both antibody-producing plasma cells (*4,5,7*) and regulatory cells (*6*). In vivo models are necessary to test whether some chemicals are able, by some mechanism, to suppress the local immune response in the gastrointestinal tract. Such systems are currently being tested in this and other laboratories. Besides direct immunosuppression, long-term studies of indirect immunosuppression (toxicity to lymphoid-forming organs, hemopoietic organs, or chemically induced hormonal imbalances) are also necessary. Possible consequences of loss of local immunity come mainly from studies of selective IgA deficiency syndrome and are reviewed elsewhere (*3,33,34*).

The incidence of autoimmune disease in persons with selective IgA deficiency is very high (*2,3*). Temporary loss of immune exclusion could lead to autoimmune disorders. Antigenic materials crossing the normally exclusive intestinal barrier could induce production of autoantibodies directed, as a result of cross reactivity, to self antigens. Thus, loss of immune exclusion could contribute to an increased incidence of diseases such as systemic lupus erythematosus, rheumatoid arthritis, and thyroiditis. Increased uptake of antigenic material may also sensitize an individual to that substance so that, on second exposure to the antigen at a later time, an allergic reaction may occur (reviewed in *22*). Gastrointestinal allergies present a complex clinical picture and are poorly understood; epidemiological data are therefore difficult to obtain. The normal flora of the gut consists of potentially toxigenic bacteria and viruses; temporary increase in gut permeability due to a local immunosuppressive event could allow these organisms or their toxins to establish infection or to elicit gastrointestinal disturbances. In vivo studies with mouse models have shown that immunosuppressive drugs facilitated the invasion of *Pseudomonas aeruginosa* across the intestinal barrier (*24*), resulting in the death of the mouse. Malignancies have also been connected with selective IgA deficiency (*3*). There are several mechanisms by which the body screens for abnormal cells; chemicals affecting a variety of cells or cellular interactions of the immune system could disrupt this normal immunologic screening. A full discussion of all possible consequences of loss of local gut immunity cannot be presented here. The above examples represent only a partial list of the possible consequences of ingestion of an immunosuppressive compound. Few if any epidemiological studies have been done correlating food additive intake with any of the disorders presented.

Another aspect of local immunity that must not be overlooked is the interferon system. Interferons act on the host cell to prevent virus replication and are a major part of the body's defense against viral infections (reviewed in *13,15*). Interferons are produced in response to provocation of any cell by virus or viral nucleic acid (Type I or virus type interferon), or by lymphocytes in response to specific antigens or non-specific mitogens (Type II or immune interferon). The two types of interferons differ chemically, and much more is known about the virus-type interferon. Both types of interferon are immunosuppressive (*16,26*), and immune interferon relates quantitatively to suppressor T-cell activity (*17*). The role of interferons as immunoregulatory molecules has not been ascertained, but it was recently shown that a food additive metabolite could block production of immune interferon and suppressor cell activation concomitantly (*6*). Interferons also express their antiviral function along mucosal surfaces, and much remains to be done to determine the effects of ingested chemicals on production and function of interferons.

In summary, chemicals that can react with any

component (lymphoid cell or soluble mediator) of the immune response require closer scrutiny to determine their immunosuppressive potential. Several compounds have been shown to possess immunosuppressive activity in an in vitro mouse system (*4-7*). In vivo systems must be utilized to determine whether there is a correlation between in vitro results and in vivo immunologic events, particularly in the gastrointestinal tract. As pointed out in a recent review by Vos (*30*), the importance of the immune system has thus far been underestimated in toxicologic research; the few chemicals that have been shown to be immunosuppressive may be only the "tip of the iceberg."

REFERENCES

1. Allen, J. C. 1976. Infection and the compromised host. The Williams and Wilkins Co., Baltimore.
2. Ammann, A. J., and R. Hong. 1970. Selective IgA deficiency and autoimmunity. Clin. Exp. Immunol. 7:833-838.
3. Ammann, A. J., and R. Hong. 1973. Selective IgA deficiency. pp. 199-214. *In* E. R. Stiehm and V. A. Fulginiti (eds.). Immunologic disorders in infants and children. W. B. Saunders Company, Philadelphia.
4. Archer, D. L., J. A. Bukovic-Wess, and B. G. Smith. 1977. Inhibitory effect of an antioxidant, butylated hydroxyanisole, on the primary in vitro immune response. Proc. Soc. Exp. Biol. Med. 154:289-294.
5. Archer, D. L., J. A. Bukovic-Wess, and B. G. Smith. 1977. Suppression of macrophage-dependent T-lymphocyte function(s) by gallic acid, a food additive metabolite. Proc. Soc. Exp. Biol. Med. 156:465-469.
6. Archer, D. L., and H. M. Johnson. 1978. Blockade of mitogen induction of the interferon lymphokine by a phenolic food additive metabolite. Proc. Soc. Exp. Biol. Med. 157:684-687.
7. Archer, D. L., B. G. Smith, and J. A. Bukovic-Wess. 1978. Use of an in vitro antibody-producing system for recognizing potentially immunosuppressive compounds. Int. Arch. Allergy Appl. Immunol. 56:90-93.
8. Bridges, B. A. 1976. Short term screening tests for carcinogens. Nature 261:195-200.
9. Calderon, J., J. M. Kiely, J. L. Lefko, and E. R. Unanue. 1975. The modulation of lymphocyte functions by molecules secreted by macrophages. I. Description and partial biochemical analysis. J. Exp. Med. 142:151-163.
10. Calderon, J., and E. R. Unanue. 1975. Two biological activities regulating cell proliferation found in cultures of peritoneal exudate cells. Nature 253:359-361.
11. Chen, C., and J. G. Hirsch. 1972. The effects of mercaptoethanol and of peritoneal macrophages on the antibody-forming capacity of nonadherent mouse spleen cells in vitro. J. Exp. Med. 136:604-617.
12. Gill, D. M., D. J. Evans, Jr., and D. G. Evans. 1976. Mechanism of activation of adenylate cyclase in vitro by polymyxin-released, heat labile enterotoxin of *Escherichia coli*. J. Infect. Dis. 133:s103-s107.
13. Gressor, I. 1977. On the varied biologic effects of interferon. Cell. Immunol. 34:406-415.
14. Hampton, C. M., and R. L. Myers. 1976. Effects of cyclamate calcium on the immune response in rabbits. Arch. Environ. Health 31:51-53.
15. Johnson, H. M., and S. Baron. 1976. Interferon: Effects on the immune response and the mechanism of activation of the cellular response. CRC Crit. Rev. Biochem. 4:203-227.
16. Johnson, H. M., and S. Baron. 1976. Interferon as the mediator of the suppressive effect of some interferon inducers in the in vitro immune response. IRCS Med. Sci. 4:187.
17. Johnson, H. M., J. E. Blalock, and S. Baron. 1977. Separation of mitogen-induced suppressor and helper cell activities during inhibition of interferon production by cyclic AMP. Cell. Immunol. 33:170-179.
18. Lemke, H., and H. G. Opitz. 1976. Function of 2-mercaptoethanol as a macrophage substitute in the primary immune response in vitro. J. Immunol. 117:388-395.
19. Lotzova, E., and E. R. Richie. 1977. Promotion of incidence of Adenovirus type 12 transplantable tumors by carrageenan, a specific antimacrophage agent. J. Nat. Cancer Inst. 58:1171-1172.
20. Mishell, R. I., and R. W. Dutton. 1967. Immunization of mouse spleen cell cultures from normal mice. J. Exp. Med. 126:423-442.
21. Ogra, P. L., and D. T. Karzon. 1969. Distribution of poliovirus antibody in serum, nasopharynx and alimentary tract following segmental immunization of lower alimentary tract with polio-vaccine. J. Immunol. 102:1423-1430.
22. Parker, C. W. 1977. Immune responses to environmental antigens absorbed through the gastrointestinal tract. Fed. Am. Soc. Exp. Biol. 36(5):1732-1735.
23. Pierce, C. W., B. M. Johnson, H. E. Gershon, and R. Asofsky. 1971. Immune responses in vitro. III. Development of primary IgM, IgG, and IgA plaque-forming cell responses in mouse spleen cell cultures stimulated with heterologous erythrocytes. J. Exp. Med. 134:395-416.
24. Schook, L. B., L. Carrick, Jr., and R. S. Beck. 1976. Murine gastrointestinal tract as a portal of entry in experimental *Pseudomonas aeruginosa* infections. Infect. Immun. 14:564-570.
25. Smith, B. G., and H. M. Johnson. 1975. The effect of staphylococcal enterotoxins on the primary in vitro immune response. J. Immunol. 115:575-578.
26. Sonnenfeld, G., A. Mandel, and T. C. Merigan. 1977. The immunosuppressive effect of Type II mouse interferon preparations. on antibody production. Cell. Immunol. 34:193-206.
27. Spiers, R. S., R. W. Benson, B. J. Knowles, and D. Roberts. 1978. Models for assessing the effect of toxicants on immunocompetence in mice. II. Effect of cyclophosphamide on the antibody responses to Type III pneumococcal polysaccharide and tetanus toxoid in BALB/c female mice. J. Env. Pathol. Toxicol. In press. 1978.
28. Thomson, A. W., A. R. Wilson, W. J. Cruickshank, and A. H. Jeffries. 1976. Evidence for a selective cytotoxic effect of carrageenan on cells of the immune system in vivo and in vitro. Experentia 32:525-526.
29. Tomasi, T. B., Jr. 1976. The immune system of secretions. p. 161. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.
30. Vos, J. G. 1977. Immune suppression as related to toxicology. CRC Crit. Rev. Toxicol. 5(1):67-101.
31. Waksman, B. H., and Y. Namba. 1976. On soluble mediators of immunologic regulation. Cell. Immunol. 21:161-176.
32. Walker, W. A. 1976. Host defense mechanisms in the gastro-intestinal tract. Pediatrics 57:901-916.
33. Walker, W. A., and K. J. Isselbacher. 1974. Uptake and transport of macromolecules by the intestine: Possible role in clinical disorders. Gastroenterology 67:531-550.
34. Walker, W. A., and K. J. Isselbacher. 1977. Intestinal antibodies. N. Engl. J. Med. 297:767-773.
35. Walker, W. A., K. J. Isselbacher, and K. J. Bloch. 1972. Intestinal uptake of macromolecules: effect of oral immunization. Science 177:608-610.
36. Walker, W. A., M. Wu, K. J. Isselbacher, and K. Bloch. 1975. Intestinal uptake of macromolecules. III. Studies on the mechanism by which immunization interferes with antigen uptake. J. Immunol. 115:854-861.
37. Walker, W. A., M. Wu, K. J. Isselbacher, and K. J. Bloch. 1975. Intestinal uptake of macromolecules. IV. The effect of pancreatic duct ligation on the breakdown of antigen and antigen-antibody complexes on the intestinal surface. Gastroenterology 69:1223-1229.
38. Warshaw, A. L., W. A. Walker, and K. J. Isselbacher. 1974. Protein uptake by the intestine: evidence for absorption of intact macromolecules. Gastroenterology 66:987-992.
39. Yardley, J. H., D. F. Keren, S. R. Hamilton, and G. H. Brown. 1978. Local (Immunoglobulin A) immune response by the intestine to cholera toxin and its partial suppression with combined systemic and intraintestinal immunization. Infect. Immun. 19:589-597.

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Salmonella/Mammalian-Microsome Test to Detect Chemical Mutagens

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ABSTRACT

With few exceptions, chemical mutagens are carcinogens. A number of short-term microbial pre-screening procedures for detection of potential chemical mutagens are now available. One of these, the *Salmonella*/mammalian-microsome assay (Ames test), has proven to be highly reliable in predicting what chemicals are potential carcinogens. The test is designed primarily to identify which chemicals are mutagens, so that priorities can be established for further testing in long-term animal carcinogenicity studies. This report describes what the *Salmonella* test is, and how the test has been used as a tool to detect mutagens in our food and drinking water. The usefulness of the test in detecting mutagenic metabolites in human feces and urine is also discussed briefly.

More than 200 years ago, chimney soot was suggested to be a carcinogen (*36*); it was the first environmental substance to be so implicated. Since that time, scientists have been investigating the mechanisms of the development of cancer, but much remains to be discovered. However, epidemiologic studies have provided and are increasingly providing evidence that environmental factors do have an important role in the induction of cancer in humans.

Although some physical agents, such as ionizing and nonionizing radiation, have been directly implicated in tumor development, most human cancers are believed to be caused by exposure to a variety of carcinogenic chemicals. Such exposure may be occupational, social, dietary, or cultural. Liver and lung tumors have a high incidence among workers in the vinyl chloride and asbestos industries, respectively, and are well-documented examples of the risks of occupational exposure to chemicals (*22,39*). The influence of social, dietary, and cultural factors on carcinogenesis is evidenced by the wide variation in incidence of some types of cancer in genetically similar populations living in different geographic areas. As populations move to different areas and become accustomed to new environments, their risk increases for developing the types of cancer prevalent in the regions of their relocation (*7,10*).

Scientists believe generally that 20 to 30 years is the expression period for chemically induced cancer and that such cancer reflects the environmental influences and cultural habits of that time. Since World War II, new substances have been entering the environment in increasing numbers each year without having been tested for their potential carcinogenic activity. Of the 2 million known chemicals, only a few thousand have been tested for their tumor-inducing activity. Moreover, many of the long-term carcinogenicity studies in laboratory animals that have been used most frequently to evaluate these chemicals are now believed to be inadequate (*13*).

A number of short-term microbial mutagenesis assays are now available. They have a high predictive value for mammalian carcinogenesis and are particularly well suited for rapid screening of chemicals (*3,8,17,41*). Many of these tests are based on the use of microorganisms to detect agents that react with and modify DNA; this detection is important because ample evidence suggests that DNA damage leads to mutational events in all living organisms. A cellular genetic change is important to man because it may result in tumor induction (somatic mutation theory of carcinogenesis). With the increasing testing for mutagenic activity of chemicals--including known carcinogens and noncarcinogens--in microbial assays systems has emerged a pattern of high correlation between carcinogenicity and mutagenicity of chemicals (*26,35*). Thus, short-term microbial screening tests are useful in identifying which chemicals are mutagens. One microbial test that has proven to be highly reliable in predicting what chemicals are potential carcinogens is the *Salmonella*/mammalian-microsome test, developed by Dr. Bruce Ames and colleagues at the University of California at Berkeley (*2,3,5,6,18,27*).

THE TEST SYSTEM

The test consists of using several standard, specially constructed strains of *Salmonella typhimurium* that are

unable to grow in the absence of histidine because of a specific mutation in the histidine operon. When these histidine-dependent cells are grown on a plate of minimal medium containing a trace of histidine, only those cells that revert to histidine independence (*his*$^+$) are able to form colonies. The small amount of histidine allows all the plated bacteria (about 10^8) to undergo a few cell divisions; in some cases, this growth is essential for mutagenesis to occur (*47*). The *his*$^+$ revertants are easily scored as colonies against the slight background of growth. The spontaneous mutation frequency of each tester strain is relatively constant, but when a chemical mutagen is added to the agar, the mutation frequency is significantly increased.

Some carcinogenic chemicals, either of the aromatic amino or polycyclic hydrocarbon type, are inactive unless they are metabolized to active forms. In animals and man, enzyme systems in the liver or other organs (e.g., lung or kidney) are capable of metabolizing a large number of these chemicals to carcinogens. Some of these intermediate metabolites are very potent mutagens in the *Salmonella* test (*6*). Therefore, an important aspect of mammalian metabolism is included in the test by addition of a mammalian microsomal activation system (usually rat liver) to the petri plate.

Tester strains

The five standard bacterial tester strains are described in Table 1. Strain TA1535 has the missense mutation *hisG46* (*14*) and is reverted to histidine independence by many mutagens that cause base-pair substitutions (e.g., β-propiolactone). Strain TA1537 has the frameshift mutation *hisC3076* and is sensitive to some acridines and benzanthracenes, whereas strain TA1538 with the frameshift mutation *hisD3052* is particularly sensitive to other frameshift mutagens such as 2-nitrosofluorene. The mutations in these two frameshift mutants have been studied at the molecular level. Strain TA1537 has a run of cytosine bases at the site of the mutation (*2*), and strain TA1538 has a repetititve sequence of alternating cytosine-guanine bases near the site of the histidine mutation (*15*).

Strains TA100 and TA98 are derived from strains TA1535 and TA1538, respectively, by the introduction of the resistance transfer factor plasmid pKM101. Resistance transfer factor plasmids (R-plasmids) are transmissible, extrachromosomal genetic elements capable of conferring resistance to one or more antibacterial drugs on their hosts. Some R plasmids have been shown to enhance physically or chemically induced mutation rate (*20,29-31*). Plasmid pKM101 was derived from the R plasmid R46 and provides resistance to the antibiotic ampicillin. (Its parent plasmid provides resistance to three other drugs--streptomycin, tetracycline, and sulfonamide.)

