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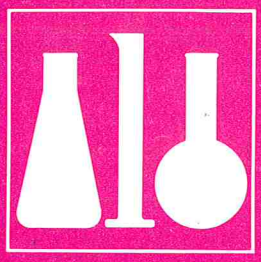

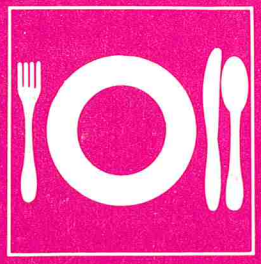

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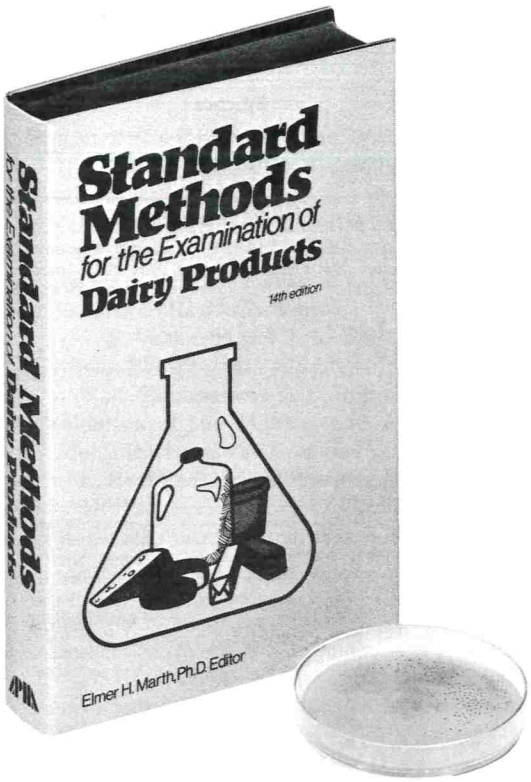

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Antiviral Effectiveness of Grape Juice

D. O. CLIVER* and K. D. KOSTENBADER, JR.

Food Research Institute (Department of Food Microbiology and Toxicology), Department of Bacteriology, and World Health Organization Collaborating Centre on Food Virology, University of Wisconsin, Madison, Wisconsin 53706

(Received for publication August 8, 1978)

ABSTRACT

Grape juice inactivated human enteroviruses, but not parainfluenza type 1 (Sendai) virus, in vitro. The effect was not one of aggregation or of degradation of the virus surface. Some of the inactivated virus adsorbed specifically to host cells, but did not infect them. Most of the inactivated virus could be reactivated by treatment with polyethylene glycol. Grape juice-inactivated virus and coproantibody-neutralized virus were both reactivated by contents of porcine stomach and duodenum, which suggests that ingestion of such viruses would lead to intestinal infection. Grape juice-inactivated virus was efficiently reactivated by human blood serum. Ingested grape juice has not been shown likely to prevent or modify human enterovirus infections.

In a series of reports, Konowalchuk and Speirs (*5,6,7*) described an in vitro activity against viruses that was common to several fruits and vegetables and their products. They called the phenomenon "inactivation" (and so shall we), although it differs from most known modes of virus inactivation in that the virus particle is apparently not degraded in the process. Much of their attention was devoted to grapes, grape juices, and wines. The prospect that these foods might prevent or cure virus infections in humans attracted a good deal of attention in the popular and scientific press. The coverage ranged from serious to clearly frivolous.

Given the hopes that might have been built on these reports, we thought it important to try to confirm the findings of Konowalchuk and Speirs and to assess the significance of these findings to human health. Our work was done almost exclusively with heat-pasteurized Concord grape juice. In its simplest hypothetical mode of action, grape juice might exert its protection locally in the digestive tract by inactivating virus before or after ingestion. Alternately, the active substances from the grape juice would have to pass into the bloodstream to prevent virus from being transported in the blood or to reach and protect remote sites of virus infection, such as the respiratory tract. We chose not to consider any means of administration of the grape juice other than ingestion.

MATERIALS AND METHODS

Viruses, cultures, and reactive substances

Enteroviruses used for these experiments were poliovirus type 1 (PO1) strain CHAT (obtained from the American Type Culture Collection), coxsackievirus type B3 (CB3) strain Nancy (obtained from T. G. Metcalf, University of New Hampshire), and echovirus type 6 (EC6) strain D'Amori (obtained from the American Type Culture Collection). The respiratory virus used was parainfluenza type 1, strain Sendai (obtained from the American Type Culture Collection). Porcine enterovirus type 3, strain ECPO-6, was obtained from E. H. Bohl, Ohio Agricultural Research and Development Station.

Tissue culture lines used were Buffalo green monkey kidney (BGM; obtained from International Biological Laboratories), HeLa (obtained from Wisconsin State Laboratory of Hygiene, Madison), Vero (obtained from Flow Laboratories), and minipig kidney (MPK; obtained from the American Type Culture Collection). All cultures were passed and maintained in our laboratory by methods described previously (*2*).

Except as otherwise noted, PO1 and CB3 were titered on either HeLa or BGM tissue cultures using Heberling's agar overlay (*2*). EC6 was titered on Vero cultures using a medium based upon Eagle's MEM prepared with Hanks' solution; supplemented with nonessential amino acids, 1% whole milk, 2% fetal calf serum, 50 mM $MgCl_2 \cdot 6\ H_2O$, and 0.1% DEAE dextran; solidified with 1% Ionagar No. 2; and containing 0.0015% neutral red. ECPO-6 was titered on MPK using a solid maintenance medium based upon Eagle's MEM prepared with Earle's solution, supplemented with another 1× MEM amino acids and 5% fetal calf serum; solidified with 0.7% Noble agar; and containing 0.002% neutral red. Sendai virus was titered on BGM cells using a crystal violet staining procedure (CVF); briefly this involved incubating the cells under solid maintenance medium (Eagle's MEM + 10% fetal calf serum, solidified with 1% Noble agar) for 5-7 days, removing the agar, and then staining with crystal violet fixative (1% NaCl, 26% ethanol, 0.5% crystal violet, and 5% formaldehyde). It had been previously determined (unpublished data) that Sendai virus would not replicate in PK 15, HeLa, or Vero tissue cultures, but would replicate in either BGM or MPK tissue cultures. It had also been determined that plaques could be demonstrated by the CVF procedure but would not be produced by more conventional procedures involving neutral red in the overlay medium.

Whole, pure, heat-pasteurized Concord grape juice was supplied by Welch Foods Inc. Polyethylene glycol (PEG; m.w. 20000) was obtained from Union Carbide. Enocianina grape skin extract was supplied by K. M. Green, Coca-Cola Inc., Atlanta, GA. Feces containing coproantibody were supplied by T. E. Minor, University of Wisconsin, Madison. Human pooled blood serum was obtained from the Wisconsin State Laboratory of Hygiene.

Inactivation and reactivation

PO1, CB3, and EC6 were each inoculated into undiluted grape juice, grape juice diluted to 10%, deionized water, and glycine-HCl buffer (pH 3.3); Sendai virus was inoculated into undiluted grape juice only. All viruses were inoculated at a final level of 5×10^3 plaque-forming units (PFU)/ml. All test suspensions were held at 4 C for 4 h except Sendai virus, which was held at 4 C for approximately 18 h. After the reaction time, all solutions were titered in appropriate tissue cultures, using appropriate agar overlays.

To test the effects of temperature on inactivation, CB3 was inoculated at a final level of 5×10^3 PFU/ml into undiluted grape juice, grape juice diluted to 10%, and deionized water. Each sample was divided into three subsamples, and subsamples were reacted for 4 h at 4, 25, and 37 C. Each subsample was then titered in HeLa tissue cultures.

To test reactivation of inactivated virus by PEG, we first inoculated PO1 into both undiluted grape juice and deionized water. After

reaction for 18 h at 4 C, each sample was divided into three subsamples. One set of subsamples of both juice and water was mixed with an equal volume of 1% PEG (final concentration 0.5%), a second set of subsamples was mixed with an equal volume of 5% PEG (final concentration 2.5%), and a third set of subsamples was mixed with an equal volume of deionized water. After 1 h reaction at room temperature (ca. 25 C), all subsamples were titered using BGM tissue cultures. The same procedure was used to test for reactivation of Sendai virus, except the 5% PEG concentration was omitted.

Reactivation of inactivated virus was also tested by inoculating human blood serum with either grape juice-inactivated PO1 or uninactivated PO1 contained in phosphate-buffered saline (PBS); deionized water control samples were also inoculated with both preparations of virus. After incubation for 30 min at room temperature, all samples were assayed for virus by the plaque technique on HeLa tissue culture.

Grape skin extract

Enocianina grape skin extract powder was suspended in deionized water at levels of 0.1, 1, and 5%. CB3 was incubated with these suspensions, and some of the 1% suspension was further reacted with 1% PEG, as described above. The samples were assayed in HeLa cultures by the plaque technique.

Mode of inactivation

PO1 was labelled with ^{32}P as described previously (*8*). To test whether the protein coat of inactivated virus had been modified so that the virus could no longer adsorb to a Millipore filter (*3*), we reacted labelled PO1 with both undiluted grape juice and deionized water for 4 h at 4 C. Both suspensions were then filtered through a 25-mm diameter 0.22-μm porosity Millipore GS membrane filter. To test whether the grape juice had caused aggregation of virus into more or less stable complexes, we substituted a 25-mm diameter Nuclepore polycarbonate membrane filter with a nominal pore size of 50 nm (less than two virus diameters) for the Millipore filter. Radioactivity of both filtered and unfiltered samples was counted on a Baird-Atomic GM scaler: two planchets were counted twice, an average count calculated, and the average count corrected for background.

To examine whether adsorption of inactivated virus to cells still occurred, we reacted ^{32}P-labelled PO1 with both undiluted grape juice and deionized water for 4 h at 4 C; the juice and water were then split into two subsamples. One set of subsamples was reacted for 1 h at 25 C with minipig kidney cell suspensions (MPK); this reaction was a negative control, as PO1 does not adsorb to MPK cells. The other set of subsamples was reacted for 1 h at 25 C with HeLa cell suspensions; PO1 normally will adsorb to HeLa cells. After the reaction time the cell suspensions were centrifuged for 15 min at 1000 rpm, the supernatant fluids were removed, and the packed cells were resuspended. Radioactivity of the resuspended cells, the cell supernatant fluids and suspensions not reacted with cells were counted.

Protection of digestive tract

As human enteroviruses appear to initiate their infections in the last segment of the small intestine, the ileum (*1*), we wanted to determine whether ingested PO1 which had been inactivated by grape juice would regain its infectivity before reaching the ileum. Untreated virus and PO1 which had been neutralized by coproantibody were included as bases for comparison. The swine digestive tract, a close analog of that of man, served as the source of the materials with which the virus preparations were treated.

PO1 was inactivated by grape juice by reacting the virus with undiluted grape juice for 18 h at 4 C. Uninactivated PO1 was diluted in PBS and held for 18 h at 4 C. Coproantibody was extracted from a child's stool specimen by preparing a 20% (w/v) suspension in PBS, homogenizing for 3 min, centrifuging at 7500 rpm for 20 min, and then heating the supernatant fluid at 56 C for 30 min (*9*). After filtration at 0.20-μm porosity (Gelman GA-8) to remove bacteria, the supernatant fluid was reacted with PO1 and ECPO-6 for 6 h at 37 C: 99.8% of the PO1, but none of the ECPO-6, was neutralized (thus showing the presence of coproantibody). Coproantibody-neutralized PO1 was finally prepared by reacting PO1 with the coproantibody preparation for 18 h at 4 C.

The pigs used in these experiments were obtained from a local farm when 1 day old. They were raised with littermates in a heated nursing incubator until 3½ wk old; during this time they were fed a mixture of Cadco milk replacer and powdered milk. The pigs were then held for an additional 2 wk in individual isolator cages and were fed a diet of processed cheese, powdered milk, peanut oil, eggs, oatmeal, and bananas, supplemented with vitamins and salt; they had unlimited access to water.

The first digestive tract experiment was a preliminary test of the experimental procedures. A pig that had not been fed for 24 h but that had access to water was sacrificed by electrocution. The abdominal cavity was opened, and sections of stomach, duodenum, and large intestine were tied off and removed. The sections were opened, and contents were taken from each. Deionized water control samples were also prepared. Each sample was inoculated with either grape juice-inactivated or "normal" (uninactivated) PO1, and all samples were incubated for 30 min at room temperature (ca. 25 C). To remove both bacteria and intestinal contents that would interfere with culture inoculation, the samples were treated ultrasonically for 5 min, diluted 1:100 with PBS, filtered simultaneously through Miracloth (a nonwoven toweling; Chicopee Mills, New York) and a Gelman 1.2 μm-porosity (GA-4) membrane filter by positive pressure, and finally filtered by positive pressure through a Gelman 0.20-μm porosity (GA-8) membrane filter. Each sample was then assayed for virus by the plaque technique on HeLa tissue cultures.

The second digestive tract experiment was a confirmation and expansion of the first experiment. Due to watery intestinal contents in the first experiment, the pig used in the second experiment was fed 5 h before being sacrificed. Samples of six segments of the pig's digestive tract (stomach, duodenum, jejunum, ileum, cecum, and descending large intestine) were collected in the same way as for the first experiment. These and the deionized water controls were reacted with each of three different PO1 preparations: (*a*) virus in PBS, (*b*) virus inactivated by grape juice, and (*c*) virus neutralized by coproantibody. As before, the samples were all held for 30 min at room temperature. However, due to some problems with removal of interfering materials in the first experiment, the samples were processed somewhat differently. After the reaction time, the samples were diluted 1:100 in PBS, and were then homogenized chilled for 2 min. Each sample was then filtered in succession through Gelman membrane filters of 5.0-, 1.2-, 0.8-, and 0.45-μm porosity (GA-1, GA-3, GA-4, and GA-6, respectively). The 5.0-μm filter was 47 mm in diameter, and filtration was done by vacuum; the other filters were 25 mm in diameter, and filtration was done by positive pressure. Finally, each sample was filtered by positive pressure through a Gelman 25-mm diameter 0.20-μm porosity (GA-8) membrane filter. Although there was still some extraneous material left, this was a great improvement over the procedure used in the first experiment. The samples were then titered as previously described.

RESULTS

Inactivation and reactivation

There was no difference between the acid buffer and the deionized water (pH approx. 7.0) results for any of the three viruses tested (Table 1); thus acidity is not a cause of the inactivation phenomenon. More than 99% of both PO1 and EC6 was inactivated in both undiluted and 10% grape juice. CB3 showed > 99% inactivation in undiluted grape juice, but only 76% of CB3 was inactivated by a 10% grape juice solution.

There was little difference between samples at different temperatures for CB3 virus (Table 2), and the results of the 4 C samples closely agreed with the results obtained in the first CB3 experiment (Table 1). Thus the temperatures tested were not a factor in viral inactivation of grape juice.

TABLE 1. *Inactivation of enteroviruses by grape juice.*

Sample suspension	Virus		
	P01	CB3	EC6
Grape juice, undiluted	0[a]	0	0
Grape juice, diluted to 10%	0	21	0
Deionized water	84	88	71
pH 3.3 buffer	86	93	70

[a]Total of plaques recorded among four culture flasks per sample suspension.

TABLE 2. *Effect of temperature upon inactivation of CB3 virus by grape juice.*

Sample suspension	Temperature		
	4 C	25 C	37 C
Grape juice, undiluted	0[a]	0	0
Grape juice, diluted to 10%	21	20	19
Deionized water	83	93	90

[a]Total of plaques recorded among four culture flasks per sample suspension.

Reactivation of inactivated PO1 by PEG is shown in Table 3. Of the inactivated virus, 61% was reactivated by 0.5% PEG; about the same amount of virus was reactivated by 2.5% PEG (68%). Thus most of the virus is not irreversibly inactivated by grape juice; furthermore, at PEG concentrations tested, an increase of PEG does not cause additional reactivation of virus.

Results of both inactivation and reactivation of Sendai virus are in Table 4. This was the first tested virus against which grape juice was virtually ineffective (14% inactivation); others (Table 1) had undergone >99% inactivation under similar conditions. The small grape juice effect was completely reversed by PEG.

The results of the reactivation experiment with human blood serum are shown in Table 5. As can be seen from comparing these results with those in Table 3, human blood serum causes greater reactivation ($\geq$ 94%) than PEG of PO1. Given this degree of reactivation, it is clear that the antiviral substances, which would presumably occur in the serum if present in the blood, could not be detected simply by adding virus to the blood. Other means of detecting these substances in blood could only be undertaken if the nature of the substances were more precisely known.

TABLE 3. *Reactivation by PEG of grape juice-inactivated P01 virus.*

Sample suspension	PEG concentration(%)		
	0	0.5	2.5
Grape juice, undiluted	0[a]	51	61
Deionized water	83	84	90

[a]Total of plaques recorded among four culture flasks per sample suspension.

TABLE 4. *Inactivation by grape juice and reactivation with PEG of Sendai virus.*

Sample suspension	PEG concentration (%)	
	0	0.5[a]
Grape juice, undiluted	82[b]	57
Deionized water	95	51

[a]Additional two-fold dilution of virus suspension, as compared with 0 PEG concentration.
[b]Total of plaques recorded among four culture flasks per sample suspension.

TABLE 5. *Reactivation by human blood serum of grape juice-inactivated P01 virus.*

Sample suspension	Reactivation		
	None	Deionized water	Serum
Grape juice, undiluted	0[a]	0	88
PBS	99	102	94

[a]Total of plaques recorded among four culture flasks per sample suspension.

Grape skin extract

Results of the experiments with grape skin extract are in Table 6. It is difficult to find a valid basis on which to compare these results with those reported above for virus inactivation by grape juice because there is no convenient conversion factor by which to relate the skin extract to the whole grape juice. Although each of the suspensions of skin extract inactivated substantially more CB3 than did the 10% solution of grape juice reported above (93.9% for 0.1% skin extract vs. 78% for 10% juice), the degree of reactivation by PEG was essentially the same as was seen with virus inactivated by grape juice.

TABLE 6. *Inactivation by grape skin extract and reactivation with PEG of CB3 virus.*

Effect tested	Grape skin extract (%)	Virus titer (PFU/ml)	Effect (%)
Inactivation (no PEG)	0	4100	—
	0.1	250	93.90
	1	4	99.90
	5	1	99.98
Reactivation (0.5% PEG)[a]	0	2600	—
	1	1600	62

[a]Additional two-fold dilution of virus suspension, relative to those with no PEG.

Mode of inactivation

More than 95% of ^{32}P-labelled PO1 was adsorbed during filtration through a 0.22 μm porosity Millipore GS membrane filter, either before (1123 CPM/ml vs. 52 CPM/ml) or after (1233 CPM/ml vs. 55 CPM/ml) inactivation by grape juice. This adsorption is a property of the coat protein surface of the virus particle, which evidently is not modified greatly by grape juice inactivation.

Essentially none of the ^{32}P-labelled PO1 was retained by a 50-nm porosity Nuclepore filter, either before (1050 CPM/ml vs. 1131 CPM/ml) or after (1254 CPM/ml vs. 1236 CPM/ml) inactivation by grape juice. Thus aggregation is not what the grape juice does to the virus.

The results of the cell adsorption studies are in Table 7. As expected, there was no adsorption of either inactivated or "normal" virus to MPK cells (97% of radioactivity remained in the supernatant fluid). "Normal" virus adsorbed to the HeLa cells somewhat better than expected (82%). However, there was significant adsorption of inactivated virus to HeLa cells (17%). Although the adsorption was much lower than that of "normal" virus, it was significantly higher than that of the negative (MPK) controls. These results

TABLE 7. *Uptake of grape juice-inactivated P01 by cells.*

Sample suspension	Cells	Fraction	
		Supernatant fluid (CPM/ml)	Resuspended cells (CPM/ml)
Grape juice, undiluted	None	599	—
	MPK	540	12
	HeLa	568	120
Deionized water	None	599	—
	MPK	569	18
	HeLa	131	585

indicate that, whereas most of inactivated virus is blocked from cell receptor sites by the active substances in grape juice, ca. 20% of the virus remains able to adsorb. It is unknown why this 20% fails to express itself in plaques.

Protection of digestive tract

The data for the experiments involving reactivation of inactivated and neutralized virus by contents of the digestive tract are in Table 8. Reactivation of virus inactivated by grape juice occurred in all samples (except the deionized water control) and ranged from 42 to 82%. Although reactivation of coproantibody-neutralized virus did not take place in contents of jejunum, ileum, or large intestine, it would be expected that virus reactivated in the stomach or duodenum would infect upon reaching the ileum. Thus the data show that virus inactivated by grape juice or neutralized by coproantibody would still present a health hazard due to reactivation in the intestinal tract.

DISCUSSION

The findings of Konowalchuk and Speirs (*5,6,7*) have been confirmed. We worked only with Concord grape juice, but they found the virus inactivation phenomenon to be a generic property of grapes and grape products. They reported that enteroviruses, as well as members of other virus groups which we did not test, are inactivated by grape juice. We also found that the Sendai strain of parainfluenza virus type 1, which they did not test, is virtually refractory to the effect of grape juice.

The loss of infectivity titer that occurs when enteroviruses are treated with grape juice is neither the result of aggregation nor of modification of the particle's surface sufficient to interfere with its nonspecific adsorption to a cellulose nitrate membrane filter. In fact, a portion of the treated virus adsorbs specifically to host cell receptors, even though it cannot then initiate a replicative cycle.

The finding that most of the virus can be reactivated by PEG and other substances makes it clear that the particle is not being degraded in the process of inactivation. The active substances are said by Konowalchuk and Speirs (*5,6*) to be polyphenols of low to moderate molecular weight, derived principally from the skin of the grape. They tested several pure compounds of this class and showed them to inactivate enteroviruses.

We have tried to assess the significance of this phenomenon to human health. We wished to learn whether ingested grape juice was likely to be able to prevent or modify virus infections in man. The experimental approaches were indirect, in that no human subjects were used, but were generally pertinent to this question.

Because grape juice is a food, it seemed most reasonable to look first for protection of the digestive tract. Contents of various portions of the digestive tract were obtained from young swine: their digestive tract is quite like that of man, and they had been fed on human foods to heighten the analogy. Under conditions which we think are representative of those inside the digestive tract, extensive virus reactivation took place with contents from each segment. Coproantibody-neutralized virus was included because the neutralization process is also known to be reversible and not to degrade the virus. Reactivation of coproantibody-neutralized virus differed in some specifics, but it was clear that neither coproantibody nor grape juice was likely to prevent enterovirus infection of the human intestines.

If ingested grape juice were going to act against viral infections at other sites in the body, the active substances would probably have to be absorbed from the digestive tract and transported via the bloodstream to these

TABLE 8. *Reactivation of grape juice-inactivated and coproantibody-neutralized P01 by contents of pigs' digestive tracts.*

Tested suspension	Experiment number	Virus treated with				
		PBS	Grape juice		Coproantibody	
		Plaque count	Plaque count	Reactivation (%)	Plaque count	Reactivation (%)
Input	1	96[a]	0	—	—	—
	2	94	0	—	0	—
Stomach	1	92	50	54	—	—
	2	96	48	50	48	50
Duodenum	1	101	56	55	—	—
	2	99	59	60	41	41
Jejunum	2	98	50	51	0	<1
Ileum	2	95	55	58	0	<1
Cecum	2	103	43	42	81	79
Large intestine	1	88	72	82	—	—
	2	96	70	73	0	<1
Deionized water	1	96	0	<1	—	—
	2	96	0	<1	0	<1

[a]Total among four flasks per sample suspension.

alternate locations. The straightforward approach to detecting these substances in blood, direct testing with virus, proved not to be feasible because blood serum reactivates inactivated virus with unequaled efficiency. Any other approach to detecting these substances in transit in the blood would require much more exact knowledge of their nature than now exists.

Sendai virus was selected as a potential model virus because it produces lethal respiratory infections in mice under controlled experimental conditions (*4*). The question of whether ingestion of grape juice could produce effective levels of active substances in the murine respiratory tract was mooted by the inability of grape juice to inactivate the Sendai virus.

These laboratory experimental approaches have failed to produce evidence that ingested grape juice would be valuable for antiviral prophylaxis or therapy in man. We think the potential purchaser would do best to continue to judge the product on its merits as a food.

ACKNOWLEDGMENTS

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Drug Residues in Dairy Culls Target of New USDA Program

Dairymen should keep up-to-date records on drugs they administer to dairy cows. If they don't, they can be asking for trouble when they cull antibiotic treated cows.

A U.S. Department of Agriculture program to detect drug residues in cull animals sold for slaughter goes into effect early in 1979. Under the "swab test on premises" (STOP) program, dairymen can be fined or can face charges if the cows they sell are found to contain illegal levels of drug residues in their tissues. Federal meat inspectors will be using a new detection technique to find drug residues before carcasses leave the slaughterhouse.

"Dairymen must read drug labels, keep track of the dates they administer medications and not sell cattle before the withdrawal time indicated on the label," says Allan N. Bringe, University of Wisconsin-Extension dairy scientist. "The dairyman is responsible for cull cows marketed being free of residues."

The USDA estimates that about 7,000 cows going to market each year contain residues of drugs used by farmers to treat various illnesses and injuries, including mastitis. The department doesn't object to farmers marketing animals that have been treated or that have disorders, but it does insist that they market only when the animals are free of residues.

The drugs most frequently found in illegally high amounts are the antibiotics, penicillin and dihydrostreptomycin.

One effect of carelessness in drug use is lower prices for cull cows. Carcasses in which high levels of residues are found must be condemned. Current cull cow prices are in the $30-$40 per hundredweight range.

Bringe says the old test used to detect residues took 14 days to confirm specific residues. A new swab test technique will find antibiotic residues in an animal's kidneys within a few hours. If residues are found, further lab tests will be carried out. If the level exceeds U.S. Food and Drug Administration (FDA) tolerances, the carcass will be condemned.

"The risk for dairymen who send cull animals to market in violation of residue tolerances is now much higher," says Bringe. "It won't take long for packers to learn which producers or animal dealers are marketing cows with violative levels. Packers may refuse to buy animals from individuals who consistently violate the law."

Under the new program, federal inspectors will report incidents of violation to the FDA, which has the authority to investigate and take action. If problems continue, new and tougher laws on antibiotic use could be imposed.

Bringe urges dairymen to review their present use of drugs for disease prevention and control. "Ask yourself if the drugs are really needed. If you're convinced they are, be sure to follow proper procedures and insist those who work with your animals also follow them. Buy medications from reputable suppliers and sell animals to reputable dealers.

"Good records should have the highest priority," says Bringe. "Keep track of what dose was given to which

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Evaluation of Fluorescent Light on Flavor and Riboflavin Content of Milk Held in Gallon Returnable Containers [1]

JONATHAN C. HOSKIN and PAUL S. DIMICK*

Department of Food Science, The Pennsylvania State University, University Park, Pennsylvania 16802

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ABSTRACT

Five 1-gal. retail containers were evaluated for their protection of homogenized milk against development of light-induced flavor and degradation of riboflavin. These were clear polycarbonate, tinted polycarbonate, high-density polyethylene, and glass returnable containers and an unprinted fiberboard non-returnable container. All containers were held in a commercial sliding door display case at 7 ± 1 C illuminated to 1076 lx with a fluorescent lamp up to 72 h. Sensory evaluation was conducted by a trained panel using hedonic 9-point scoring and magnitude estimation scale techniques. Riboflavin was determined by the fluorometric method. An evaluation of the containers demonstrated that there was a significant difference ($P < .05$) in preference and degree of light-induced flavor between the milk held in clear polycarbonate and glass compared to the control milk after 12 h of exposure. Milk held in high-density polyethylene was significantly different in preference from the unexposed control following 12 h when evaluated by the hedonic method; however, 24 h of exposure were needed to demonstrate a significant difference in the degree of light-induced flavor using the magnitude estimation technique. The tinted polycarbonate container, which is fabricated with a blocking agent that inhibits transmission of light at 380-480 nm, provided the milk with greatest protection of the returnable containers against development of the off-flavor. Milks exposed in fiberboard and also milks in the five containers held in the dark were not significantly different from the unexposed control. The milks held in glass demonstrated significant losses in riboflavin following exposure.

Home-delivery of fluid milk has decreased steadily with a concurrent increase in milk sold through local retail stores, the latter now amounting to about 94% of sales in the Federal Order Markets (*9*). Along with these changes in marketing, fluid milk may not be consumed for up to 9 days following processing. A major flavor defect and possible factor in nutrient loss is attributed to the extended exposure of milk to fluorescent lights used to illuminate dairy display cases. Several reviews (*5,13,15*) discuss the concern over photochemically-induced changes which are associated with the protein, lipid and vitamin components of milk. Many factors may influence the overall reaction such as light source, wave length, intensity, exposure time, and temperature as well as the influence of the container material.

The gallon milk container has become the most popular size since 1974 and was estimated to account for 44% of the fluid milk market in 1976 (*9*). The objectives of this study were to evaluate five 1-gal. retail containers for their protection of homogenized milk against development of light-induced flavor and degradation of riboflavin.

MATERIALS AND METHODS

Containers

Table 1 describes the five 1-gal. milk containers used in this study. Total light transmission values of the various containers were obtained by observing the light readings inside and ouside each container using a Model 756 Weston meter with a light source of cool white fluorescent lamps (F40CW). Spectral transmission characteristics over the wavelength range of 380-750 nm of the four returnable container materials were measured with a Beckman Model 25 spectrophotometer and of the non-returnable fiberboard material with a Pye Unicam SP8-100 spectrophotometer (Fig. 1).

Samples and treatment

Mixed herd milk routinely supplied to the University Creamery was used in this study. The raw milk was pasteurized at 75.5 C for 16 sec, homogenized at 155 kg/cm² (2500 psig), cooled to 6 C and transferred into the various 1-gal. milk containers with a minimum of exposure to light. All containers were washed, rinsed, sanitized and dried just before filling. The filled containers were randomly placed in a commercial double sliding door display case held at 7 ± 1 C. The size of the display case restricted the number of containers to be evaluated; therefore, the containers were grouped into two trials. During each trial the containers were evaluated twice. The display case was illuminated by cool white fluorescent lamps (F40CW) mounted parallel to the shelves to produce 1076 ± 50 lx at the vertical surface of the containers. Milk from each type of container was examined for flavor and riboflavin content after exposure to fluorescent light for 0, 3, 6, 12, 24, 48 and 72 h. The milk was not agitated during storage. Unexposed milk samples in the various containers tested were also sampled at 3, 24 and 72 h.

At each testing period the containers were mixed by inversion, samples were transferred to 30-ml cups in dim light and then presented to the panel members within 15 min. The trained taste panel consisted of 11 women, all of whom had from 2 to 10 years of experience in organoleptic evaluations of numerous food products. Before the study three training sessions were conducted. Preference evaluation was obtained by using a 9-point hedonic scale (1, dislike extremely; 9, like extremely) (*8*). The magnitude estimation scale technique (*10*) was also employed to quantify light-induced flavor intensity. In this procedure the panel member is instructed to assign values in proportion to the perceived intensity of light-induced flavor. The reference (control) stimulus was assigned an arbitrary value of 10 and all judgements of intensity were based on this control, i.e., if the light-induced flavor intensity was perceived as twice that of the reference its score would be 20. The raw scores were averaged, divided by 10 (reference value), and computed as the ratio score.

Riboflavin was determined in duplicate by the Official Final AOAC fluorometric procedure (*1*) using a Coleman Model 12C photofluorometer. To monitor possible microbial post-pasteurization contamination of milk in the various containers, Standard Plate Count procedures were conducted (*14*).

The data were computer-analyzed using the Statistical Analysis

[1]*Authorized for publication on June 5, 1978 as paper no. 5527 in the journal series of the Pennsylvania Agricultural Experiment Station.*

System (2) to determine analysis of variance and the Duncan's multiple range test.

RESULTS AND DISCUSSION

Results of the taste panel evaluation of homogenized milk from the five types of containers exposed to fluorescent light and the controls are presented in Tables 2 and 3. The milk held in the non-returnable fiberboard container did not change significantly in preference or degree of light-induced flavor intensity from the unexposed control throughout the 72 h of exposure. The protective effect of this container material is due to the low total transmission properties (Table 1) and the blocking of the 380-480 nm wavelength region (0.2-0.4%T, Fig. 1) of the light spectrum. Strong absorption in the 380-480 nm region by the vitamin, riboflavin, promotes the photocatalytic degradation of the amino acid, methionine to methional (*6,11*); a major contributor to light-induced flavor.

A comparison of the four returnable containers demonstrated that the yellow-tinted polycarbonate (P.C. tinted) material provided the greatest protection against development of light-induced flavor. This packaging material is fabricated with a blocking agent that inhibits absorption of light in the 380-480 nm region (Fig. 1) thus limiting absorption by riboflavin. In Trial I no significant differences in preference (hedonic) or degree of light-induce flavor (ratio) were noted between the milks held in P.C. tinted containers and the unexposed control until 72 h of exposure (Table 2). Results of Trial II demonstrated a significant difference in preference between the milk held in P.C. tinted and the control at 24 h and the intensity of light-induced flavor was significant when compared to the control at 48 h of exposure (Table 3). Milk packaged in P.C. tinted containers afforded greater protection against development of the off-flavor when compared to both glass and high density polyethylene (HDPE) which were significantly different from the unexposed control milks after only 12 h of exposure. Milks held in the clear polycarbonate (P.C. clear) containers had about the same protection as milks in the glass and HDPE containers. Figures 2-5 graphically illustrate the change in panel members responses in hedonic and ratio scores for milk held in the containers tested over the exposure treatment. The data demonstrate that after 24 h of exposure the flavor acceptability of milk packaged in glass, P.C. clear and HDPE containers drastically decreased to approximately 3 (hedonic score) with a concurrent increase in light-induced flavor intensity of 2-4 times that of the corresponding control. Analysis of data demonstrated that the hedonic scores and ratio scores as measured by the magnitude estimation technique were correlated at $P < .05$.

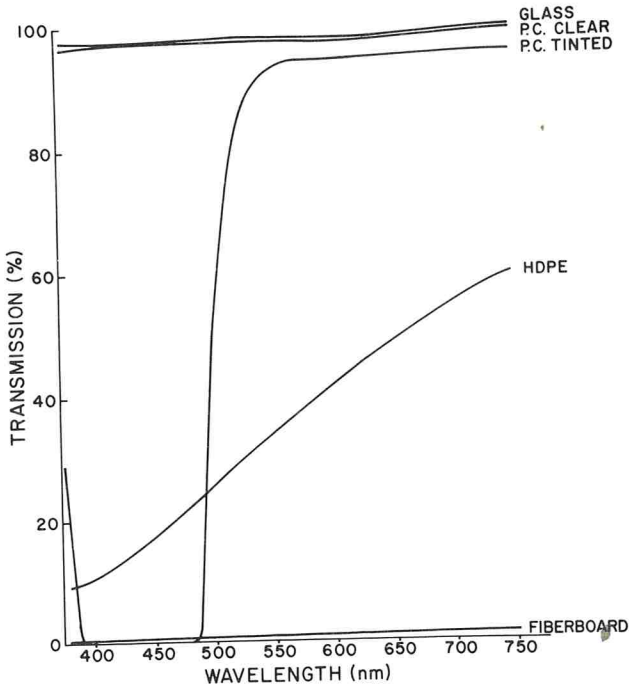

Figure 1. *Spectral transmission of one-gallon milk containers.*

The total transmission values of the container material were not an indicator of the protective effect against light-induced flavor development. Even though the HDPE container permits only 58% total light transmission (Table 1) as compared to 75% for the PC tinted and 92% for the glass container; transmission of light in the 380-480 nm region is critical. Glass, P.C. clear, and HDPE containers transmit 95%, 48-63%, and 10-24% light, respectively in this region and therefore are less protective than the P.C. tinted container which totally blocks these critical wavelengths. Recent studies (*3*) using titanium dioxide as a blocking agent in polyethylene containers demonstrated that this coloring material is not effective in preventing the light-induced flavor. Light transmission values of 7-30% at 450 nm were found using 0.5-2% TiO_2 and thus allow light penetration in the critical area of the spectrum.

To determine if the container material had any

TABLE 1. *Description and characteristics of one-gallon milk containers.*

Container	Type	Trade name[a]	Thickness (mm)	Transmission[b] (%)
Clear flint glass	Returnable	—	3.4	92
Clear polycarbonate	Returnable	Merlon	1.5	90
Tinted polycarbonate	Returnable	Merlon	1.5	75
High density polyethylene	Returnable	Polytrip	1.7	58
Unprinted fiberboard	Nonreturnable	Pure-Pak	0.7	4

[a]The use of trade names is for description only and does not imply endorsement by The Pennsylvania State University.
[b]Light transmission with 40-w cool white fluorescent lamp source measured inside and outside of container using Model 756 Weston meter.

TABLE 2. *Mean hedonic and ratio scores[a] by container over time of fluorescent light exposure - Trial I.*

Container	Exposure time (h)													
	0		3		6		12		24		48		72	
	Hedonic	Ratio	Hedonic	Ratio	Hedonic	Ratio	Hedonic	Ratio	Hedonic	Ratio	Hedonic	Ratio	Hedonic	Ratio
Control	6.1a	1.2a	6.6a	0.9a	6.1a	1.1a	6.6ab	0.8ab	6.9a	1.2a	6.9a	1.0a	6.9a	0.8a
Fiberboard	6.3a	0.9a	5.9a	1.1a	6.0a	0.9a	6.8a	0.7a	7.1a	1.1a	6.8a	0.9a	6.4a	1.2ab
P.C. tinted	6.3a	1.1a	5.7a	0.9a	6.4a	0.7a	5.9abc	1.2ab	6.0a	1.4a	5.6a	1.6a	4.7b	2.4bc
P.C. clear	6.3a	1.3a	5.7a	1.1a	5.6a	0.9a	5.0c	1.1ab	3.3b	3.2b	3.1b	4.3b	2.9c	3.9c
Glass	6.1a	1.2a	6.4a	0.5a	6.4a	1.2a	5.1bc	1.5b	3.3b	3.5b	2.3b	4.0b	2.6c	4.2c

[a]Means within columns with same letter are not significantly different at $P < .05$ (n = 22).

TABLE 3. *Mean hedonic and ratio scores[a] by container over time of fluorescent light exposure - Trail II.*

Container	Exposure time (h)													
	0		3		6		12		24		48		72	
	Hedonic	Ratio	Hedonic	Ratio	Hedonic	Ratio	Hedonic	Ratio	Hedonic	Ratio	Hedonic	Ratio	Hedonic	Ratio
Control	6.9a	1.0a	6.6a	1.3a	6.5a	1.0a	7.1a	0.8a	7.0a	1.0a	7.1a	0.8a	6.5a	0.7a
Fiberboard	6.8a	1.1a	6.2a	1.1a	6.2a	0.9a	6.4ab	1.1a	6.2a	0.9a	6.3a	1.1a	5.6a	1.1a
P.C. tinted	6.6a	1.0a	6.4a	1.0a	6.5a	1.1a	6.1abc	1.1a	4.3b	1.6ab	4.5b	2.6b	3.4b	2.5b
P.C. clear	6.7a	1.4a	5.8a	1.1a	6.2a	1.3a	4.9bc	1.1a	3.1b	2.6b	3.9bc	3.1b	2.5b	3.4b
HDPE	6.3a	1.4a	5.9a	1.2a	6.0a	1.1a	4.7c	1.5a	3.2b	2.4b	2.8c	3.1b	2.6b	3.2b

[a]Means within columns with same letter are not significantly different at $P < .05$ (n = 22).

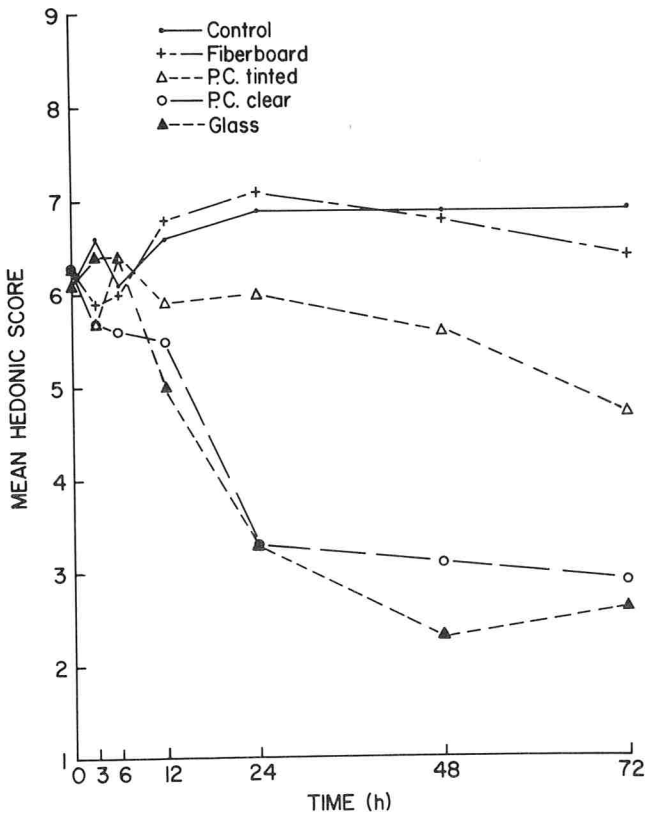

Figure 2. *Mean hedonic flavor scores from trained taste panel members for milk exposed to fluorescent light in one-gallon containers - Trial I.*

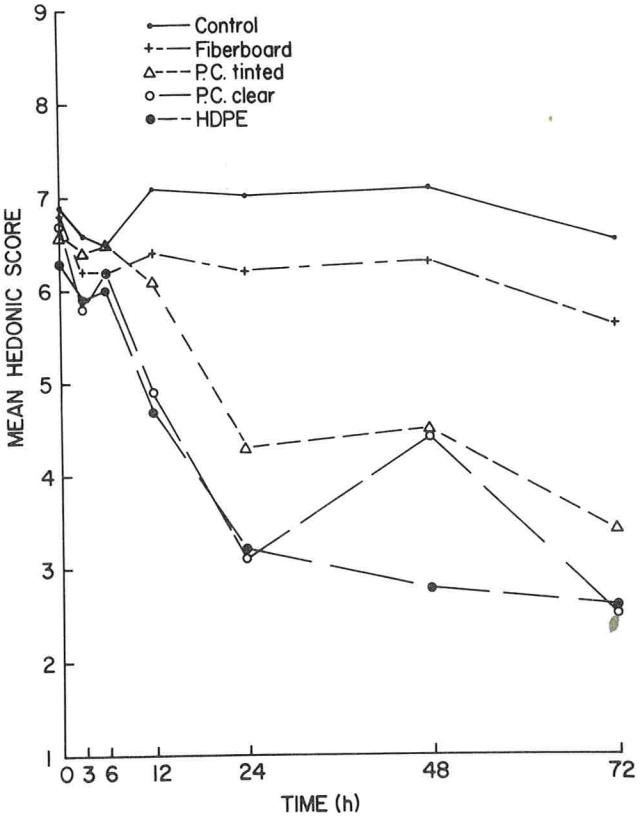

Figure 4. *Mean hedonic flavor scores from trained taste panel members for milk exposed to fluorescent light in one-gallon containers - Trial II.*

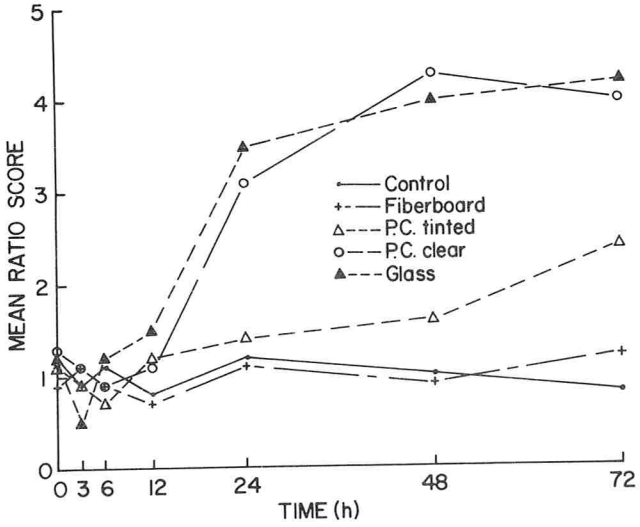

Figure 3. *Mean ratio scores from magnitude estimation by trained taste panel members for milk exposed to fluorescent light in one-gallon containers - Trial I.*

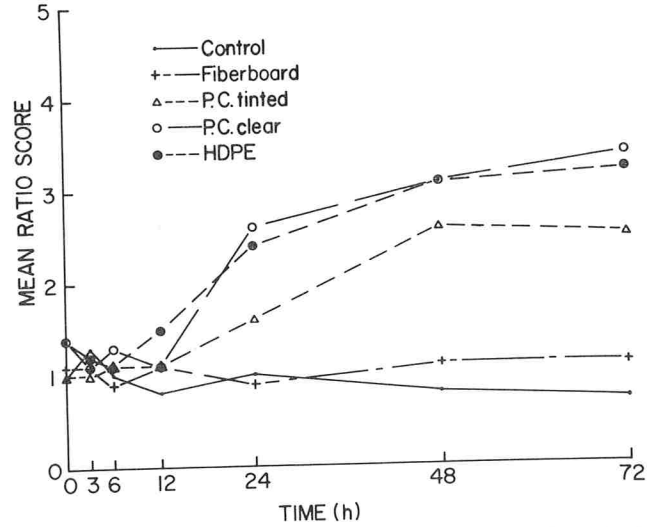

Figure 5. *Mean ratio scores from magnitude estimation by trained taste panel members for milk exposed to fluorescent light in one-gallon containers - Trial II.*

influence on the responses by the panel members, unexposed samples were held throughout the test period and organoleptically evaluated. No significant differences in flavor were found between any container when measured by the previously described methods. Standard Plate Counts of all samples revealed no abnormal microbial contamination during the test period.

The mean concentration of riboflavin in the homogenized milk in glass was significantly ($P < .05$) lower than the control milk at 24 and 72 h of exposure. This amounted to a 27% loss in riboflavin at 72 h of exposure. No significant differences were noted in riboflavin concentration of milk held in the other containers over time of exposure. At 72 h, however, greater percent losses of the vitamin in milk held in P.C. clear containers (-13%) were noted when compared to the milk held in

the HDPE (-10%), fiberboard (-10%) and P.C. tinted (-6%) containers. No significant differences were observed in the riboflavin content of milks held in the dark in various containers over time.

Losses of the vitamin in milk have been reported in glass and polyethylene half-gallon containers (*4*) using the same illumination levels (1076 lx) and significant decreases in riboflavin concentration occurred after 48 h. Higher illumination levels (2200 lx) have been reported (*3*) to promote losses as high as 18% after 48 h in plastic jugs.

It is evident from this study and others related to the problem of light-induced flavor development in milk, that the packaging material is a key to the protection of quality of fluid market milk under present marketing conditions. With the increase in use of transparent or translucent returnable containers the necessity to limit the critical wavelengths of light is paramount.

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animal and when it was given. That goes for any medication given by you, your employees or your veterinarian. Also note on the record the date it will be safe to sell milk from the animal or to sell the animal for slaughter. These dates are determined by counting the number of days indicated on the label as a safe withdrawal period from the last administration of the drug."

Bringe says both intramammary infusion and injectable drugs can cause tissue residues, so don't misuse either. If you have questions about how to use a drug, ask your veterinarian or dealer about it.

You'll also need a good way to identify animals that have been treated. There are several good ways, says Bringe, including tail tape, leg bands or dye marks. If it's possible, isolate these animals until the withdrawal period is over.

The USDA points out that label instructions can change, so always check labels for dosage and withdrawal times each time you use a medication. Also, use only the dose recommended for the species and size of animal being treated.

For treatment to be effective, the dose must be administered correctly. Use the right needle size and pick out the injection site carefully. Unless you do, the animal may suffer an adverse reaction, or the treatment may be ineffective.

Avoid "double dosing," Bringe urges. Twice as much isn't twice as good. Double dosing is using both an injectable and an oral dose at the same time or giving twice as much of a dose. Either can cause illegally high residues.

Keep your veterinarian informed about the composition of any drugs you administer to your animals, and always ask him about the safe date to market animals he treats.

Using sound herd management techniques to prevent dairy cow disease problems can reduce the difficulties dairymen experience with medications, Bringe points out. These techniques include providing adequate housing, yards and bedding, following correct milking procedures, and making sure milking equipment is up to standards.

"Safe meat, like safe milk, is the farmer's legal responsibility," Bringe says. "It's just good business to insure that cull cows sold are free of antibiotic residues."

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Need for a Working-Factor in Evaluating Freezing Point Results on Raw Milk Samples[1]

V. S. PACKARD* and R. E. GINN

Department of Food Science and Nutrition, University of Minnesota, St. Paul, Minnesota 55108 and Dairy Quality Control Institute, Inc., St. Paul, Minnesota 55113

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ABSTRACT

The legal upper base for taking action in cases of water adulteration of milk is generally accepted as - 0.525°H. Henningson (*2*), in a study of 660 samples of milk known to contain no added water and representing 22 states in the U.S. and four Canadian provinces, found (at the 95% confidence limit) only 1% probability of a naturally-occurring milk sample falling above that level. Yet at a mean value of - 0.540°H, as noted in this same study, such base allows for 3% added water for the "average" dairy farm. The present study, made on samples without knowledge of their purity (lack of added water), tends to confirm this potential. At the same time it suggests the likelihood that, on a practical basis, a "working factor" set at some lower value could be useful to the dairy industry in coping with the problem of added water in milk.

Freezing point of milk is a universally accepted method of evaluating raw milk for presence of added water. In the latest major study of normal freezing values, Henningson (*2*) surveyed 660 milk samples taken over much of North America. All samples were determined not to contain added water before being taken for analysis. The mean freezing point of these naturally-occurring milk samples was found to be −0.5404 C, with a standard error of 0.0003 C. The standard deviation was - 0.00676 C. From this information, a base freezing point value was computed, which included 99% of the observed values at 95% confidence. This value was taken as 2.326 standard deviations from the mean, and was found to be - 0.525 C. In other words, no more than 1% of naturally-occurring milk samples would be expected to fall above this value. Putting it positively, 99% of samples found to have freezing points above - 0.525 C could rightfully be expected to contain some added water. This base of - 0.525 C has since become the AOAC standard (*1*). As such, it serves a regulatory function, being the basis upon which legal action may be taken. However, such base also provides for the presence of nearly 3% added water in milk supplies approximating the mean value of - 0.5404 found in this study: 0.5404 − 0.5247 = 0.0157; 0.0157 ÷ 0.5404 × 100 = 2.9%. So while a base of - 0.525 C serves as a reasonable value for taking legal action, it is too high to serve a useful quality control function. On a practical basis, a ceiling of - 0.525 C tends to become a "tolerance" level. There is need, therefore, to set a working level, a point at which field action is taken, at some lower value. It was to establish this working standard that the five dairy organizations joined in this study.

But there is still another point of confusion which must be broached. That confusion centers on the standard on which freezing point determinations are made. Most modern-day cryoscopes are calibrated and operate on degrees Hortvet, not degrees centigrade. The literature, in which both measures are cited, becomes no less confusing in this regard. In fact there is good reason to believe that many values given as degrees centigrade were measured in degrees Hortvet. So at the outset it is essential to note that the cryoscopes used in this study were all factory and/or laboratory-calibrated in degrees Hortvet. And, as AOAC (*1*) recognizes, the standard of - 0.525 C, listed in this Journal as the upper level of tolerance in water-adulteration of milk, is in fact a standard measured in degrees Hortvet, not centigrade. The authors restate this for sake of clarity, to emphasize again the need for confirming the basis upon which measurements are made on milk, and to suggest the need to establish a permanent standard in degrees centigrade.

MATERIALS AND METHODS

Five dairy industry organizations operating in and around the state of Minnesota pooled freezing point values obtained during the summer, fall and early winter of 1977 on raw milk samples in working plants

[1]*This project was a joint effort involving the Minnesota Department of Agriculture, St. Paul, MN, Associated Milk Producers, Inc., New Ulm, MN, Kraft, Inc., Melrose, MN, Land O'Lakes, Inc., Minneapolis, MN, Mid America Dairymen, Inc., St. Paul, MN., and Dairy Quality Control Institute, Inc., St. Paul, MN. Scientific Journal Series Paper No. 10374, Minnesota Agricultural Experiment Station, St. Paul, MN 55108.*

under their supervision. All samples were tested on semi-automatic cryoscope devices, standardized and calibrated by the individual plant or organization. Herd milk samples analyzed in this study represented Minnesota and adjoining areas of Wisconsin, Iowa, North Dakota, and South Dakota. A total of 10,852 milk samples were tested and the data subjected to computer evaluation. No attempt was made to procure milk known not to contain water. These were merely samples checked in the routine work of the laboratories represented.

Precision Systems, Inc. (60 Union Ave., Sudbury, Mass. 01776) has worked out formulas for calculating degrees Hortvet from degrees centigrade and vice versa. Where centigrade values are presented parenthetically in this work, one of these formulas was used to compute the value. This one is given as follows:

$$°C = \frac{(0.1915 \times - °H) - 0.0004785}{0.199}$$

The formula for converting centigrade to Hortvet is:

$$°H = \frac{(0.199 \times - °C) + 0.0004785}{0.1915}$$

RESULTS AND DISCUSSION

Table 1 summarizes findings of this study. A total of 10,852 raw milk samples were analyzed for freezing point using semi-automatic cryoscopes of the Precision Systems, Inc./Advance type. All were calibrated in degrees Hortvet. Respectively, the different organizations analyzed 353, 1017, 666, 990, and 7,826 samples. Mean freezing point values ranged from -0.539°H (-0.521 C) to -0.544°H (-0.526 C) for the various organizations. The grand mean was found to be -0.544°H. The standard deviation for all samples was 0.0072°H. Skewness, the degree to which the curve of all values tends to tail off to the left or right, averaged -0.519. The negative value indicates a tailing to the left. Kurtosis, a measure of flatness or sharpness of the curve, was found to average 0.258. A positive value actually implies a narrow curve, but this average value is perhaps misleading. As a matter of fact only one organization (number 2) included in this study showed a positive value for this statistic. All the rest showed negative readings, but only slightly so. If there is meaning in a positive result, it is that the milk supplies are either of very uniform freezing point or the quality control program in prevention of added water is very effective, or both. In any event, most of the organizations showed freezing point curves tending toward flatness.

It has already been stated that no attempt was made to secure samples known not to contain water. This point is repeated here, in drawing a comparison between these results and those of Henningson (*2*). With no added water, milks in the Henningson study averaged -0.5404 C (centigrade is indicated, as reported by the investigator). In this study the average value was −0.544° Hortvet. Understanding both measurements to be degrees Hortvet, the value obtained in the present work (with no special control exerted to obtain unwatered samples) is actually slightly lower than the former. While some samples no doubt contained some added water, an observed mean of -0.544°H (-0.526 C) suggests the true mean to be at least -0.540°H (-0.540 C, as reported by Henningson). This value, -0.540°H, is in fact -0.522 C.