Comparison of a variety of R plasmids as to their ability to enhance chemical mutagenesis in some of the *Salmonella* tester strains revealed that plasmid pKM101 was generally superior in increasing the mutation frequency (*27*). The introduction of this plasmid into the test system has enabled detection of new classes of carcinogens previously not detected as mutagens with the original strains [e.g., the nitrofuran food additive AF2 [2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide]]. In addition, the plasmid-carrying strains are much more sensitive to some carcinogens previously detected as weak mutagens (e.g., benzo(*a*)pyrene). The exact mechanism involved in the plasmid-mediated enhanced mutagenesis is not known. However, indirect evidence suggests that plasmid pKM101 carries genes that code for additional DNA repair enzymes that are involved in the inducible error-prone (SOS) DNA repair pathway. (*46,48*).

In addition to having mutations in the histidine operon, all the indicator strains have two additional mutations, namely, *rfa* and *uvrB*. The *rfa* mutation leads to a defective lipopolysaccharide layer that coats the bacterial surface. This mutation causes an increase in the permeability of the cell wall, thereby allowing more mutagens to penetrate the cell. The *uvrB* gene is needed for excision repair of DNA damage by certain chemical or physical agents. A deletion mutation that covers the *gal-chl-bio-uvrB* region of the chromosome results in elimination of the excision repair system, which enhances the strains' sensitivity to some mutagenic chemicals (*1*).

TABLE 1. *Genotype of the bacterial tester strain.*[a]

TA1535	TA1537	TA1538	TA98	TA100
missense mutation *hisG46*	frameshift mutation *hisC3076*	frameshift mutation *hisD3052*	frameshift mutation *hisD3052*	missense mutation *hisG46*
			plasmid pKM101	plasmid pKM101

[a]All tester strains carry the following additional mutation:
uvrB: defective excision (accurate) repair of damaged DNA lesions;
rfa: increased permeability of the cell wall (LPS)
Plasmid pKM101 increases the sensitivity of the strains to certain chemical mutagens, probably by providing additional enzymes involved in error-prone DNA repair.

The standard plate incorporation assay

This quantitative test consists of adding the test chemical, about 10^8 bacteria, and mammaliam liver homogenate (optional) to 2 ml of melted top agar (0.6%) supplemented with 0.05 mM histidine and 0.05 mM biotin. This mixture is poured onto a petri plate containing minimal medium (Fig. 1). When the thin agar overlay has set, the plates are incubated for 2 days at 37 C and the number of histidine revertant colonies are scored (Fig. 2). Different concentrations of the chemical are usually tested to obtain a dose-response curve. At the higher doses, the number of revertant colonies may decrease due to toxicity of the chemical.

The spot test

This is a qualitative test. It differs from the standard plate incorporation assay in that the test chemical is applied to the center of the minimal medium plate when

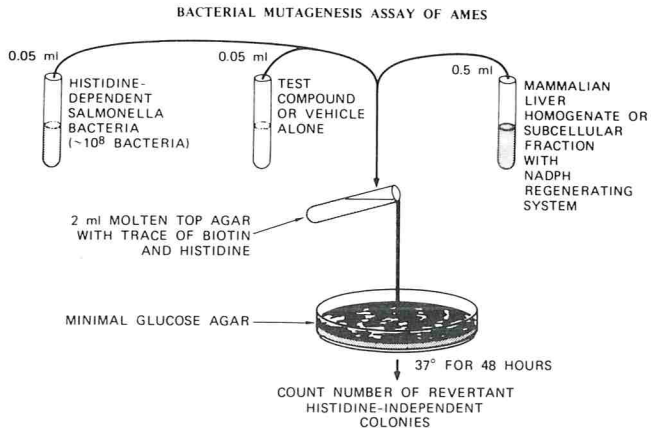

Figure 1. *Experimental procedure for testing chemicals in the standard plate incorporation assay.*

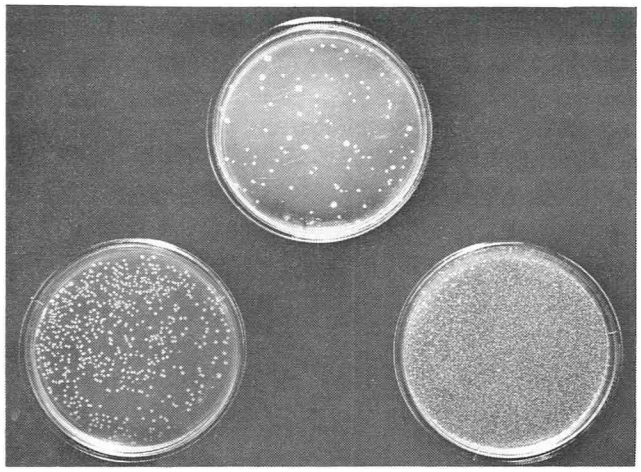

Figure 2. *The standard plate incorporation assay. Mutagenic activity of ethyl methanesulfonate (EMS) in strain TA100. Top plate: spontaneous revertants; left bottom plate: 10 mg of EMS; right-bottom plate: 50 mg of EMS.*

the overlay agar containing the bacteria (with or without metabolic activation) has set. As the chemical diffuses out into the agar, a concentration gradient is formed. Growth of revertant colonies around the center spot indicates a positive result (Fig. 3). The advantages of using this test are: (*a*) a small amount of the chemical is needed (usually less than 1 mg), and (*b*) few plates are needed. However, only compounds that diffuse readily can be tested in this way.

Testing of volatile liquids and gases

The standard plate incorporation assay and the spot test are not suitable for testing volatile liquids and gases, but these can be tested in desiccators. Plates are prepared as described for the standard plate incorporation assay, but no test chemical is added. Plates, the lids having been removed, are then placed side by side on a perforated ceramic shelf in the desiccator. For volatile liquids, a known volume is placed in a glass petri plate attached to the center bottom of the shelf. The desiccator is sealed and placed on a magnetic stir plate in a 37 C incubator. A magnetic stirrer with vanes, placed in the

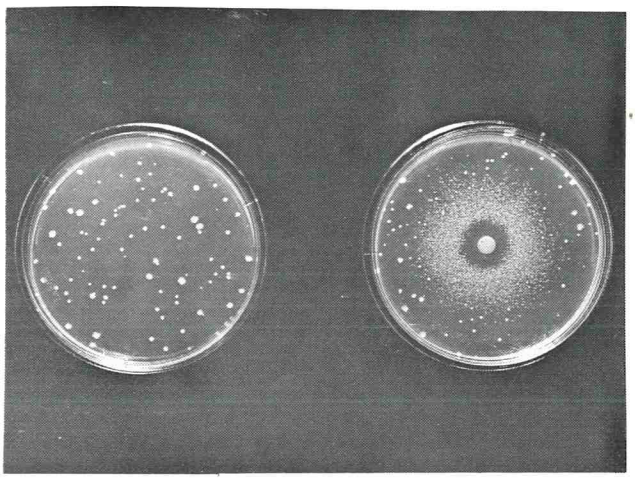

Figure 3. *The spot test. Ethyl methane sulfonate (EMS) induced histidine revertants of strain TA100. Left plate: spontaneous revertants; right plate: 10 mg of EMS.*

base of the desiccator, ensures adequate dispersion of the chemical. With gases, a known volume of the test gas is introduced into the desiccator after evacuation of the air. The pressure inside the desiccator is then normalized by letting air in through a sterile cotton plug. Plates are left in the desiccator for about 8 h and then removed. Their lids are replaced and they are further incubated at 37 C for another 42 h.

RELIABILITY OF THE TEST

The test has proven to be about 85% accurate in detecting chemical carcinogens as mutagens and is equally accurate in predicting that a non-mutagenic compound is noncarcinogenic. In one study by McCann et al. (*24,26*) 313 chemicals were tested with one or more of the five standard tester strains. Of 179 known carcinogens, 157 (87.7%) were found to be mutagenic; of 117 non-carcinogens, 101 (86.3%) were found to be nonmutagenic. Of 17 compounds for which no carcinogenicity data were available, 11 (65.7%) were found to be mutagenic. In another study conducted by Sugimura et al. (*44*), a total of 232 chemicals were tested using one or more of the *Salmonella* tester strains. Of the 98 chemicals that are known carcinogens, 90 (91.8%) were found to be mutagenic. Of the 47 chemicals known to be noncarcinogenic, 36 (76.6%) were nonmutagenic. Of 87 compounds for which no carcinogenicity data were available, 50 (57.4%) were mutagenic. False-positive or false-negative results may be obtained for a variety of reasons, some of which are discussed below.

False-positives

One of the interesting findings that has come out of *Salmonella* assay is that there is a million-fold range in mutagenic potency of chemicals. For instance, one nanomole of 1,2-epoxybutane gives rise to less than one histidine revertant, whereas one nanomole of AF2 gives rise to more than 20,000 revertant colonies. If a nonmutagenic compound contained only a trace amount

of a very potent mutagen, a false positive result could easily be obtained. For instance, the noncarcinogen 7-hydroxy-2-acetyl-aminofluorene was found to be weakly mutagenic in the *Salmonella* test by Donahue et al. (*11*). Further study using high-pressure liquid chromatography revealed the presence of a mutagenic impurity, probably the potent carcinogen 2-acetyl-aminofluorene.

Weak carcinogens are not easily detected as carcinogens in animal carcinogenicity tests, due to statistical limitations inherent in such studies. However, weak carcinogens are quite frequently detected as weak mutagens in the *Salmonella* test (e.g., styrene oxide and β-naphthylamine) (*24,26*).

False-negatives

False-negative results may be obtained because some chemicals are not activated by mammalian liver enzymes, but by the natural mammalian bacterial flora. Although the liver is indeed a very important metabolic organ, it is not always realized that a great deal of metabolism occurs in the gastrointestinal tract, which is filled with bacteria, primarily, anaerobes (which outnumber the aerobes by a factor of one thousand). These anaerobes are active in metabolizing chemical carcinogens to their active forms. McCoy et al. (*28*) demonstrated that the *Salmonella* mutagenicity assay can be coupled to cell-free extracts from anaerobic bacteria to activate chemical mutagens. They used extracts of *Clostridium perfringens* and *Bacteroides fragilis* to activate the potent carcinogen and mutagen 2-aminofluorene. This is a very important finding since recent studies have indicated a relationship between diet and the incidence of certain cancers, such as stomach and colon cancer. It should be pointed out, however, that 2-aminofluorene also gives a positive response in the *Salmonella* test when tested in the presence of rat liver enzymes.

The cancer-causing hormone diethylstilbesterol is not mutagenic in the *Salmonella* test, probably because hormones do not interact directly with DNA. They may exert their carcinogenic effect through an indirect, non-mutagenic event. It is also possible that some "false-negative" results may, in fact, not be false. Some animal carcinogencity studies have been shown to be inadequate because of poor control experiments (*13*) or because enormous doses of a test chemical were given to the animals, which raises the question about possible mutagenic contaminants. For example, acetamide is carcinogenic in rats but is nonmutagenic in the *Salmonella* test. In one animal study, rats were given a diet containing more than 1% acetamide for 1 year. However, it is not known how pure the test compound was (*24*).

OVERLAPPING OF CARCINOGENS AND MUTAGENS

Over the years, as microbial mutagenesis assays have been improved, a pattern of high correlation between carcinogencity and mutagenicity of chemicals has emerged (*24,26,35,44*). As late as 1960, 4-nitroquinoline-1-oxide was one of the few chemical carcinogens that was also identified as being mutagenic (*32*). The mutagenicity of well-known carcinogens such as dimethylnitrosamine was not demonstrated until 1962 (*34*), 2-acetylamino-fluorene until 1968 (*21*), benz(a)anthracene until 1972 (*4*), and N, N-dimethyl-4-aminoazobenzene until 1975 (*49*). Butyl-N-(4-hydroxybutyl) nitrosamine was shown to be a carcinogen in 1964, but was not identified as a mutagen until 1975 (*12*). Conversely, the mutagen N-methyl-N'-nitro-N-nitrosoguanidine was not identified as a carcinogen until 1966 (*23,38,42*). It is now generally accepted that most chemical carcinogens are mutagens. With further improvements in microbial tester strains and metabolic activation, the exceptions will continue to decrease. However, certain classes of carcinogens will probably never be detected as mutagens. As already mentioned, hormones may exert their carcinogenic effect through a nonmutagenic event.

DETECTION OF CHEMICAL MUTAGENS IN FOOD

A classical example used to put emphasis on the importance of microbial tests in the detection of chemical carcinogens is the Japanese food additive AF2. This food additive was used extensively in Japan from 1965 until recently as an antibacterial food additive in a wide variety of common food products such as soybean curd and fish sausage. It was found to be negative in tests for carcinogenicity in rats in 1962 and 1964. Additional animal carcinogenicity testing in mice in 1971 also gave negative results. In 1973, however, AF2 was found to be highly mutagenic in a strain of *Escherichia coli* (*16,19*) and was also detected as a potent mutagen by the *Salmonella* test in the newly constructed strain TA100 carrying plasmid pKM101. (*27*). When subsequently examined in other eukaryotic organisms, AF2 was found to be mutagenic in yeast and Neurospora (*33*). All these reports on the mutagenicity of AF2 led to new animal carcinogenicity tests that were more extensive than the previous ones. These tests revealed that AF2 is indeed a carcinogen. Based on these reports, the Japanese government immediately banned the use of AF2 as a food additive and all products already containing AF2 were removed from the market. Since the expression time for cancer in humans is usually between 20 and 30 years, it is too early to tell whether there will be an increase in the cancer rate in Japan as a consequence of the general use of this food additive.

Mycotoxins

Aflatoxin B_1, a mycotoxin produced by *Aspergillus flavus*, is a very potent liver carcinogen. Aflatoxin B_1 is found in contaminated corn and peanuts. It is a potent mutagen in the *Salmonella* test. For instance, 100 ng of this toxin gives rise to about 1,000 histidine revertants per plate with strain TA100 (*25*). Presence of the plasmid pKM101 significantly enhances the mutagenic effect.

Strain TA1535, the patent strain of TA100, barely detects this chemical as a mutagen. A variety of other mycotoxins produced by fungi that contaminate grains have also been found to be mutagenic in the *Salmonella* test. An example is fusarenon X, produced by *Fusarium nivale,* which contaminates wheat, rice, and corn. PR-toxin, produced by *Penicillium roqueforti*, has also been detected as a mutagen with the *Salmonella* tester strain TA100. *P. roqueforti* has been detected as a contaminant in wheat flour and pickles. Some of these mutagenic mycotoxins have been shown to be carcinogenic in animal studies; others have not yet been tested for carcinogenicity.

Methylene chloride

Until recently, trichloroethylene, which is a weak mutagen and carcinogen, was used in the decaffeination of coffee. When it was found to be carcinogenic in animal studies done at the National Cancer Institute, it was replaced by the related chemical methylene chloride. However, the latter is also a mutagen in the Ames test (*40*). No reports on the carcinogenicity of methylene chloride have been published. Also, no data are available on the residual levels of this compound in decaffeinated coffee.

Pyrolysis products

Sugimura and colleagues at the National Cancer Research Institute in Japan used the *Salmonella* assay in a survey of possible causative agents of human cancer, especially in foods (*43*). They found that smoke from broiled fish and meat have high mutagenic activity and that the charred surfaces of broiled fish and steak also have fairly high mutagenic activity. Apparently, it is the pyrolysis of proteins and amino acids that gives rise to the high mutagenicity of various substances. Further examination revealed that pyrolysis products of tryptophan had the highest mutagenic activity. Pyrolysates of sugars such as glucose, arabinose, fructose, sorbitol, and glucosamine were also found to have mutagenic activity.

Nitroso compounds

Of all the groups of known chemical carcinogens, the nitroso compounds are likely to be the major causative agent in the induction of human gastric cancer associated with consumption of food. Nitroso compounds have been shown to be potent carcinogens in animal studies, but there is as yet no direct linking them with human cancer. The *Salmonella* test detects many nitroso compounds (but not nitrosamines) quite readily as mutagens, but caution should be used in evaluating the results of such tests. The *Salmonella* tester strains contain nitro reductase enzymes that convert many nitroso compounds to their active forms. However, Rosenkranz and Speck (*37*) found that mammalian liver enzymes can also convert nitroso compounds to their active forms.

Preformed nitrosamines found in food are usually present at low levels--somewhere around 10 parts per billion or less. The one most commonly found is dimethylnitrosamine, a potent carcinogen, which can be present in hot dogs, ham, and luncheon meat. Another nitroso compound commonly found in cured meats is nitrosoproline. Although this compound is not carcinogenic, upon heating it is converted to the carcinogenic substance nitrosopyrolidine. Thus, raw bacon does not contain the carcinogen, but cooked bacon does. More important sources of a variety of nitrosamines are amines and nitrites. It is standard practice to cure meat with nitrate, which is converted to nitrite by bacterial reduction. The nitrite then interacts with blood pigment to form a stable red-brown color, thereby increasing the attractiveness of the meat.

Many drugs and food additives are nitrosatable amines. The drug aminopyrine, for instance, which until a few years ago was commonly used in this country as an analgesic, reacts readily with nitrous acid. One of the products formed is the potent carcinogen and mutagen dimethylnitrosamine. In one study in which rats were fed aminopyrine and nitrite for 6 months, the incidence of liver tumors was 100%. These tumors were found to be identical to those induced in animals that had been fed dimethynitrosamine (*45*).

In Western countries, cancer of the colon has a high mortality rate, and the disease seems to be associated with the diet. Bruce and colleagues at the Ontario Cancer Institute in Canada examined the possibility that human feces might contain mutagenic chemicals (*9*). Using the *Salmonella* test, they found that feces of four normal individuals who were on a typical Western diet contained mutagenic activity. None of the subjects took any medication or vitamin supplements. After extraction, fractionation, and some purification of the feces, mutagenic activity was assayed with tester strain TA100. Mutagenic response curves were obtained for eight consecutive fecal samples and no metabolic system was needed to induce the mutagenic response. After further purification of the samples by high-pressure liquid chromatography, the mutagenic activity was attributed to the presence of a nitroso compound.

Drinking water

Another important aspect of our diet that deserves special attention is drinking water. For hundreds of years, drinking water has been a topic of interest as a source of human disease. Cholera and typhoid fever come to mind immediately as diseases associated with impure water. In many cities in the United States, chlorination of drinking water has virtually eliminated these diseases. However, a large number of organic chemicals, including alkyl halides, have been identified in finished drinking water. Many alkyl halides are believed to be formed as a result of chlorination.

Research into the relationship of cancer to drinking water is still in its infancy. However, there is concern that widespread contamination of drinking water with minute amounts of chemical carcinogens might increase the incidence of cancer. Over 500 organic chemicals have

been identified in drinking water in the United States. Some of these compounds are known carcinogens, but no carcinogenicity data are available on most of them. Simmon et al. (*40*) reported that 34% of 71 compounds tested were found to be mutagenic. However, the selection of chemicals from the list of 500 may have biased the outcome of the test results. But it can be predicted that at least 10% of the chemicals found in drinking water will turn out to be mutagenic. Some examples of mutagenic chemicals that are carcinogenic and that are present in finished drinking water are bromodichloromethane, vinyl chloride, bromoform, dichloroacetonitrile, and benzo(a)pyrene. Since many of these organic chemicals are present in very low concentrations in drinking water, it is difficult to assess the role, if any, of these mutagenic and/or carcinogenic chemicals in the induction of human cancer.

Mutagens in urine.

Ames and colleagues at the University of California at Berkeley have adopted the *Salmonella* test to examine mutagenic activity in the urine. They found, for instance, that urine from cigarette smokers has mutagenic activity, but urine from nonsmokers or from cigarette smokers who do not inhale was not mutagenic (*50*). These researchers suggested that the *Salmonella* test be used for monitoring exposure to dangerous chemicals by examining human urine for mutagenic metabolites.

CONCLUSION

Over the past few years the *Salmonella* test has proven to be highly reliable in predicting which chemicals are potentially hazardous to humans. However, this test should be used NOT as a final test, but to establish priorities for further testing in other available systems. If chemicals were properly tested and evaluated, greater control over their general use could be exercised and perhaps most human cancers could be prevented or their incidence could be reduced.

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REFERENCES

1. Ames, B. N. 1971. The detection of chemical mutagens with enteric bacteria. pp. 267-282. In A. Hollaender (ed.) Chemical mutagens: Principles and methods for their detection. Plenum Press, New York.
2. Ames, B. N., F. D. Lee, and W. E. Durston. 1973. An improved bacterial test system for the detection and classification of mutagens and carcinogens. Proc. Nat. Acad. Sci. USA 70:782-786.
3. Ames, B. N., J. McCann, and E. Yamasaki. 1975. Method for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. Mutation Res. 31: 970-973.
4. Ames, B. N., P. Sims, and P. L. Grover. 1972. Epoxides of carcinogenic polycyclic hydrocarbons are frameshift mutagens. Science 176:47-49.
5. Ames, B. N., E. G. Gurney, J. A. Miller, and H. Bartsch. 1972. Carcinogens as frameshift mutagens: Metabolites and derivatives of 2-acetylamino-fluorene and other aromatic amine carcinogens. Proc. Nat. Acad. Sci. USA 69:3128-3132.
6. Ames, B. N., W. E. Durston, E. Yamasaki, and F. D. Lee. 1973. Carcinogens are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection. Proc. Nat. Acad. Sci. USA 70:2281-2285.
7. Bailar, J. C. 1965. Distribution of carcinoma of esophagus, stomach and large bowel. pp. 3-14. In W. J. Burdette (ed.) Carcinoma of the alimentary tract. University of Utah Press, Salt Lake City, Utah.
8. Bridges, B. A. 1972. Simple bacterial systems for detecting mutagenic agents. Lab. Pract. 21:413-419.
9. Bruce, W. R., A. J. Varghese, R. Furrer, and P. C. Land. 1977. A mutagen in the feces of normal humans. pp. 1641-1646. In H.H. Hiatt, J. D. Watson, and J. A. Winsten (eds.) Origins of human cancer. Cold Spring Harbor Laboratory, New York.
10. Doll, R. 1967. Worldwide distribution of gastrointestinal cancer. Nat. Cancer. Inst. Monogr., 25:173-190.
11. Donahue, E. V., J. McCann, and B. N. Ames. 1978. Detection of mutagenic impurities in carcinogens and noncarcinogens by high-pressure liquid chromatography and the *Salmonella*/microsome test. Cancer. Res. 38:431-438.
12. Druckrey, H., R. Preussmann, S. Ivankovic, C. H. Schmidt, H. D. Mennel, K. W. Stahl. 1966. Selektive Erzeugung von Blasenkrebs an Ratten durch Dibutyl- und N-Butyl-N-butanol(4)nitrosamin. Z. Krebsforsch. 66:280-290.
13. Gillette, P. 1974. Cancer and the environment (II): Groping for new remedies. Science 186:242-245.
14. Hartman, P. E., Z. Hartman, R. C. Stahl, and B. N. Ames. 1971. Classification and mapping of spontaneous and induced mutations in the histidine operon of *Salmonella*. Adv. Genetics 16:1-34.
15. Isono, K., and J. Yourno. 1974. Chemical carcinogens as frame-shift mutagens: *Salmonella* DNA sequence sensitive to mutagenesis by polycyclic carcinogens. Proc. Nat. Acad. Sci. USA 71:1612-1617.
16. Kada, T. 1973. *Escherichia coli* mutagenicity of furylfuramide. Japan. J. Genet. 48:301-305.
17. Kada, T., Y. Sadaie, and K. Tutikawa. 1972. In vitro and host-mediated "rec-assay" procedures for screening chemical mutagens; and phlozine, a mutagenic red dye detected. Mutation Res. 16:165-174.
18. Kier, L. D., E. Yamasaki, and B. N. Ames. 1974. Detection of mutagenic activity in cigarette smoke condensates. Proc. Nat. Acad. Sci. USA 71:4159-4163.
19. Kondo, S., and H. Ichikawa-Ryo. 1973. Testing and classification of mutagenicity of furylfuramide in *Escherichia coli.* Japan. J. Genet. 48:295-300.
20. MacPhee, D. G. 1973. *Salmonella typhimurium hisG46* (R-Utrecht): Possible use in screening mutagens and carcinogens. Appl. Microbiol. 26:1004-1005.
21. Maher, V. M., E. C. Miller, J. A. Miller, and W. Szybalski. 1968. Mutations and decreases in density of transforming DNA produced by derivatives of carcinogens 2-acetylaminofluorene and N-methyl-4-aminoazobenzene. Mol. Pharmacol. 4:411-426.
22. Maltoni, C. 1977. Vinyl chloride carcinogenicity: An experimental model for carcinogenesis studies, pp. 119-146. In. H. H. Hiatt, J. D. Watson, and J. A. Winsten (eds.) Origins of human cancer. Cold Spring Harbor Laboratory, New York.
23. Mandell, J. D., and J. Greenberg. 1960. A new chemical mutagen for bacteria, 1-methyl-3-nitro-1-nitrosoguanidine. Biochem. Biophys. Res. Commun. 3:575-577.
24. McCann. J., and B. N. Ames. 1976. Detection of carcinogens as mutagens in the *Salmonella*/microsome test: Assay of 300 chemicals: discussion. Proc. Nat. Acad. Sci. USA 73:950-954.
25. McCann, J., and B. N. Ames. 1977. The *Salmonella*/microsome mutagenicity test: Precidictive value for animal carcinogenicity. pp. 1431-1450. In H. H. Hiatt, J. D. Watson, and J. A. Winsten (eds.) Origins of human cancer. Cold Spring Harbor Laboratory, New York.
26. McCann, J., E. Choi, E. Yamasaki, and B. N. Ames. 1975. Detection of carcinogens as mutagens in the *Salmonella*/microsome test: Assay of 300 chemicals. Proc. Nat. Acad. Sci. USA 72:5135-5139.

27. McCann, J., N. E. Spingarn, J. Kobori, and B. N. Ames. 1975. Detection of carcinogens as mutagens: Bacterial tester strains with R factor plasmids. Proc. Nat. Acad. Sci. USA 72:979-983.
28. McCoy, E. C., W. T. Speck, and H. S. Rosenkranz. 1977. Activation of a procarcinogen to a mutagen by cell-free extracts of anaerobic bacteria. Mutation Res. 46:261-264.
29. Mortelmans, K. E. 1975. Effect of R plasmids of *Salmonella typhimurium:* UV-killing, UV-mutagenesis and spontaneous mutability. Ph.D. Thesis, Stanford University.
30. Mortelmans, K. E., and B. A. D. Stocker. 1976. Ultraviolet light protection, enhancement of ultraviolet light mutagenesis, and mutator effect of plasmid R46 is *Salmonella typhimurium*. J. Bacteriol. 128:271-282.
31. Mortelmans, K. E., and B. A. D. Stocker. 1978. Segregation of the mutator property of plasmid R46 from its ultraviolet-protecting property. Mol. Gen. Genetics (accepted for publication).
32. Nakahara, W., F. Fukuoka, and T. Sugimura. 1957. Carcinogenic action of 4-nitroquinoline-N-oxide. Gann. 48:129-137.
33. Ong. T., and M. M. Shahin. 1974. Mutagenic and recombinogenic activities of food additive furylfuramide in eurkaryotes. Science 184:1086-1087.
34. Pasternak, L. 1962. Mutagenische Wirking von Dimethylnitrosamine bei *Drosophila melanogaster*. Naturwissenschaften 49:381.
35. Poirier, L. A., and V. F. Simmon. 1976. Mutagenic-carcinogenic relationships and the role of mutagenic screening tests for carcinogenicity. Clin. Toxicol. 9:761-771.
36. Pott, P. 1775. Cancer scrot. p. 63. In Chirurgical observations. Hawes, Clark and Collins, London.
37. Rosenkranz, H. S., and W. T. Speck. 1975. Mutagenicity of metronidasole: Activation by mammalian microsomes. Biochem. Biophys. Res. Commun. 66:520-525.
38. Schoental, R. 1966. Carcinogenic activity of N-methyl-N-nitroso-N'-nitroguanidine. Nature 209:726-727.
39. Selikoff, I. J. 1977. Cancer risk of asbestos exposure. pp. 1765-1784. In H. H. Hiatt, J. D. Watson, and J. A. Winsten (eds.) Origins of human cancer. Cold Spring Harbor Laboratory, New York.
40. Simmon, V. F., K. Kauhanen, and R. G. Tardiff. 1977. Mutagenic activity of chemicals identified in drinking water. pp. 249-258. In D. Scott, B. A. Bridges, and F. H. Sobels (eds.) Progress in genetic toxicology. Elsevier/North-Holland Biomedical Press.
41. Slater, E. E., M. D. Anderson, and H. S. Rosenkranz. 1971. Rapid detection of mutagens and carcinogens. Cancer. Res. 31:970-973.
42. Sugimara, T., M. Nagao, and Y. Okada. 1966. Carcinogenic activity of N-methyl-N'-nitro-N-nitrosoguanidine. Nature 210: 962-963.
43. Sugimura, T., M. Nagao, T. Kawachi, M. Honda, T. Yahagi, Y. Seino, S. Sato, N. Matsukura, T. Matsushima, A. Shirai, M. Sawamura, and H. Matsumoto. 1977. Mutagens-carcinogens in food, with special reference to highly mutagenic pyrolytic products in broiled foods. pp. 1561-1575. In H. H. Hiatt, J. D. Watson, and J. A. Winsten (eds.), Origins of human cancer. Cold Spring Harbor Laboratory, New York.
44. Sugimura, T., S. Sato, M. Nagao, T. Yahagi, T. Matsushima, Y. Seino, M. Takeuchi, and T. Kawachi. 1976. Overlapping of carcinogens and mutagens. pp. 191-215. In P. N. Magee, S. Takayama, T. Sugimura, and T. Matsushima (eds.) Fundamentals in cancer prevention. University Park Press, Baltimore, Maryland.
45. Taylor, H. W., and W. Lijinsky. 1975. Tumor induction in rats by feeding aminopyrine or oxytetracycline with nitrite. Int. J. Cancer. 16:211.
46. Walker, G. C. 1978. Isolation and characterization of mutants of the plasmid pKM101 deficient in their ability to enhance mutagenesis and repair. J. Bacteriol. 133:1203-1211.
47. Witkin, E. M. 1956. Time, temperature, and protein synthesis: A study of ultraviolet-induced mutations in bacteria. Cold Spring Harbor Symp. Quant. Biol. 21:123 (208, 209, 506, 507).
48. Witkin, E. M. 1974. Thermal enhancement of ultraviolet mutability in a *tif-1 uvrA* derivative of *Escherichia coli* B/r: Evidence that ultraviolet mutagenesis depends upon an inducible function. Proc. Nat. Acad. Sci. USA 71:1930-1934.
49. Yahagi, T., M. Degawa, Y. Seino, T. Matsushima, M. Nagao, T. Sugimura, and Y. Hashimoto. 1975, Mutagenicities of carcinogenic azo dyes and their derivatives. Cancer Letters 1:91-96.
50. Yamasaki, E., and B. N. Ames. 1977. The concentration of mutagens from urine by XAD-2 adsorption. Cigarette smokers have mutagenic urine. Proc. Nat. Acad. Sci. USA 74:3555-3559.

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Mutagenic Analysis as a Means of Detecting Carcinogens in Foods[1]

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ABSTRACT

The Ames test is a valuable screening procedure for environmental carcinogens which are believed to be responsible for a large part of the cancer incidence in the U.S. Mutagens originally detected in bacterial nutrient containing "beef extract" have now been detected in commercial beef extract and in commercial foods containing beef extract. The mutagens did not occur in uncooked beef tissue or in beef stock. The mutagens were formed when beef stock was extensively boiled to produce beef extract. The same mutagens (as evidenced by their chromatographic behavior) were found when ground beef hamburgers are cooked at temperatures in excess of 150-200 C. Well-done hamburgers cooked on an electric frying pan or hot plate, or an electric hamburger cooker (cooking temperatures 190-300 C) contained the mutagens. Hamburgers cooked under the heating element of an electric broiler, or in a microwave oven (but not on the "browning tray") contained no significant mutagenic activity. Mutagen content increased with cooking time, and was concentrated in the outer surface of the cooked hamburgers. These results need to be interpreted in terms of the known relationships between mutagenic activity toward *Salmonella* and carcinogenic activity toward laboratory animals and between the latter and the risk of cancer in people. The results show that the mutagens which have been found in beef extract and in cooked hamburgers represent a possible risk of cancer of unknown magnitude to people who ingest them. The results also show how the nutritional benefits of hamburgers can be enjoyed without incurring this risk, by a suitable choice of cooking methods.

Recognition that a considerable proportion of the incidence of cancer in the United States results from exposure to environmental carcinogens has stimulated interest in the rapid detection of such substances. The development by Ames (*1*) of a rapid means of detecting mutagens has considerably enhanced the opportunities for such studies. In the Ames test, certain strains of *Salmonella* that are genetically incapable of synthesizing the histidine which they require for growth are inoculated onto culture plates containing nearly histidine-free nutrient. When the plates are incubated, only those bacteria which mutate and become genetically capable of synthesizing histidine will grow and produce visible colonies. The number of such colonies produced per test plate is a measure of the rate of mutation. If a substance capable of inducing mutations (a mutagen) is added to such a test plate, the normal background rate of mutation is considerably enhanced. Some substances are mutagenic only after they are metabolically transformed by the mixed-function oxidases that are present in microsomes. For that reason the Ames test is usually carried out both in the absence and presence of such a microsome preparation (prepared from rat liver).

The applicability of the Ames test to detection of carcinogens is based on a long-held (but nonetheless controversial) theory that transformation of a normal cell into a cancerous one is basically a genetic change -- a mutation which alters the inherited characteristics that control cell growth. This theory suggests that chemicals which cause mutations in bacteria should also cause cancer in animals. If this relationship could be relied upon it would be possible to detect carcinogenic substances by determining their mutagenic effect on bacteria, such as *Salmonella*, without going through the expensive and slow process of testing them for their ability to cause cancer in laboratory animals.