Using one standard deviation from the mean to represent 67% of the observations, these milk samples would put the upper base level at -0.537°H (-0.519 C). Since some added water no doubt was in fact present, and since the cryoscopes themselves provide a certain degree of variability, a reasonable "working" standard would appear to be -0.540°H (-0.522 C). Certainly some field activity is warranted when a base which includes nearly two-thirds of the samples in the population is exceeded. In this study 65.6% of the samples showed freezing points above -0.540°H (-0.522 C) by actual measurement. Field work at such point should result in a much more carefully monitored program of water control than one based on an upper level of -0.525°H (-0.508 C). Again, this survey found only 2.5% of milk supplies with freezing points above this value. To concentrate field efforts on those few farms fails to consider the great majority of milk supplies where need likewise exists to control water addition. So while an upper level of -0.525°H (-0.508 C) serves as a sound legal basis for taking action against gross adulteration, a lower freezing point base would better serve a quality control function. For this reason the several organizations represented by this study intend to commence a quality control effort based on the lower (-0.540°H) (-0.522 C) standard. Other processors operating within the same milk supply area will be invited to join in the effort. Others might well wish to do so, and the data included herein would suggest it to be a wise policy.

But beyond the immediate practical implications of this work, the authors wish to re-assert the fact of

TABLE 1. *Summary of findings of freezing point determinations on 10,852 samples of milk tested by cryoscopes.*

Organization	No. of samples	Mean °H[1]	Standard deviation	Skewness[2]	Kurtosis[3]
1	353	-.543	.0080	-.378	-.473
2	1,017	-.542	.0056	-.781	2.071
3	666	-.539	.0073	-.082	-.433
4	990	-.542	.0079	-.307	-.385
5	7,826	-.544	.0070	-.599	-.475
Grand Total	10,852	-.544	.0072	-.519	.258

[1]Degrees Hortvet.
[2]A negative value implies a tailing off to the left.
[3]A negative value implies a flat curve.

confusion over the two measurement standards, degrees centigrade and degrees Hortvet. They solicit support in attempting to clarify the obvious confusion that exists. They further wish to urge adoption of the centigrade measure as the U.S. standard. To make such adjustment in present automated equipment is not difficult. It simply requires use of the same salt standards for calibration, with equipment set at the equivalent centigrade reading. Results are then readable directly in centigrade. Although such conversion might cause confusion, at least for a time, the final adoption of a correct standard of measure can only lead to less confusion in the long run. The authors and the members of this informal committee feel the time for making such change is now overdue.

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Soybean Phosphatides and Blood Lipids of Primates[1]

R. A. ROBBINS and A. L. BRANEN*

Department of Food Science and Technology, Washington State University, Pullman, Washington 99164

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ABSTRACT

Ten pigtail monkeys (*Macaca nemestrina*) were used to study the effects of soybean phosphatides on blood lipid levels. Two monkeys were maintained on a control low-fat diet and eight monkeys were made hypercholesterolemic by feeding a diet containing 16% butter oil and 1% cholesterol for a 12-week period. Soybean phosphatides were administered to the animals by infusion or incorporation into the diet. All animals were placed on low-fat diets after 12 weeks and maintained on this diet for 5 weeks. Incorporation of 3% soybean phosphatides into the butter oil-cholesterol diet before the animals became hypercholesterolemic or a twice weekly infusion of 5 ml of a 10% emulsion of soybean phosphatides after the animals were hypercholesterolemic partially prevented increases in plasma total cholesterol, cholesterol esters and lipid phosphate induced by the butter oil-cholesterol diets. Alterations in the blood plasma lipoprotein patterns resulting from the butter oil-cholesterol diet were also prevented by these methods of phosphatide administration. Switching the animals to a diet containing phosphatides after the animals were already hypercholesterolemic was not effective in preventing alterations in blood lipid levels or lipoprotein patterns. Blood triglyceride levels were not affected by any of the treatments. The phosphatide treatments had no significant effect on the rate of decrease of blood lipid levels after placing the hypercholesterolemic animals on a low fat-low cholesterol diet. Studies of the turnover of phosphatidyl choline indicate that this phospholipid or its component parts is/are rapidly absorbed and that 10% of the radioactivity of a single oral dose of the phospholipid is released from the tissues of monkeys with a half life of about 2 days. Prior treatment with phosphatides appears to delay excretion of phosphatidyl choline. Possible mechanisms of the action of phosphatides on blood lipids are discussed.

Commercial soybean lecithin is a mixture of phospholipids which is widely used as an emulsifier in food products (*7*). Although lecithin commonly refers to phosphatidyl choline, commercial soybean lecithin usually contains 20% phosphatidyl choline, 20% phosphatidyl ethanolamine and 22% inositol phosphatides (*7*). Oral consumption of this mixture of phosphatides is often recommended for lowering blood cholesterol levels and decreasing the incidence of atherosclerosis (*15*). Morrison (*15*) and Pottenger and Krohn (*17*) have found that the blood cholesterol levels of hypercholesterolemic patients were decreased by oral consumption of phosphatides. Lowering of blood cholesterol by phosphatides, however, has not been demonstrated in controlled animal experiments, although the intravenous infusion of phosphatides has been reported to decrease the levels of hepatic and aortic cholesterol in rabbits and baboons and to result in a regression of atherosclerosis (*1,2,5,13,19*).

It was the purpose of this study to determine the influence of oral consumption or intravenous infusion of phosphatides on the blood lipid levels of monkeys receiving a high fat-high cholesterol diet in a controlled experiment. Monkeys, because of their phylogenetic relationship to man, should give a physiological response similar to that found in man.

MATERIALS AND METHODS

Phosphatides

The phosphatide granules (Centrolex, Central Soya Company, Inc.) used in this study were 95% soybean phosphatides composed of roughly equal amounts of phosphatidyl choline, phosphatidyl ethanolamine and inositol phosphatides (*3*). The phosphatides preparation used for infusion (Lexinol-Cal, American Lecithin Company) was an alcohol-soluble fraction obtained from the phosphatide granules.

Animals

Ten sexually mature female pigtail monkeys (*Macaca nemestrina*) weighing approximately 5 kg were used in this study. They were confined in specially designed chairs and exercised every 4 weeks by caging them in their individual cages for 2 days.

Preparation of diets

The composition of the diets is shown in Table 1. The monkeys were made hypercholesterolemic by feeding them an experimental diet containing 16% butter oil and 1% cholesterol. Three percent soybean phosphatides (Controlex, Central Soya Company, Inc.) were incorporated into the phosphatide diets so that 3 g of phosphatides were ingested daily. The diets were made isocaloric by varying the amounts of starch and cellulose. Water was added to the dry ingredients and biscuits were formed and then dried overnight at 55 C. The monkeys were fed 50 g of the diet twice daily. The animals also received one Vigran multi-vitamin tablet (Squibb Products Company) and 1/2 of an apple or orange daily.

Preparation of lecithin infusions

Ten grams of Lexinol-Cal (American Lecithin Company), which is the alcohol-soluble fraction of the phosphatide granules, was blended with 90 ml of saline solution and dialyzed for 24 h. The infusion was then autoclaved at 121 C for 15 min. Five ml were administered biweekly via the saphenous vein.

Preparation of radioactive dosages

For dose by infusion, 2 μCi of uniformly labeled [^{14}C]phosphatidyl choline was added to 3 ml of corn oil and then added to 10 ml of the 10% phosphatide solution used for infusions. As described above, a 5-ml dose (0.5 μCi) was given to each monkey.

For oral dose 10 μCi of [^{14}C]phosphatidyl choline was mixed with 30 g of phosphatide granules, 45 g of corn oil and 25 ml of water. Ten milliliters (1 μCi) were then given to each monkey by stomach intubation.

[1]*Scientific Paper No. 4694, Washington Agriculture Experiment Station, Pullman, WA 99164, Project No. 0216.*

Experimental design

Monkeys were fed the control low fat diet for 2 weeks to acclimate the animals to the type of diets used in the study and to provide control blood lipid levels. The monkeys were divided into five groups of 2 monkeys each according to the experimental design shown in Table 2. This consisted of a high-fat phase (Phase I) and a low-fat phase (Phase II).

Phase I. Animals in group A were fed the control low-fat diet, animals in group B were fed the control high-fat diet and animals in group C were fed the high-fat diet containing phosphatides. Animals in group D were fed the control high-fat diet for 5 weeks then placed on a high-fat diet containing phosphatides. Animals in group E were also fed the control high-fat diet, but were given infusions of phosphatides biweekly after the sixth week. Five milliliters of blood were drawn weekly from the femoral vein of each animal following a 15 to 17-h fasting period.

Phase II. In Phase II the 5 groups of monkeys used in Phase I were placed on low-fat diets. One monkey in each of groups A through E from Phase I was placed on the control low-fat diet while the other monkey was placed on a low-fat phosphatides diet. Both monkeys in group E were placed on the control low fat diet, but continued to get the biweekly infusions of phosphatides. During the 5 weeks of Phase II, blood was drawn from each monkey's femoral vein after a 15 to 17-h fasting period.

Phosphatidyl choline turnover. During the eleventh week of Phase I a single dose of [^{14}C]phosphatidyl choline was given to the monkeys in groups A through D by stomach intubation and to group E by intravenous injection. Blood was drawn from the femoral vein of each monkey at 6, 24 and 72 h after administration and urine and feces were collected daily. Radioactivity was monitored in the plasma, urine and feces by use of scintillation counting.

Treatment of blood samples

The blood was drawn into a vacutainer containing EDTA and was immediately placed on ice until it could be separated. The blood plasma was separated from the erythrocytes by the method of Turner and Rouser (*21*) and immediately frozen and stored at - 20 C. Upon thawing, the lipids were extracted by the method of Carlson (*6*) and stored in chloroform at - 20 C.

Lipid analysis

The lipid extracts were assayed for free and total cholesterol by the method of Crawford (*9*), for triglycerides by the method of Carlson (*6*) and for lipid phosphorus by the method of Morrison (*16*). Electrophoresis of lipoproteins was carried out using the Canalco Redi-disc (Canalco Company).

Analysis of radioactivity

One-half milliliter of plasma was added to 10 ml of Aquasol (New England Nuclear). One milliliter of each sample of urine collected over a 24-h period was added to 10 ml of Aquasol. The daily output of feces was ground with 30 g of celite, packed in a glass column, and the radioactivity eluted with 550 ml of chloroform. One milliliter of the chloroform eluant was added to 10 ml of Aquasol. The vials were counted for a 10-min period in a Packard Automatic TriCarb Spectrometer (Downers Grove, IL). Counts per minute were corrected to disintegrations per minute with standard quench correction curves using a channels ratio (*8*).

TABLE 1. *Composition of diets.*

Ingredients	Control low-fat (%)	Control high-fat (%)	High-fat + phosphatides (%)	Control + phosphatides (%)
Chow[a]	58.7	58.7	58.7	58.7
Fat[b]	2.0	16.0	16.0	2.0
Cholesterol	0	1.0	1.0	0
Starch	38.0	5.0	0	33.0
Cellulose[d]	0	18.0	20.0	2.0
Minerals[e]	0.8	0.8	0.8	0.8
Vitamins[f]	0.5	0.5	0.5	0.5
Phosphatides[g]	0	0	3.0	3.0

[a]Purina Monkey Chow 25, Ralston Purina, Co., St. Louis, Missouri.
[b]Butter oil.
[c]Corn Starch, Nutritional Biochemical Corporation, Cleveland, Ohio.
[d]Alphacel, Nutritional Biochemical Corporation.
[e]Salt Mixture XIV, Nutritional Biochemical Corporation.
[f]Vitamin Diet Fortification Mixture, Nutritional Biochemical Corporation.
[g]Centrolex, Central Soya, Chicago, Illinois.

TABLE 2. *Experimental design*[a].

	High-fat phase			Low-fat phase		
Group No.	Treatment	Length of experiment (wks)	No. of animals	Treatment	Length of experiment (wks)	No. of animals
A	Control low fat diet	12	2	Control low fat diet Low fat + phosphatides	5	1 1
B	Control high fat diet	12	2	Control low fat diet Low fat + phosphatides	5	1 1
C	High fat + phosphatides diet	12	2	Control low fat diet Low fat + phosphatides diet	5	1 1
D 1	Control high fat diet	5	2	Control low fat diet Low fat + phosphatides diet	5	1 1
2	Followed by high fat + phosphatides diet	7	2			
E 1	Control high fat diet	6	2	Control low fat diet + phosphatides infusions biweekly	5	2
2	Control high fat diet + phosphatides infusions biweekly	6	2			

[a]The experiment consists of a high fat phase followed by a low fat phase in which all diets are isocaloric.

Data evaluation

The data were subjected to a statistical analysis using analysis of variance for split plot designs for groups with unequal replications (*20*).

RESULTS

Phase I.

Cholesterol levels. The plasma cholesterol levels of all monkeys fed a diet containing 16% butter oil and 1% cholesterol increased more than 10 times during Phase I of the experiment at a rate of 100 mg per week (Table 3). The plasma cholesterol level of the monkeys maintained on the control low fat-low cholesterol diet did not significantly change.

Incorporation of soybean phosphatides into the diet did not prevent the increase in plasma total cholesterol levels. However, when compared to the animals on the control high-fat diet for 10 and 11 weeks, the levels of those monkeys maintained on the diet containing phosphatides from the start of the experiment were significantly lower ($P < .05$). Levels of those animals receiving phosphatide injections were significantly lower ($P < 0.05$) than the levels of animals on the control high fat diet at 10, 11 and 12 weeks. When the high-fat diet containing phosphatides was given to the monkeys after they had been on the control high-fat diet for 5 weeks, the cholesterol levels were never significantly lower than the control high-fat diets.

Free cholesterol and cholesterol ester levels. Free cholesterol and cholesterol ester levels of the monkeys maintained on the high-fat diet also increased during Phase I (Table 4). The high-fat diets resulted in wide variations in the free cholesterol levels of the monkey groups. In week five, before the treatment with the phosphatides, the free cholesterol levels of the monkeys in groups receiving a delayed phosphatide diet or phosphatides injections were significantly higher ($P < .05$) than the control high-fat group. After treatment with phosphatides, the free cholesterol levels decreased to the same level as the level of control high-fat diet group. It appeared that these fluctuations in free cholesterol levels

TABLE 3. *Plasma cholesterol levels of monkeys treated with soybean phosphatides.*

Stage of treatment (wk)	Control low-fat diet (mg/100 ml)	Control high-fat diet (mg/100 ml)	High-fat + phosphatides diet[a] (mg/100 ml)	Phosphatide injection[b] (mg/100 ml)	Delayed high-fat + phosphatides diet[c] (mg/100 ml)
0	106.8 ± 24.3	109.9 ± 49.5	90.5 ± 5.2	115.4	108.7 ± 12.9
1	97.0 ± 31.9	76.8	119.4 ± 13.6	122.1 ± 27.4	110.2 ± 21.0
2	99.4 ± 9.8	208.5	260.3 ± 28.1	207.1 ± 1.5	338.9
3	147.3 ± 38.3	275.2 ± 98.4	340.9 ± 89.1	400.1 ± 6.1	355.8 ± 81.5
4	135.5 ± 29.0	341.9	330.2 ± 93.4	398.0 ± 39.6	
5	144.9 ± 26.3	436.2 ± 22.9	531.6 ± 32.5	514.1 ± 44.9	646.2 ± 25.7
6	151.3 ± 7.8	660.6 ± 57.9	630.4 ± 129.5	667.9 ± 156.4	817.9 ± 214.7
7	124.0 ± 10.0	797.6	659.4 ± 127.8	624.5 ± 171.4	895.6 ± 214.6
8	105.6 ± 2.3	797.9	726.2 ± 67.9	630.3 ± 196.8	1040.0
9	175.9 ± 18.8	861.3 ± 87.5	769.4 ± 65.7	647.4 ± 8.2	919.2 ± 228.9
10	151.0 ± 2.2	1135.9 ± 79.8	843.3 ± 170.8#	854.9 ± 242.1#	893.4 ± 141.5
11	149.4 ± 5.0	1359.5 ± 184.7	991.0 ± 151.8#	985.5 ± 271.5#	1241.8 ± 7.0
12	164.0 ± 40.0	1072.2 ± 203.6	990.3 ± 11.7	762.0 ± 337.2#	1155.1 ± 131.9

Note: Values given as the mean ± standard deviation except when only one aminal was tested. Those marked with a # differ significantly ($P < .05$) from the control high-fat diet.

[a]Soybean phosphatides incorporated into diet so that each animal received 3 g of lecithin daily.

[b]Animals received control high-fat diet with 5 ml intravenous injection of 10% phosphatide emulsion.

[c]Animals received control high-fat diet for 5 weeks, then high-fat + phosphatides diet (3 g of phosphatide daily).

TABLE 4. *Plasma free and esterified cholesterol levels of monkeys treated with soybean phosphatide.*

Stage of treatment (wk)	Cholesterol	Control low-fat diet (mg/100 ml)	Control high-fat diet (mg/100 ml)	High-fat + phosphatides diet[a] (mg/100 ml)	Phosphatides injection[b] (mg/100 ml)	Delayed high fat + phosphatides diet[c] (mg/100 ml)
1	Free	29.4 ± 4.3	24.8 ± 11.6	30.9 ± 5.7	31.4 ± 6.3	22.7 ± 0.4
	Esters	67.6 ± 27.6	60.2	88.6 ± 7.8	90.7 ± 21.1	87.5 ± 20.6
3	Free	49.1 ± 1.6	78.4 ± 28.3	99.6 ± 32.8	140.0 ± 98.7	108.4 ± 22.1
	Esters	98.3 ± 36.7	196.8 ± 70.1	241.3 ± 56.3	260.0 ± 92.8	247.4 ± 59.4
5	Free	57.0 ± 9.4	142.0 ± 46.0	216.5 ± 5.0	325.3 ± 110.9#	367.5 ± 107.7#
	Esters	88.0 ± 35.7	294.3 ± 68.9	315.2 ± 27.5	188.8 ± 66.0	278.8 ± 82.0
7	Free	31.5 ± 2.0	240.5 ± 50.1	205.2 ± 36.4	207.8 ± 117.8	317.1 ± 195.1
	Esters	92.6 ± 12.0	557.2 ± 223.0	454.2 ± 91.4	416.7 ± 53.6	578.5 ± 19.5
9	Free	47.2 ± 4.2	229.8 ± 54.0	212.4 ± 0.6	168.1 ± 15.0	415.9 ± 72.8#
	Esters	128.8 ± 14.7	631.5 ± 33.5	557.0 ± 66.3	479.3 ± 23.2	503.3 ± 301.7
11	Free	44.0 ± 7.7	267.4 ± 24.5	312.0 ± 193.2	197.7 ± 130.7	291.2 ± 25.0
	Esters	105.4 ± 2.7	1092.2 ± 209.2	679.0 ± 41.4#	787.8 ± 140.8#	950.6 ± 18.0

Note: Values given as the mean ± standard deviation except when only one animal was tested. Those marked with a # differ significantly ($P < .05$) from the control high fat diet.

[a]Soybean phosphatides incorporated into diet so that each animal received 3 g of phosphatides daily.

[b]Animals received control high-fat diet with 5-ml intravenous injection of 10% phosphatide emulsion biweekly.

[c]Animals received control high-fat diet for 5 weeks then high-fat + phosphatides diet (3 g of phosphatides daily).

were due to the individual animal differences.

Although few significant differences occurred in the cholesterol ester levels of the monkeys treated with phosphatides, the animals treated with phosphatides generally had lower levels than those on the control high-fat diet. The cholesterol ester levels of the group receiving a high-fat diet containing phosphatides or receiving phosphatide injections were significantly lower ($P < .05$) than those of the animals on the high-fat diet at week 11.

Lipid phosphate levels. The plasma lipid phosphate levels of all animals on the high-fat diets increased during Phase I; however, animals on the high-fat plus phosphatide diet from the start or receiving phosphatide injections had significantly lower lipid phosphate levels ($P < .05$) than the control high-fat group in weeks 9 and 12 (Table 5). The lipid phosphate levels of the animals on the delayed phosphatide diet were never significantly ($P < 0.05$) different than the control high-fat groups. Lipid phosphate levels of the animals on the high-fat plus phosphatide diet or receiving injections of phosphatides, were closer to the normal and were not significantly ($P < 0.05$) different than the levels of the control low-fat animals until the 11th week.

Triglyceride levels. The plasma triglyceride levels of the monkeys had a wide range of variation and no significant change resulted from the high-fat diets or the phosphatide treatments (Table 6).

Lipoprotein patterns. The lipoprotein patterns of the blood plasma as determined by electrophoresis are shown in Fig. 1. The *a*-lipoprotein layer disappeared from the plasma of the monkeys on the control high fat diet while the *β*-lipoprotein layer increased. When the animals received infusions of phosphatides or received diets containing phosphatides from the beginning of the high-fat treatment, the *a*-lipoprotein layer was again present. The lipoprotein pattern of plasma from animals receiving a delayed phosphatide diet was nearly identical to that of animals on the control high fat diet.

Phosphatidyl choline

At the end of Phase I when a single oral dose of [^{14}C]phosphatidyl choline was administered to the monkeys, about 17% of the radioactivity was recovered in

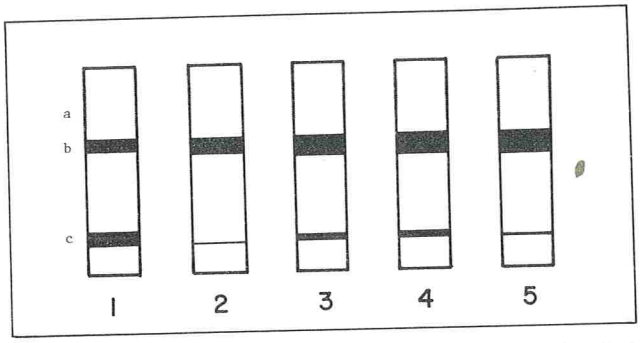

Figure 1. *Gel electrophoresis of monkey plasma at end of phase I. 1, Control low-fat diet; 2, High-fat diet; 3, High-fat + phosphatides diet; 4, Delayed phosphatides diet; 5, Phosphatide injection; a, chylomicron fraction; b, β-lipoprotein fraction; c, a-lipoprotein fraction.*

TABLE 5. *Plasma lipid phosphate levels of monkeys treated with phosphatides.*

Stage of treatment (wk)	Control low-fat diet (mg/100 ml)	Control high-fat diet (mg/100 ml)	High-fat + phosphatides diet[a] (mg/100 ml)	Phosphatides injection[b] (mg/100 ml)	Delayed high-fat + phosphatides diet[c] (mg/100 ml)
1	30.7 ± 6.6	32.2 ± 25.2	30.4 ± 4.4	29.5 ± 14.8	16.4
3	46.3 ± 8.3	37.5 ± 4.3	51.4 ± 24.7	47.4 ± 16.3	47.7 ± 7.6
5	36.1 ± 3.7	53.9 ± 18.5	38.6	42.1 ± 16.9	56.9 ± 9.3
7	36.6 ± 0.5	112.1 ± 67.0*	77.6 ± 2.5	76.2 ± 14.6	97.0 ± 38.8*
9	34.7 ± 3.6	102.1 ± 63.4*	60.5 ± 13.4#	53.3 ± 14.5#	84.2 ± 20.9*
11	38.1 ± 0.4	121.2 ± 11.5*	83.3 ± 15.0*	86.6 ± 35.6*	114.8 ± 5.9*
12	37.7 ± 7.7	155.5*	93.9 ± 5.9#*	98.8 ± 15.2#*	107.6 ± 11.2*

Note: Values given as the mean ± standard deviation except when only one animal was tested. Those marked with a # differ significantly ($P < .05$) from the control high fat diet. Those marked with a * differ significantly ($P > .05$) from the control low fat diet.
[a]Soybean phosphatides incorporated into diet so that each animal received 3 g of phosphatides daily.
[b]Animals received control high-fat diet with 5 ml intravenous injection of 10% phosphatide emulsion biweekly.
[c]Animals received control high-fat diet for 5 weeks, then high fat + phosphatides diet (3 g of phosphatides daily).

TABLE 6. *Plasma triglyceride levels of monkeys treated with soybean phosphatides.*

Stage of treatment (wk)	Control low-fat diet (mg/100 ,ml)	Control high-fat diet (mg/100 ml)	High fat + phosphatides diet[a] (mg/100 ml)	Phosphatides injection[b] (mg/100 ml)	Delayed high-fat + phosphatides diet[c] (mg/100 ml)
0	349.8 ± 71.1	310.2 ± 68.3	309.2 ± 47.8	355.6 ± 218.6	317.2 ± 41.3
2	322.9 ± 20.1	253.6 ± 73.5	278.0 ± 92.5	304.8 ± 1.1	304.0 ± 42.3
4	466.2 ± 125.8	429.4	486.0 ± 128.1	438.9 ± 3.4	375.7 ± 27.9
6	279.0 ± 15.6	315.9 ± 151.6	319.6 ± 54.2	263.1 ± 63.3	320.4
8	303.2 ± 214.1	340.7 ± 141.8	162.7 ± 66.5	210.8 ± 28.3	357.4 ± 61.5
10	177.8 ± 34.0	271.8 ± 10.4	163.0 ± 7.0	197.1 ± 18.2	210.4 ± 97.7
12	198.0 ± 23.2	350.2 ± 65.8	268.5 ± 30.2	208.5 ± 73.2	368.2 ± 55.3

NOTE: Values given as the mean ± standard deviation except when only one animal was tested.
[a]Soybean phosphatides incorporated into diet so that each animal received 3 g of phosphatides daily.
[b]Animals received control high-fat diet with 5 ml intravenous injection of 10% phosphatides emulsion biweekly.
[c]Animals received control high fat diet for 5 weeks then high-fat + phosphatides diet (3 g of phosphatides daily).

the urine and 31% was recovered in the feces (Table 7). Most of the radioactivity excreted was recovered in the first 3 days. The percentage of the single dose recovered in the feces was greatest in the control low-fat diet and delayed phosphatide diet groups. A major portion of the single oral dose of radioactivity was excreted in both the urine and feces within the first 24 h after dosing. The remaining portion of radioactivity was excreted more slowly. The identity of the compounds containing radioactivity and excreted in the urine and feces was not determined and they could have been metabolized products of the phosphatidyl choline and not the phosphatidyl choline molecule itself.

Since the bulk of the excreted radioactivity following dosing with radioactivity phosphatidyl choline appeared on the first or second day and progressively diminished over time, the excretion products may undergo enterohepatic recirculation (*10*). If one assumes that the release of the material was from that circulation, it would follow first order kinetics, that is:

$$dU/dt = -dS/dt = kS \qquad [1]$$

where U is the amount excreted in the urine, S is the amount in the body, and $S_0 = U + S$. This equation may be transformed to

$$dU/dt = S_0 k e^{-kt} \qquad [2]$$

If U_t is the amount excreted in the urine in the time interval (t-1, t), then

$$\log_e U_t = \log_e S_0 (e^k - 1) - kt \qquad [3]$$

A plot of $\log_e U_t$ against t is shown in Fig. 2. U_t is the average value of the daily excretion in urine and t is time. In all instances these curves show that there was a period of rapid excretion of phosphatidyl choline in the urine followed by a slower release. The rate of excretion of the groups treated with phosphatides was similar to the control low-fat diet group while the excretion of the control high-fat group was decidely more rapid. In all instances about 10% of the single oral dose was released from the tissues with a half life of about 2 days. Although the curve of the injected single dose closely resembles the other curves, infused phosphatidyl choline appears to be metabolized differently than intubated phosphatidyl choline and does not appear to follow first order kinetics.

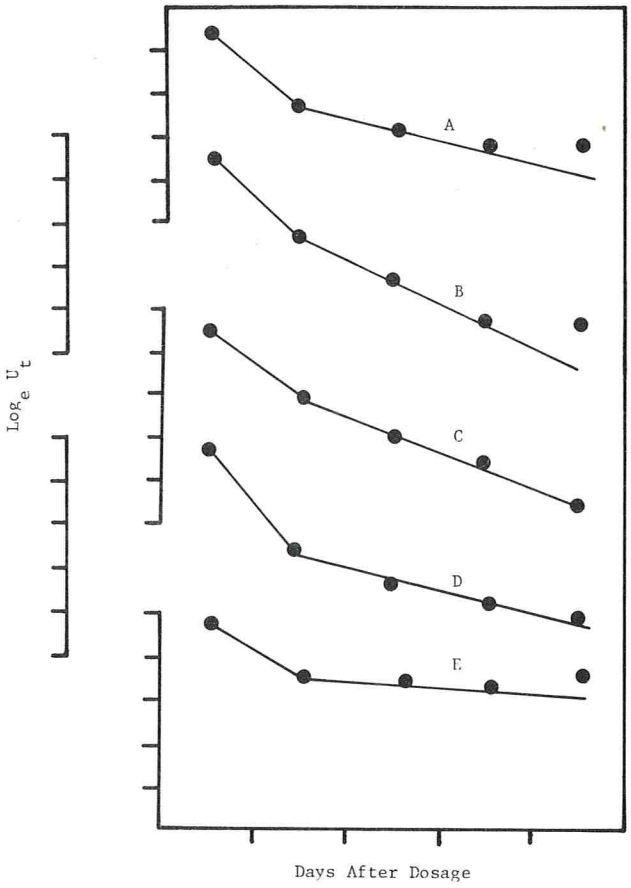

Figure 2. *Excretion rate of [^{14}C]phosphatidyl choline.* A, Low-fat diet; B, High-fat diet; C, High-fat + phosphatides diet; D, Delayed *phosphatide diet; E, Phosphatide injection.*

TABLE 7. *Excretion of ^{14}C in urine and feces of monkeys given a single dose of intubated or injected (^{14}C)phosphatidyl choline.*

Diet	No. of animals	Urine at day 1	2	3	4	5	Total	Feces at day 1	2	3	4-7	Total
		(Percentage of stomach intubated dose excreted daily)[a]										
Control low fat	1	11.1	2.4	1.1	0.4	0.4	15.4	14.2	13.5	1.7	0.3	29.7
Control high fat	2	13.2	2.3	0.8	0.3	0.3	16.9	8.1	9.9	6.2	1.5	25.7
Control high fat[b] + phosphatides	2	13.4	2.6	1.0	0.6	0.5	18.1	5.8	10.5	9.7	2.4	28.4
Delayed high fat[c] + phosphatides	2	15.7	1.5	0.7	0.4	0.3	18.6	13.8	11.2	13.1	1.3	39.4
Average		13.6	2.1	0.9	0.4	0.3	17.4	10.5	11.3	7.7	1.4	30.8
		(Percentage of injected dose excreted daily)[d]										
Control high fat[e]	2	16.6	5.1	4.5	3.6	5.4	35.1	4.2	8.1	12.8	4.6	29.7

[a]One μCi of [^{14}C]phosphatidyl choline in a 10% phosphatides emulsion was administered to each animal.
[b]Soybean phosphatides incorporated into diet so that each animal received 3 g of phosphatides daily.
[c]Animal received control high-fat diet for 5 weeks, then high-fat + phosphatides diet for 7 weeks.
[d]One-half μCi [^{14}C]phosphatidyl choline in a 10% phosphatides emulsion was administered to each animal.
[e]Animals received control high-fat diet with 5 ml intravenous injection of 10% phosphatides emulsion biweekly.

Most of the radioactivity that was given to the monkeys by intubation reached the blood by the end of the first 24-h period; then blood levels of radioactivity slowly decreased for the remainder of the 7 day period (Table 8). The monkeys maintained on the high-fat diets had greater blood levels of radioactivity than did the monkeys maintained on the control low-fat diet. This indicated that animals receiving a high-fat diet had greater absorption of phosphatidyl choline. The level of radioactivity in the blood of animals given an injected dose decreased rapidly during the first 24 h and reached levels only slightly above background after 7 days.

Phase II

Following removal of the animals from the high-fat diets, the levels of plasma total cholesterol, free cholesterol, cholesterol esters, phospholipids and triglycerides returned to control levels in all animals after 5 weeks. No differences in response occurred in the animals due to the initial treatment in Phase I or the various treatments used in Phase II. Lipoprotein patterns also returned to normal after 5 weeks.

DISCUSSION

The pigtail monkeys used in this study were very susceptible to hypercholesterolemia when fed a cholesterol-enriched high-fat diet. With a dietary cholesterol intake of 1 g per day, the levels of plasma cholesterol increased 10 times the initial levels; levels of cholesterol esters increased to nine times the initial levels; levels of free cholesterol increased to four times the initial levels; and levels of lipid phosphate increased to four times the initial levels. Plasma triglyceride levels were not significantly changed. The animals had cloudy plasma, no pre-β-lipoprotein layer, and showed no change in triglyceride levels indicating a Type I or Type III hyperlipoproteinemia (*11*).

Many workers have observed the beneficial effects of lecithin or phosphatide mixtures in preventing alterations of lipid metabolism resulting from a high-fat diet (*1,2,12,15*). The results reported herein confirm that phosphatides do affect the lipid metabolism of monkeys given a high fat-high cholesterol diet by partially preventing alterations in lipid levels and lipoprotein patterns. The incorporation of 3% phosphatides into the high-fat diet from the start of the study or infusions of phosphatides after the animals had reached cholesterol levels of 500 to 600 mg%, resulted in significantly lower plasma total and esterified cholesterol, lower lipid phosphate levels and more normal lipoprotein patterns. Incorporation of phosphatides into the diet after the animals had reached high lipid levels did not prevent further changes in the lipid levels or the lipoprotein patterns.

The effect of phosphatides in maintaining lipid levels and lipoprotein patterns closer to those of the control low-fat animals could be attributed to the lipotropic action of the phosphatides. Because of the hydrophobic paraffin chains which are miscible with hydrophobic lipids and the hydrophilic choline, phosphoryl and other groups which are miscible with water, phosphatides undoubtedly could complex with the lipids causing a dispersion of particles which are easily transported in the blood. Other workers have reported on the ability of lecithin to prevent fatty livers and have attributed the cholesterol-lowering effect of phosphatides to their lipotropic action (*1,2,18*).

The high quantities of polyunsaturated fatty acids present in the phosphatides could also account for decreased blood cholesterol levels. Harper (*11*) and Adams et al. (*1*) have indicated that cholesterol esters of polyunsaturated fatty acids are more rapidly metabolized by the liver and other tissues resulting in increased cholesterol turnover and excretion rates. Welle et al. (*22*) hypothesized that the process of cholesterol esterification produces cholesterol-linoleate which could have the effect of increasing the excretion of cholesterol via the feces. The retention of high-density lipoproteins in animals receiving phosphatides could be related to this cholesterol esterification. The high-density lipoproteins are the major sources of the phosphatidyl choline used as a substrate by lecithin: cholesterol acyltransferase (LCAT) (*14*). It is possible that the phosphatides were incorporated into the high-density lipoproteins and therefore maintained this fraction and also provided a source of unsaturated fatty acids for esterification of cholesterol. These polyunsaturated fatty acid esters of cholesterol would then be readily broken down resulting

TABLE 8. *Radioactive counts in one milliliter of blood plasma of monkeys given a single dose of [^{14}C]phosphatidyl choline.*

Diet	No. of animals	6 h	24 h	72 h	7 days
		(CPM recovered from dose given by stomach intubation)[a]			
Control low fat	1	54.5	69.1	36.9	9.7
Control high fat	2	102.8	132.4	70.0	40.5
Control high fat + phosphatides	2	109.8	114.5	62.2	31.8
Delayed phosphatides[c]	2	100.0	84.4	40.6	23.6
		(CPM recovered from dose given by intravenous injection)[d]			
Control high fat + phosphatide injection[e]	2	22.4	7.1	4.4	3.2

[a]One μCi of [^{14}C]phosphatidyl choline in a 30% phosphatides emulsion administered to each animal.
[b]Phosphatides incorporated into diet so that each animal received 3 g of phosphatides daily.
[c]Animals received control high-fat diet for 5 weeks, then high-fat + phosphatides diet (3 g of phosphatides daily).
[d]One-half μCi of [^{14}C]phosphatidyl choline in a 10% phosphatide emulsion was administered to each animal.
[e]Animals received control high fat diet with 5-ml intravenous injection of 10% phosphatides emulsion biweekly.

in a lowering of cholesterol ester levels. The delayed phosphatide diet may not have been effective in lowering cholesterol since the mechanism for formation of high-density lipoproteins in the liver had been altered by the high-fat diet and thus phosphatides were not incorporated into these lipoproteins. Intravenous injection of phosphatides, however, would still allow phosphatides to be available in the serum for use by LCAT for formation of cholesterol esters. The above hypothesis could account for the results of Welle et al. (*22*) who observed that lecithin had no effect on lipoproteins of Type II hyperlipoproteinemia. Since in Type II hyperlipoproteinemia the serum is not deficient in high-density lipoproteins, one would not expect, based on our results, any effect on this lipoprotein pattern by phosphatides. Further experiments are needed, however, to verify these observations. This does indicate the necessity of determining lipoprotein patterns in all experiments designed to determine the influence of diet on blood lipid levels.

The rapid return of blood lipid levels to control levels when placed on a low-fat diet indicates that the high cholesterol diet accounted for the high blood cholesterol levels observed in this study. Thus, these superficial levels of cholesterol may provide different information than what is found otherwise. It would appear, however, that soybean phosphatides may be important in controlling alterations in blood lipid levels which result from intake of a high fat-high cholesterol diet.

Ansell and Hawthorne (*4*) have reported that dietary lecithin is absorbed whole or in part via the lymph system and then enters the circulatory system via the thoracic duct. Studies on the turnover of phosphatidyl choline indicate that high levels of phosphatidyl choline or its breakdown products remain in the blood for a considerable time following ingestion (Table 8). This would allow the phosphatidyl choline sufficient time for incorporation into the lipoproteins. The delayed excretion of phosphatidyl choline observed in animals given a phosphatide diet or phosphatide infusion could have resulted from incorporation of phosphatidyl choline or its component parts into the lipoproteins. Future studies should determine if the absorbed phosphatidyl choline is associated with the lipoproteins.

In summary, this study has indicated that the administration of soybean phosphatides to primates may prevent alterations in blood lipid levels resulting from feeding a high fat-high cholesterol diet. However, to be effective, it appears that the phosphatides must either be incorporated into the diet before the animal becomes hypercholesterolemic or be directly injected into the blood stream. These effects of the phosphatides are apparently related to the transport of lipids in the body, but further studies are needed to verify these effects.

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Evaluation of the Minitek and API 20E Systems for Identification of *Yersinia enterocolitica*

L. RESTAINO,* G. S. GRAUMAN, W. A. McCALL and W. M. HILL

Armour Food Research Center
15101 North Scottsdale Road
Scottsdale, Arizona 85260

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ABSTRACT

The basic 12-disk Minitek system (14 tests) and the API 20E system were evaluated for identification of *Yersinia enterocolitica* strains isolated at various locations in the world. The API 20E system correctly identified all the *Y. enterocolitica* strains while the Minitek system identified 80% (20/25) of the strains. Four of the five cultures misidentified by Minitek were atypical strains isolated from vacuum-packaged meats. These four cultures were identified as *Citrobacter freundii* by Minitek due to a rhamnose-positive reaction. Minitek and API 20E are rapid and convenient systems which could prove to be beneficial for identifying *Y. enterocolitica* strains.

The awareness of *Yersinia enterocolitica* as a human pathogen has increased in the last 5 to 10 years. Extensive research has been done in several European countries (*25*), Japan (*1,31,32*), and Canada (*30*), but the seriousness of *Y. enterocolitica* infection in the United States has been ascertained only recently. *Y. enterocolitica* has been isolated from human as well as nonhuman sources. From 1968-1975, Bissett (*2*) obtained 24 human isolates of *Y. enterocolitica* in which the major clinical symptoms of the patients consisted of abdominal pain, diarrhea and fever. *Y. enterocolitica* has been isolated from water in the United States (*15*) and Europe (*19,21*). Serotype 0:17, the same serotype isolated from non-mesenteric clinical samples (*4*), has been isolated in a survey of vacuum packaged fresh beef (*13*).

Y. enterocolitica has been isolated from various other foods such as lamb, ice cream, and raw milk (*13,24,26*) and animals including the swine, canine, rodent and cow (*17,18,29*). Because similar serotypes of *Y. enterocolitica* have been isolated from humans, animals and foods, a mode of transmission of *Y. enterocolitica* to humans from contaminated foods or infected animals could be possible (*3,22*). In 1976, the first reported outbreak of foodborne illness in the United States implicating *Y. enterocolitica* occurred among school children in Oneida County, New York (*23*). Chocolate milk was determined to be the common vector of transmission in this outbreak. Serotype 0:8 was isolated from children and chocolate milk.

Rapid and reliable identification of *Y. enterocolitica* is of importance to the clinical, food or environmental microbiologist. The API 20E and Minitek systems have proven to be extremely valuable for rapidly identifying members of the *Enterobacteriaceae* family isolated from clinical samples (*14,16,20,28*). Recently, the applicability and accuracy of both miniaturized systems have been evaluated for identifying members of the *Enterobacteriaceae* isolated from foods. By using comminuted beef, pork or turkey, Guthertz and Okoluk (*12*) showed that the API 20E and Minitek systems correctly identified 96.1 and 78.3%, respectively, of the enteric strains. Further, Cox and Mercuri (*6*) demonstrated that the API 20E correctly classified 82% of the *Enterobacteriaceae* strains isolated from various foods and food products. However, for *Y. enterocolitica*, a member of the *Enterobacteriaceae*, there are insufficient data to determine the feasability of using Minitek or API 20E systems for its identification from clinical or food samples. The present study was done to determine the accuracy of the basic 12-disk Minitek and API 20E systems for identifying typical and atypical strains of *Y. enterocolitica*.

MATERIALS AND METHODS

Bacterial strains

The antigen type, origin and contributor of the 25 *Y. enterocolitica* strains used in this study are listed in Table 1. Stock cultures were maintained on Difco brain heart infusion (BHI) slants and stored at 4 C.

Conventional methods

Each strain was inoculated into 10 ml of BHI broth and incubated for 24 h at 25 and 37 C. After incubation, the broths were centrifuged and the pellet was resuspended in 10 ml of sterile distilled water. The washed cells were refrigerated at 4 C for no longer than 1 h until use. All the strains were inoculated into the following media: 1% tryptone broth, motility-ornithine decarboxylase medium (*8*), Christensen urea slants, MR-VP broth, Simmons citrate, triple sugar iron agar slants, gelatin, malonate broth, lysine decarboxylase and arginine dihydrolase semisolid media, and phenylalanine agar. Kovac reagent was used to detect the presence of indole in the tryptone tubes. Beta-galactosidase was detected from triple sugar iron slants (*11*). One percent

TABLE 1. Yersinia enterocolitica *strains tested.*

Antigen	Origin	Contributor
Unknown	Human (face)	ATCC 9610
Unknown	Human (blood)	ATCC 23715
0:8	Human (blood)	ATCC 27729
0:8	Stream water	ATCC 27739
0:8	Human, United States	E. J. Bottone
0:17	Human, United States	
0:17	Human, United States	
0:3[a]	Human, Canada	S. Toma
0:8	Human, Canada	
0:9	Unknown, Canada	
0:6,30	Human, Canada	
0:4,32	Human, Canada	
0:5,27	Human, Canada	
0:16	Stream water, U.S.	S. Harvey
0:3	Swine, Japan	M. Tsubokura
0:3	Swine, Japan	
0:5	Swine, Japan	
0:5	Swine, Japan	
0:17	Vacuum-packed meats United States	C. Vanderzant
0:17	Vacuum-packed meats United States	
Nontyped	Vacuum-packed meats United States	
0:20	Vacuum-packed meats United States	
Nontyped	Vacuum-packaged meats United States	
0:3[b]	Human, Europe	H. H. Mollaret
0:9[c]	Human, Europe	

[a]Phage type 9b. Different from European 0:3 serotype.
[b]Nilehn's biotype 4.
[c]Nilehn's biotype 2.

concentration of the following carbohydrates was sterilized in Difco phenol red broth base: glucose, mannitol, inositol, sorbitol, rhamnose, raffinose, salicin, meliboise, lactose, amygdalin, sucrose, trehalose, adonitol and dulcitol. Ten percent solutions of xylose and arabinose were filter sterilized and 0.5-ml volumes were pipetted into 4.5 ml of sterile broth base. The inoculated media were incubated at 25 and 37 C, and reactions were read after 2, 4 and 7 days. Differential charts and schemes presented in Ewing (*9*), Darland et al. (*7*), Bottone (*3*), and Brenner et al. (*5*), were used for identification of all strains.

Bacterial preparation for miniaturized systems

Each strain was inoculated into BHI and incubated at 25 C for 24 h. After incubation, a loopful of cells was aseptically streaked onto Difco Plate Count Agar and incubated at 25 C for 24 h.

API 20E system

The tests in the API 20E system were done according to manufacturer's instructions with some slight variations. Three to six colonies from Plate Count Agar were transferred into 10 ml of sterile distilled water and vortexed until the cells were fully dispersed. Using a sterile Pasteur pipette, the suspension was inoculated onto an API 20E test strip containing the 20 standard biochemical tests. The test strip was incubated at 37 C for 18-24 h. After incubation, appropriate reagents were added and the reactions recorded. All strains were identified to the species level by using the API Profile Recognition System.

Minitek system

In this study, the 12 disks chosen were the basic set recommended by the manufacturer for the differentiation of *Enterobacteriaceae*. These disks included dextrose/nitrate, hydrogen sulfide/indole, ornithine, urea, lysine, arabinose, rhamnose, inositol, phenylalanine, citrate, *O*-nitrophenyl-β-D-galactopyranoside (ONPG), and malonate. These substrates in the Minitek system were used following manufacturer's suggestions with a slight modification. Two Minitek inoculum broths (1.0 ml per vial) were aseptically transferred into a third vial. From each plate count medium, three to six colonies were dispersed into the inoculum broth (3.0 ml) and vortexed. With the aid of a Miniek pipette gun, 0.05-ml volumes were dispensed into each well. The plates were incubated at 37 C for 18-24 h. The addition of reagents and interpretation of the results for the 14 tests were followed according to manufacturer's recommendations. The BBL Minicoder was used for identification of each strain.

RESULTS AND DISCUSSION

Twenty-five typical and atypical strains of *Y. enterocolitica* isolated from human and nonhuman sources were obtained from various geographical locations throughout the world (Table 1). Nine atypical strains were used in this study which utilized rhamnose, raffinose, melibiose or citrate. Typical strains included the most predominant serotypes isolated in the United States [0:8 (*3*)], Europe [0:3 and 0:9 (*25*)], Canada [0:3, phage type 9b (*30*)], and Japan [0:3 (32)]. Before this study, biochemical results from conventional methods identified all the strains as *Y. enterocolitica* (data not presented).

By using the Minicoder and following the manufacturer's recommended identification procedure, the results from the 12 Minitek disks correctly identified 80% (20/25) of the strains. As indicated in Table 2, four of the five strains misidentified by Minitek were atypical strains isolated from vacuum-packaged meats (*13*). In contrast to the atypical strains isolated by Bottone et al. (*4*) and Harvey et al. (*15*) which produce acid from rhamnose at 25 C, the vacuum-packaged meat isolates utilize rhamnose at 25 and 37 C (*13*). Therefore, the Minitek sysem identified these four strains as *Citrobacter freundii* due to their rhamnose- and inositol-positive reactions at 37 C. By using the 12 recommended Minitek disks for the identification of *Enterbacteriaceae*, Chester and Evans (Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, C168, p. 305) correctly identified all 33 *Y. enterocolitica* strains representing four DNA relatedness groups based on reactions for rhamnose, raffinose, medibiose, sucrose and citrate. However, the Minicoder system was not flexible enough to account for the atypical rhamnose-utilizing isolates from the vacuum-packaged meats, and consequently incorrectly identified them in this study.

TABLE 2. Y. enterocolitica *strains not identified by Minitek*[a].

Antigen type[c]	Origin	Incorrect identification	Incorrect reactions on Minitek[b]
0:5	Swine, Japan	*Escherichia coli*	Urease -
0:17	Vacuum packaged meats, U.S.	*Citrobacter freundii*	Rham.+, ino.+
0:17	Vacuum packaged meats, U.S.	*Citrobacter freundii*	Rham.+, ino.+
0:20	Vacuum packaged meats, U.S.	*Citrobacter freundii*	Rham.+, ino.+
Nontyped	Vacuum packaged meats, U.S.	*Citrobacter freundii*	Rham.+, ino.+

[a]All strains were correctly identified by the API 20E system.
[b]Abbreviations: rham: rhamnose; ino.: inositol.
[c]The strain isolated from swine in Japan was a typical *Y. enterocolitica* strain whereas the isolates from vacuum-packaged meats represented atypical strains.

The other strain not identified as *Y. enterocolitica* by Minitek was the 0:5 serotype isolated from swine in Japan. The Minitek system identified this typical strain as *Escherichia coli* (Table 2). This disagreement was caused by a false-negative urease reaction which has been shown to occur with other *Enterobacteriaceae* members for Minitek (*27*).

The API 20E correctly identified all the *Y. enterocolitica* strains (Table 2). The atypical isolates from vacuum-packaged meats failed to use rhamnose on the API 20E strips at 37 C (unpublished data). Consequently these atypical strains displayed a biochemical reaction scheme resembling a typical *Y. enterocolitica* incubated at 37 C. Therefore, the API Profile Recognition System correctly identified all the atypical and typical strains of *Y. enterocolitica*.

Y. enterocolitica is a human pathogen that can grow anaerobically at refrigerated temperatures (4 C) in foods (*22,24*). An evaluation of *Y. enterocolitica* as a microorganism displaying public health significance in foods cannot be ascertained until more information is accumulated pertaining to its incidence in foods. Recently a rapid increase of atypical isolates has occurred in clinical (*4*), environmental (*15,19,21*) and food samples (*13,26*). Both typical and atypical strains have been recovered from stool cultures of patients who had acute abdominal disease (*3*). Therefore, a rapid and accurate method for identifying the increasing atypical as well as typical strains could prove valuable to the clinical and food microbiologist. The Minitek and API 20E are designed to be convenient and rapid diagnostic systems. The ability of either of these systems to accurately identify *Y. enterocolitica* strains could be very advantageous. In this study, the 12 basic Minitek disks representing 14 tests identified 93.8% of the typical *Y. enterocolitica* strains. When identifying the nine atypical strains, the Minitek system correctly differentiated only 55% of these strains. However, by supplementing the 12 basic Minitek disks with two conventional tubed tests consisting of Voges-Proskauer and motility incubated at 25 and 37 C, possibly these atypical isolates which were misidentified as *C. freundii* could be correctly identified as *Y. enterocolitica*. The API 20E system accurately identified 100% of the typical and atypical strains of *Y. enterocolitica* used in this study. The data presented in this paper indicate that Minitek and API 20E systems could prove beneficial for identifying *Y. enterocolitica* strains.

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Journal of Food Protection

FOODSERVICE COMMITTEE

The Foodservice Committee was formed in December 1977, at the request of the *Journal* Editor and charged with the following responsibilities:

1. To identify timely foodservice topics that should be discussed in the *Journal of Food Protection*.
2. To identify authors with the needed expertise to prepare papers on the various topics.
3. To assist with the solicitation of manuscripts and to encourage people doing research in foodservice to submit appropriate research papers to the *Journal*.

Membership on the committee consists of: C. Dee Clingman, Chairman, Director, Food Protection Programs, National Institute for the Foodservice Industry; K. J. Baker, R. S., Senior Food Consultant, Food and Drug Administration; Ruth S. Dickie, R.D., Department of Continuing Medical Education, University of Wisconsin; Dorothy Ellis, R.P.Dt., Food Technology Division, George Brown College; Dave Hartley, Director, Public Health, National Automatic Merchandising Association; Earl Helmreich, Food Protection Unit, Ohio Department of Health; Fred Mitchell, Chief, Hotels, Resorts & Restaurants, Minnesota Department of Health; Roy Moser, Extension Food Technologist, University of Hawaii; Robert Pickenpack, Director, Product Safety, General Mills, Inc.; Barry Preswick, Quality Assurance Supervisor, McDonald's Corporation; Thomas Schafer, Director, Quality Control & Sanitation, Pizza Hut, Inc.; Oscar Snyder, Associate Prof., Department of Food Science and Nutrition, University of Minnesota; Gail Terreri, Microbioloist, Arthur Treacher's Fish and Chips; Nan Unklesbay, Assistant Prof., Department of Food Science and Nutrition, University of Missouri; James C. White, Professor, Hotel Administration, Cornell University.

The first official activity of the committee was the completion of an Idea Explosion Form by each committee member. This activity was accomplished through written correspondence. Over one hundred ideas and comments were received by the Chairman as the result of this inquiry.

On August 14, 1978, the committee held its first meeting in Kansas City, Missouri in conjunction with the IAMFES annual meeting. The minutes of the meeting are contained below. These minutes contain specific committee recommendations and are the initial activities of the committee.

It is anticipated that future committee activities will be completed largely through written correspondence. However, a committee meeting is planned for early 1979, and again in August 1979, in Orlando.

COMMITTEE MINUTES

The committee was called to order at 2:00 PM on Monday, August 14, 1978, in Kansas City. Those in attendance were K. J. Baker, Dee Clingman, Pat Franks, Robert Pickenpack, Gail Terreri, and Nan Unklesbay.

1. It was requested that the Chairman obtain a breakdown, if possible, of IAMFES membership as far as the major disciplines are concerned (regulatory, industry, education, student, etc.). The Chairman indicated that he would attempt to obtain this information through the IAMFES Executive Secretary.
2. It was generally felt that an index or catalog of foodservice articles which appeared in the *Journal* be published. Such an index would aid in the location of articles and serve as a reference to the committee on future article solicitation. Mr. K. J. Baker volunteered to research past issues of the *Journal* from five years ago to present and compile an indexed, cross referenced catalog of foodservice articles by general topic. A tentative completion for this project is November 1, 1978.
3. It was proposed that the committee be considered as a publication review committee for all articles submitted to the *Journal* on foodservice.
4. It was agreed that all committee members should request relevant articles from speeches which are given at various organizational functions and relate to foodservice food protection.
5. It was recommended that the area of continuing education be reviewed as its application within the general structure of the *Journal*. Items such as quizzes, essay type examinations, or additional technical information should be examined.
6. It was recommended that a student award and an individual membership award be established and presented each year for the best overall article on foodservice food protection. Annual winners would be determined by the committee. The awards would be presented at the IAMFES Annual Meeting during the awards banquet or in the foodservice section. Further discussion on this matter will be taken up with the *Journal* Editor and the IAMFES Executive Board.
7. The following areas were discussed with reference to future *Journal* articles on foodservice food protection:
   A. Within one general topic area there could be a number of articles written in this topic area by different authors at different technical levels.
   B. There appears to be a need to encourage more management and marketing level people, who control purse strings within corporate structures, to share their concerns, fears, and doubts regarding foodservice food protection.
   C. The areas of product liability and the legal aspects of consumer food protection should be addressed from a legal standpoint.
   D. Summaries of the Food and Drug Administration Compliance Reports should be submitted to the *Journal* for publication.
   E. Articles on the quality assurance hierarchy within various companies as well as the philosophy of these organizations should be described for the readership.
   F. Articles should be solicited and encouraged based on the Center for Disease Control's listing of the most common factors involved in foodborne illness.
   G. It was generally felt that the incorporation of more photographs would enhance the visual appearance of articles.
8. The following recommendations apply to the *Journal* design and management:
   A. There was some concern regarding the design of the Table of Contents for the *Journal*. Some committee members felt that it was difficult to scan the *Journal* rapidly for relevant articles. It was suggested that the Table of Contents be organized more effectively so that the reader can rapidly find their area of interest. An idea was to insert adjacent

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Journal of Food Protection Vol. 42, No. 2, Pages 124-125 (February, 1979)

A Research Note

Temperatures of Water in Household Refrigerators[1]

R. B. MAXCY

Department of Food Science and Technology University of Nebraska, Lincoln, Nebraska 68583

(Received for publication May 8, 1978)

ABSTRACT

Temperatures of water exemplifying liquid foods in household refrigerators were determined. The mean of a.m. and p.m. observations with 11 different refrigerators was 3.6 C. Only two refrigerators showed a significant difference between a.m. and p.m. temperatures. Both the variance and range were surprisingly low in view of the limited previous published data. These results indicated household refrigerators have received too much condemnation for inadequate protection of quality of foods.