Regardless of disagreements over the theoretical link between mutagenesis and carcinogenesis, it has been found empirically that the Ames test is a reliable means of detecting carcinogens. When substances already known to cause cancer in laboratory animals and those which are known to be noncarcinogens are tested in the Ames system, it is found that from 86 to 97% of the mutagenic substances are also carcinogenic toward laboratory animals (*2,5,14*). Thus the Ames test is an effective way of detecting a substance which is very likely to be carcinogenic toward laboratory animals, and which therefore may represent a risk of cancer to people who are exposed to it.

A number of laboratories, including our own, have consistently observed a minor anomaly in the Ames

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Salmonella mutagenesis system--that the presence of the microsomal preparation used to activate certain mutagens causes a small increase in the background rate of mutation of *Salmonella* strain TA1538. The effect usually increases the background or control rate of mutation to about 30-40 mutant colonies per test plate. However, since tests of active substances usually yield several hundred or more mutant colonies per test plate, the phenomenon does not interfere with interpretation of the Ames test.

On investigating this phenomenon we discovered that it is due to the presence of mutagens in the bacterial nutrients commonly used to culture the *Salmonella* in this system (*15*). We also found that the mutagens are present in a series of such nutrients which contain "beef extract," a common constituent of bacterial nutrients. Beef extract is prepared (usually in abattoirs) by boiling down beef stock (obtained by boiling minced beef tissue briefly in several times its weight of water and removing the tissue fat) to 20% or less of its original volume. The result is a dark brown paste, which is commercially available and is used not only in bacterial nutrients, but also in a number of food preparations such as bouillon cubes and gravy bases. About 4 million pounds of beef extract are produced in the United States annually, mostly for human consumption. Our studies also showed that the mutagens present in bacterial nutrients which contain beef extract are active toward *Salmonella* strain 1538, but only when the microsome preparation is present. Thus, like a number of other mutagens, the beef extract mutagens are not inherently mutagenic but require activation by the microsomal mixed-function oxidase system.

Given that bacterial nutrients from a wide variety of sources contain the mutagens, it appeared from these studies that the substances are commonly present in beef extract. Three possible origins of the mutagens need to be considered: (*a*) that they are natural constituents of beef tissue, (*b*) that they are environmental pollutants which are commonly taken up by cattle, or (*c*) that they are formed during the process of preparing beef extract from beef tissue.

Accordingly, we have undertaken experiments to determine which of these possible origins can account for the presence of mutagens in beef extract. As will be seen from the experiments described below, we have found that the mutagens are neither normal constituents of beef tissue nor ubiquitous pollutants but are formed when beef stock is boiled down to produce beef extract. These experiments also show that the same mutagens are produced when ground beef is cooked, in the form of an ordinary hamburger, in an electric frying pan or an electric home hamburger-cooking appliance, in which a critical temperature of 150-200 C is exceeded (*6*). We have also found the mutagens in several commercial food preparations which include beef extract as a constituent. As a result, these experiments raise the possibility that the mutagens detected in beef extract and in cooked hamburgers may represent a risk of cancer in people.

MATERIALS AND METHODS

Materials

Samples of commercial beef extract were obtained from Difco (Bacto Beef Extract, Difco Laboratories). Batches of lean meat were obtained from local stores, ground and used throughout the experiments. Beef stock was made in the laboratory by mixing ground beef with twice its volume of distilled water, followed by heating for 20 min at 40 C and finally by boiling for 10 min. The meat was removed by filtration through a muslin cloth, and the resultant beef stock kept in a 4-C cold room for 12 h. The solidified fat was then removed and the solution was filtered through glass wool and Whatman filter paper (no. 21). "B-V Concentrate" and "Maggi Bouillon Cubes" were bought in local supermarkets.

Methods

Mutagenesis assay. Mutagenesis assays were carried out according to the conventional Ames procedure (*1*). All samples were tested with and without PCB-induced rat liver microsome preparation ("S-9"). After 48 h of incubation at 37 C, the number of revertant colonies on duplicate plates was counted. All results are reported as the averages of the duplicate plates. Results obtained in the absence of the microsome preparation were uniformly negative and are not reported here. The values of mutagenic activity reported in the paper, unless otherwise specified, were obtained with strain TA1538 in the presence of a microsome preparation. An eventual increase in the number of colonies in the presence of organic extracts derived from biological materials can a priori be explained both by an increase of the mutation rate and by the contamination of organic extract with traces of histidine. The effects described in this paper are not due to the presence of histidine for the following reasons: (*a*) replica plating of test plates showed that the colonies were comprised of revertant, histidine-positive cells; (*b*) the effect consistently required the presence of the microsome preparation, a condition not required for the influence of histidine on colony formation and (*c*) control experiments showed that the extraction and chromatographic procedures which we employed eliminated histidine from the material finally added to the test plates.

Mutagenic activity of beef extracts and foods. Beef extract was dissolved in water and acidified (to pH 2.0) with HCl. Protein was then precipitated by adding ammonium sulfate to saturation; the sample was then filtered through glass wool and extracted twice with methylene chloride. The aqueous phase was adjusted to pH 10 with ammonium hydroxide and extracted three times with methylene chloride and the pooled basic extracts evaporated to dryness. The residue was taken up in 0.25 ml of DMSO and added to the plates. Beef stock was analyzed with the same procedure. Hamburgers were made in the laboratory from lean ground beef, cooked in commercial cooking appliances for variable times and analyzed with the following procedure. The patties were at first homogenized in a Waring blender in two volumes of distilled water. The homogenate was then adjusted to pH 2.0 with HCl and ammonium sulfate was added to saturation. It was then filtered through glass wool. The acidified filtrate was extracted twice with equal volumes of methylene chloride and the latter discarded. The aqueous phase was then adjusted to pH 10 with ammonium hydroxide and extracted three times with methylene chloride. The methylene chloride extracts were dried with anhydrous sodium sulfate and evaporated to dryness under vacuum at 40 C. The residue was taken up in a known volume of chloroform, from which various aliquots were taken for mutagenesis testing. The same procedure was used for analyzing "B-V Concentrate" and bouillon cubes.

Chromatographic fractionation. Aliquots of chloroform solutions prepared as indicated above were applied to Gelman (ITLC-SG) sheets, and separated with different solvent systems. One-cm zones of the

developed chromatograms were extracted with chloroform:methanol (90:10), dried, the residue taken up in DMSO and tested with strain TA1538 in the presence of a microsome preparation. The number of mutant colonies produced by each zone, plated against zone position, describes the chromatographic distribution of the mutagenic material of the sample.

RESULTS

A series of samples of commercial beef extract (Bacto Beef Extract, Difco Laboratories) have been analyzed for mutagen content. An approximately linear dose-effect relationship was observed with *Salmonella* strains TA1538 and TA98 (Fig. 1), strains which respond preferentially to frame-shift mutations.

The control value (i.e., with no sample present) was 25 colonies per plate. In the absence of microsomes, the number of mutant colonies per plate increased from a control value of 10 to a value of 25 at 0.5 g of beef extract per plate (an effect of doubtful significance). Mutagenic analyses of thin-layer chromatograms showed that the mutagens extracted from commercial beef extract are indistinguishable from those extracted from bacterial nutrients which contain beef extract.

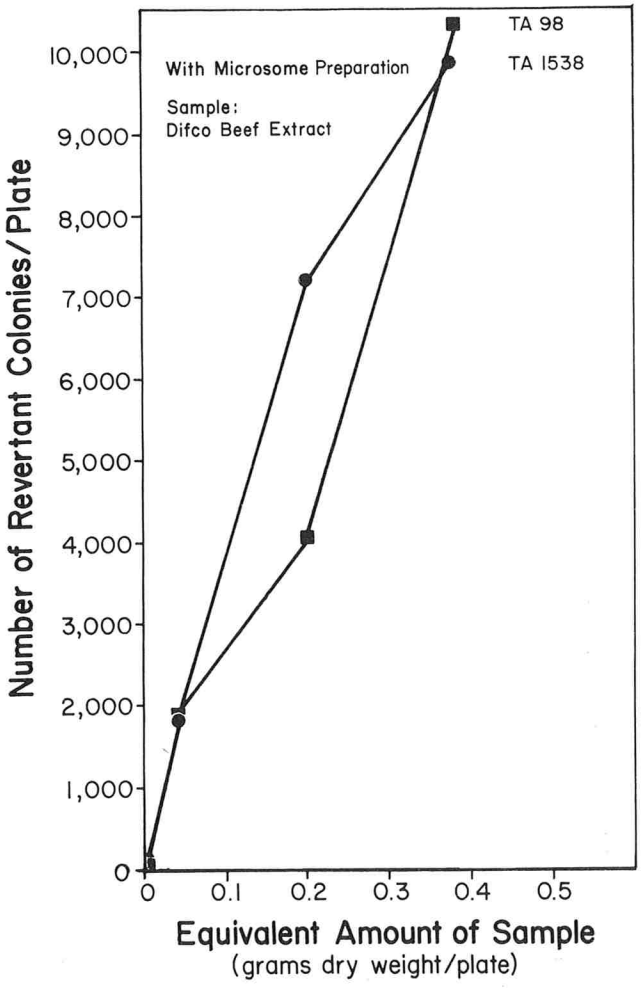

Figure 1. *Dose-response curves obtained with increasing amounts of material extracted with methylene chloride from Difco beef extract in the presence of a microsome preparation. Tester strains: TA98 and TA1538.*

When beef stock was tested by the procedure used in the preceding experiment, no mutagenic activity was detected, suggesting that the mutagens are formed when beef extract is produced by boiling down beef stock. Accordingly, an experiment was carried out in which samples of beef stock were analyzed for mutagen content over a 13-h period of boiling. The water content and the O.D. at 295 nm, which is proportional to the concentration of the products of the Maillard reaction (also known as browning reaction) (*4*) were followed as well (Fig. 2). Over the first 8 h of boiling the mutagen content (per unit of dry weight) increased gradually, then increased more rapidly when the water content fell below 50% of the original value to yield 1572 mutant colonies per plate per 0.7 g of dry weight after 13 h of boiling. This level of activity is about half of that obtained from commercial beef extract. The optical density at 295 nm increased approximately in parallel with the falling water content. These results show that the mutagens were not present in either beef tissue or beef stock, but were formed by reactions which occur during the heating and evaporation that took place when beef stock was boiled down to produce beef extract.

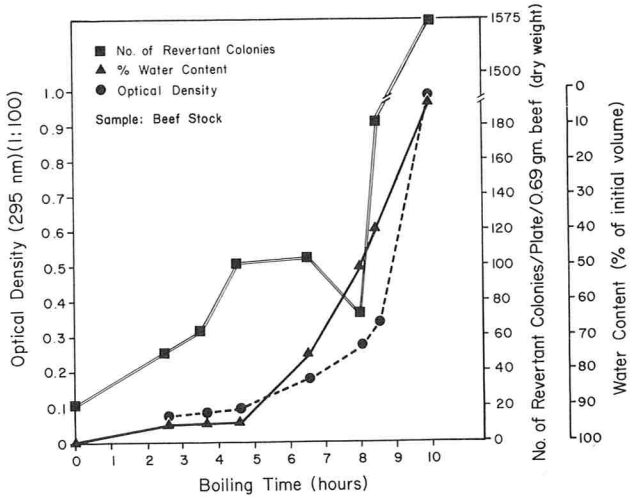

Figure 2. *Variation of mutagenic activity (determined on strain TA1538; microsome preparation present), optical density at 295 nm (dilution 1:100), and water content of beef stock as a function of boiling time.*

The foregoing results suggested the possibility that the mutagens might be formed when beef tissue itself is heated during cooking. Accordingly, 100-g samples of lean ground beef were cooked in various ways, as conventional hamburgers, extracted and the extracts tested for mutagenic activity, as described above. The overall results obtained from the largest amount of sample tested (usually representing 25-g of dry weight of beef) are summarized in Table 1.

TABLE 1. *Mutagenic activity of uncooked hamburgers, hamburgers cooked for different times in different cooking appliances and of fat drippings from cooking hamburgers.*

Appliance	Cooking time (minutes)	Degree of cooking	Amount of sample[a]	Mutant colonies per plate[b]
--	--	Uncooked	35 g	41
Microwave oven	10	Well done	25 g	51
Electric	5	Rare	25 g	55
broiler	10	Well done	25 g	78
Electric	1	Rare	25 g	166
frypan	3	Well done	25 g	692
Electric	1.5	Rare	25 g	737
hamburger	5.5	Well done	25 g	1721
cooker	5.5	Well done	-	776 (fat drippings[c])
Electric			35 g	1157 (outside 3 mm layer)
hot plate	10	Well done	35 g	60 (remaining internal portion)

[a]Dry weight of the original sample applied to the plate (in grams). Dose-response curves were made for each sample, but only the values for the largest amount of sample tested are shown.
[b]Control plates had an average of 38 colonies per plate.
[c]The amount of fat drippings tested was equivalent to that obtained from 25 g of dry weight of hamburger.

A series of such experiments yielded the following main results.

(*a*) No mutagenic activity (i.e., results not significantly greater than the controls) was observed for any of the samples in the absence of the microsome preparation.

(*b*) Uncooked lean ground beef yielded no significant mutagenic activity when extracts were tested with the microsome preparation present.

(*c*) When samples of the same batch of ground beef were cooked for increasing lengths of time in a commercial electrically heated home hamburger-cooking appliance, the mutagenic activity increased (in the presence of microsomes) with cooking time, from 737 mutants (per 25-g sample) for 1.5 min of cooking (rare) to 1721 mutants per test plate for 5.5 min of cooking (well done).

(*d*) Mutagenic activity also was produced when lean hamburger patties were cooked in an electrically heated frying pan and, again, the activity of a well done hamburger was considerably greater than that of a rare one. However, when ground beef was cooked (whether rare or well done) in a microwave oven, or under the element of an electrically heated broiling oven, no significant mutagen production occurred.

(*e*) When commercial ground beef (about 30% fat) was cooked in the electrically heated home hamburger-cooking appliance (well done) and the meat and the melted fat analyzed separately, there was somewhat more mutagenic activity (per gram of hamburger) in the fat than in the meat. However, when beef fat alone was cooked (*7*) very little mutagenic activity was found; thus the mutagens appear to be formed in the beef tissue and are partly dissolved in the fat during cooking.

(*f*) The mutagens produced in hamburgers cooked on a conventional electric hot plate were restricted to the surface. This was determined by freezing the cooked hamburgers in liquid nitrogen and removing about 3 mm of the outside layer with a band saw. The outer layers and the central section of the hamburgers were then analyzed separately. As shown in Table 1, the surface material exhibited considerable mutagenic activity, while the interior of the hamburger contained an insignificant level of activity.

These results suggest that the critical factors which determine the amount of mutagen produced in cooked ground beef are the temperature and the time of cooking. In both the electric hamburger-cooking appliance and the electric frying pan, the meat is in contact with metal at temperatures around 200 C. Given the metal's high heat conductivity, the surface of the hamburger quickly approaches this temperature; this has been confirmed by thermocouple measurements. In a microwave oven the meat is cooked by the heat absorbed from the microwave radiation by the liquid water which it contains. The cooking temperature therefore cannot exceed 100 C, which we have confirmed by thermocouple measurements. When a hamburger is cooked in an electric broiler, with the heating element *above* the meat, cooking is achieved by radiant energy and by the surrounding air temperature (from which heat transfer to the meat is relatively poor). Thermocouple measurements of the surface temperature of a hamburger patty 2 inches below the broiler heating element reach only 110-150 C in the course of a 10-min cooking period (well done). From these data we can infer that the mutagens are formed during the time required to produce a well-done hamburger when the cooking temperature exceeds some critical value, between 150 and 200 C. This conclusion also explains why the interior of a well done hamburger cooked on an electrically heated grill contains no significant amount of mutagens, for the interior temperature is only 80 C. In sum, these results show that, depending on quite common variations in cooking procedure, hamburgers may contain significant amounts of the mutagens or none at all.

As a consequence of these results, it seemed useful to carry out tests on food products containing beef extract. When two such products, "B-V Concentrate" and

"Maggi Bouillon Cubes," were tested as described above, dose-response curves showed that they contained mutagens active on *Salmonella* strains TA1538 and TA98 in the presence of the microsome preparation. Thin layer chromatograms (see Fig. 3) showed that the mutagens of Difco beef extract, Difco nutrient broth, and of these commercial products are indistinguishable in their chromatographic behavior. Figure 4 shows that this is also true of the mutagens prepared from beef extract and cooked hamburgers.