Refrigeration is important to protect the organoleptic quality of food and to protect it from public health hazards. Highly perishable foods require refrigeration from the time of production to the time of consumption. The household refrigerator is an important item in this sequence of protection as considerable storage time is involved.

Few data are available to establish actual environmental conditions within household refrigerators. van Walbeek et al. (*4*) indicated without giving data that they found the temperature in household refrigerators to range from -0.5 to 10 C. No details were given on the experimental observations such as thermometer location in the refrigerator, opening and closing regimen, air or product temperature, etc. Torrey and Marth (*3*) made extensive observations on two household refrigerators with emphasis on location of the sensing device and time relative to opening and closing the refrigerator. Their results showed extreme variations of air temperature from 1.7 to 20.2 C when comparing pre-opening temperatures to the maximum obtained during an opening and use situation. Recovery time to the pre-opening temperature was less than 30 min. These observations were made for information on surface temperature and mold growth. Liquid or solid food with mass of 100 g or more might logically show less variation in temperature.

Observations on the common temperatures to be expected in liquid foods such as milk in household refrigerators should be useful in directing the emphasis of quality protection of foods.

METHODS

Graduate students, the author, and technicians skilled in collection of scientific data made observations on their home refrigerator. They were furnished a test thermometer checked with a Bureau of Standards thermometer. Instructions were for the test thermometers to be placed in 100-200 ml of water and stored in the refrigerator for single daily a.m. and p.m. observations. The former was to be before normal major activity associated with the use of the refrigerator, and the latter was to include the warming effects of normal opening and closing activity. Observations with each refrigerator were over a period of 7-8 days. The whole series of observations extended over a number of months. Ambient room temperature for the various refrigerators was in the approximate range of 20 to 35 C.

RESULTS AND DISCUSSION

Results of observations with 11 refrigerators are shown in Table 1. The mean temperature for all observations was 3.6 C, which was considerably lower than would have

[1]*Published as Paper No. 5540, Journal Series, Nebraska Agricultural Experiment Station.*

TABLE 1. *Observations of temperature in °C of water in household refrigerators.*

	Time of day					
	a.m.			p.m.		
Refrigerator	Mean	Standard Deviation	Range	Mean	Standard Deviation	Range
1	4.9	.78	2.0	4.9	.73	2.5
2	1.6	.35	1.0	2.8	2.14	6.5
3	3.6	.45	1.0	3.4	.53	1.0
4	4.9	.75	2.0	4.9	.79	2.0
5	4.1	.66	2.0	4.9	.38	1.3
6	3.3	.27	.5	3.4	.56	1.5
7	2.1	.64	1.5	3.0	.89	2.5
8	4.5	0	0	4.5	0	0
9	4.4	.82	1.5	4.9	1.1	2.5
10	3.5	.87	2.0	3.9	1.1	3.5
11	1.0	.55	1.0	1.5	.46	1.0
Average	3.5		1.3	3.8		2.2

been expected in light of the reports of van Walbeek et al. (*4*) and Torrey and Marth (*3*). The relatively stable temperature was probably attributable to the mass of water in which the thermometer was immersed. The mean p.m. temperature was only 0.3 C higher than the mean a.m. temperature though the differences were highly significant ($P < .01$) as determined by an analysis of variance. Likewise, the differences between refrigerators was highly significant ($P < .01$). On an individual basis only two refrigerators, Numbers 5 and 7, showed a significantly greater mean p.m. than mean a.m. temperature. The range of temperatures for the individual refrigerators was surprisingly low, especially when the data of Table 1 were considered without a single p.m. reading for refrigerator Number 2.

The above results indicate that household refrigerators are not as significant a factor in food quality deterioration as some other factors in the distribution, marketing, and storage chain. For example, Hankin et al. (*1*) in 1975 showed that 53% of milk samples in retail market dairy cases were 4.4 to 7.2 C and 5% were over 7.2 C. Some samples were exposed to these conditions for a number of days. Such temperature abuse has been shown to be extremely deleterious to quality and hastens subsequent microbial growth during secondary abuse stages such as room temperature storage (*2*). Thus, factors other than home refrigerator storage likely are more important than commonly considered.

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Committee Report, con't from p. 123

to the title in the Table of Contents a specific symbol to denote that article's area. For example, two forks crossed could relate to foodservice, a milk can to milk, a test tube to research, etc.

B. When an issue contains a number of articles in any one area, the front cover of the *Journal* should highlight that theme or area of interest.

C. Areas of research or future articles dealing with foodservice food protection could be listed in the *Journal* to entice individuals to look for these topics in future issues.

It was generally felt that a future meeting of the committee would be beneficial if a mutual time and date could be arranged. No date was set.

The meeting adjourned at 4:15 PM.

Respectfully submitted,

C. Dee Clingman
Chairman

Floyd Bodyfelt Biography, con't from p. 186

Membership in professional organizations and trade associations include IAMFES, Inc; secretary-treasurer of the Oregon affiliate of IAMFES; Oregon representative to the IAMFES Affiliate Council; American Dairy Science Ass'n.; Institute of Food Technologists; Oregon Section of IFT; campus coordinator of the Oregon Dairy Industries Ass'n.

Floyd serves as a consultant to Farrell's Ice Cream Parlour-Restaurants and is currently the university chairman of the production committee for the National Ice Cream Retailers Ass'n.

In 1976-1977, Floyd exchanged professional duties and homes with David K. Bandler, Cornell University, for a 12 month period.

He and his wife have four children, Marcy, 17, Julie, 15, Clark, 13, and Kyle, 11.

Bob Marshall Biography, con't from p. 186

ness Section and was Association Program Chairman. He is advisor to the National F.F.A. on their Milk Quality and Dairy Foods Contest. He is State Superintendent of that contest.

He is a member of Sigma Xi, Gamma Sigma Delta, Omicron Delta Kappa, Alpha Zeta, Phi Eta Sigma, IAMFES, ADSA, IFT, and the National Mastitis Council. He received the Faculty-Alumni Award from the University of Missouri Alumni Association, the Junior Faculty Award from Gamma Sigma Delta, and was made Honorary State Farmer by the Missouri F.F.A. and Honorary American Farmer by the National F.F.A. In 1975 he received the Milk Industry Foundation Teaching Award and in 1976 the Dairy Research Inc. Research Award through the American Dairy Science Association.

Journal of Food Protection Vol. 42, No. 2, Pages 126-130 (February, 1979)

Quality of Restructured Beef Steaks After Refrigerated and Frozen Storage[1]

H. W. OCKERMAN* and C. S. ORGANISCIAK[2]

Animal Science Department,
The Ohio State University, Columbus, Ohio 43210 and
The Ohio Agricultural Research and Development Center,
Wooster, Ohio 44691

(Received for publication May 11, 1978)

ABSTRACT

This experiment evaluated a procedure for converting forequarter beef tissue into a fabricated steak-like product and to test its refrigerated and frozen storage characteristics. The restructured steaks were manufactured by tumbling thin slices of beef arm chuck roast with salt (2%) and added water (3%) and then passing this product through a mechanical patty machine. Refrigerated (4 ± 2 C) samples were evaluated at 0, 3, 5 and 10 days of storage. Frozen (- 23 ± 3 C) samples were evaluated after 0, 1, 2, and 3 months of storage. An acceptable steak-like product was manufactured; however its raw visual appearance and raw odor deteriorated quickly and its cooked palatability characteristics deteriorated with both types of storage, probably due to oxidation.

The concept of restructuring meat is not new and has its origin in loaf and casing-type products that were cooked to bind the tissues. Meat patties are also an attempt to form larger pieces of meat from ground product in the uncooked state. These types of products, however, have a texture that is characteristic of comminuted meat. Recently, many technological advances and processing combinations have been used in attempts to make the restructured product more closely resemble a solid piece of meat from the textural standpoint (*3-6,11,13,14,20,22*).

Mandigo (*13*) identified some of the advantages of restructuring meat which included better utilization of lower value meat resources and increased processor control over physical and chemical composition of the product. The flake cutting method for preparing meat for restructuring increases cohesiveness and bind (*5*) when compared to a ground product. Flaked-cut patties can be used in the place of ground patties when a coarser grind and a more steak-like texture are desired. Flaked-cut patties display greater cohesiveness and bind and have a less greasy organoleptic quality than ground patties (*21*). Flaked products have also been shown to possess greater water holding capacity (*12*) than ground patties.

Tumbling or massaging is a rather recent technique (*10,17,18,19,23,25,27-29*) for mechanically agitating muscle tissue and extracting a protein exudate which will improve binding characteristics. This method can be used to bond together pieces of meat. Adding salt in the amount of at least 0.6% helps extract salt-soluble proteins to the meat surface (*31*). As salt level and massaging times increase, the amount of protein in the exudate increases (*26,30*). Salt at 2% appears to be optimal (*27*).

The objective of this research was to utilize the tumbling technique as part of a process for fabrication of restructured meat and to evaluate this product during both refrigerated and frozen storage.

MATERIALS AND METHODS

Preliminary research evaluated such factors as beef tissue types (round, chuck, shank tissue from Standard through Choice grades), chilling temperatures (-3, 0, 3, 6, and 9 C), grinding (4.7-, 9.5- and 22.2-mm plates) and slicing (1, 3, 6 and 9 mm) to alter meat particle sizes, salt (0, 1, 2 and 3%) and moisture levels (0, 1, 3, and 5% addition), tumbling duration (10 min/h for 1, 3, 6, 9 and 12 h), and patty-forming techniques (hand and machine patties of 75-, 110- and 140-g sizes). The most successful (fresh appearance and organoleptic characteristic) combinations were used in this research project.

This experiment used muscle tissue from U.S. Good grade beef arm chuck roasts which weighed approximately 4.5 kg. This tissue was cooled to - 3 ± 1 C and sliced on a Hobart Slicer to a thickness of 3 ± 0.5 mm. As nearly as possible, slicing was accomplished perpendicular to the muscle fiber, yielding approximately 75% perpendicular slices and the remaining 25% varying from this position to parallel to the muscle fiber.

To the raw meat, 2% salt and 3% water (based on meat block) were added and all ingredients were mixed together and placed in a tumbler constructed at Ohio State University. This equipment was manufactured from a stainless steel drum (56-cm diameter and 85-cm depth) containing three baffles. The tissue was tumbled in a 2 ± 2-C cooler, for 10 min out of each hour, for 6 h, at 12 revolutions per min.

The tumbled beef tissue was formed into 24 oval-shaped patties (140 ± 5-g weight, 14.7-cm length, 10.2-cm width, and 1.2-cm thick) by

[1]*Approved for Publication as Journal Article 8-78 of The Ohio Agricultural Research and Development Center, Wooster, Ohio 44691.*
[2]*Ministry of Agriculture, Vet. Dept., 30 Wspolna, Warsaw, Poland.*

a Hollymatic Patty Machine, Model #500. Salt and proximate analyses were done by modified (*16*) AOAC methods (*1*) on each production lot.

Patties were packaged three per Barrier Bag (Cry-O-Vac Type B-620 Barrier Bag -- water vapor transmission, 0.5 - 0.6 g per 645 cm^2 in 24 h at 38 C and 100% relative humidity; oxygen transmission, 30-55 cc per m^2 in 24 h at 23 C and one atmosphere) and the air was evacuated by a Cry-O-Vac laboratory vacuum pump (Model Number CV-U) to 450 ± 25 mm of mercury. The bags were closed using a Poly-Clip Applicator Unit (Type SCH2210) and were visually checked after storage.

From each of the six production lots, a sample of three patties (140 ± 5-g each) for each sampling period were packaged together and stored for 0, 3, 6 and 10 days at 4 ± 2 C and an equal number of samples (patties) from the same production lot were frozen at - 23 ± 3 C, stored for 0, 1, 2 and 3 months in a freezer at - 23 ± 3 C and thawed (5 h at 20 ± 3 C) before evaluation and cooking.

After being stored under these conditions, patties were evaluated by a six-member trained panel to determine the individual levels of each character of quality such as color, odor, and cohesiveness of the raw product. All panel members had at least 5 years of meat panel experience and were given samples, during training, of the extremes of all factors to be evaluated. The evaluation scale ranged from 1 (light, stale meat odor, not cohesive) to 10 (dark, fresh meat odor, very cohesive). After each storage treatment, the raw beef tissue was examined for aerobic and anaerobic organisms.

Aerobic organisms were determined by standard techniques (*2,16*) using Tryptone Glucose Extract Agar and incubating at 25 C for 48 h. Anaerobic organisms were also determined by standard techniques using Baltimore Biological Laboratory (BBL) Anaerobic Agar and incubating in a CO_2 atmosphere at 25 C for 5 days. In both instances the number of organisms were expressed as the log of the number per gram of tissue.

Rancidity of the raw tissue was determined by TBA analysis (*16*) and pH was measured with a Beckman Expandomatic SS-2 pH meter (*16*).

Patties were broiled (approximately 5 min first side, 3 min second side) in a Hotpoint Broiler (Model #201B17), to an internal temperature of 65 ± 2 C and each character of quality was evaluated for visual acceptance, cohesiveness, odor, texture, tenderness, flavor, juiciness and general acceptability by the same trained panel (different time) using the same 1 (stale appearance, not cohesive, stale meat odor, crumbly texture, extremely tough, stale flavor, dry, unacceptable) to 10 (fresh appearance, very cohesive, fresh meat odor, solid meat texture, extremely tender, fresh flavor, very juicy, acceptable) scale.

This experiment was replicated six times and the data were analyzed by Harvey's (*9*) Least Squares Analysis of Data with Unequal Subclass Frequency.

RESULTS AND DISCUSSION

Non-stored product characteristics

Analysis of the raw patties indicated their composition was as follows: protein 16.9 ± 0.9%, fat 14.7 ± 4.1%, moisture 65.9 ± 3.8%, ash 2.9 ± 0.4% and salt 2.1 ± 0.4%. The results of this research indicate that an acceptable product was produced by the tumbling method of manufacturing restructured steaks (Table 1). All quality attributes of the raw product immediately after manufacture were in the range normally expected for steak-like products when compared with previous steak taste panels from this same laboratory and with the same panel members. The uncooked color darkened ($P < 0.05$) during freezing-thawing and the uncooked odor scores also decreased (more stale) at the $P < 0.05$ level. All other uncooked product attributes remained relatively constant during freezing-thawing. Cooking the non-stored product resulted in a very acceptable steak-like product. Cohesiveness and odor were improved by cooking (Table 1). Other characteristics of the cooked product (visual acceptability, steak texture, tenderness, flavor, juiciness and general acceptability) were in a range considered acceptable for cooked steak products (previous steak panels, same laboratory). Freezing and thawing (Table 1) had no real effect on any of the cooked quality attributes.

TABLE 1. *Least square means and analysis of variance comparison of steaks before (refrigerated storage, 0 time) and after freezing and thawing (frozen storage, 0 time).*

Parameters evaluated	Ref. storage 0-days Least square mean	Change during freezing and thawing[a]
Uncooked color[b]	6.63	+ 1.08 *
Uncooked odor[c]	7.23	- 1.16 *
Uncooked cohesiveness[d]	7.50	- 0.29 NS
Cooked visual acceptability[e]	8.05	+ 0.12 NS
Cooked cohesiveness[f]	8.05	+ 0.22 NS
Cooked odor[g]	8.02	- 0.05 NS
Cooked steak texture[h]	7.22	+ 0.02 NS
Cooked tenderness[i]	8.10	- 0.20 NS
Cooked flavor[j]	8.20	- 0.35 AS
Cooked juiciness[k]	7.75	- 0.30 NS
Cooked general acceptability[l]	8.10	- 0.17 NS
Uncooked aerobic (log/g)	5.31	+ 0.03 NS
Uncooked anaerobic (log/g)	3.47	+ 0.55 NS
Uncooked TBA number	2.28	+ 2.72 AS
Uncooked pH	5.60	0.00 NS

[a] NS = non-significant; AS = approach significance, 10% level.
* = significance at 5% level.
[b] 1 = light, 10 = dark.
[c] 1 = stale meat odor, 10 = fresh meat odor.
[d] 1 = very cohesive, 10 = not cohesive.
[e] 1 = stale appearance, 10 = fresh appearance.
[f] 1 = not cohesive, 10 = very cohesive.
[g] 1 = stale meat odor, 10 = fresh meat odor.
[h] 1 = crumbly texture, 10 = solid meat texture.
[i] 1 = extremely tough, 10 = extremely tender.
[j] 1 = stale flavor, 10 = fresh flavor.
[k] 1 = dry, 10 = very juicy.
[l] 1 = unacceptable, 10 = acceptable.

Stored product characteristics

Uncooked product evaluation. Uncooked product color scores increased (darker) significantly (Table 2) during refrigerated ($P < 0.05$) and frozen storage ($P < 0.01$). The largest increases occurred during freezing-thawing and in early stages of refrigerated storage. This darkening in raw product color agrees with results of Schwartz et al. (*24*) for flake-cut restructured pork. Gokalp (*7*) found little color change in a vacuum-packed ground beef patty (no NaCl) that had been stored at - 22 C for 135 days. Uncooked product color scores from the refrigerated product showed a positive correlation (Table 3) with raw TBA values ($P < 0.01$). The same was true with frozen patties ($P < 0.05$). The increase in TBA values (Table 2) suggests fat oxidation during storage. The literature (*8,15*) suggests this was probably accelerated by the salt and retarded by vacuum packaging.

Uncooked product odor became more stale ($P < 0.05$) during refrigerated storage (Table 2). The same decrease was noted with the frozen product up to 60 days, after

TABLE 2. *Least squares mean for variables during storage.*

Days	Refrigerated storage, days				Frozen storage, days			
	0[a]	3	6	10	0[a]	30	60	90
Uncooked color[b]	6.63^{Q*}	7.55	7.98	8.27	7.71^{L**}	8.56	8.36	8.65
Uncooked odor[c]	7.23^{Q*}	5.51	4.88	4.36	6.07^{L**}	4.86	4.18	4.31
Uncooked cohesiveness[d]	7.50	7.51	7.56	7.35	7.21	7.23	7.01	7.26
Cooked visual acceptability[e]	8.05	8.51	8.23	8.26	8.17^{L*}	8.36	8.11	7.86
Cooked cohesiveness[f]	8.05	8.41	8.43	8.20	8.27^{L*}	8.23	7.96	7.81
Cooked odor[g]	8.02^{C*}	8.06	7.33	7.55	7.97^{L**}	7.68	7.01	6.71
Cooked steak texture[h]	7.55^{Q*}	7.81	7.75	7.11	7.57^{L*}	7.71	7.43	7.00
Cooked tenderness[i]	8.10	7.80	7.76	7.60	7.90^{L*}	7.80	7.58	7.23
Cooked flavor[j]	8.20^{L**}	7.90	7.41	6.76	7.85^{L*}	7.18	6.96	6.73
Cooked juiciness[k]	7.75	7.80	7.58	7.21	7.45	7.51	7.85	6.98
Cooked general acceptability[l]	8.10^{L**}	8.08	7.71	7.36	7.93^{L**}	7.61	7.48	6.90
Uncooked aerobic (log/g)	5.31^{L**}	5.60	6.02	6.49	5.34	5.51	5.40	5.41
Uncooked anaerobic (log/g)	3.47	4.14	4.79	4.14	4.02	4.65	4.33	4.47
Uncooked TBA number	2.28^{Q*}	6.25	8.31	9.50	5.00	5.56	4.80	6.23
Uncooked pH	5.60	5.60	5.58	5.55	5.60^{C*}	5.66	5.93	5.75

[a]Significance: L = linear, Q = quadratic, C = cubic, * = 5% level of significance, ** = 1% level of significance.
[b]1 = light, 10 = dark.
[c]1 = stale meat odor, 10 = fresh meat odor.
[d]1 = very cohesive, 10 = not cohesive.
[e]1 = stale appearance, 10 = fresh appearance.
[f]1 = not cohesive, 10 = very cohesive.
[g]1 = stale meat odor, 10 = fresh meat odor.
[h]1 = crumbly texture, 10 = solid meat texture.
[i]1 = extremely tough, 10 = extremely tender.
[j]1 = stale flavor, 10 = fresh flavor.
[k]1 = dry, 10 = very juicy.
[l]1 = unacceptable, 10 = acceptable.

which the values stabilized and remained relatively constant. These lower panel uncooked product odor scores during refrigerated storage (Table 2) are in accordance with increased TBA values. The correlation (Table 3) with TBA was negative ($r = -0.79$, $P < 0.01$) during refrigerated storage and was also negative ($r = -0.25$) during frozen storage but not at a significant level. Uncooked TBA values (Table 2) increased ($P < 0.05$) during refrigerated storage of the restructured samples. The uncooked TBA values in the frozen samples fluctuated over time. This suggests that the decrease in odor desirability is at least partially due to the presence of products from oxidative processes that occurred during storage.

Aerobic bacteria counts increased ($P < 0.01$) with refrigerated product storage time, but remained relatively unchanged in frozen samples (Table 2). The difference is most likely due to storage temperature differences. Anaerobic counts increased in the refrigerated product up to the sixth day, then decreased. Anaerobic counts remained relatively stable in the frozen product.

Cohesiveness was very acceptable as rated by the panel. The product approached a solid piece of meat in appearance and texture. Cohesiveness values remained relatively constant during both refrigerated and frozen storage (Table 2).

Cooked evaluation. Cooking the tissue gave higher (Table 2) visual acceptability scores than those of uncooked tissue of the refrigerated product. Cooking slightly lowered visual acceptability scores in the freezer-stored product. An explanation for this difference was the darkening ($P < 0.05$) of color during freezing (Table 1). Cooking greatly improved low uncooked product odor scores; however, it did not change the general downward trend that occurred over storage time (Table 2).

Cohesiveness scores were slightly higher (Table 2) for the cooked than the uncooked product. As with raw samples, length or type of storage had little or no effect on cohesiveness values.

In both the refrigerated and frozen phase, cooked steak texture decreased slightly (Table 2) during storage but remained very acceptable for all samples evaluated. There appeared to be no real difference between cooked steak texture scores for these two types of storage procedures. As would be expected, steak texture scores were positively and significantly correlated ($P < 0.01$) with cooked product cohesiveness scores (Table 3).

Cooked product tenderness scores (Table 2) from the refrigerated product remained relatively constant during storage; while the scores for frozen products decreased slightly but again remained in an acceptable range (previous steak panel, same laboratory).

Mean scores for steak texture (Table 2), indicate that this new method of forming steak-like product resulted in a product with a desirable steak texture. Furthermore, this desired texture was accompanied by an acceptable degree of tenderness.

Longer storage time for refrigerated and frozen products (Table 2) decreased cooked product flavor scores. This trend was more pronounced in the non-frozen samples. Flavor scores of the refrigerated product correlated ($P < 0.01$) with TBA values ($r =$

TABLE 3. *Correlation values*[a].

Characteristic	Uncooked pH	Uncooked TBA	Uncooked anaerobic	Uncooked aerobic	Cooked general accept.	Cooked juiciness	Cooked flavor	Cooked tenderness	Cooked steak texture	Cooked odor	Cooked cohesiveness	Cooked visual accept.	Uncooked cohesiveness	Uncooked odor	Uncooked color
Uncooked color	-0.03	0.71**	0.45*	0.59**	-0.48*	-0.09	-0.70**	-0.35	-0.32	-0.44*	0.28	0.13	0.03	-0.75**	
Uncooked odor	0.22	-0.79**	-0.35	-0.43*	0.39	0.34	0.60**	0.44*	0.07	0.41*	-0.28	-0.26	0.23		-0.61**
Uncooked cohesiveness	0.27	0.05	-0.15	0.01	0.35	0.44*	0.21	0.48*	-0.07	0.09	0.51**	0.15		0.24	0.19
Cooked visual acceptability	0.02	0.17	0.22	0.01	0.29	-0.06	-0.06	0.03	0.24	-0.04	0.52**		0.24	0.32	-0.28
Cooked cohesiveness	-0.17	0.44*	0.33	0.03	0.27	0.16	-0.15	0.14	0.28	-0.07		0.69**	0.28	0.53**	-0.27
Cooked odor	0.04	-0.39	-0.19	-0.07	0.38	0.40*	0.54**	0.17	0.11		0.59**	0.44*	0.16	0.74**	-0.47*
Cooked steak texture	-0.15	-0.08	0.06	-0.31	0.39	0.03	0.27	-0.04		0.51**	0.67**	0.58**	0.39	0.44*	-0.31
Cooked tenderness	0.30	-0.34	-0.39	-0.61**	0.51**	0.43*	0.60**		0.73**	0.43*	0.34	0.20	0.29	0.34	-0.12
Cooked flavor	0.22	-0.61**	-0.42**	-0.59**	0.74**	0.60**		0.46*	0.44*	0.72**	0.42*	0.33	0.37	0.77**	-0.40*
Cooked juiciness	0.29	-0.14	-0.23	-0.13	0.50*		0.30	0.57**	0.42*	-0.03	-0.02	0.22	0.40*	-0.01	0.16
Cooked general acceptability	0.17	-0.42*	0.01	-0.48*		0.50*	0.79**	0.57**	0.68**	0.73**	0.50*	0.64**	0.36	0.62**	-0.45*
Uncooked aerobic	0.11	0.52**	0.52**		0.09	0.15	0.12	0.32	0.19	0.03	-0.01	-0.14	-0.06	-0.09	0.23
Uncooked anaerobic	0.01	0.26		0.82**	-0.07	-0.02	-0.12	0.07	0.01	-0.06	-0.05	-0.11	-0.55	-0.20	0.28
Uncooked TBA	-0.25		0.45*	0.53**	-0.05	0.12	-0.02	0.03	-0.02	-0.08	0.08	0.02	0.30	-0.25	0.47*
Uncooked pH		0.04	-0.11	-0.09	-0.14	0.12	-0.09	-0.14	-0.08	-0.25	-0.05	-0.05	0.17	-0.36	0.23

[a]Upper-left = refrigerated; lower-right = frozen.
* - 5% level of significance
** - 1% level of significance

-0.61), uncooked product anaerobic ($r = -0.42$; $P < 0.05$) and uncooked product aerobic ($r = -0.59$; $P < 0.01$) counts (Table 3). One or a combination of these might explain the decrease in flavor acceptability.

Cooked product juiciness scores remained relatively constant and at a desirable level (compared with previous steak results, same laboratory) during both refrigerated and frozen storage (Table 2). General acceptability scores decreased ($P < 0.01$) during both types of storage (Table 2).

This restructured product is very acceptable in the fresh state. However, rapid deterioration occurs in uncooked product color and odor and the stored product is also rated lower with longer storage time when cooked product odor, flavor, and general acceptability are compared. Fat oxidation, based on TBA values, is an important factor in this deterioration. If the product cannot be used quickly it appears that phosphates or antioxidants will be necessary for an acceptable shelf life.