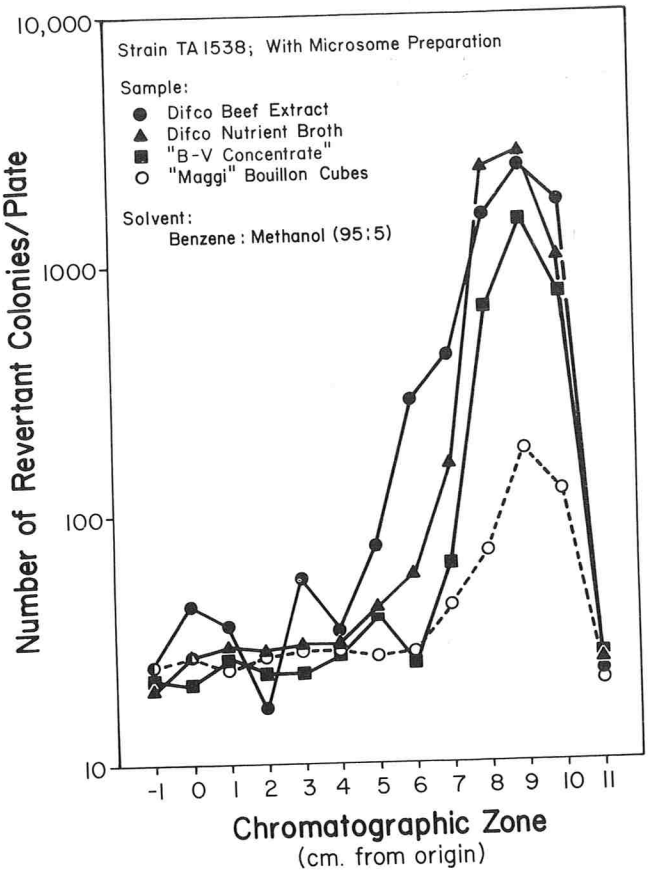

Figure 3. *Thin-layer chromatographic fractionation in benzene-methanol (95:5) of the mutagenic material of Difco beef extract, Difco nutrient broth, "BV Concentrate" and "Maggi" bouillon cubes. Chromatogram zones were extracted and analyzed for mutagenic activity as described in the text.*

The mutagens from commercial beef extract have been further purified by column chromatography on silica gel, fractionation on LH-Sephadex columns and by high-pressure liquid chromatography, using mutagenic assay as a means of following the process. The most highly purified preparations yielded two peaks of equal mutagenic activity when fractionated on the Sephadex column [using hexane: chloroform:methanol (70:25:5) as a solvent] or by means of high pressure liquid chromatography on a silica gel column, using a mixture of ethyl acetate and methanol (0 to 100% gradient) as solvents. One of these subfractions exhibited an ultraviolet absorbancy maximum at 264 nm, and the

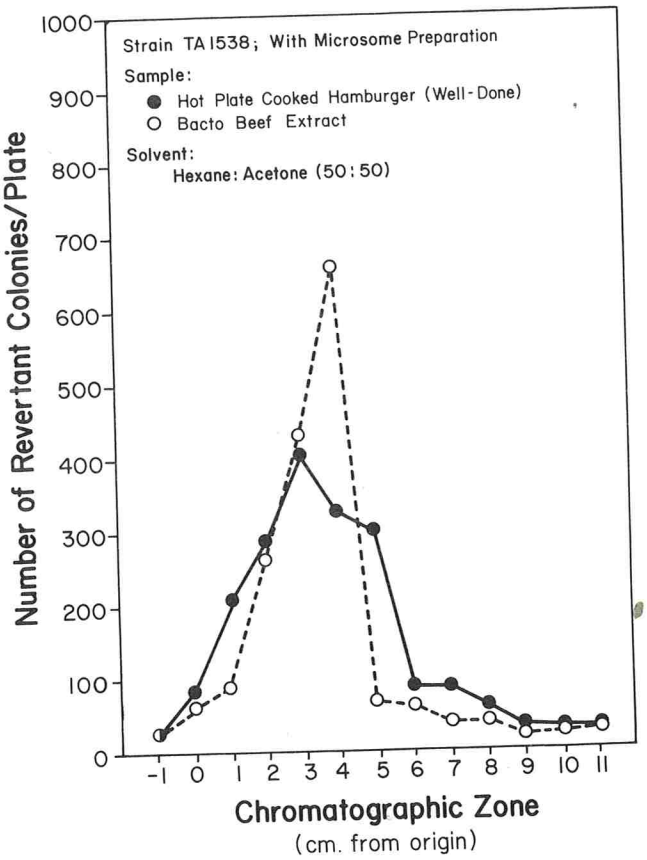

Figure 4. *Chromatographic fractionation of methylene chloride extracts of Bacto Beef Extract (Difco) and of hot plate-cooked hamburgers (well done). Solvent system: Hexane-acetone (50:50).*

other at 275 nm.

The mutagenic activity of preparations partially purified from beef extract was stable during extensive reflux boiling at pH 12 or pH 2. The mutagens in such preparations are soluble in water and are extracted from aqueous solution by organic solvents such as methylene chloride at a basic pH. When a purified preparation is treated with nitrous oxide, it becomes *inherently* mutagenic (i.e., no longer requiring microsomal activation). These characteristics suggest that the mutagen's molecules contain an amino group. Further efforts to purify and identify the mutagens from beef extract are under way.

The most highly purified preparations thus far available, tested on strain TA1538 in the presence of the microsome preparation produce about 5000 mutant colonies per plate per μg. By comparison the potent carcinogen 2-acetylamino fluorene produces about 500 mutant colonies per plate per μg, and the substance with the highest reported specific activity in the Ames test, aflatoxin B_1 (also a potent carcinogen), produces about 22,600 mutant colonies per plate per μg. The beef extract mutagens appear, therefore, to be extremely active.

Several investigations have reported that a carcinogen, benzo(a)pyrene, which is mutagenic in the Ames test, is formed when meats are cooked over an open flame (*10*).

Recently a team of Japanese researchers have reported finding mutagenically active material (in the Ames system) in the charred surface of meats cooked at high temperatures (*13,14*). They conclude that this material is probably similar to mutagens formed when amino acids (*11*) or proteins (*12*) are pyrolyzed at temperatures in excess of 300 C. Accordingly, we have compared the behavior of benzo(a)pyrene, the mutagens formed from pyrolyzed amino acids and the mutagens found in beef extract and in hamburgers cooked in an electrically heated appliance in thin-layer chromatograms. The peaks of mutagenic activity exhibited by beef extract and cooked hamburgers are readily distinguishable from those of both benzo(a)pyrene, and from the mutagens produced by pyrolysis of a mixture of 18 amino acids or of protein. It should be noted as well that the mutagens are formed by pyrolysis of amino acids and proteins only at temperatures in excess of 300 C. In contrast, the mutagens which we have detected in beef extract are readily formed at temperatures which do not exceed 105 C, and the mutagens which we have detected in cooked hamburgers are formed at temperatures between 150 and 200 C. These are temperatures which are readily encountered in common cooking procedures. For example, the French culinary preparation "glace de viande" is produced in the same way as beef extract by boiling down an aqueous stock prepared from beef (and including as well other meats and vegetables) until it is reduced to one-tenth of its original volume. Similarly, the surface temperatures of hamburgers cooked in an electrically heated frying pan or hamburger-cooking appliance do not exceed about 200 C.

DISCUSSION

It is evident from the foregoing results that mutagens which are active in the Ames system can occur in certain foods: beef extract; foods, such as beef bouillon cubes which contain beef extract; hamburgers cooked at surface temperatures of 150-200 C. These mutagens are distinct from benzo(a)pyrene and those produced from the pyrolysis of amino acids and proteins at temperatures above 300 C. Unlike the latter, which occur in the special conditions associated with cooking over open flames, the mutagens which we have detected in foods are produced under conditions readily encountered in common cooking procedures such as the boiling down of beef stock and the cooking of hamburgers in an electrically heated frying pan. It is therefore reasonable to expect these mutagens to occur in diets typical of the U.S. population. It is useful to consider the implications of this fact, especially with respect to the risk of cancer that may be associated with the presence of these mutagens in the diet.

In assessing these implications we need to consider (*a*) the relevance of mutagenicity in the Ames test to the carcinogenicity of a substance toward laboratory animals, (*b*) the relevance of the carcinogenicity of a substance toward laboratory animals to its ability to induce cancer in people, and (*c*) the scientific and practical steps that are suggested by these considerations and the fact that substances mutagenic in the Ames system are present in certain common foods.

There has now been considerable experience in testing known compounds with the Ames mutagenesis system, and it is possible to derive from these results an assessment of its reliability in detecting carcinogens. In relation to mutagenic tests of unknown materials, such as those described above, the relevant issue is the reliability with which a positive mutagenic result is evidence that carcinogenic material is present. Thus Ames et al. (*2*) have reported that 92% (157/171) of the substances which are mutagenic in the *Salmonella* test are also carcinogenic. In our laboratory we have found (*5*) that of 42 compounds that were found to be mutagenic in the Ames system, only one was not carcinogenic (98%). Sugimura et al. (*14*) have found a value of 86% (121/140) for the mutagens on the Ames test which are also carcinogenic. These data show that the probability that a compound which is mutagenic in the Ames test will also be carcinogenic toward laboratory animals is between 86 and 98%.

The relevance of mutagenicity as an indication of carcinogenicity is sometimes questioned on theoretical grounds — for example, that the theory neoplasia is a consequence of a cellular mutation is unproven, or that cancers do not, after all, occur in bacteria. In this connection, it is useful to note that the reliability of the test in no way depends on any theoretical relationship between mutations and cancer. Its reliability derives only from *empirical* results such as those summarized above.

On these empirical grounds, therefore, we must regard the occurrence of active mutagens in beef extract and cooked hamburgers as evidence that these substances are very likely to act as carcinogens when tested against laboratory animals. The practical implications of this fact are illustrated by recent experience with at least two environmental agents. For example, several years ago, Japanese investigators showed that a chemical substance ("AF-2," 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide) widely used in that country to preserve foods, was mutagenic in the Ames system. As a result, the Japanese government was urged to ban the use of AF-2 in foods. The government refused to order the ban, but did carry out tests on laboratory animals. Two years later, after considerable expense, it was established that AF-2 causes cancer in laboratory animals, and it was banned. Obviously, if this action had been taken on the basis of the mutagenesis experiments, the risk of cancer could have been reduced 2 years earlier. In the same way Ames' discovery that a number of hair dye constituents are mutagenic (*3*) was followed by the later evidence that at least one of these substances is carcinogenic in laboratory animals (*9*).

The evidence cited above shows that mutagenicity is significantly correlated with carcinogenicity *toward*

laboratory animals. What, then, is the relevance of the occurrence of mutagens in beef extract and hamburgers to the incidence of cancer in *people* who might ingest them? The relationship between animal tests and the risk of cancer to people is a complex and still controversial issue. The difficulties involved in deriving conclusions about the risk of cancer to people from animal studies include (*a*) that animal tests involve doses much greater than those usually encountered in the human population; and (*b*) that species and strains of laboratory test animals differ greatly in their susceptibility to carcinogens.

With respect to the first of these problems, it should be noted that what is of concern in the human population would be an increase in the relatively low incidence of cancer, which is about 3 per 1,000 per year in the U.S. population. An increase to an incidence of 10 per 1,000, which would be regarded as a disastrous medical problem, would be very difficult to detect in an animal experiment without using huge, impractical numbers of animals. The only practical way to test a substance is to administer it at a concentration sufficiently high to induce a statistically meaningful number of cancers in a reasonable number of test animals. Such a test then indicates that the substance is capable of inducing cancer in a susceptible animal. Its relevance to people depends on the assumption of a more or less linear relation between dose and response, and that some people will be as susceptible as the test animals to the substance.

With respect to the last point, it is important to take into account the very considerable variability of susceptibility to carcinogens that is evident in human populations. In contrast, the animals used in laboratory cancer tests are selected and bred for their *uniformity* of response to carcinogens. Usually the most responsive strains are used in a test. Taking these factors into account, then, we can conclude that when a laboratory experiment shows that a substance significantly increases the number of test animals that develop cancer, that substance is likely to cause cancer (assuming dose-response linearity) in *some fraction* of the human population.

Given these considerations, it is reasonable to conclude that a substance which is carcinogenic toward laboratory animals is likely to represent some risk of cancer to an exposed human population, assuming a more or less linear relation between dose and response. While the scientific evidence for this assumption remains debatable, it is usually deemed prudent--given the seriousness of the disease--to accept it as a guide to efforts to assess the risk of cancer to people. Present regulations regarding exposure to carcinogens, such as the Delaney Amendment of the Food and Drug Administration, and the carcinogen regulations recently proposed by the Occupational Safety and Health Administration (*8*) are based on this approach. Thus, in the proposed OSHA regulations, evidence that a substance causes cancers in two different species of animals (or in one species if it is also active in a short-term test such as *Salmonella* mutagenesis) is deemed sufficient to initiate emergency action to reduce workplace exposure to the substance. In these proposed regulations evidence that a substance is mutagenic (for example in the Ames system) is regarded as a warning of possible carcinogenicity and an indicator of the need for animal tests.

When deciding what to do about any environmental threat to health, the expected hazard must be balanced against the benefit of the activity that produces it. Striking that balance is not a scientific process, but a matter of social or personal judgement. In the case of hamburgers, we can look at the situation in the following way. First, consider a similar case in which there is *no* benefit associated with the hazard, for example, the red dyes that have been added to the casing of some brands of hot dogs to give them a "pleasing" color. Since the only value of a hot dog is as a food and the dye does not contribute to that value, there is no benefit, but only a hazard, in using the dye[2]. Hence if the dye is eliminated, whatever risk of cancer is associated with it (even if this is but poorly known) can be reduced *at no loss in benefit.* But hamburgers have a very important value, because they are a convenient and inexpensive way to add meat to the diet. At this stage of our knowledge it would make little sense to suggest that because we have found mutagens in hamburgers cooked in certain ways that people should generally give up eating hamburgers. This would mean giving up an important benefit before we known how serious a risk is involved.

Although we have not yet studied other cooked meats, the same reasoning would apply to them, insofar as their mutagen content is also likely to be affected by different cooking procedures. Nevertheless, for the reasons already discussed, there is reason to believe that there may be *some* risk, of an as yet unknown magnitude, in eating products which contain the mutagens that we have detected in beef extract and in hamburgers cooked in certain ways. Therefore, it would be useful to reduce this risk if that could be done *without* giving up any of the benefit. Our results show that there are ways of doing that. Thus a hamburger can be prepared essentially free of the mutagens if it is broiled *under* the heat source instead of grilled in a frying pan, in an electric hamburger-cooking appliance or in a similar appliance. Alternatively, if the hamburger is cooked in such an appliance, the potential risk can be reduced by more than 90% by cooking it rare.

In sum, our results on the occurrence of mutagens in beef extract and hamburgers should be regarded as a *warning* that these substances represent a risk of unknown magnitude to the people who are exposed to them. Fortunately, our results also show that regardless of how large or small it eventually turns out to be, the risk

[2] *In fact, the dye may diffuse in the meat during cooking procedures, and the casing is eaten anyway by most of the people.*

can be reduced to zero by choosing an appropriate cooking procedure.

It is evident that the results reported above raise a number of questions that require further investigation. First it is important to determine the molecular structure of the mutagens and the reactions responsible for their formation in beef extract and cooked beef. These data would help to determine what cooking procedures are likely to produce the mutagens and suggest modifications that reduce the mutagen content. This information, together with analyses of foods other than beef for the mutagens, would provide a basis for assessing their occurrence in the diet, eventually enabling studies regarding the correlation of dietary mutagen content with the incidence of cancer in various populations. Once a purified mutagen is in hand it would be important, of course, to determine its toxicological and carcinogenic properties in test animals. Such data will eventually provide an evaluation of the risk of cancer to human populations from the mutagens which have thus far been detected in beef extract and cooked beef. Nevertheless, for the reasons cited earlier, the present data already indicate that *some* risk may be involved. Fortunately, the present data also show how this risk can be substantially reduced and even eliminated — steps which need not wait upon further elaboration of our initial findings.