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Thermal Resistance of *Vibrio parahaemolyticus* in Clam Homogenate

R. P. DELMORE, JR.* and F. D. CRISLEY

Department of Biology, Northeastern University, 360 Huntington Avenue, Boston, Massachusetts 02115

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ABSTRACT

Nine strains of *Vibrio parahaemolyticus* from widely varied sources were exposed to heat in sterile clam homogenates. D-values were determined at temperatures ranging from 49 to 55 C for four strains, and at 51 C for the remaining five strains. The D-values in minutes, corrected for thermal lag and lethality during thermal lag, were 0.39 to 0.70 at 49 C, 0.26 to 0.54 at 51 C, 0.12 to 0.31 at 53 C, and 0.03 to 0.24 at 55 C. Values of z in degrees Centigrade determined for four strains were 5.6 to 12.4. One strain was found to be significantly more resistant at 53 and 55 C.

Although a number of reports (*4,10,13*) indicate that the thermal resistance of *Vibrio parahaemolyticus* is quite low, few of the available data are suitable for thermal process calculation. Process-directed data are useful both for proper treatment of foods and often for a better understanding of the epidemiology of a seafood-borne disease outbreak, since such foods require very little cooking if their attractiveness is to be preserved and also are frequently prepared in large quantities for social occasions. Seafoods have been responsible for outbreaks of *V. parahaemolyticus* food poisoning in the United States (*2,8*). Furthermore, this lack of readily reproducible data leaves open the question of the existence of unusually resistant strains. For example, *Salmonella senftenberg* has been found to have a higher heat resistance than most other salmonellae (*1*).

Beuchat (*3*) reported D_{35C} values ranging from 2.8 to 4.0 min at pH 7.0 in Trypticase Soy Broth containing 3% NaCl, and 0.9 to 1.5 min at pH 5.0, indicating that thermal sensitivity is related to the pH of the suspending medium. Covert and Woodburn (*6*) reported heat resistance to be increased in fish homogenate as compared to broth, and to be greater in broth and homogenate containing added NaCl. Thermal resistance studies have been reported for suspensions of cells in phosphate buffer (*4*), Trypticase Soy-salt-crabmeat homogenates (*10*), and shrimp homogenates (*13*). Although valuable in confirming the low heat resistance of the organism, these data are inadequate for thermal process calculation since either the heating menstruum was not sufficiently representative of actual food conditions or no measurements of heat penetration were included. In this study, we employed a clam homogenate to simulate a seafood environment as closely as possible, and measured the rate of heat penetration into the food to correct for thermal lag and lethality that occurs within this time.

MATERIALS AND METHODS

V. parahaemolyticus strains 14E, 30E, and 58E were isolated from Cape Cod soft shell clams in a previous study (*9*). Dr. Morris Fishbein, Food and Drug Administration, Washington, D.C., kindly furnished strain 5C4, an enteritis case isolate, and strains 5A52B and 7A82K, isolated from bluefish and sea trout respectively. Strains KC843 (isolated from an unknown Japanese source) and C3474 (isolated from lobster meat in Massachusetts) were obtained from Dr. V. R. Dowell, C.D.C., Atlanta, Ga. Strain 6-IBOH-421 was furnished by Drs. Baross and Liston, Institute of Food Technology, College of Fisheries, University of Washington.

Stock cultures of all strains were maintained at room temperature (21-22 C) on slants of Trypticase Soy Agar containing 3% NaCl (TSAS).

Fresh cultures were prepared for each experiment by performing two successive transfers from a stock slant on TSAS. The final subculture was made on a TSAS slope in an 8-ounce oval bottle which was incubated at room temperature for 20-22 h. The cells were successively washed from the surface with sterile phosphate-buffered-saline (PBS) (*5*), shaken thoroughly to break clumps of cells, and adjusted to a concentration of 10^8 cells/ml with a Coleman Universal Spectrophotometer at 580 nm.

The inoculated clam homogenate was prepared as follows: A carton of frozen minced sea clams was allowed to thaw and the clam meat was separated into 250-g samples. Each sample was placed in a separate blender jar. The jars were covered with aluminum foil, autoclaved for 30 min at 121 C, and kept refrigerated at 4 C until used. Random samples were periodically checked for sterility. When a sample was to be used, the sterile clam meat was blended into a homogenate on a Waring blender. Small measured amounts of sterile PBS not exceeding 40 ml were added until the homogenate blended uniformly without adhering to the sides of the jar. The pH of the final blended homogenate was consistently between 6.2 and 6.4. The amount of test culture added varied with the amount of PBS needed, but was always 1% of the final total weight of the sample. This resulted in a concentration of approximately 10^6 cells per gram, as determined by control plating of untreated samples. After addition of the test culture the homogenate was mixed thoroughly with a sterile tongue depressor.

The modified grease-gun apparatus for dispensing the inoculated homogenate was previously reported by Crisley et al. (*7*).

For each experiment 60 thick-walled thermal-death-time tubes, 100 × 10 mm, were distributed into five Wasserman racks, 12 tubes per rack allowing two extra for breakage. Each rack was covered with aluminum foil and autoclaved for 30 min at 121 C. The TDT-tubes were filled aseptically one at a time with each tube receiving the amount of food delivered by one stroke of the gun. Washers added to the pressure-piston adjusted the amount of each delivery to 1.0 ± 0.1 gram.

After all the tubes in the racks were filled, each tube was sealed in an oxy-gas flame, allowed to cool briefly to avoid cracking, and the completed racks of tubes were submerged in a waterbath set at 14 C. All tubes were allowed to equilibrate at this temperature for at least 10 min. After equilibration all five racks were removed from the cold waterbath and plunged simultaneously into a second waterbath heated

to the desired test temperature. Timing was begun at the moment of submersion and at each selected time interval one rack was withdrawn from the test bath and returned immediately to the cold bath to be cooled for at least 10 min.

Bacterial inoculum control cultures were made from two tubes selected at random immediately after filling. The contents were removed aseptically and serially diluted to 1:10,000 in PBS. Plate counts were made on the dilutions using TSAS and Thiosulfate Citrate Bile-salts Sucrose (TCBS) Agar. To insure that no significant multiplication occurred during sealing and cooling, two sealed tubes were maintained in the cold bath during the entire heat-treating and recovery process after which their contents were removed, serially diluted, and plated as above. In all instances the food was found to contain between 8×10^5 and 2×10^6 cells per gram.

Each heat-treated TDT-tube was examined for survivors. The tubes were scored on a motor-driven glass-tubing cutter, snapped open, and the contents were transferred to tubes of Trypticase Soy Broth containing 3% NaCl and .004% Bromthymolblue indicator using an inoculating needle wound into a small coil. The recovery tubes were incubated at 35 C and examined at 24-h intervals for 7-14 days. Tubes showing a color change from green to yellow were recorded as positive for survivors. Several positive tubes from each time-treatment group were confirmed by either gram-staining or by plating on TCBS.

The rate of heat penetration was determined essentially by the procedure of Angelotti et al. (*1*). The internal temperature of the homogenate was recorded every 8 sec with a Bristol temperature recorder set at a chart speed of 4½ inches per minute. Heat penetration curves were drawn for each test temperature, and lethality curves were constructed for each strain at each test temperature as previously described (*1*). Each exposure time in all tests was corrected by subtracting the appropriate correction factor for thermal lag and lethality during lag.

D-values with 95% confidence limits were computed using the probability method of Schmidt (*11*). From these data z values with 95% confidence limits were determined by two-variable linear regression with a Hewlett-Packard 9810A Calculator.

RESULTS AND DISCUSSION

Survival of four strains of *V. parahaemolyticus* (14E, KC843, 5C4, and 6-IBOH-421) in clam homogenate at four temperatures is shown in Table 1. Duplicate experiments were done on most strains at 49, 51, and 53 C; a single experiment was done on each strain at 55 C. A third experiment was done on strain 6-IBOH-421 at 49 C. The survival of five other strains (7A82K, 30E, 58E, C3474, and 5A52B) at 51 C only is shown in Table 2.

The data for each strain at a single temperature were combined and D-values with 95% confidence limits were computed. A typical calculation using the probability method of Schmidt is shown in Table 3 for strain 14E at 49 C. The exposure times used have been corrected for thermal lag and lethality during lag.

Table 4 lists D-values at four temperatures and

TABLE 1. *Survival of four strains of* Vibrio parahaemolyticus *in clam homogenate at four temperatures. Values are the number of recovery tubes out of ten showing growth through 14 days incubation.*

Temp. (C)	Exposure time (min)	Strain			
		14E	KC843	5C4	6-IBOH-421
49	2	10,–[1]		10, –	
	4	8,9	7,–	5,–	5,5,3
	5	– ,9			– ,0,1
	6	1,5	2,3	2,8	0,1,0
	7	–,0	2,1		–,0,0
	8	0,0	1,1	1,3	0,–,–
	9		–,0		
	10	0,–	0,0	0,0	0,–,–
51	1	10,–			
	2		8		
	3	9,–			3,5
	3.5				–,3
	4	–,9	3	3	0,0
	4.5				–,0
	5	4,3		0	0,0
	6	–,0	0	0	1,–
	7	0,0		0	
	8	–,0	0	0	1,–
	9	0,–			
53	1				10,10
	1.5				–,9
	2	10	4,6	9,–	5,3
	2.5		–,2	–,5	–,1
	3	6	0,0	2,1	0,0
	3.5		–,0	–,0	
	4	0	0,0	0,0	0,–
	4.5			–,0	
	5	0	0,–	0,–	0,–
	6	0	0,–	0,–	
55	1	10	10	10	10
	1.5	10	2	9	5
	2	10	0	3	2
	2.5	6	0	0	0
	3	0	0	–	0

[1] Indicates this time interval not used in this particular experiment.

TABLE 2. *Survival of five strains of* Vibrio parahaemolyticus *in clam homogenate at 51 C. Values are the number of recovery tubes out of ten showing growth through 14 days incubation.*

Exposure time (min)	Strain				
	7A82K	30E	58E	C3474	5A52B
3	9,10	8	7	5	
4					0
5	–,2				
6	1,–	1	0	0	0
7	–,0				
8					0
9	1,0	1	0	0	
10					0
11	–,0				
12	0,–	0	0	0	0

z-values for four strains plus D-values for four more strains at 51 C only. No D-value could be computed for strain 5A52B because no positive recovery tubes were obtained at the exposure times used. Therefore this strain is no more heat resistant at this temperature than the other strains.

Strain 14E showed the highest heat resistance at the higher test temperatures. The D-values for this strain at 53 and 55 C (0.31 and 0.24 min, respectively) were significantly higher than those of the other strains tested at these temperatures, and the D-value at 51 C was also comparatively high (0.54 min). Strain 7A82K also had a D_{51} of 0.54 min. Strain 5C4 showed the highest heat resistance at 49 C with a D_{49} of 0.70 min. A thermal process calculation designed to eliminate *V. parahaemolyticus* from seafood should utilize these D-values, at least until a more resistant strain at these temperatures is found.

A comparatively low D-value (0.03 min) was observed at 55 C for strain KC843 which may be unusually heat sensitive at this temperature. Only the D-values obtained at the other three test temperatures were used to compute z for this strain.

In the improved general method of process calculation (*12*) z-values are often used. A z-value represents the number of degrees the temperature must be increased to lower the D-value by a factor of ten. For vegetative cells z-values are usually around 10 F ± 2 (5.5 C ± 1.1) for temperatures within the range 54-70 C (*12*). This encompasses only the highest test temperature (55 C) used for *V. parahaemolyticus*. The higher resistance at the higher temperatures demonstrated by strain 14E

TABLE 3. *Typical calculation of D-value using the probability method of Schmidt* (11) *for* Vibrio parahaemolyticus *strain 14E at 49 C.*

Corrected exposure time (min)	# tubes		m[1]	n[2]	m+n	P[3]	LD_{50}[4]	D[5]	95%CL_D[6]
	+	–							
0.5	10	0	42	0	42	.023			
2.5	17	3	32	3	35	.097			
3.5	9	1	15	4	19	.238			
4.5	6	14	6	18	24	.730			
5.5	0	10	0	28	28	.970			
							4.05	0.66	0.61 0.70

[1]The cumulative number of positive samples starting with the longest exposure time.
[2]The cumulative number of negative samples starting with the shortest exposure time.
[3]The probability of a sample being negative at that particular exposure time. P = n + 1/m + n +2.
[4]The corrected exposure time that should cause 50% of the tubes to be negative, obtained from plotting values of P on probability paper.

[5] Obtained from the following formula (*11*): $D = \frac{LD_{50}}{\log 10^6 - \log 0.69}$

[6] 95% confidence limits for D obtained from the following formula (*11*): $95\%CL_D = \frac{LD_{50} \pm 95\%CL}{\log 10^6 - \log 0.69}$

$95\%CL = 1.96 \times 2s/\sqrt{2N}$ where 2s is the difference between the exposure times corresponding to P = .16 and .84; and N is the total number of tubes in groups showing partial survival.

TABLE 4. *D^1- and z^2-values for* Vibrio parahaemolyticus *strains.*

Strain	D, LCL[3], UCL[4] at test temperature				z,	LCL,	UCL
	49 C	51 C	53 C	55 C			
14E	.66 .61 .70	.54 .48 .59	.31 .22 .40	.24 .18 .29	12.4	5.6	19.3
KC843	.63 .54 .72	.29 .16 .43	.12 .09 .15	.03 .02 .05	5.6	3.3	7.8
5C4	.70 .58 .82	.37 .26 .49	.21 .18 .23	.11 .08 .15	7.5	7.0	8.0
6-IBOH-421	.39 .35 .43	.26 .21 .32	.13 .10 .15	.09 .06 .13	9.4	4.6	14.2
7A82K		.54 .45 .62					
30E		.50 .35 .66					
58E		.37 .17 .58					
C3474		.26 .02 .50					
5A52B		*					

*Could not be determined from the data, see text.
[1]D is the time in minutes required of heating at a certain temperature to cause a 90% reduction in viable cells.
[2]z is the number of degrees the temperature must be increased to lower the value of D tenfold.
[3]Lower 95% confidence limit.
[4]Upper 95% confidence limit.

resulted in an exceptionally high z-value of 12.4 C. This value should be considered in the improved general method.

These data not only corroborate previous reports of the overall low resistance to heat of *V. parahaemolyticus* but also are suitable for use in thermal process calculation.

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Dick Brazis Biography, con't from p. 187

A registered microbiologist, Dick has specialist certificates in Public Health and Sanitation Microbiology of The American Board of Microbiology. His memberships in organizations include IAMFES, Inc.; ASM, APHA, NMC (member of Board of Directors), IFT, AFDOUS, ADSA, American Academy of Sanitarians (diplomat) and Nebraska Dairy Industry Association.

Dick has served as Chairman of the Applied Laboratory Methods Committee of IAMFES since 1964 and has been a member of the Farm Methods Committee for several years. He has assisted in the preparation of four editions of Standard Methods for the Examination of Dairy Products (SMEDP).

Awards which he has received include the PHS Commendation Medal (1963), the Citation Award of IAMFES (1975), FDA Group Award of Merit (1976), "Toastmaster of the Year" in Cincinnati (1976), Kentucky Colonel (1972), and service as a member of Intersociety Council on SMEDP (14th edition).

He has been married 26 years to Rosemary Wiggs Brazis and they have a son and daughter, Bill, a sophomore at Southeast Missouri State University and Barbara, a junior high student.

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Inactivation of Milkborne Foot-and-Mouth Disease Virus at Ultra-High Temperatures[1]

H. R. CUNLIFFE*, J. H. BLACKWELL, R. DORS and J. S. WALKER

Plum Island Animal Disease Center, Federal Research Science and Education Administration, U.S. Department of Agriculture, P.O. Box 848, Greenport, New York 11944

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ABSTRACT

Milk from cows with foot-and-mouth disease containing $10^{3.7}$ to $10^{6.4}$ plaque-forming units of virus/ml was exposed to several ultra-high temperature treatments for 2-5 sec. Results indicated that the virus in such milk could be reliably inactivated when held at 148 C for 3 sec or longer.

Many reports indicate that milk obtained from foot-and-mouth disease (FMD) virus-infected cows (*4,10-13,15*) and the derived milk products such as cheese (*1*) and casein (*8*) may be insidious sources of infectious FMD virus. For example, FMD virus can survive conventional high temperature-short time (HTST = 71.7 C/15 sec) pasteurization of whole milk, skim milk, cream and cellular debris from the milk of experimentally infected dairy cows (*3*). Similarly, cheese products made from milk of FMD virus-infected cows contained infectious virus for extended periods after curing and storage (*2*). We have shown persistence of infectious FMD virus up to 42 days in heat-dried casein produced by acid precipitation of HTST-pasteurized skim milk from infected cows (*7,8*).

Therefore, milkborne FMD virus may pose a serious threat to FMD-free countries, and reliable procedures are needed to inactivate FMD virus in milk. Rapid technological advances associated with the application of ultra-high temperature (UHT ⩾ 130 C/2 sec) procedures, enabled production of sterile milk with a long shelf life at ambient temperatures (*16*). Therefore, milk from cows infected with FMD virus was treated at different times and temperatures, and inactivation of FMD virus is reported.

[1]*Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.*

[2]*Conde 600 Milking Machine Unit with pneumatic pulsator, and S. S. RHT pail; Conde Milking Machine Co., Inc., Sherrill, NY.*

MATERIALS AND METHODS

Experimental design

Results of experiments with bacterial spores reported by Westhoff and Doores (*16*) suggested to us that a temperature range of 102 to 149 C would include the heat inactivation conditions for FMD virus. Therefore, milkborne FMD virus was exposed to 102, 123, 130, 138 or 148 C for 2 to 5 sec — 1 to 5 replicates for each temperature.

Immediately after UHT treatments, milk was cooled in a tap water bath (7 C), transferred directly into sterile plastic bottles and stored at 4 C.

Virus inoculum

The virus used was FMD virus, type A, subtype 3, strain Mecklenburg (FMD, A-3M). It was prepared as a 5% suspension of bovine vesicular material in fetal bovine serum. Virus inoculum ($10^{6.3}$ PFU/ml) for dairy cows was 1 ml intravenously and 1 ml each in the right fore and left hind quarters with a teat cannula and syringe.

Cattle

Groups of three nonregistered Holstein dairy cows were milked twice daily with a portable automatic milking machine.[2] Milk from each group was pooled during collection, and morning and evening pools were combined when necessary. Housing of cattle was described in detail previously (*11*).

Steers used for infectivity testing were nonregistered Herefords about 18 months old. Housing and maintenance of the steers have been described elsewhere (*5*).

Ultra-high temperature apparatus

An indirect heat exchanger raised the milk temperature to the desired treatment levels. The heat exchanger (Fig. 1) consisted of a steel pressure tank containing 20.56 m of coiled stainless-steel tubing with an inside diameter of 7.5 mm. The heat exchange coil contained 908 ml of fluid. Milk was pumped through the exchanger by a variable-speed, stainless-steel, positive displacement centrifugal pump. Before addition of infectious milk, the apparatus was adjusted to the desired temperature by recycling distilled water and adjusting steam pressures. Variations of steam pressure and pump speed provided the various temperatures and transit times. Milk volume varied from 8-15 liters/experiment.

The apparatus was decontaminated between experiments by thorough rinsing with large volumes of tap water followed by a 5-min recycling of hot (150 C) 2% NaOH. The NaOH was then flushed from the system with running tap water, which was replaced by 10 liters of distilled water. The distilled water was recycled and heated to 150 C for 5 min and allowed to remain in the system until the next experiment.

Assay methods

A sample of pooled milk from each milking was assayed for FMD virus titer; standard plaque procedures were used. Plaque titers (PFU/ml) were determined for milk pools before and after

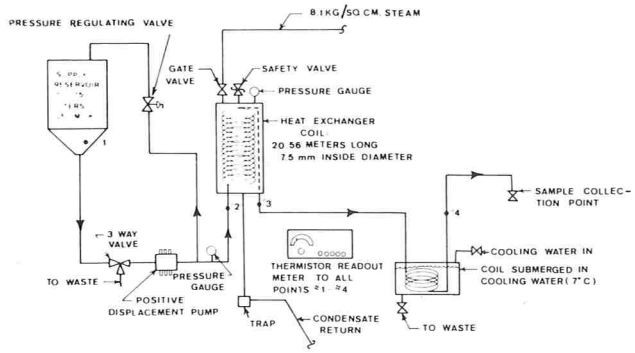

Figure 1. *Schematic view of Ultra-High Temperature apparatus.*

experimental treatments. The inoculum (0.1 ml) was allowed to adsorb to drained primary bovine kidney cell cultures for 1 h at 37 C before it was overlayered with 0.6% gum tragacanth in culture fluid (*6*).

Infectivity of UHT-treated milk samples was assayed in two steers each by inoculation with 2 ml of sample distributed over the dorsal surface of the tongue in 20 intradermolingual (IDL) needle tracks (*7*) and with 48 ml intramuscularly; thus, 100 ml of each sample was inoculated into steers. The IDL route was used because of its optimal sensitivity (*8*) for detecting very small amounts of FMD virus. Sera from steers that did not develop FMD 14 days after test inoculations were assayed for virus neutralizing antibodies as previously described (*9*).

RESULTS

All infected dairy cows were febrile and viremic, and their milk contained relatively high titers of FMD virus, 1 day after infection. Clinical signs of a vesicular disease were not detectable until the second day.

Results in Table 1 indicate that most pools of infective milk had substantial FMD virus titers immediately before UHT treatment. Six pools between 102 and 138 C retained infectivity for cattle after UHT treatments. However, only one UHT-treated pool infected cell cultures (#3, Table 1).

The data also show that FMD virus rapidly lost its infectivity for cattle at treatments above 130 C (Table 1). Only one of five milk samples treated at 138 C was infective for cattle, and infectious FMD virus was not detected in two samples heated at 148 C. Virus neutralizing antibodies were not detected in sera of test steers which did not react to inoculations with UHT-treated milk samples.

DISCUSSION

Large amounts of FMD virus can be found in cows' milk before FMD is even suspected (*4*). Data in Table 1 show that UHT treatment at 148 C for at least 3 sec can inactivate FMD virus in milk from infected dairy cows. Further studies have been designed to refine UHT inactivation conditions for FMD virus in cows' milk. Thus, UHT treatment of fresh, whole milk in the dairy industries can eliminate regional or international distribution of infectious FMD virus in dairy products.

Although organoleptic qualities of UHT-treated milk were not studied here, reports in the literature (*16*) indicate that UHT-treated milk can be a palatable food. Current research in several countries involves removal of objectionable odors and flavors caused by UHT treatments of milk (*14*). Use of UHT-treated milk in the cheese industry may present technical problems involving loss of coagulability. We expect that such problems can be resolved by applied research. We have also produced dried casein from UHT-treated (148 C) milk according to methods described previously (*8*) and have observed no manufacturing difficulties.

The versatility of UHT technology is apparent when one considers that mobile UHT units can be rapidly moved into an outbreak area and attached to existing bulk milk processing equipment. Mobile units would provide a practical alternative for safe disposal of infectious milk in an FMD outbreak and would salvage a valuable source of high-quality nutrients for man.

TABLE 1. *Infectivity of foot-and-mouth disease virus infected cows' milk before and after ultra-high temperature treatments.*

UHT pool	Temp. (C)	Time[a]	Cultured cell infectivity[b]		Cattle Infectivity[c]
			Before heat	After heat	
1	102	2-3	6.1	< 0.0	+
2	123	2-3	5.1	< 0.0	-
3	123	2-3	6.3	0.92	+
4	123	2-3	6.2	< 0.0	+
5	130	2-3	4.9	< 0.0	-
6	130	2-3	6.3	< 0.0	+
7	130	2-3	5.6	< 0.0	+
8	138	2-3	6.4	< 0.0	+
9	138	2-3	5.2	< 0.0	-
10	138	2-3	5.4	< 0.0	-
11	138	2-3	5.2	< 0.0	-
12	138	2-3	3.7	< 0.0	-
13	148	2-3	4.4	< 0.0	-
14	148	2-3	5.2	< 0.0	-

[a]Time in seconds that milk was held at indicated temperature.
[b]Values are $\log_{10}$ plaque-forming units/ml; < 0.0 = < 1 PFU/ml.
[c]+=one or more steers developed FMD after inoculation of UHT milk;
- =steers failed to develop signs of FMD during 14 days after test inoculation, and 14-day sera did not contain virus neutralizing antibody to FMDV A-3M.

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Ice Cream's Contents List Won't Indicate Its Quality

Next July ice cream will become one of the first "standard of identity" foods requiring a list of ingredients on the package. The U.S. Food and Drug Administration (FDA) will continue to regulate the ingredients in ice cream, however.

The standard of identity guarantees consumers that all ice cream contains minimum amounts of certain ingredients, according to William Winder, food scientist at the University of Wisconsin-Madison (UW-Madison).

At first glance, the FDA's description of the minimum composition of ice cream appears simple:

At least 10 percent of ice cream must be milk fat. At least 20 percent must be milk solids which includes milk fat, protein, lactose (milk sugar) and minerals.

A gallon of ice cream must weigh at least 4.5 pounds. This controls the amount of air the producer may whip into the ice cream to increase its volume.

A gallon of ice cream must contain at least 1.6 pounds of food solids or digestible ingredients. The remainder can be water.

All ice cream contains emulsifiers to prevent the fat and water from separating, and stabilizers to help maintain ice cream's smooth texture. Emulsifiers and stabilizers together can't exceed five-tenths of 1 percent of the final product.

One reason why the price and quality of ice creams vary is that producers have a long list of acceptable ingredients to choose from, says Winder.

For example, producers can choose from several sources of milk solids, including nonfat dry milk solids or whey to comply with the standard of identity. Whey solids, which can constitute only one-fourth of the milk solids not fat, cost about one-fourth as much as non-fat dry milk solids, but contain less protein.

Producers can also choose from a list of sweeteners, emulsifiers, stabilizers and milk fat sources.

Some ice creams contain more than the minimum amounts of milk fat and solids. And some producers whip in less air than the standards allow.

"Ice cream grading is unique among dairy products," notes Winder.

"Butter, for example, is graded for quality. The grades AA, A and B listed on butter containers clearly identify the quality of butter. These grades also help explain price differences to consumers," he adds.

Ice cream is not graded for quality. Instead, the standard of identity establishes the minimum quantity of the ingredients used in ice cream. The producer can move up from the minimum level, but there's no grading scale to indicate he has done so.

Winder doesn't foresee ice cream grading in the near future. Although he thinks grading, if done properly, would make it easier for consumers to compare ice creams, he points out that it would also increase the cost.

If you equate quality with price in ice cream, you could be missing out on some very good competitively priced ice cream.

"Talking about price and ingredients only scratches the surface of what constitutes quality ice cream. Processing, handling and flavoring are also important to quality. The fact is that there is quality ice cream on the market today that is competitively priced," Winder concludes.

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A Critical Evaluation of Automatic Bacterial Colony Counters

M. H. BRODSKY*, B. W. CIEBIN and D. A. SCHIEMANN

Ontario Ministry of Health, Laboratory Services Branch
Environmental Bacteriology, Box 9000, Terminal A,
Toronto, Ontario M5W 1R5, Canada

(Received for publication May 16, 1978)

ABSTRACT

Four automatic colony counters (ACC), 3M Model 620 and Artek Models 480, 870 and 880, were evaluated for their precision and accuracy in counting bacterial colonies in pour-plates prepared using raw and pasteurized milk samples. The automatic colony counters were precise, labor saving devices, but not one of the ACC units approached our acceptability criterion that 90% of the ACC counts fall within ± 10% of the corresponding manual count. Some parameters of experimental design and instrument calibration which may significantly influence the response and performance of the automatic counters are discussed.