REFERENCES

1. Ames, B. N., J. McCann, and E. Yamasaki. 1975. Methods for detecting carcinogens and mutagens with *Salmonella*/mammalian microsomes mutagenicity test. Mutat. Res. 31:347-364.
2. McCann, J., E. Choi, E. Yamasaki, and B. N. Ames. 1975. Detection of carcinogens as mutagens in the *Salmonella*/microsome test:assay of 300 chemicals. Proc. Nat. Acad. Sci. USA 72:5135-5139.
3. Ames, B. N., H. O. Kammen, and E. Yamasaki. 1975. Hair dyes are mutagenic: identification of a variety of mutagenic ingredients. Proc. Nat. Acad. Sci. USA 72:2423-2427.
4. Burton, H. S., and D. J. McWeeny. 1962. Sulphur dioxide and Maillard type reactions. pp. 217-225. In: Food science and technology. Gordon and Breach Science Publisher.
5. Commoner, B., J. I. Henry, J. Gold, M. J. Reding, and A. Vithayathil. 1976. Reliability of bacterial mutagenesis techniques to distinguish carcinogenic and noncarcinogenic chemicals. U.S. Environmental Protection Agency, Washington, D.C., Report No. EPA-600/1-76-022.
6. Commoner, B., A. Vithayathil, P. Dolara, S. Nair, P. Madyastha, and G. Cuca. 1978. Formation of mutagens in beef and beef extract during cooking. Science 201:913-916.
7. Dolara, P., B. Commoner, A. Vithayathil, G. Cuca, E. Tuley, P. Madyastha, S. Nair, and D. Kriebel. 1978. The formation of mutagens in heated beef stock and cooked ground beef. Mutat. Res. (Submitted).
8. Federal Register, Vol. 42, No. 192. Tuesday, October 4, 1977. p. 54148.
9. IARC Monographs on the evaluation of the carcinogenic risk of chemicals to man. Volume 16, 1978. IARC, Lyon, France.
10. Lijinsky, W., and P. Shubik. 1964. Benzo(a)pyrene and other polynuclear hydrocarbons in charcoal broiled meat. Science 145:53-55.
11. Matsumoto, T., D. Yoshida, S. Mizusaki, and H. Okamoto. 1977. Mutagenic activity of amino acid pyrolyzates in *Salmonella typhimurium* TA98. Mutat. Res. 48:279-286.
12. Matsumoto, T., D. Yoshida, S. Mizusaki, and H. Okamoto. 1978. Mutagenicity of the pyrolyzates of peptides and proteins. Mutat. Res. 56:281-288.
13. Nagao, M., M. Honda, Y. Seino, T. Yahagi, and T. Sugimura. 1977. Mutagenicity of smoke condensate and the charred surface of fish and meat. Cancer Letters (Amst.) 2:221-226.
14. Sugimura, T., M. Nagao, T. Kawachi, M. Honda, T. Yahagi, Y. Seino, S. Sato, and N. Matsukura. 1977. Mutagen-carcinogens in food, with special reference to highly mutagenic pyrolytic products in broiled foods. pp. 1561-1578. In: Origins of human cancer. Cold Spring Harbor Laboratory.
15. Vithayathil, A., B. Commoner, S. Nair, and P. Madyastha. 1978. Isolation of mutagens from bacterial nutrients containing beef extract. J. Toxicol. Environ. Health 4:189-202.

Coming Events, con't from p. 1028

ogy Dept., University of California, Davis, CA 95616. 916-752-0980.

June 5, 1979. PENNSYLVANIA SANITARIANS ASSOCIATION, Annual Meeting. Keller Conference Center, Pennsylvania State University, University Park Campus, State College, PA 16801. Contact: Sid Barnard, Pennsylvania State University.

June 10-13, 1979. INSTITUTE OF FOOD TECHNOLOGISTS 39th ANNUAL MEETING AND FOOD EXPO. Alfonso J. Cervantes Convention & Exhibition Center, St. Louis, MO. Contact: C. L. Willey, Institute of Food Technologists, Suite 2120, 221 N. LaSalle St., Chicago, IL 60601.

Aug. 29-31, 1979. FOURTH INTERNATIONAL IUPAC SYMPOSIUM ON MYCOTOXINS AND PHYCOTOXINS. Co-sponsored by World Health Organization and Swiss Society for Analytical and Applied Chemistry. Lausanne, Switzerland. For participation and poster presentation, contact: Prof. P. Krogh, Dept. of Veterinary Microbiology, School of Veterinary Medicine, Purdue University, West Lafayette, IN 47907 or Prof. D. Reymond, IUPAC, Case postale 88, 1814 La Tour de Peilz, Switzerland.

Sept. 18-20, 1979. WESTPACK. Convention Center, Anaheim, CA.

Journal of Food Protection Vol. 41, No. 12, Pages 1004-1005 (December, 1978)

Report of the Editor
Journal of Food Protection
1977-1978

REVIEW OF VOLUME FORTY

Volume 40 of the journal was the first that bore the title, *Journal of Food Protection*. This title seems to have been well received by authors and readers. The change in title has not had a negative effect on the flow of manuscripts during 1977 or 1978. In fact, it may have had a positive effect since the number of submitted manuscripts has increased appreciably during 1977 and 1978 as compared to the number received in earlier years when the journal was titled *Journal of Milk and Food Technology*.

Volume 40 contained about the same number of pages as Volume 39 (Table 1) but it contained about 30% more papers (175) than were in Volume 39 (134). This was possible because less pages in Volume 40 than in Volume 39 were devoted to equipment standards and association affairs. Also, Volume 40 contained only a few lengthy review papers and so space could be devoted to more short papers. Volume 40 set another record in that it contained not only more papers but also more research papers (136) than appeared in any earlier volume of the journal. This represents an increase of approximately 450% during the 10-year period, 1967-1977 (Table 1).

Details on composition of Volume 40 are in Table 1. These data can be compared with similar information for Volumes 39, 38, 37, 36, 34, and 30. Volume 30 is included as a base line since the undersigned became Editor of the journal at that time.

Of the 175 papers in Volume 40, approximately 63% considered topics related to foods other than milk or milk products, 35% dealt with milk or milk products, and 2% discussed topics that cannot be included in either of these major categories. Hence the trend continues that greatest growth in number of papers comes in subject matter not related to the dairy industry. Lack of adequate funding for dairy-oriented research continues to be a problem and is reflected in continued decrease in number of papers dealing with this subject.

TABLE 1. *Summary of contents of* Journal of Food Protection *(1977) and* Journal of Milk and Food Technology *(1967-1977).*

Item	Volume 30 (1967)	Volume 34 (1971)	Volume 36 (1973)	Volume 37 (1974)	Volume 38 (1975)	Volume 39 (1976)	Volume 40 (1977)
1. Total pages, including covers	512	728	752	752	868	948	944
2. Total papers published	64	102	108	102	136	134	175
3. Research papers							
a. Number	30	67	65	72	100	94	136
b. Pages	137	288	284	330	402	400	532
c. Percent of total pages	26.7	39.5	37.7	43.9	46.3	42.2	56.4
4. General interest papers-technical							
a. Number	11	24	31	21	26	28	25
b. Pages	47	150	208	160	178	188	125
c. Percent of total pages	9.2	20.6	27.7	21.2	20.5	19.8	13.2
5. Equipment standards							
a. 3-A, pages	9	40	17	41	25	32	22
b. E-3-A, pages	—	30	—	—	2	18	—
c. Percent of total pages	1.7	9.6	2.2	5.4	3.1	5.2	2.3
6. General interest papers-nontechnical							
a. Number	23	11	12	9	10	12	14
b. Pages	72	46	49	29	46	57	48
c. Percent of total pages	14.1	6.3	6.5	3.8	5.2	6.0	5.0
7. Association affairs							
a. Pages	64	45	84	75	67	65	52
b. Percent of total pages	12.5	6.3	11.2	9.9	7.7	6.8	5.5
8. News and events							
a. Pages	51	17	4	0	26	36	39
b. Percent of total pages	9.9	2.3	0.5	0.0	3.0	3.7	4.1
Percent of pages-technical material, including standards	37.6	69.7	67.6	70.5	69.9	67.3	71.9
Percent of pages-nontechnical material	36.5	14.9	18.2	13.7	16.0	16.7	14.6
Percent of pages-covers, adds, index, etc.	25.9	15.4	14.2	15.8	14.1	16.0	13.5

PRESENT STATUS OF VOLUME FORTY-ONE

The first six issues of Volume 41 (1978) consisted of 524 pages, including covers. This compares with 436, 456, and 264 pages in the first six issues of Volumes 40, 39, and 30 (1967), respectively. Contents of the first six issues of Volume 41 included 61 research papers, 28 technical papers of general interest and 7 nontechnical papers of general interest. This compares with 65, 13 and 7 papers in these same categories in Volume 40. The first six issues of Volume 41 contained a total of 96 papers compared with 85 papers in the same issues of Volume 40.

Awaiting publication on July 1, 1978 were 71 research papers, 16 technical papers of general interest, and 5 nontechnical papers of general interest. This compares with 66, 11, and 5 papers in the same categories on July 1, 1977. Additionally, on July 1, 1978 34 research papers were being reviewed or revised. To insure prompt publication the remaining issues of Volume 41 will contain approximately 100 papers. This will result in publication of approximately 200 papers in Volume 41 which probably will, for the first time, exceed 1,000 pages. Hence Volume 41 will again demonstrate growth both in number of published papers and number of pages.

Several changes were made in the journal and they went into effect with Volume 41. Included are: (*a*) introduction of an easier-to-read contents page, (*b*) printing of month, volume, and pages near the spine of each issue, (*c*) introduction of brief write-ups on equipment and supplies, and (*d*) placing an asterisk after the name of an author of a paper who should be contacted for reprints or other information about the paper.

INTERNATIONAL CHARACTER OF THE JOURNAL

Volume 40 contained papers by authors from the following countries outside of the United States: Canada, Egypt, Finland, India, Ireland, South Africa, Spain, Sweden, Switzerland, The Netherlands, and Venezuela. Thus far, Volume 41 has contained papers by authors from Canada, Finland, India, Israel, and Turkey in addition to the United States. The journal continues to be enriched by these contributions from outside of the United States. It is hoped that more authors from beyond the borders of the United States will consider the journal as a medium for publication of their research findings.

SPECIAL COMMITTEES

During 1977-1978 two committees were established to aid the journal in carrying out its mission. The first committee, headed by Dr. L. R. Beuchat, serves to identify topics that should be discussed through appropriate review papers. This committee has submitted a list of topics to the Editor. Members of the committee include: Drs. F. M. Clydesdale, R. T. Marshall, J. A. Troller, C. Vanderzant, and H. M. Wehr.

The second committee is headed by C. D. Clingman and is addressing itself to how needs of the foodservice industry can be met by the journal. Working with Clingman are: K. J. Baker, Prof. Ruth S. Dickie, Dorothy Ellis, D. Hartley, F. Hegele, E. Helmreich, F. Mitchell, Prof. R. Moser, B. Preswick, T. Schafer, Dr. O. P. Snyder, Dr. Nan Unklesbay and Dr. J. C. White.

Additional committees are to be appointed to consider the needs of in-the-field sanitarians and dairy fieldmen.

EDITORIAL BOARD

The Editorial Board currently consists of 50 U.S. and Canadian scientists in academic, governmental, and industrial laboratories. Members of the Board need to be reappointed in 1978. Dr. C. K. Johns has indicated his desire to be relieved of duties as a member of the Editorial Board. Dr. Johns has served on the Editorial Board continuously since the journal was founded in 1937 — a total of 41 years. Additionally, for about 2 years Dr. Johns has checked proofs of papers and thus has helped immeasurably to eliminate typographical and other errors from the journal. The efforts of Dr. Johns and the other members of the Editorial Board are gratefully acknowledged.

The following persons who are not members of the Editoral Board reviewed manuscripts during 1977: S. E. Barnard, R. L. Bradley, Jr., A. L. Branen, H. E. Calbert, R. G. Cassens, S. R. Cecil, C. D. Clingman, C. L. Duncan, R. R. Eitenmiller, B. A. Glatz, A. H. W. Hauschild, A. W. Hayes, W. S. LaGrange, R. C. Lindsay, T. E. Minor, V. S. Packard, L. H. Schultz, D. A. Stuiber, S. R. Tatini and B. Walker. Thus far during 1978 the following persons, who are not members of the Editorial Board, have reviewed manuscripts: M. S. Bergdoll, R. L. Bradley, Jr., F. W. Bodyfelt, H. E. Calbert, D. O. Cliver, A. H. W. Hauschild, A. W. Hayes, H. W. Jackson, W. S. LaGrange, R. C. Lindsay, D. B. Lund, M. E. Matthews, A. J. Maurer, T. E. Minor, M. Morgan, V. S. Packard, O. Snyder, A. K. Stersky, D. A. Stuiber, E. C. D. Todd, G. S. Torrey, N. Unklesbay and D. F. Wessley. Their help is acknowledged and appreciated.

Respectfully submitted,

ELMER H. MARTH
Editor
Journal of Food Protection

Journal of Milk and Food Technology
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*Asterisk designates person to whom inquires regarding the paper should be addressed.

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General Interest

SUBJECT INDEX

3-A Sanitary Standards For Sifters For Dry Milk and Milk Products

Number 26-01

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

It is the purpose of the IAMFES, USPHS, and DIC in connection with the development of the 3-A Sanitary Standards Program to allow and encourage full freedom for inventive genius or new developments. Dry milk and dry milk products sifter specifications heretofore or hereafter developed which so differ in design, material and fabrication, or otherwise as not to conform with the following standards, but which in the fabricator's opinion, are equivalent or better, may be submitted at any time for the consideration of the IAMFES, USPHS, and DIC.

A.

SCOPE

A.1

These standards cover the sanitary aspects of sifters used for processing dry milk and dry milk products.

A.2

In order to conform with these 3-A Sanitary Standards, sifting equipment for dry milk and dry milk milk products shall comply with the following design, material, and fabrication criteria.

B.

DEFINITIONS

B.1

Product: Shall mean dry milk or dry milk products.

B.2

Product Contact Surface: Shall mean all surfaces that are exposed to the product, or from which other materials may drain, drop or be drawn into the product.

B.3

Non-Product Contact Surface: Shall mean all other exposed surfaces.

C.

MATERIALS

C.1

Product contact surfaces shall be of stainless steel of the AISI 300 series[1] or corresponding ACI[2] types (See Appendix, Section E), or metal which under conditions of intended use is at least as corrosion resistant as stainless steel of the foregoing types, and is non-toxic and non-absorbent, except that:

C.1.1

Rubber and rubber-like materials may be used for balls, bonded or removable gaskets, flexible connectors, and inspection port covers and parts used in similar applications.

C.1.2

Rubber and rubber-like materials when used for the above specified applications shall comply with the applicable provisions of the 3-A standard for rubber and rubber-like materials, Number 18-00.

C.1.3

Plastic materials may be used for screening media, screen frame assemblies, balls, bonded or removable gaskets, flexible connectors, and inspection port covers and parts used in similar applications.

C.1.4

Plastic materials when used for the above specified applications shall comply with the applicable provisions of the 3-A standard for plastic materials, Number 20-08.

C.1.5

Bonded rubber and rubber-like materials and bonded plastic materials having product contact surfaces shall be of such composition as to retain their surface and conformation characteristics when exposed to the conditions encountered in the environment of intended use and in cleaning and bactericidal treatment.

C.1.6

The final bond and residual adhesive, if used, of bonded rubber and rubber-like materials and bonded plastic materials shall be non-toxic.

C.1.7

Cotton, linen, silk, or synthetic fibers may be used for flexible connectors and screening media. These

[1] *The data for this series are contained in the following reference: AISI Steel Products Manual, Stainless & Heat Resisting Steels, December 1974, Table 2-1, pp. 18-19. Available from: American Iron & Steel Institute, 1000 16th Street, N.W., Washington, DC 20036.*

[2] *Alloy Casting Institute Division, Steel Founders' Society of America, 20611 Center Ridge Road, Rocky River, OH 44116.*

materials shall be non-toxic, relatively insoluble, easily cleanable, and shall not impart a flavor to the product.

C.1.8
Solder shall be non-toxic and non-absorbent.

C.2
Non-product contact surfaces shall be of corrosion-resistant material or material that is rendered corrosion-resistant. If coated, the coating used shall adhere. Non-product contact surfaces shall be relatively non-absorbent, durable and cleanable. Parts removable for cleaning having both product contact and non-product contact surfaces shall not be painted.

D.
FABRICATION

D.1
Product contact surfaces, except those of screens, shall be at least as smooth as a No. 4 ground finish on stainless steel sheets free of imperfections such as pits, folds and crevices. (See Appendix, Section F). The use of stainless steel sheets with a No. 2B finish free of imperfections such as pits, folds and crevices in the fabricated form for product contact surfaces is limited to dry product contact surfaces.

D.2
Permanent joints in metallic product contact surfaces shall be flush and continuously welded. Welded areas on product contact surfaces shall be at least as smooth as a No. 4 ground finish on stainless steel sheets free of imperfections such as pits, folds and crevices.

D.3
Solder may be used to fill the joints where the screen is attached to the frame.

D.4
Woven stainless steel wire or woven materials provided for in C.1.3 and in C.1.7 may be used for screening media.

D.5
Appurtenances having product contact surfaces shall be easily removable for cleaning or shall be readily cleanable in place.

D.6
Product contact surfaces shall be easily accessible, visible for inspection, and readily cleanable, either when in an assembled position or when removed. Removable parts shall be readily demountable.

D.7
Gaskets:

D.7.1
Gaskets having a product contact surface(s) shall be removable or be bonded so as to be smooth and easily cleanable.

D.7.2
Bonded rubber and rubber-like gaskets and bonded plastic gaskets shall be bonded in such a manner that the bond is continuous and mechanically sound, and so that when exposed to the conditions encountered in the environment of intended use and in cleaning and bactericidal treatment, the rubber and rubber-like material or the plastic material does not separate from the base material.

D.7.3
Grooves in gaskets shall be no deeper than their width and the minimum radius of any internal angle shall be not less than 1/8 inch unless the gasket is readily reversible for cleaning.

D.8
Gasket retaining grooves in product contact surfaces for removable gaskets shall not exceed 1/4 inch in depth and, except those for standard O-Rings smaller than 1/4 inch, shall be at least 1/4 inch wide.

D.9
Radii:

D.9.1
Internal angles of 135° or less on product contact surfaces shall have radii of not less than 1/4 inch, except that:

D.9.1.1
The radii in gasket retaining grooves for removable gaskets, except for those for standard 1/4 inch and smaller O-Rings, shall be not less than 1/8 inch.

D.9.1.2
The radii in grooves for standard 1/4 inch O-Rings shall be not less than 3/32 inch and for standard 1/8 inch O-Rings shall be not less than 1/32 inch.

D.10
The edges of openings in the cover shall extend upward at least 3/8 inch. Openings not continually in use shall be provided with removable covers having a downward flange of at least 1/4 inch.

D.11
There shall be no exposed threads on product contact surfaces.

D.12
Means shall be provided for the prompt, continuous removal of rejected material.

D.13
Supports:

D.13.1
The means of supporting a sifter shall be one of the following:

D.13.1.1
With legs: Legs shall be smooth, have no exposed threads and shall be of sufficient length to provide a clearance between the lowest fixed point of the sifter and the floor of at least 6 inches. Legs made of hollow stock shall be sealed.

D.13.1.2
Mounted on a slab or island: The base of the sif-

ter shall be such that it may be sealed to the mounting surface (See Appendix, Section G).

D.13.1.3

Mounted on a pedestal: The base of the pedestal shall comply with D.13.1.2 or if the sifter will not be mounted on a slab or island, the pedestal shall be provided with adjustable legs that (1) comply with D.13.1.1 and (2) provide the clearance required in D.13.1.1.

D.13.1.4

Mounted on wall: The design of a sifter to be mounted on a wall shall be such that there will be at least a clearance of 4 inches between the wall and the nearest part of the sifter.

D.13.1.5

Suspended: When the means of suspending a sifter is a cable, the cable ends should be concealed or enclosed. When means of suspending a sifter is other than a cable, the supports shall be smooth and have no exposed threads or other conditions which may provide areas which are difficult to clean.

D.14

A guard(s) required by a safety standard that will not permit accessibility for cleaning and inspection in place shall be designed so it (they) can be removed without tools.

D.15

Non-product contact surfaces shall be smooth, free of pockets and crevices and be readily cleanable and those to be coated shall be effectively prepared for coating.

APPENDIX

E.

STAINLESS STEEL MATERIALS

Stainless steel conforming to the applicable composition ranges established by AISI[1] for wrought products, or by ACI[2] for cast products, should be considered in compliance with the requirements of Section C.1 herein. Where welding is involved the carbon content of the stainless steel should not exceed 0.08%. The first reference cited in C.1 sets forth the chemical ranges and limits of acceptable stainless steels of the 300 series. Cast grades of stainless steel corresponding to types 303, 304, and 316 are designated CF-16F, CF-8, and CF-8M respectively. These cast grades are covered by ASTM[3] specifications A296-68 and A351-70.

F.

PRODUCT CONTACT SURFACE FINISH

Surface finish equivalent to 150 grit or better as obtained with silicon carbide, is considered in compliance with the requirements of Section D.1 herein.

G.

SLABS OR ISLANDS

When the sifter is designed to be installed on a slab or island, the dimensions of the slab or island should be such that the base of the equipment will extend beyond the slab or island at least 1 inch in all horizontal directions. The slab or island should be of sufficient height so that the bottom of all product connections are not less than 4 inches above the floor. The surface of the slab or island should be coated with a thick layer of waterproof mastic material, which will harden without cracking. The junction of the equipment base and the slab or island should be sealed.

H.

SCREEN SIZES

For the general guidance of sifter manufacturers and the dry milk industry, the following screen size openings may be considered as recommended openings to result in satisfactory screening of the listed dry milk products:

Product	Maximum Sieve Opening	
	mm.	inches (approx.)
Nonfat dry milk	0.707	.027
Dry whole & dry buttermilk	1.19	.047

It is recognized that larger screen size openings may be necessary for sifting certain special dry milk products (such as "instant" products) and for desired classification of products on the basis of different particle sizes. Openings referred to above are based on general experience as to what constitutes satisfactory screening to remove product lumps or foreign materials, and also on ability of most currently used sifters to successfully sift dry milk products through such size openings without excessive loss of fine product into the "reject material" outlet. (Other factors also affect such loss, such as percent of "open area" in screen used, uneven flow rates to the sifter, ratio of screening surface area to dryer capacity, amount and kind of mechanical energy applied to the screening surface, sifter design and construction, and nature of dry milk product being sifted.)

Screen openings dimensions may be obtained by any desired combination of wire thickness and number of wires per inch. For instance, if the screening surface is made of stainless steel woven wire, the .027 inch opening might be obtained by using 24 x 24 mesh market grade screen cloth made of wire .014 inch thick (about 45% open area) or by using 30 x 30 bolting cloth screen made of wire .0065 inch (about 65% open area) or by many other mesh-wire thickness combinations. These combinations allow a wide choice to obtain desired balance between screen strength and percent open area. If materials other than stainless

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[3]*Available from American Society for Testing and Materials, 1916 Race St., Philadelphia, PA 19103.*

steel are used to construct the screening surface, similar combinations may be employed to achieve desired opening size.

I.

RECOMMENDATIONS FOR CLEANING DRY MILK SIFTERS

I. DAILY CLEANING PROGRAM-The procedures set forth below should be followed as a daily cleaning program.

1. Completely dismantle and thoroughly vacuum or dry brush all product contact surfaces of the dry milk sifter. Reassemble as soon as finished and make every effort to keep all parts dry.

2. Check sifter screen(s) for broken or displaced wires (threads) and for other openings around the frame of the screen, which might permit the passage of unsifted product. Other parts of the sifter, including ball trays and balls, if used, should also be inspected for condition. Any necessary repair or replacement should be made as soon as possible.

3. Flexible rubber or cloth socks at the inlet and outlets of the sifter should be thoroughly cleaned daily, following the procedures as recommended for the sifter. At this time socks should be closely examined for holes, cracks or other damage. (To facilitate removal for cleaning, use of easily removable fastening devices are recommended.)

4. Thoroughly vacuum or dry brush clean all external parts of the sifter, including the sifter frame and drive mechanism.

II. *WEEKLY CLEANING PROGRAM*-The procedures set forth below should be followed at weekly intervals.

1. Completely dismantle as in I. above, remove all loose, dry milk, then rinse all parts with clear water and follow by a thorough hand brushing of all parts using a general purpose dairy cleaner. Rinse thoroughly to remove all evidence of cleaning solution or soil. It is recommended that hot water (170° F. or above) be used for rinsing in order to sanitize the equipment and to aid the subsequent drying. Allow all parts to air dry completely prior to reassembly. The wet wash should be done more frequently if necessary and should be done after each use if the sifter is not being used on a daily basis. After cleaning, drying, and reassembly the powder outlet should be protected against recontamination.

III. *GENERAL RECOMMENDATIONS*

1. Vacuum cleaning is preferred to brush cleaning or cleaning with air under pressure as it decreases the dust drift problem to other areas of the plant.

2. Brushes or vacuum cleaner fittings used for cleaning product contact surfaces should not be used for cleaning non-product contact surfaces or for other uses which might result in contamination. Such brushes and special fittings should be stored in an enclosed cabinet when not in use. (For protection and housekeeping considerations, such cabinets preferably should be made of non-wood construction and should have open mesh metal shelving.)

These standards are effective January 14, 1979, at which time the "3-A Sanitary Standards for Sifters for Dry Milk and Dry Milk Products, Number 26-00," are rescinded and become null and void.

3-A Sanitary Standards for Equipment for Packaging Dry Milk and Dry Milk Products

Number 27-01

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

It is the purpose of the IAMFES, USPHS, and DIC in connection with the development of the 3-A Sanitary Standards program to allow and encourage full freedom for inventive genius or new developments. Dry milk and dry milk products packaging equipment specifications heretofore or hereafter developed which so differ in design, material, and fabrication, or otherwise as not to conform with the following standards, but which in the fabricator's opinion are equivalent or better, may be submitted at any time for the joint consideration of the IAMFES, USPHS, and DIC at any time.

A.

SCOPE

A.1

These standards cover the sanitary aspects of equipment for performing the functions of holding, forming, dispensing, filling, weighing, closing, and/or sealing containers, and all parts which are essential to these functions when they are performed as an integral part of the packaging operation. These standards do not pertain to the container nor to a duct(s) which is not a part of the packaging equipment.

A.2

In order to conform with these 3-A Sanitary Standards, equipment for packaging dry milk, and dry milk products shall comply with the following design, material and fabrication criteria.

B

DEFINITIONS

B.1

Product: Shall mean dry milk and dry milk products.

B.2

Container: Shall mean a packaging enclosure holding the product, including multiwall bags.

B.3

Holding, Opening, Forming and Dispensing Equipment: Shall mean all equipment for holding, opening, forming, and dispensing the empty containers.

B.4

Filling Equipment: Shall mean the equipment for mechanically filling the container with the product.

B.5

Closing and Sealing Equipment: Shall mean the equipment for mechanically closing and sealing the container.

B.6

Surfaces:

B.6.1

Product Contact Surfaces: Shall mean all surfaces which are exposed to the product, surfaces from which other materials may drain, drop or be drawn into the product or into the container, and surfaces that touch the product contact surfaces of the container.

B.6.2

Non-Product Contact Surfaces: Shall mean all other exposed surfaces.

B.7

Engineering Plating: Shall mean plated to specific dimensions or processed to specified dimensions after plating[1].

C.

MATERIALS

C.1

Product contact surfaces shall be of stainless steel of the AISI 300 series[2] or corresponding ACI[3] types (See Appendix, Section E), or metal which under conditions of intended use is at least as corrosion-resistant as stainless steel of the foregoing types and is non-toxic and non-absorbent, except that:

C.1.1

Bearings may be made of metal covered with an engineering plating of nickel, chromium or equally corrosion-resistant, non-toxic material.

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[1] *QQ-C-320 B-Federal Specification for Chromium Plating (Electrodeposited) June 17, 1974, 40c. QQ-N-290 A-Federal Specification for Nickel Plating (Electrodeposited) November 12, 1971, 20c. Both documents available from: Business Service Center, General Services Administration, Seventh and D Sts., SW, Washington, D.C.*

[2] *The data for this series are contained in the following reference: AISI Steel Products Manual, Stainless & Heat Resisting Steels, December 1974, Table 2-1, pp. 18-19. Available from: American Iron & Steel Institute, 1000 16th St., NW, Washington, D.C. 20036.*

[3] *Alloy Casting Institute Division, Steel Founders' Society of America, 20611 Center Ridge Road, Rocky River, OH 44116.*

C.1.2
Those surfaces of holding, forming, opening, dispensing, closing, or sealing equipment which touch the product contact surfaces of the container or from which contaminants may drain, drop or be drawn into the container may be made of a non-toxic, non-absorbent metal that is corrosion-resistant under conditions of intended use or may be made of metal made corrosion and wear-resistant by a covering of an engineering plating of chromium or nickel or an equally corrosion and wear-resistant non-toxic metal.

C.1.3
Rubber and rubber-like materials may be used for container opening, dispensing, and closing parts, filling nozzles, flexible connectors, plungers, bonded or removable gaskets, diaphragms, shields, filling valve members, seals and parts used in similar applications.

C.1.4
Rubber and rubber-like materials, when used for the above specified applications, shall comply with the applicable provisions of the 3-A standard for rubber and rubber-like materials, Number 18-00.

C.1.5
Plastic materials may be used for container holding, opening, forming, dispensing, and closing parts, filling nozzles, flexible connectors, plungers, bonded or removable gaskets, diaphragms, shields or guards, filling valve members, covers, seals, diverting aprons and parts used in similar applications.

C.1.6
Plastic materials when used for the above specified applications shall comply with the applicable provisions of the 3-A standard for plastic materials, Number 20-08.

C.1.7
The final bond and residual adhesive, if used, of bonded rubber and rubber-like materials and bonded plastic materials shall be non-toxic.

C.1.8
Rubber and rubber-like materials and plastic materials used for bonded gaskets having product contact surfaces, shall be of such composition as to retain their surface and conformation characteristics when exposed to the conditions encountered in the environment of intended use and in cleaning and bactericidal treatment.

C.1.9
Silver soldered or brazed areas and silver solder or braze material shall be non-toxic and corrosion resistant.

C.1.10
Single service gaskets of a sanitary type may be used on parts which must be dissassembled for cleaning.

C.2
Non-product contact surfaces shall be of corrosion-resistant material or material that is rendered corrosion-resistant. If coated, the coating used shall adhere. Non-product contact surfaces shall be relatively non-absorbent, durable and cleanable. Parts removable for cleaning having both product contact and non-product contact surfaces shall not be painted.

D.
FABRICATION

D.1
Product contact surfaces shall be at least as smooth as a No. 4 ground finish on stainless steel sheets free of imperfections such as pits, folds and crevices. (See Appendix, Section F). The use of selected stainless steel sheets with a No. 2 B finish free of imperfections such as pits, folds and crevices in the fabricated form for product contact surfaces is limited to dry product contact surfaces.

D.2
Permanent joints in metallic product contact surfaces shall be flush and continuously welded. If it is impractical to weld, they may be silver soldered or brazed. An exception is made to the foregoing for product connections which may have rolled-on sanitary pipeline ferrules or flanges. Welded or silver soldered or brazed areas of product contact surfaces shall be at least as smooth as a No. 4 ground finish on stainless steel sheets free of imperfections such as pits, folds and crevices.

D.3
The minimum thickness of engineering plating shall be 0.0002-inch for all product contact surfaces, except that when the parts listed in C.1.2 that are to be plated are other than stainless steel, the minimum thickness of the engineering plating shall be 0.002-inch.

D.4
Product contact surfaces shall be easily accessible, visible for inspection, and readily cleanable, either when in an assembled position or when removed. Removable parts shall be readily demountable.

D.5
Product contact surfaces shall be self-draining or self-purging except for normal clingage.

D.6
Product hoppers integral with the filler shall be equipped with dust-tight covers, gasketed if necessary, and have drop flanges which overlap the rim of the hoppers by at least 3/8 inch. The edges of openings in the hopper cover shall extend upward at least 3/8 inch or be fitted with a permanently attached sanitary pipeline connection conforming to D.15. Openings in the hopper cover, except those fitted with a permanently installed sanitary pipeline connection, shall be provided with dust-tight cov-

ers, gasketed if necessary, having a downward flange of not less than 1/4 inch so designed as to prevent contaminants from entering the hopper. Covers shall be self-draining.

D.7

The filling equipment shall be so designed that adjustments necessary during the operation can be made without raising or removing the hopper cover(s).

D.8

Gaskets:

D.8.1

Gaskets having a product contact surface(s) shall be removable or be bonded so as to be smooth and easily cleanable.

D.8.2

Bonded rubber and rubber-like gaskets and bonded plastic gaskets shall be bonded in such a manner that the bond is continuous and mechanically sound, and so that when exposed to the conditions encountered in the environment of intended use and in cleaning and bactericidal treatment, the rubber and rubber-like material or the plastic material does not spearate from the base material.

D.8.3

Grooves in gaskets shall be no deeper than their width and the minimum radius of any internal angle shall be not less than 1/8 inch unless the gasket is readily reversible for cleaning.

D.9

Gasket retaining grooves in product contact surfaces for removable gaskets shall not exceed 1/4 inch in depth and, except those for standard O-Rings smaller than 1/4 inch shall be at least 1/4 inch wide.

D.10

Radii:

D.10.1

Internal angles of 135° or less on product contact surfaces shall have radii of not less than 1/4 inch, except that:

D.10.1.1

Where smaller radii are required for essential functional reasons, such as those in filler nozzles and screw conveyors, the radii shall be not less than 1/32 inch.

D.10.1.2

The radii in gasket retaining grooves for removable gaskets, except those for standard 1/4 inch and smaller O-Rings shall be not less than 1/8 inch.

D.10.1.3

The radii in grooves for standard 1/4 inch O-Rings shall be not less than 3/32 inch and for standard 1/8 inch O-Rings shall be not less than 1/32 inch.

D.11

Covers, diverting aprons, shields or guards shall be provided and shall be so designed and located so as to prevent contaminants from draining or dropping into the container or product, or onto product contact surfaces.

D.12

Where lubrication is required, the design and construction of the equipment shall be such that the lubricant cannot leak, drain, be forced, or be drawn into the product or onto product contact surfaces. Lubricated bearings shall be located outside the product contact surface with at least 1 inch clearance between the product contact surface and the bearing.

D.13

There shall be no exposed threads on product contact surfaces.

D.14

A shaft seal, if provided, shall be of a packless type, sanitary in design, with all parts accessible for cleaning.

D.15

Sanitary fittings and connections, if used, shall conform with the applicable provisions of the 3-A standard for sanitary fittings, Number 08-17, as amended.

D.16

Coil springs having product contact surfaces shall have at least 3/32 inch openings between coils including the ends when the spring is in a free position. Coil springs shall be readily accessible for cleaning and inspection.

D.17

Equipment for producing air under pressure and/or air piping which is supplied as an integral part of the filling equipment shall comply with the applicable provisions of the 3-A accepted practices for supplying air under pressure, Number 604-03.