The plate count remains one of the most basic but tedious techniques employed in dairy microbiology. While many factors influence the Standard Plate Count during the course of pour-plate preparation, technician counting proficiency is regarded as the greatest and most uncontrollable source of error (*2,12*). *Standard Methods for the Examination of Dairy Products* (*1*) states that analysts should be able to duplicate their counts on the same plate to within 5% and the counts of other analysts to within 10%. Recent studies suggest that these restrictions may be unrealistic. Snyder (*12*) reported on an unpublished study conducted by the American Public Health Association in 1975 which found that the variation among technicians counting the same plates was ± 24%. Fowler et al. (*4*) found that analysts could reproduce their own counts only within ± 7.71% and the counts of others within ± 18.19%. Fruin and Clark (*5*) also reported that analysts were within 5% of the true count on only 68% of the plate counts.

The need to improve the precision and speed of the counting technique, coupled with advanced electronic technology, led to the evolution of automatic bacterial colony counters (ACC). The principles of the automatic counting system are described in other papers (*10,12,13*). In 1957, Mansberg (*9*) described the first practical ACC for use with the pour-plate technique. He noted certain limitations and precautions to be observed when using the automated system: the scanning resolution was only 0.5 mm and needed substantial improvement; preparation of pour plates for use with the automatic counter required greater care to ensure even distribution of the inoculum and to avoid scratches and agar defects (using a mechanical rotator was suggested); certain types of colonies, e.g. "spreaders" and "pulls," could not be accurately counted; the counter was sensitive to plate orientation; the molded rim of the petri dish interfered with the automatic counter's ability to enumerate colonies growing on or near the meniscus of the agar where it contacted the sides of the petri dish. This latter interference had to be optically masked, but a correction factor for the masking could be applied.

An improved version of Mansberg's counter was evaluated by Malligo in 1965 (*8*). Using pure cultures of *Serratia marcescens* and *Bacillus subtilis* var. *niger*, Malligo determined that the manual and ACC techniques had the same precision; however, the ACC had a stable bias which could be corrected by interpolating from a linear regression equation derived from the combined data of both pure culture studies. Malligo used spread plates to minimize dispersion of colonies to the outer edges of the agar and thereby reduce the number of colonies developing within the masked area.

Within the past decade, a number of more sophisticated ACC systems have been developed and evaluated. The 3M Brand Automatic Colony Counter, Model 620, was evaluated for counting variability by Packard and Ginn (*11*) and more recently for accuracy by LaGrange et al. (*7*). Packard and Ginn concluded that 3M counts were somewhat more variable than the manual counts. The overall mean values of the 3M colony counts were comparable to those obtained by the manual technique, but averaged 2.33 counts higher. LaGrange et al. reported that the 3M counter tended to underestimate the number of colonies per plate. Both studies suggested the application of correction factors for adjusting the automated counts based on interpolation from linear regression equations.

Goss et al. (*6*) evaluated the Artek 870 colony counter for sensitivity, precision and accuracy by comparing the counts obtained by two technicians with an Artek count on the same plates. Their data showed that surface and subsurface colonies of 0.3-mm size or larger were counted with good precision but that the Artek 870 uniformly underestimated the manual count. A scatter plot of the plate count data on a logarithmic scale indicated a linear relationship between the manual and automated counts.

Fleming and O'Conner (*3*) examined the Artek 480 counter and the Foss Biomatic 16900 electronic counter using raw milk samples. They observed that the automatic counters overestimated the plate counts relative to the manual method, but were more precise than the corresponding manual counts. Fruin and Clark (*5*) evaluated the accuracy of the Artek 480 colony counter by comparing manual and automated counts with the true count obtained by the use of photographs. They commented on a number of errors associated with the experimental design of studies used to evaluate ACC's and the need for a more standardized and sophisticated approach. Lack of awareness of these sources of error, including improper plate orientation, irregularities in agar surfaces, air bubbles in the medium, spreaders and extremely larger surface colonies could influence the results and lead to erroneous conclusions. After these possible sources of error had been eliminated, the data from Fruin and Clark indicated that the Artek 480 underestimated plate counts when the density exceeded 100 colonies per plate.

It is evident from the literature that the results of ACC evaluations were often contradictory, even when similar units were examined by different investigators. Within the past 4 years we examined the performance of a number of ACC units. This paper reports on our evaluation of four ACC systems. Some parameters of experimental design and instrument calibration which may significantly influence the response and performance of the automatic counters are discussed.

MATERIALS AND METHODS

Instrument description and calibration

Four ACC systems, supplied by the manufacturers, were evaluated: 3M Brand Automatic Bacterial Colony Counter (3M Company, New Business Ventures Division, St. Paul, Minnesota, 55101); Artek models 480, 870 and 880 (Artek Systems Corporation, Farmingdale, New York, 11735).

The 3M 620 was equipped with a fixed-position plate support stage designed to allow the petri plates to be "nestled" in the correct position for counting. The masked area and compensation factor were factory-set at 17%. The size threshold was empirically adjusted to exclude all particles less than 0.2 mm in diameter as a "noise" factor was introduced at more sensitive settings. According to the manufacturer, this "noise" factor is created by the inability of the scanning system to distinguish between the background and low density colonies or particles when the medium/colony contrast is minimized at maximum threshold settings.

The Artek 480 had a flat support stage with two adjustable plate-guide arms. The plates had to be centrally positioned and the guide arms secured with tightening screws. The diaphragm was manually adjusted to mask the rim of the petri plate. The correction factor (CF) was calculated, according to the manufacturer's instructions, as the percent difference between the arithmetic mean count of 20 plates (30-300 counting range) determined manually and by the ACC without compensation. For our study, the CF for the Artek 480 was calculated to be + 14%. The Artek 480 was also equipped with a sensitivity threshold dial which was adjusted to obtain a maximum response without introducing a "noise" factor.

The Artek 870 was similar in design and operation to the Artek 480 except that it was equipped with an electronic aperture for controlling the counting area. The CF and sensitivity threshold settings were determined as for the Artek 480.

The Artek 880 was originally equipped with a plate support stage similar to that for the 480, but this was changed to a "nesting" type stage at our request. The CF was calculated as the percent difference between the total area of the plate surface and the actual counting area controlled by the electronic aperture. For our study, a CF of 11% was determined. The size threshold discriminator was set to exclude all particles less than 0.2 mm in diameter. An added feature of the Artek 880 was a separate sensitivity threshold adjustment with two "tuning windows" in the viewing screen. Maximum sensitivity was obtained for each counting series by increasing the threshold until the upper window began flashing and then slowly reducing the threshold until the flashing ceased.

Source of samples and preparation of pour plates

Pasteurized and raw milk samples submitted to the laboratory for routine bacteriological examination were used to prepare pour plates for the comparative studies. Each sample was diluted appropriately in Butterfield's phosphate buffer (*1*) to obtain 30 to 300 colonies per plate. Standard Methods Agar (BBL) was prepared in the Agarmatic Bench-Top Agar Sterilizer (New Brunswick Scientific Co., Inc., New Brunswick, New Jersey) and dispensed in 12-15-ml volumes per pour plate. Plastic petri plates (100 × 15 mm, Fisherbrand Cat. 8-757-13) without stacking rings were used throughout the study. The plates were incubated for 48 ± 4 h at 32 C before counting.

Counting procedures

All plates were visually screened to eliminate any plates with colony counts less than 30 or greater than 300, and any showing uneven or rippled agar or scratched plastic surfaces. Each acceptable pour plate was counted manually by three different technicians using Quebec colony counters and hand tallies. Only those plates whose colony counts agreed within 10% of the median manual count were acceptable for comparative counting with the 3M 620, Artek 480 and Artek 870. Only those plates whose manual counts agreed within 5% of the median count were used for comparative counting with the Artek 880. Each plate was counted three times on the automatic counters using 90° rotation between counts.

Performance criteria and statistical analysis

The performance of each instrument was statistically evaluated by linear regression analysis of the scatter plot data obtained from the $\log_{10}$ of the mean counts. The overall regression data and the data from low (30-100), middle (101-200) and high (201-300) counting ranges of each comparative study were subjected to Analysis of Variance (ANOVA). The null hypothesis, Ho: m(Slope) = 0, was applied so that Ho would be rejected and we could assume that a linear regression existed if F was significant (*14*). ACC performance was further evaluated by calculating the percentage of ACC counts deviating by more than ± 10% of the mean manual count. We set for our laboratory a performance criterion of 90% agreement for acceptance of an automatic colony counter.

RESULTS

Figure 1 displays the scatter plot data of a comparison between the manual counts and the corresponding 3M counts. Table 1 presents a statistical

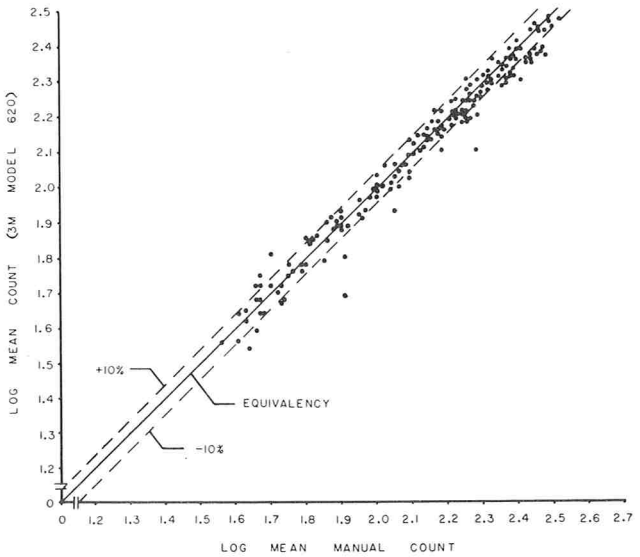

Figure 1. *Scatter plot relating plate counts determined by 3M 620 to manual technique. Linear regression equation: 3M 620 count = 0.069 + (0.960 × manual count).*

TABLE 1. *Linear regression evaluation of data comparing colony counts by 3M 620 automatic colony counter with manual counts.*

Parameters	Counting range			
	30-100	101-200	201-300	30-300
n[1]	64	81	28	173
Manual: $\overline{x}_g$[2]	63.4	147	246	117
3M 620: $\overline{x}_g$	62.5	141	230	113
r[3]	0.933	0.914	0.841	0.986
m[4]	0.957	0.939	0.791	0.960
b[5]	0.071	0.116	0.470	0.069
ANOVA (F)[6]	S	S	NS	S
EMS[7]	0.002	0.001	0.001	0.002
No. unacceptable ACC counts[8]	16	12	14	42
(%)	(25.0%)	(14.8%)	(50.0%)	(24.3%)

[1]n, number of observations.
[2]$\overline{x}_g$, geometric mean.
[3]r, correlation coefficient.
[4]m, slope of regression line.
[5]b, Y intercept.
[6]F, S - significant; NS - not significant.
[7]EMS, error mean square.
[8]Unacceptable arithmetic mean counts by ACC exceed ± 10% of corresponding mean manual count.

summary of the 3M linear regression data obtained for three arbitrary counting ranges, 30-100, 101-200, 201-300, and overall, 30-300. All the correlation coefficients (r) were significant ($a = 0.05$), indicating that the counts obtained by the 3M were meaningfully related to the corresponding manual counts. Analysis of the regression coefficients (m or slope) by ANOVA showed that there was a linear relationship between the two counting techniques overall; however, the F value was not significant in the 201-300 counting range indicating a non-linear relationship in this range. Although the overall arithmetic mean count obtained by the 3M 620 fell within ± 10% of the mean manual count, 24.3% of the individual 3M counts exceeded the 10% limit of the corresponding manual count.

Figure 2 shows the scatter plot data of a comparison between $\log_{10}$ Artek 480 and manual counts. Table 2 is a statistical summary of the linear regression data displayed in Fig. 2 and the data from a second study comparing the manual counts with those obtained by the 3M 620. The correlation coefficients for the manual versus the Artek 480 were significant ($a = 0.05$); however, analysis of the regression coefficients by ANOVA for each counting range and overall were not significant, indicating that the relationship between the manual and Artek 480 counts was not truly linear. Furthermore, although the overall arithmetic mean count obtained by the Artek 480 fell within ± 10% of the arithmetic mean manual count, 47.7% of the individual mean Artek counts fell outside the ± 10% limit for the corresponding manual counts. The results for the 3M 620 in this study were similar to those in Table 1.

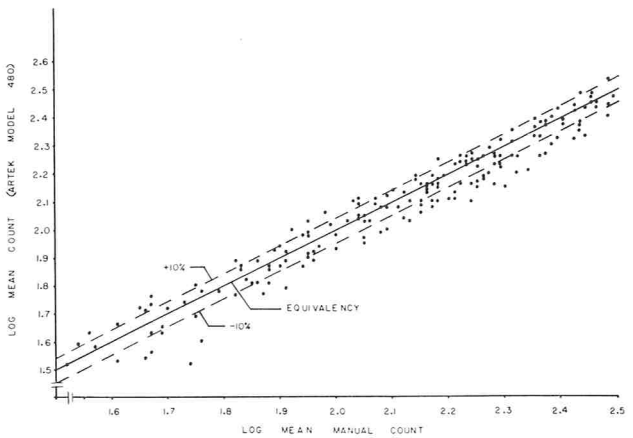

Figure 2. *Scatter plot relating plate counts determined by Artek 480 to manual technique. Linear regression equation: Artek 480 counts = 0.148 + (0.924 × manual count).*

Fig. 3 illustrates, by scatter plot, the relationship between the corresponding Artek 870 and manual counts. Table 3 presents a statistical summary of the data from the Artek 870 study as well as data from additional studies of the 3M 620 and Artek 480 with the same manual counts for comparison. The correlation coefficients for the Artek 870 versus the manual counts were all statistically significant. Analysis of the regression coefficients by ANOVA illustrated that a linear relationship existed between the automated and manual counts within the individual arbitrary counting ranges but not overall. A comparison of the arithmetic mean counts indicated close agreement between the Artek 870 and manual technique but 36% of the individual counts by the Artek 870 varied by more than 10% of the corresponding manual counts.

The statistical conclusions for the 3M 620 data in this study were similar to those presented for the data from Tables 1 and 2, except that a non-significant F value was calculated for the 30-100 colony count range in addition to the previously observed 201-300 range. Also the number of individual counts by the 3M unit which varied

TABLE 2. *Linear regression evaluation of data comparing colony counts by 3M 620 and Artek 480 automatic colony counters with manual counts.*

Parameters	Counting range 30-100	101-200	201-300	30-300
n[1]	60	71	22	153
Manual: $\bar{x}_g$[2]	62.0	146	249	113
3M 620: $\bar{x}_g$	63.4	141	234	111
r[3]	0.913	0.892	0.866	0.982
m[4]	0.857	0.891	0.831	0.924
b[5]	0.266	0.222	0.378	0.148
ANOVA (F)[6]	S	S	NS	S
EMS[7]	0.003	0.001	0.001	0.002
No. unacceptable 3M 620 counts[8]	23	10	10	43
(%)	(38.3%)	(14.1%)	(45.5%)	(28.1%)
Artek 480:				
$\bar{x}_g$	59.9	138	227	107
r	0.858	0.793	0.671	0.965
m	0.875	0.861	0.878	0.946
b	0.209	0.273	0.253	0.086
ANOVA	NS	NS	NS	NS
EMS	0.005	0.003	0.004	0.004
No. unacceptable Artek 480 counts	31	31	11	73
(%)	(51.7%)	(43.7%)	(50.0%)	(47.7%)

1,2,3,4,5,6,7 and 8 - See Table 1.

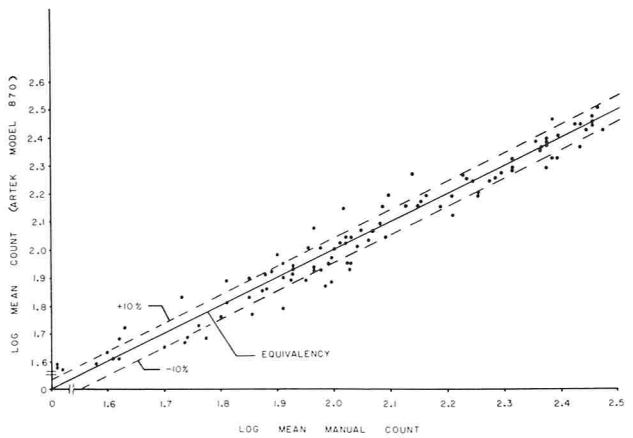

Figure 3. *Scatter plot relating plate counts determined by Artek 870 to manual technique. Linear regression equation: Artek 870 count = 0.475 + (0.936 × manual count).*

by more than 10% of the manual counts increased to 37.3% although the overall mean counts remained comparable.

Similarly, the data for the Artek 480 presented in Table 3 indicated a change in machine performance as a non-significant F value was determined for all three counting ranges but not overall. The overall arithmetic mean counts remained comparable to the mean manual counts, but only 37.3% of the individual counts exceeded ± 10% of the corresponding manual counts, a drop of 12% compared to the results in Table 2.

Figure 4 displays the scatter plot data of the comparison between the $\log_{10}$ Artek 880 and manual

TABLE 3. *Linear regression evaluation of data comparing colony counts by 3M 620, Artek 480 and Artek 870 automatic colony counters with manual counts.*

Parameters	Counting range 30-100	101-200	201-300	30-300
n[1]	26	25	24	75
Manual: $\bar{x}_g$[2]	57.8	140	249	124
3M 620 $\bar{x}$[3]	60.7	137	233	122
r[4]	0.955	0.962	0.762	0.991
m[5]	0.916	0.856	0.810	0.918
B[6]	0.170	0.300	0.427	0.166
ANOVA (F)[6]	NS	S	NS	S
EMS[7]	0.002	0.001	0.001	0.001
No. unacceptable 3M 620 counts[8]	13	5	10	28
(%)	(50.0%)	(20.0%)	(41.7%)	(37.3%)
Artek 480:				
$\bar{x}_g$	60.5	138	245	124
r	0.953	0.900	0.663	0.987
m	0.893	0.837	0.857	0.943
b	0.201	0.343	0.335	0.120
ANOVA	NS	NS	NS	S
EMS	0.002	0.002	0.002	0.002
No. unacceptable Artek 480 counts	13	8	7	28
(%)	(50.0%)	(32.0%)	(29.2%)	(37.3%)
Artek 870:				
$\bar{x}_g$	60.9	144	242	126
r	0.951	0.847	0.668	0.986
m	0.903	0.811	0.771	0.936
b	0.526	0.751	0.869	0.475
ANOVA	NS	NS	NS	S
EMS	0.002	0.003	0.002	0.002
No. unacceptable Artek 870 counts	12	7	8	27
(%)	(46.2%)	(28.0%)	(33.3%)	(36.0%)

1,2,3,4,5,6,7 and 8 - See Table 1.

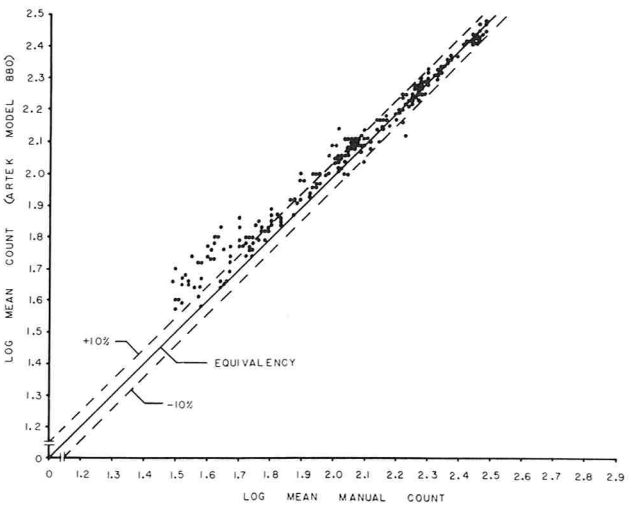

Figure 4. *Scatter plot relating plate counts determined by Artek 880 to manual technique. Linear regression equation: Artek 880 count = 0.304 + (.866 × manual count).*

counts. This figure clearly indicates a closer agreement between manual and Artek 880 data with plate counts of more than 100 colonies per plate. The statistical summary of the data in Table 4 confirmed this observation. A non-significant F value was calculated for

Table 4. *Linear regression evaluation of data comparing plate counts by Artek 880 automatic colony counter with manual counts.*

Parameters	Counting range 30-100	101-200	30-300
n[1]	105	130	235
Manual: $\overline{x}g$[2]	54.6	163	100
r[3]	0.941	0.982	0.990
m[4]	0.826	0.913	0.866
b[5]	0.375	0.203	0.304
ANOVA (F)[6]	NS	S	S
EMS[7]	0.002	0.001	0.001
No. unacceptable ACC counts[8]	70	18	88
(%)	(66.7%)	(13.9%)	(37.5%)

1,2,3,4,5,6,7 and 8 - See Table 1.

related counts in the 30-100 range but not for the 101-300 range or the overall data. Eighty-eight out of 235 (37.4%) of the individual arithmetic mean counts exceeded ± 10% of the manual counts; however, 79.1% of these counts occurred in the 30-100 range and all were underestimated values.

DISCUSSION

The determination of the "true count", against which the performance of the ACC is measured, is critical to every ACC evaluation. Fruin and Clark (*5*) suggested that an evaluation based on a simple comparison between ACC and manual counts was not valid due to the recognized imprecision of replicate technician counts. In our investigation, certain precautions were taken to obtain pour plates with precise counts for comparative counting purposes. Plates which contained "spreaders" or "pulls", air bubbles, scratches or other artifacts were excluded because such plates were subject to individual interpretations which the ACC's were incapable of making. Furthermore, we used three different analysts for counting and rejected any plate whose counts could not be reproduced within 10% or 5% as previously described. Care was also taken to ensure that a representative number of "true count" plates in the low (30-100), middle (101-200) and high (201-300) plate count ranges were included in each evaluation.

Despite these precautions, not one of the ACC units approached our acceptability criteria that 90% of the ACC counts fall within 10% of the corresponding manual count. In separate studies, 24.3% (Table 1), 28.1% (Table 2) and 37.3% (Table 3) of the 3M 620 counts exceeded the 10% limit; 47.7% (Table 2) and 37.3% (Table 3) of the Artek 480 counts were unacceptable; 37.3% (Table 3) of the counts by the Artek 870 were in excess of the 10% limit; and 37.5% (Table 4) of the Artek 880 counts were unacceptable. The inability of these ACC counts to meet our acceptability criteria occurred in spite of the overall mean counts of each ACC being comparable to the mean manual counts.

Analysis of variance of the linear regression data for each study revealed other serious deficiencies of the ACC systems. A non-significant F value, indicating a non-linear relationship between the ACC and manual counts, was calculated for different counting ranges for each unit. This finding indicated that ACC counting accuracy was not just a function of population density, but reflected the influence of other parameters. The inherent problem of having to mask the rim of the petri dish and consequently determine the correct compensation for bacterial colonies excluded from the counting area has been a major concern. Applying correction factors based on statistical considerations was originally recommended by Mansberg (*9*). Malligo (*8*), Fruin and Clark (*5*) and Van Reusel et al. (*13*) suggested that the corrected counts for the ACC be determined by extrapolation from a linear regression equation derived from ACC versus manual counts over a series of counting ranges. Every ACC examined in our laboratory, however, employed a simple multiplicity factor to correct the ACC count. The 3M 620 had a fixed masked loss of 17% with a corresponding built-in correction factor. The Artek 480, 870 and 880 had adjustable camera diaphragms or electronic apertures with flexible compensation factor dials. Correction of the ACC counts based only on uniformly applied multiplicity factors, regardless of the calculation method used, is predicated on false assumptions. This simple approach assumes that the response of the ACC is just a function of plate population density when, in fact, the ACC count is also influenced by colony distribution, plate orientation, medium/colony contrast, instrument calibration and the intrinsic resolution of the scanning system (*13*).

The low count range (30-100) data from the Artek 880 study were re-evaluated (data not shown) using ACC counts reduced by 11% (compensation factor). The percentage of Artek 880 counts which fell within ± 10% of the corresponding mean manual values increased from 33% (compensated) to 61% (without compensation). The improved accuracy of the low range counts, without compensation, illustrated the significance of colony distribution. With a small plate population, the probability of colonies developing in the masked area was low and thus a compensation factor was not required. However, the inability of the Artek 880 to accurately count 40% of the low range plates despite the removal of the compensation factor reflected the influence of the other, previously described, parameters. In addition, the confidence limits for the slope of the regression line calculated for the re-evaluated data did not include 1, indicating a lack of equivalency between the Artek 880 and the corresponding manual counts. A representative from the Artek Corporation also discovered that the upper tuning window on our Model 880 was too finely set at the factory. Consequently, noise factors and dust particles on the base plate were being sporadically counted, particularly with low range count plates, resulting in irregularly elevated counts.

Our scatter plot data showed an unbiased distribution of the ACC counts about the line of equality with the exception of the low range counts using the Artek 880. Similar distribution patterns have been demonstrated

only by Van Reusel et al. (*13*). Reports by other investigators indicated that the ACC counts were either uniformly greater than the manual counts (*4,10*) or always underestimated the manual count (*3,5,6*). Such contradictory results suggest not only variation in experimental design, but also failure on the part of the manufacturers to provide proper guidance, direction and precautionary controls in instrument calibration to the electronically naive operator.

The Artek Systems Corporation has recently introduced the Artek Compu-Print 700, a card-programmable calculator with a print-out base, which can be integrated with the Artek 880 counter to provide statistically corrected counts automatically. The value of this additional unit for improving the performance of the Artek 880 counter, particularly in the lower counting range, needs to be assessed.

Commercial ACC systems are a distinct improvement in electronic circuitry, optics and aesthetic design over the original unit described in 1957. Despite these improvements, many of the deficiencies inherent in the ACC system described by Mansberg have not been resolved. Our study concludes that ACC systems compromise accuracy for speed and precision. Although ACC systems do not have human foibles, their performance is greatly influenced by human factors.

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Growth of *Clostridium perfringens* in Three Different Beef Media and Fluid Thioglycollate Medium at Static and Constantly Rising Temperatures[1]

R. R. WILLARDSEN, F. F. BUSTA* and C. E. ALLEN

Department of Food Science and Nutrition and Department of Animal Science
University of Minnesota, St. Paul, Minnesota 55108

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ABSTRACT

Growth of an eight-strain composite of *Clostridium perfringens* was compared using rolled raw beef strips, raw and autoclaved ground beef and Fluid Thioglycollate medium. The inoculated media were exposed to either static or dynamic temperatures increasing at linear rates. The most rapid growth and shortest lag times were observed in autoclaved ground beef. Generation times in the beef media were directly related to oxidation-reduction potential. Fluid Thioglycollate medium supported the slowest growth even though it presented a favorable E_h.

Presence of *Clostridium perfringens* in food products is of major concern to the food industry. Beef has been identified as one food frequently associated with this foodborne pathogen (*5,9,10*). Hall and Angelotti (*4*) observed 70% contamination of the raw, unprocessed beef supply in the Cincinnati area. Each year, numerous cases of *C. perfringens* foodborne illness are attributed to roast beef (*2,3*). Mishandling of beef roasts after cooking is generally assumed to be responsible for growth of the organisms. Recent research has shown that *C. perfringens* can grow to large numbers in autoclaved ground beef exposed to temperature rises analogous to the interior of beef roasts during long-time, low-temperature (LTLT) cookery (*13*). This work suggested that *C. perfringens* may be able to grow in the interior of contaminated beef roasts during LTLT cooking procedures.