D.18

Supports:

D.18.1

The means of supporting the equipment shall be legs or casters, or the equipment shall be mounted on a slab or island and shall comply with the applicable provisions of the following:

D.18.1.1

Legs or casters shall provide a clearance between the lowest fixed point on the equipment and the floor of at least 4 inches when the base outlines and area in which no point is more than 12½ inches from the nearest edge of the base, or a clearance of at least 6 inches when any point is more than 12½ inches from the nearest edge.

D.18.1.2

Legs, if provided, shall be smooth with rounded ends and have no exposed threads. Legs made of hollow stock shall be sealed.

D.18.1.3

Casters, if provided, shall be easily cleanable, durable and of a size that will permit easy move-

ment of the equipment.

D.18.1.4

If the equipment is to be mounted on a slab or island, the base shall be designed (1) for sealing to the slab or island (See Appendix, Section G), and (2) to permit adequate cleaning, drainage and drying of the interior of the base.

D.19

A guard(s) required by a safety standard that will not permit accessibility for cleaning and inspection in place shall be designed so it (they) can be removed without tools.

D.20

Non-Product Contact Surfaces:

D.20.1

Non-product contact surfaces shall be smooth, free of pockets and crevices and be readily cleanable and those to be coated shall be effectively prepared for coating.

D.20.2

Panels or doors shall be provided to allow easy access without tools for the cleaning and inspection of mechanical areas of the equipment which are not dust-tight.

APPENDIX

E.

STAINLESS STEEL MATERIALS

Stainless steel conforming to the applicable composition ranges established by AISI[2] for wrought products, or by ACI[3] for cast products, should be considered in compliance with the requirements of Section C.1 herein. Where welding is involved the carbon content of the stainless steel should not exceed 0.08%. The first reference cited in C.1. sets forth the chemical ranges and limits of acceptable stainless steels of the 300 series. Cast grades of stainless steel corresponding to types 303, 304, and 316 are designated CF-16F, CF-8, and CF-8M, respectively. These cast grades are covered by ASTM[4] specifications A296-68 and A351-70.

F.

PRODUCT CONTACT SURFACE FINISH

Surface finish equivalent to that obtained with 150 grit silicon carbide, is considered in compliance with the requirements of Section D.1.

G.

SLABS OR ISLANDS

When the equipment is designed to be installed on a slab or island, the dimensions of the slab or island should be such that the base of the equipment will extend beyond the slab or island at least 1 inch in all horizontal directions. The slab or island should be of sufficient height so that the bottoms of all product connections are not less than 4 inches above the floor. The surface of the slab or island should be coated with a thick layer of waterproof mastic material, which will harden without cracking. The junction of the equipment base and the slab or island should be sealed.

These standards are effective January 14, 1979, at which time the "3-A Sanitary Standards for Equipment for Packaging Dry Milk and Dry Milk Products, Number 27-00," are rescinded and become null and void.

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[4]*Available from American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA 19103.*

News and Events

IAMFES to hold Double Election

IAMFES will hold a double election this year.

The Executive Board voted at its winter meeting Nov. 20-22 to fill the vacancy created by the resignation of Richard P. March as President-Elect by advancing each current officer one position. In effect, this leaves two vacancies, second vice-president and secretary-treasurer, to be filled by the election once the current officers advance again at the Annual Meeting in August.

To maintain the representative order of the board, the persons already nominated from education will run for second vice-president instead of secretary-treasurer. Nominations are now open for candidates from industry for the position of secretary-treasurer. Please send biographical information and a picture of your candidate to:

Harold J. Barnum, Chairman
Nominating Committee
International Association of Milk, Food and Environmental Sanitarians
736 Cloute Street
Fort Atkinson, WI 53538

The election will be held as soon as the industry candidates are named and information can be provided about all candidates in the *Journal*. Plan to look for this in the February issue.

Bailus Walker Named Recipient of 1978 Browning Prize

Bailus Walker, Jr., Ph.D., M.P.H., was awarded the 1978 Edward W. Browning Prize for his "outstanding contribution to disease prevention."

Dr. Walker, an environmental health scientist, was honored during the 106th Annual Meeting of the American Public Health Association, in Los Angeles, California, October 15-19, 1978.

The Browning Prize is presented annually in five separate fields of endeavor — environment conservation, improvement of food resources, prevention of disease, alleviation of addiction and spreading the Christian gospel. Each of the five winners receives a $5,000 honorarium and a bronze Browning medal.

The American Public Health Association presents the Prize for disease prevention, which is administered by the New York Community Trust.

After having held several environmental health posts (administrative, academic and research), Dr. Walker became administrator of the Environmental Health Administration, Department of Environmental Services, District of Columbia government, in 1972.

In the latter assignment he has conducted extensive research into environmental health conditions and their effects on inmates in jails and prisons, and his findings have resulted in publications and in court-ordered improvements of correctional institutions.

Dr. Walker is also an adjunct professor of environmental health at Howard University and a visiting professor of environmental medicine at Meharry Medical College.

Since 1975 he has served as a member of the Environmental Health Advisory Committee - Science Advisory Board - U.S. Environmental Protection Agency, and as consultant (environmental health) to the Civil Rights Division of the U.S. Department of Justice.

A native of Springfield, Tennessee, Dr. Walker did his undergraduate work at Kentucky State University and attended the University of Michigan where he received his M.P.H. degree. He was an honor graduate at the University of Minnesota with a doctorate in environmental health.

NAMA Revises Vending Machine Standard

The National Automatic Merchandising Association (NAMA) announced November 8 the publication of a proposed revision of its Vending Machine Evaluation Manual as approved by NAMA's advisory health-industry council, AMHIC, on October 6, 1978.

The revisions reflect new requirements contained in the 1978 FDA "Vending of Food and Beverages" (a major revision of the 1965 Public Health Service Vending Code) and other changes involving water supply protection and cabinet design features.

Copies of the proposed revision have been sent to all states having uniform vending sanitation regulations and to all known food and beverage vending machine manufacturers. Single copies are available free from NAMA, 7 South Dearborn Street, Chicago, Illinois 60603. Comments are invited from all interested parties. The comment period closes December 31, 1978.

Coming Events

Jan. 9, 11, 1979. FOOD PROCESSORS SANITATION WORKSHOP, Sponsored by University of California Cooperative Extension. Jan. 9 session will be held at the Holiday Inn, Park Center Plaza, 181 Almaden Blvd., San Jose, CA. Jan. 11 session will be at the Proud Bird Restaurant, 11022 Aviation Blvd., Los Angeles, CA. Contact: Paullette De Jong, Food Science and Technology Dept., Univ. of California, Davis, CA 95616, 916-752-1478.

Jan. 10-11, 1979. DAIRY PROCESSORS CONFERENCE. Sponsored by Food Science Dept., University of Wisconsin-Madison. Sheraton Inn, Madison, WI. Contact: Myron Dean, Dept. of Food Science, Babcock Hall, 1605 Linden Drive, Madison, WI 53706.

Jan. 21-24, 1979. INTERNATIONAL EXPOSITION FOR FOOD PROCESSORS. Sponsored by Food Processing Machinery and Supplies Assoc. Brooks Hall-Civic Center, San Francisco, CA. Contact: T. J. Gorman, Food Processing Machinery & Supplies Assoc., Suite 700, 1828 L St., N. W., Washington, D.C. 20036.

Jan. 24, 1979. CONNECTICUT ASSOCIATION OF DAIRY AND FOOD SANITARIANS, INC. Annual Meeting. Contact: Walter F. Dillman, Dept. of Agriculture, State Office Building, Hartford, CT 06115.

Feb. 14-15, 1979. DAIRY AND FOOD INDUSTRY CONFERENCE. Ohio State University, Contact: J. Lindamood, Dept. of Food Science and Nutrition, 2121 Fyffe Rd., Ohio State University, Columbus, OH 43210.

Feb. 25 - Mar. 2, 1979. NINTH ENVIRONMENTAL ENGINEERING IN THE FOOD PROCESSING INDUSTRY CONFERENCE. Asilomar Conference Grounds, Pacific Grove, CA. Sponsored by the Engineering Foundation. Contact: Engineering Foundation, 345 E. 47th St., New York, NY 10017, 212-644-7835. Or contact: Dr. Roy Carawan, Program Co-Chairman, 129 Schaub Hall, North Carolina State University, Raleigh, NC 27650, 919-737-2956.

Feb. 26-28, 1979. SIXTH ENERGY TECHNOLOGY CONFERENCE AND EXPOSITION. Sponsored by American Gas Association/ Gas Research Institute, Electric Power Research Institute, and Thomas Alva Edison Foundation. Sheraton Park Hotel, Washington, D.C. Contact: Martin Heavner, Energy Technology Conference, Inc., 4733 Bethesda Avenue, N.W., Washington, D.C. 20014. 301-656-1090.

Feb. 27 - Mar. 1, 1979. THIRD DOMESTIC WATER QUALITY SYMPOSIUM. Sponsored by American Society of Agricultural Engineers and 17 national co-sponsoring organizations and agencies. St. Louis, MO. Contact: American Society of Agricultural Engineers, P.O. Box 410, St. Joseph, MI 49085, 616-429-0300.

Feb. 27-28, 1979. KENTUCKY ASSOCIATION OF MILK, FOOD, AND ENVIRONMENTAL SANITARIANS, INC., Annual Meeting. Contact: Dale Marcum, Milk Control Branch, Frankfort, KY.

FEB. 27 - Mar. 2, 1979. TECH EX '79-ANNUAL WORLD FAIR FOR TECHNOLOGY EXCHANGE. Georgia World Congress Center, Atlanta, GA. Contact: E. B. Prine, Vice President, Tech Ex '79, Dr. Dvorkovitz & Assoc., P.O. Box 1748, Ormond Beach, FL 32074.

Mar. 1-2, 1979. ANNUAL FOOD TECHNOLOGY CONFERENCE. Sponsored by IFT Kansas City and St. Louis sections. Ramada Inn, Columbia, MO. Contact: S. A. Taillie, Paniplus Co., ITT Continental Baking Co., 100 Paniplus Roadway, Olathe, KS 66061.

Mar. 6-7, 1979. VIRGINIA ASSOCIATION OF MILK AND FOOD SANITARIANS, Annual Meeting. Donalson Brown Center for Continuing Education, Virginia Polytechnic Institute and State University, Blacksburg, VA. Contact: Marshall Cooper, 116 Reservoir St., Harrisburg, VA 22801.

Mar. 19-21, 1979. AMERICAN CULTURED DAIRY PRODUCTS INSTITUTE ANNUAL TRAINING SCHOOL AND JUDGING CONTEST. Hilton Inn, Columbus, OH. Contact: C. Bronson Lane, ACDPI, P.O. Box 7813, Orlando, FL 32854.

Mar. 19-23, 1979. MID-WEST WORKSHOP IN MILK AND FOOD SANITATION. Ohio State University. Contact: John Lindamood, Dept. of Food Science and Nutrition, 2121 Fyffe Road, Ohio State University, Columbus, OH 43210.

Mar. 26-28, 1979. MISSOURI ASSOCIATION OF MILK AND FOOD SANITARIANS, Annual Meeting. Lodge of the Four Seasons, Lake of the Ozarks, MO. Contact: Erwin Gadd, Bureau of Community Sanitation, Missouri Division of Health, Box 570, Jefferson City, MO 65101.

Mar. 27-29, 1979. WESTERN FOOD INDUSTRY CONFERENCE. University of California, Davis, CA. Contact: John C. Bruhn, Extension Food Technologist, Dept. of Food Science & Technology, University of California, Davis, CA, 916-752-2192.

Mar. 27-28, 1979. WESTERN FOOD INDUSTRY CONFERENCE. Freeborn Hall, University of California, Davis. Contact: Robert C. Pearl, Food Science and Technol-

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NATIONAL MASTITIS COUNCIL ANNUAL MEETING

February 19-21, 1979

EXECUTIVE INN, LOUISVILLE, KENTUCKY

The 18th Annual Meeting of the National Mastitis Council will be held Tuesday, February 19 through Thursday, February 21, 1979. Program highlights will include presentations and technology sessions on the following subjects:

SOMATIC CELLS-NATURE & ACTION

LEUKOCYTE MIGRATION DURING MASTITIS

EXPERIENCES WITH SOMATIC CELL COUNTING ON DHI

THE ROLE OF SOMATIC CELL COUNTING IN THE NATIONAL DHI PROGRAM

SOMATIC CELLS IN GOAT MILK

RELATIONSHIP OF MILK CHARACTERISTICS AND MILKING PROCEDURES TO MASTITIS

PRESENT STATUS OF MASTITIS VACCINATION

OVERVIEW OF CURRENT RECOMMENDATIONS FOR MASTITIS CONTROL AND HOW TO IMPLEMENT THEM

LABORATORY PROCEDURES IN SOMATIC CELL COUNTING

- Fossomatic
- Filter-DNA
- Coulter Counter
- Technicon

LABORATORY PROCEDURES IN ANTIBIOTIC RESIDUES

- Bacillus Stearothermophilus
- Charm Test

FARM PRACTICES

- Checking Milking Equipment
- Milking Procedures and Cow Management
- Cleaning and Sanitation
- Selling Mastitis Management

DHI SOMATIC CELL COUNTING PROGRAM

MASTITIS THERAPY

- Pharmacokinetic Principles of Intramammary Therapy
- Infection Rates and Response to Therapy in Ten Saskatchewan Dairies
- Evaluation of a Method of Treating Clinical Infections
- Field observations in Mastitis Therapy
- Discussant

For additional information or to register, contact the National Mastitis Council, 30 F Street, N.W., Washington, D.C., 20001, 202-393-6607.

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U.S.P. UNITED STATES PHARMACEUTICAL STANDARDS

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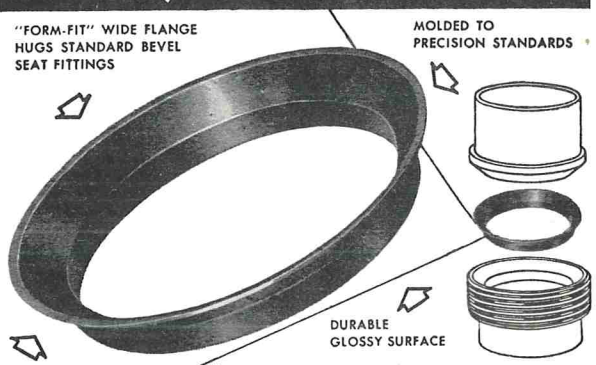

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Teat Dipping Reduces Mastitis and Increases Profits

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The development of suitable teat dips represents a significant advancement in the battle to control mastitis. Indeed, the most important single step that a dairy farmer can take to prevent this disease in his dairy herd is to dip teats after milking with a product shown by research to be safe and effective.

Bacteria on Teat Ends Mean Trouble

The rate of infection is related to the number of organisms on the teat end. Management practices that cause an increase in the organisms will result in a higher infection rate while those that reduce the population will lower the infection rate.

The primary sources of the most common mastitis bacteria are infected udders and teats. The bacteria are transmitted during milking by the milkers' hands, udder cloths, and milking machine teat cups. Only a small number of infections will occur during milking if the machines are functioning correctly and used properly. The majority will occur between milkings caused by bacteria left on the teats at the end of milking. Teat dipping with a good product will destroy most of the bacteria and will reduce the new infection rate by an average of 50%, or more. That statement is supported by data from more than 30 carefully controlled research experiments.

Teat dipping should be done as soon as practical after milking because the teat canal starts to close tightly. This process begins at the outside edge of the canal and may result in bacteria being trapped inside the canal out of reach of the dip if application is delayed.

A wide range of teat dip products is available. Most are probably effective, but dairy farmers should require manufacturers to provide satisfactory evidence.

Teat Dipping: One of Five Steps in Good Herd Health

Teat dipping should be employed as one component of a five step program that includes: (1) correct maintenance and use of milking equipment; (2) teat dipping; (3) prompt treatment of clinical cases; (4) treatment of cows at drying off; and (5) culling of mastitis problem cows.

Dairy farmers should not expect to see dramatic results from teat dipping within a short time. This is because the practice does not eliminate existing infections that are best controlled by treatment at drying off. In the long term, however, teat dipping is a highly effective method of keeping mastitis at a low level. *Dairy farmers who don't have time to dip teats after milking just don't have time to control mastitis.*

Mastitis Control Returns Profit

This disease continues to be a heavy tax on the dairy industry because of reduced milk production, ruined cows, and other costs. The average dairy farmer loses approximately 100 dollars per cow per year in reduced milk production alone—but this need not be the case.

Research conducted in the United States and several foreign countries has revealed that dairy farmers receive three to five dollars in increased profits for every dollar invested in a mastitis control program. Naturally, dairy farmers with a high level of mastitis in their herd stand to realize the greatest return.

My philosophy concerning teat dipping can best be summed up in the statement—"If I had one cow—and she had one teat—I'd dip it, after every milking." Mastitis control makes good sense. Where else can a dairy farmer receive a 300 to 500% return on his investment?