Because of the high incidence of *C. perfringens* in beef and the rapid growth in autoclaved ground beef with generation times as low as 7.1 min (*13*), growth of *C. perfringens* was evaluated in raw and autoclaved ground beef and in intact, rolled raw beef strips at both static and constantly rising temperatures. Results from these beef media were also compared to those from Fluid Thioglycollate medium, a synthetic medium commonly used to evaluate growth of anaerobic microorganisms.

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MATERIALS AND METHODS

Test organisms

C. perfringens strains NCTC 8238, NCTC 10240 and ATCC 3624 were obtained from D. A. Adams, North Carolina State University, Raleigh, NC; NCTC 8798 was obtained from R. G. Labbe, University of Wisconsin, Madison, WI; NCTC 8239, NCTC 9851, S-40 and S-45 were obtained from the culture collection, University of Minnesota, St. Paul, MN.

Culture preparations

Stock cultures were prepared by transferring a 0.1-ml inoculum into 10-ml of Cooked Meat Medium (BBL) and incubating at 37 C for 20 h. Stock cultures were maintained by aseptically adding 10% sterile glycerol (Mallinckrodt Chemical Works, St. Louis, MO) and storing at - 20 C. Test cultures were prepared by transferring 0.1 ml of a freshly-thawed stock culture to 15 ml of laboratory-prepared Fluid Thioglycollate medium (FTG) and incubating at 37 C for 20 h.

After incubation, a composite containing all eight strains was prepared by aseptically pipetting a 1.0-ml portion of 20-h-old FTG culture of each individual strain into a sterile 25 × 150-mm screw-capped test tube and mixing for 10 sec (Maxi-Mix, Model No. M-16715, Thermolyne, Dubuque, IA). The composite was diluted in 0.1% peptone to approximately 2×10^5 cfu/ml (colony forming units per milliliter). A 0.1-ml inoculum (2×10^4 total cfu) was injected with a 13-cm, 15-gauge needle into each growth medium.

Growth media

Inside-round beef roasts (3.4 kg) were purchased from a commercial supplier and sliced into 1.5-2.5-mm thick slices on a meat slicer (Globe Slicing Machine Co., Stamford, CT). The previously frozen beef roasts were partially thawed for 17-20 h at 2 C before slicing. The partially frozen roasts were of the proper texture to give uniform slicing characteristics. Roast slices were quickly refrozen at - 20 C and stored for not more than 5 weeks. Before use, roast slices were thawed and cut into strips approximately 5 cm × 7 cm. These strips were manually rolled into a cylindrical shape (approximately 1.5 × 5 cm) and inserted into a 7.5 × 18-cm Whirl-Pak sample bag (Nasco, Fort Atkinson, WI). The rolled raw beef strips were then inoculated at three separate internal locations along the cylindrical axis to facilitate internal distribution of the inoculum.

The ground beef was prepared in the laboratory from commercially-obtained beef chucks using 9.5-mm and 3.2-mm grinder plates in succession. The ground beef was frozen at - 30 C for a maximum of 8 months. Two batches (fat content 13% and 14%, pH 6.0 and 6.3) were used during this research. No differences in growth of the test organisms were observed among the two batches of fresh ground beef and the frozen and stored ground beef. A 15-g portion of ground beef was manually rolled into a cylindrical shape, tamped with a glass rod

(8-mm diameter) into 25 × 150-mm screw-capped test tubes (Pyrex No. 9825). The autoclaved ground beef medium was autoclaved at 121 C for 15 min. The tubes were allowed to cool overnight at room temperature and tempered before use. Raw ground beef medium was placed in tubes immediately before use to avoid growth of any microorganisms present.

The FTG contained Trypticase (BBL), 15 g; yeast extract (BBL), 5 g; sodium chloride (Fisher Scientific Co., Fairlawn, NJ), 2.5 g; agar (Difco), 0.75 g; L-cystine (Fisher Scientific Co., Fairlawn, NJ), 0.5 g; sodium thioglycollate (BBL), 0.5 g; distilled water, 1 liter. The FTG used for test culture-preparation was freshly prepared each week, stored in 13 × 150-mm screw-capped test tubes and steamed (100 C) for 10 min before use. FTG used for comparative studies was freshly prepared before each use and was incubated in 25 × 150-mm screw-capped tubes.

Experimental design

Rolled raw beef strips, raw and autoclaved ground beef and FTG were inoculated with an eight-strain composite of *C. perfringens*. The composite was used to minimize variability among strains. The inoculated media were exposed to either static temperatures or dynamic temperatures which increased linearly. Duplicate trials were made at each static temperature and rising temperature rate. Samples were examined at intervals no greater than 20 min.

Growth of the *C. perfringens* eight-strain composite was examined at a static temperature of 45 C in all media and at 41 C in rolled raw beef strips or in raw or autoclaved ground beef. Growth of the composite was compared in each medium at a rising temperature rate of 7.5 C/h. Growth of the composite in rolled raw beef strips was also examined at rising temperature rates of 6.0 C/h and 8.5 C/h.

Generation times at static temperatures were calculated from the slope of the regression line of the exponential phase of growth. Mean generation times during most rapid growth (MGT_{mrg}), or mean time to double the population during most rapid growth at changing temperatures, were determined according to Willardsen et al. (*13*).

The E_h was determined in each medium at a rising temperature rate of 10.5 C/h to relate the oxidation-reduction potential of each medium to the growth of *C. perfringens*.

Apparatus

A constant-temperature water bath (Blue M Model MW-1140E-1) was supplemented with a variable wattage heater-circulator (Haake Model E-52) to allow control of both static temperatures and rising temperature rates. Water bath temperatures were monitored on a strip chart temperature recorder (Honeywell Model 153X64P16-X-41). An electronic digital thermometer (Fluke Model 2100A) was used to measure all temperatures of the samples reported in this study.

Enumeration medium

The enumeration medium was laboratory-prepared Tryptose Sulfite Cycloserine (TSC) agar containing Tryptose (Difco), 15 g; yeast extract (BBL), 5 g; Soytone (Difco), 5 g; sodium meta-bisulfite (Matheson, Coleman and Bell, Norwood, OH), 1 g; ferric ammonium citrate (Mallinckrodt Chemical Works, St. Louis, MO), 1 g; agar (Difco), 20 g; distilled water, 900 ml. The TSC agar was prepared according to methods of Willardsen et al. (*13*).

Enumeration procedure

At the appropriate sampling time, each of the samples with the exception of rolled raw beef strips was aseptically transferred to an 18 × 30-cm, 3 ½-mil polyethylene Stomacher bag using a sterile 23-cm chrome letter opener. The sample was diluted to 100 ml with sterile distilled water (85 ml). Rolled raw beef strips were transferred to a sterile graduated cylinder, diluted to 100 ml with sterile distilled water and transferred to a Stomacher bag. Each sample was mascerated for 60 sec in a Colworth 400 Stomacher (*7*). Serial dilutions were made into 9.9 ml of 0.1% peptone (Difco), and a 0.1-ml portion of the appropriate dilution was spread over the surface of TSC agar and overlaid with TSC agar without egg yolk. The inoculated plates were incubated at 37 C for 24 h in anaerobe jars (BBL GasPak System) which were evacuated to 15 inches of Hg and flushed to 5 psi with a 90% N_2-10% CO_2 mixture three times.

E_h determination

The E_h of each medium during a rising temperature rate of 10.5 C/h was measured with an Orion Research pH meter (Model 601) having a platinum inlay electrode (Corning 476060) and the reference junction of a triple purpose combination glass electrode (Corning 476051). Phthalate buffer at ph 4.0 saturated with quinhydrone was used to standardize the equipment (*11*). Laboratory-prepared test tubes (30 × 100 mm) were used for electrode placement in the samples. Raw beef strips were rolled around the electrodes and both were inserted into the test tubes. FTG and raw and autoclaved ground beef were placed in test tubes and autoclaved if appropriate. The electrodes were then inserted into the media. Each medium was then inoculated with 0.1 ml of the *C. perfringens* eight-strain composite.

RESULTS

Static temperatures

Comparisons of lag times and generation times of an eight-strain composite of *C. perfringens* in rolled raw beef strips, raw and autoclaved ground beef and FTG appear in Table 1. Lag times ranged from 1.3 h in autoclaved ground beef to 2.0 h in rolled raw beef strips at 41 C. Fluid Thioglycollate medium was not examined at 41 C. Lag times observed at 45 C ranged from 1.2 h in autoclaved ground beef to 1.9 h in FTG.

Generation times observed at 41 C ranged from 8.8 min in autoclaved ground beef to 11.4 min in rolled raw beef strips. At 45 C, the generation times in raw ground beef was 8.5 min compared to 12.2 min in FTG.

Dynamic temperatures

A representative temperature-based growth curve for *C. perfringens* at constantly rising temperatures is presented in Fig. 1. The data show growth of a composite of eight strains in raw ground beef during a temperature rise of 7.5 C/h. Changes in population (cfu/g at any sampling time divided by initial cfu/g or N/N_0) are plotted against temperature. The elapsed time from initiation of heating (25 C initial temperature) is presented for comparison on the upper axis. No growth was observed during the initial temperature increase until approximately 40 C (2 h) was reached. Population

TABLE 1. *Generation times (G_t) and lag times of* C. perfringens *eight-strain composite in various media.*

Medium	41 C		45 C	
	G_t (min)[a]	lag (h)	G_t (min)	lag (h)
Autoclaved ground beef	8.8	1.3	8.9	1.2
Raw ground beef	8.9	1.8	8.5	1.4
Rolled raw beef strips	11.4	2.0	11.2	1.7
Fluid Thioglycollate medium			12.2	1.9

[a]Generation times calculated from regression lines for exponential growth.

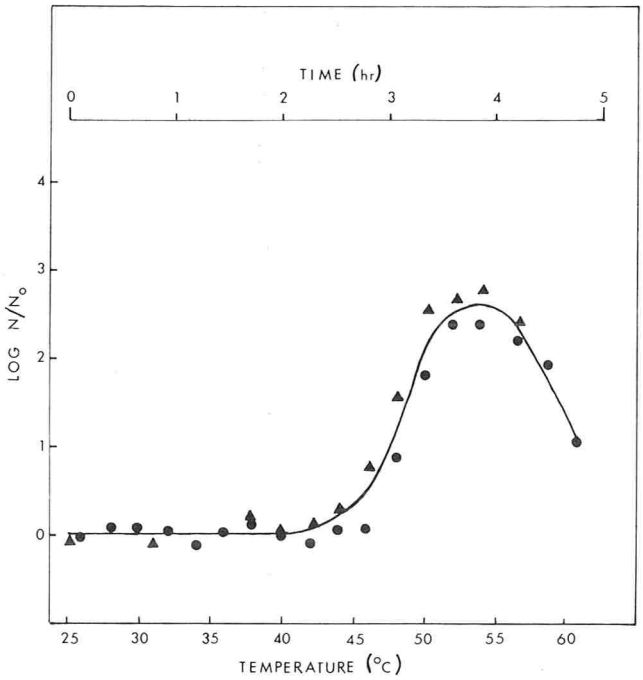

Figure 1. *Growth and survival of* Clostridium perfringens *eight-strain composite in raw ground beef at a constantly rising temperature rate of 7.5 C/h. Duplicate trials indicated by • and ▲.*

increased gradually until a maximal growth rate was observed near 48 C. Rapid growth continued to about 53 C. Temperatures above 53 C became increasingly inhibitory, and inactivation of the *C. perfringens* began at approximately 55 C. As the temperature rose above 55 C, inactivation occurred at an increasing rate.

Figure 2 presents data on growth and survival of *C. perfringens* in the experimental media at a rising temperature rate of 7.5 C/h. An initial population of approximately 10^3 cfu/g was used in all experiments. No growth was observed in any medium until temperatures of at least 36 C were reached. Growth in autoclaved ground beef was initiated near 36 C, about 1.5 h after the initial 25 C. Growth was initiated at 41 C (2.1 h) in raw ground beef and at approximately 43 C (2.4 h) in both rolled raw beef strips and FTG. Growth of *C. perfringens* began to slow at 50-51 C in all of the experimental media. No growth was observed at 53-54 C in any test media. Similar inactivation characteristics were observed in all of the beef media; however, slower inactivation appeared to take place in FTG.

The MGT_{mrg} values in autoclaved and raw ground beef at a rising temperature rate of 7.5 C/h were 8.1 and 6.2 min, respectively (Table 2). These values were similar but shorter than the generation times obtained in these media at 41 and 45 C, indicating that dynamic rising temperatures did not adversely affect growth of the organisms. The MGT_{mrg} calculated for rolled raw beef strips at 7.5 C/h (8.2 min) was also shorter than the generation times for this medium at 41 C (11.4 min) and 45 C (11.2 min). The MGT_{mrg} in FTG (11.0 min) was slightly less than the generation time observed in FTG at 45 C (12.2 min).

TABLE 2. *Mean generation times for most rapid growth (MGT_{mrg}) of* C. perfringens *eight-strain composite at selected rising temperature rates in various media.*

Growth medium	Rising temperature rate (C/h)	MGT_{mrg} (min)
Autoclaved ground beef	7.5	8.1
Raw ground beef	7.5	6.2
Fluid Thioglycollate medium	7.5	11.0
Rolled raw beef strips	7.5	8.2
	6.0	9.9
	8.5	9.6

Inside round beef roasts were ground in a manner identical to the beef chucks and prepared with and without added fat. Limited data (not presented) in a comparison of growth of *C. perfringens* in raw or autoclaved ground beef made from chuck roast or inside round with or without fat added indicated small differences in populations observed at six samplings (25, 37, 43, 48, 52, 59 C) when exposed to constantly rising temperatures of 6.0 C/h. The data obtained with ground rounds vs. ground chucks could not account for differences observed between ground chuck and rolled beef strips. When growth of *C. perfringens* in raw ground round with fat was compared to growth in strips from the same round, a relationship comparable to raw ground beef and rolled raw beef strips (Fig. 2) was observed.

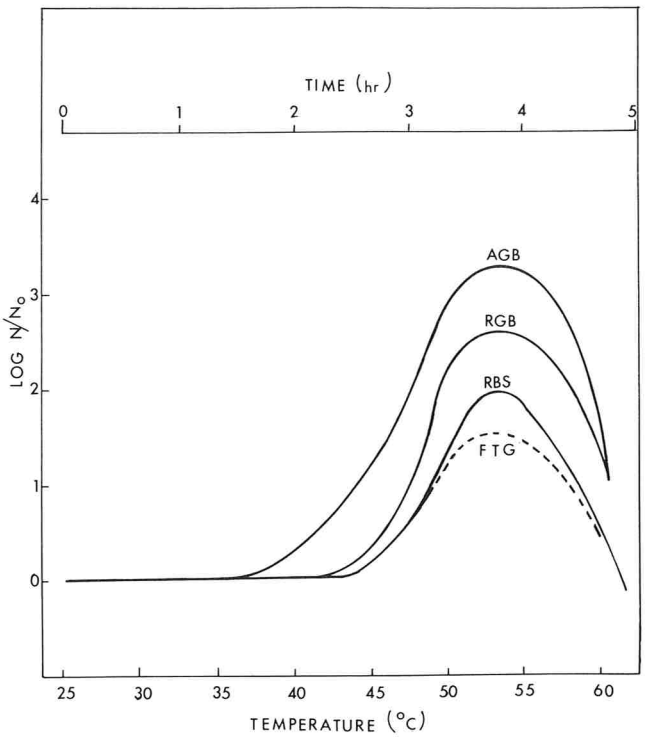

Figure 2. *Growth and survival of the* Clostridium perfringens *eight-strain composite in autoclaved ground beef (AGB) raw ground beef (RGB), raw beef strips (RBS) and Fluid Thioglycollate medium (FTG) at a rising temperature rate of 7.5 C/h.*

Growth and survival of the *C. perfringens* eight-strain composite in rolled raw beef slices were also examined at rising temperature rates of 6.0 C/h and 8.5 C/h (Fig. 3). Growth was initiated near 38 C at 6.0 C/h and 46 C at 8.5 C/h. The MGT_{mrg} was 9.9 min at 6.0 C/h and

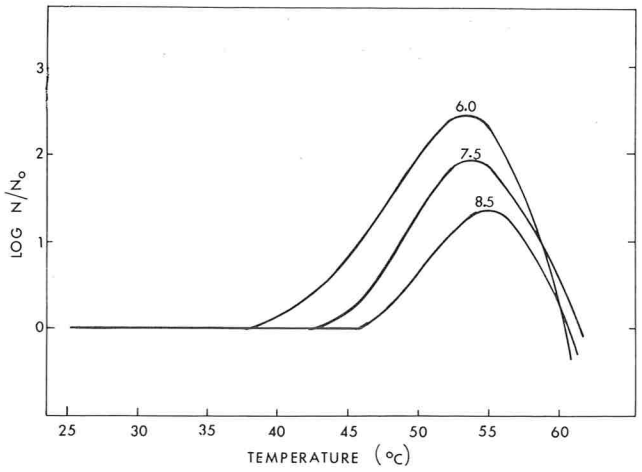

Figure 3. *The influence of three constantly rising temperature rates on the growth of the* Clostridium perfringens *eight-strain composite in rolled raw beef strips.*

9.6 min at 8.5 C/h. Growth ceased at 53, 54 and 55 C at 6.0 C/h, 7.5 C/h and 8.5 C/h, respectively. Inactivation appeared to proceed more rapidly at 6.0 C/h than at either 7.5 C/h or 8.5 C/h. Residence time at lethal temperatures was longer at slower heating rates. For instance, when 60 C is reached at rising temperature rates of 6.0 C/h and 8.5 C/h, more time was spent in the lethal temperature range with the slower rate of 6.0 C/h.

Figure 4 presents data on changes in E_h in the various inoculated media during a rising temperature rate of 10.5 C/h. The oxidation-reduction potential of both rolled raw beef strips and raw ground beef showed a definite decrease, while the oxidation-reduction potential of autoclaved ground beef and FTG remained relatively constant. The E_h of rolled raw beef strips decreased from 312 mv at 25 C to 123 mv at 62 C. In raw ground beef, the E_h decreased from 112 mv to -6 mv. The E_h of autoclaved ground beef decreased from 0 mv to -45 mv while the E_h of FTG increased from -30 mv to -15 mv. The autoclaved ground beef used for growth comparisons

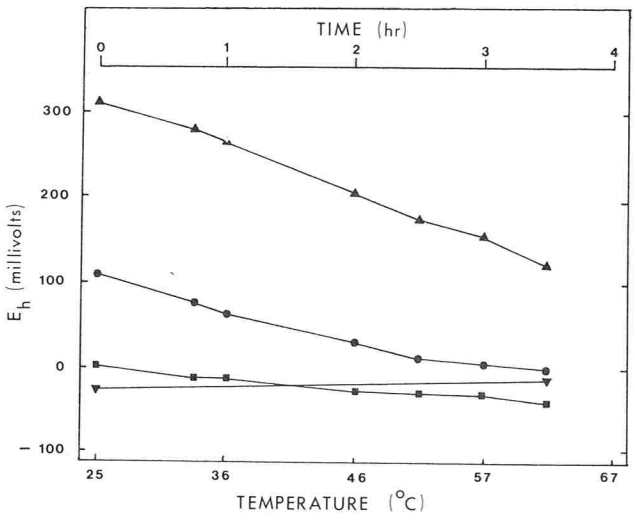

Figure 4. *The E_h of various media during a temperature rise of 10.5 C/h (autoclaved ground beef,* ■ *; raw ground beef,* ● *; raw rolled beef strips,* ▲*; Fluid Thioglycollate medium,* ▼*).*

retained a stable E_h during overnight storage at room temperature.

DISCUSSION

This study examined growth of *C. perfringens* at both static temperatures and at dynamic temperatures rising at linear rates that could represent the temperature in the interior of beef roasts cooked by long-time, low-temperature processes. Historically, growth of microorganisms has been characterized and compared at static temperatures. However, food products usually do not retain static temperatures during preparation, processing and holding.

The growth response of *C. perfringens* in rolled raw beef slices varied at identical temperatures during different rates of temperature increase. Willardsen et al. (*13*) demonstrated that growth of *C. perfringens* at any instantaneous temperature during a temperature change may not be equivalent to the growth response at the identical static temperature. This suggests that growth and survival of microorganisms in a dynamic temperature environment such as cooking or cooling of a food product may not be accurately characterized by growth and inactivation evaluations at static temperatures.

Because of the high incidence of *C. perfringens* contamination of raw beef (*1,4,5,9,10*) and the extremely rapid generation time exhibited by this organism in autoclaved ground beef (*13*), it was essential to select an environment that imitated the interior of a rolled beef roast. The rolled raw beef strips served as this environment for evaluation of *C. perfringens* growth. Rapid growth in rolled raw beefs strips was observed at the static temperatures and the dynamic temperatures examined. This approach using rolled raw beef strips is compatible with the method for containment of microbial populations in beef roasts or other food systems as reported by Willardsen et al. (*12*). Similar growth could be expected in contaminated interiors of beef roasts with an analogous temperature profile. Internal heating rates for rolled, punctured and mechanically-tenderized as well as whole beef roasts should be evaluated to prevent potential microbial hazards. The authors strongly suggest that future work evaluating microbial changes in food systems undergoing a dynamic temperature process such as cooking, cooling and reheating be done in the appropriate changing temperature environment rather than at static temperatures.

The comparison of growth in the beef media shows that autoclaved and raw ground beef supported extremely rapid growth of *C. perfringens* and that generation times in intact rolled raw beef strips were only slightly greater than in the ground beef media. Growth in FTG was slower and the lag times were longer than in any beef medium. Nutritional inadequacies or the presence of sodium thioglycollate in FTG may have prevented optimal growth.

In the beef media, generation and lag times of *C. perfringens* were directly related to the initial E_h. This suggests that the oxidation-reduction potential of the

beef media may have influenced the observed growth rates. Numerous studies on the effect of oxidation-reduction potentials on growth of *C. perfringens* have been reported; however, little agreement has been reached concerning the limiting E_h value (*6,8,11*), although it is generally assumed to be ca. 350 mv at pH 7 (*8*). These studies have shown that, within limits, the lower the E_h value, the better the growth of *C. perfringens*. The continuous drop in E_h in raw ground beef and rolled raw beef strips could be explained by a decreased oxygen solubility as temperatures increased. Autoclaved ground beef and FTG were autoclaved or steamed before use, resulting in expulsion of most of the oxygen present and generation of reducing substances.

Another explanation of the growth trends in the beef media is the physical modification each medium had undergone. Growth of the organisms was directly related to the amount of processing involved in each medium. Autoclaved ground beef, having been both ground and autoclaved, supported the most rapid growth and shortest lag times. Autoclaving had caused some expulsion of water and nutrients from the beef and denaturation of proteins. Rolled raw beef strips, which received little modification, supported the slowest growth of *C. perfringens* in the beef media tested.

Growth in FTG was slower than in the beef media although the initial E_h of FTG was lower. Even though an advantageous E_h was present in FTG, it did not present as favorable a growth environment as did beef. Because the beef media examined can realistically represent many foods and FTG cannot, beef may be a more appropriate medium for evaluation of growth of *C. perfringens* in relation to foods. However, in studies evaluating the contribution of individual food components to microbial growth characteristics, use of FTG or other synthetic media may be necessary.

The rapid growth of *C. perfringens* in the beef media at both static and dynamic temperatures indicates the potential for growth during long-time, low-temperature (LTLT) cookery of beef. During certain heating rates, a rolled or mechanically-tenderized roast with interior contamination could support growth of *C. perfringens*. If a lethal temperature is not reached in the roast, foodborne illness could occur. It was not the intention of this paper to include recommendations on safe rates for beef cookery; however, continued research and computer modeling are presently underway to select proper beef cooking parameters.

ACKNOWLEDGMENTS

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Survival of *Lactobacillus acidophilus* in a Spray-Drying Process [1]

FERNANDO ESPINA[2] and V. S. PACKARD*

Department of Food Science and Nutrition, University of Minnesota, St. Paul, Minnesota 55108

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ABSTRACT

A frozen concentrate of *Lactobacillus acidophilus* was used as inoculum for milk solids-not-fat (MSNF) reconstituted to 25 and 40% solids. Initial count of the two milks was 1.2×10^9 and 7.0×10^8 CFU/g of solids, respectively. Sublots of these two concentrates were spray-dried at 85-, 80-, and 75-C exit air temperature in a Coulter/Townley pilot dryer (vertical, venturi nozzle spray system). Survival of *L. acidophilus* was greatest at the lowest outlet air temperature investigated, and in the milk of lower solids content. At 75-C exit air temperature the count following drying was 2.6×10^7 per gram of solids at 40 percent solids, and 9.8×10^7 per gram of solids at 25% solids. Percent survival after 30 days storage under nitrogen at 4 C was 1.29 and 4.17, respectively, for the two solids levels.

Evidence is increasing regarding the potential of *Lactobacillus acidophilus* to ameliorate a number of digestive upsets (*4,5,10,11,12,14*). While the data are inconclusive, the organism in sufficient numbers appears to have the ability to aid lactose digestion among persons known to suffer a degree of lactose intolerance. While the latter problem appears not so severe as to restrict milk intake altogether, it causes various degrees of discomfort when sizeable amounts of milk are ingested on an empty stomach. Most of the world's population, Latin Americans and Asians included, are sensitive to this nutritional ailment. Around the world, in rich and poor nations alike, other digestive problems occur as a result of poor nutrition, some of which this organism might very well alleviate if ingested in regular amounts on a daily basis. For these and other reasons, "Sweet Acidophilus" milk is now marketed throughout most of the U.S. Current information suggests that an intake of about one billion viable cells of the organism daily will serve the maintenance requirements in the human body (*6,13*). To achieve this level of intake on a practical basis requires some 2-7 million viable cells per ml of fluid milk. At least this is the regulatory standard now being considered (*8*).

Obviously a fluid milk product has limited shelf life and is not readily transportable to distant markets at low cost. A dry product could better serve these needs, if sufficient numbers of *L. acidophilus* could survive both spray-drying and storage. In Chile, such a product could serve as an especially useful purpose, in that milk production is localized in the southern part of the country with much of the population located between 1,000 and 2,000 kilometers north of the supply area. Under such conditions, and for transportation over long distances, a dry product containing viable *L. acidophilus* cells could be processed in the milk supply area, then shipped to consumption centers, either to be reconstituted into a Sweet Acidophilus - type milk and sold on a "fresh" liquid basis, or for consumer reconstitution of a packaged dry product.

The main question at issue is survival of the organism during spray drying and storage in numbers and in such viable condition as to make possible the maintenance level of intake through a milk product. Apparently, work in this area has been discouraged, generally because of evidence of poor survival of *L. acidophilus* in the dry state at room temperature (*2*). It may likewise have been discouraged by past assumptions that very large numbers of organisms were necessary to achieve an appropriate ratio of *L. acidophilus* to other intestinal micro-organisms. Smaller numbers do now appear to serve the purpose adequately (*13*), if not better. This may make a spray-drying process more feasible than was formerly thought. In any event, evidence of the growing importance of this organism to the human diet appeared to the authors to offer sufficient justification to determine survival during spray-drying and short-term storage. Storage of the dry product in this study was limited to holding at refrigeration temperature (4 C).

[1] *Paper No. 10327, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul.*

[2] *Current address: Centro Tecnologico de la Leche, Universidad Austral, casilla 47, Valdivia, Chile, South America.*

MATERIALS AND METHODS

Frozen concentrate of *L. acidophilus* was obtained from Miles Laboratories, Inc., Marschall Division, (Elkhart, Ind. 46514). This culture is one currently being used in production of Sweet Acidophilus milk. Though not in any way recommended, it was necessary to store the frozen culture three months before undertaking the present study. The temperature of storage was - 40 C. No doubt some viable cells were lost during this time but sufficient numbers remained to make possible this investigation.

For ease of processing, two concentrations of milk were prepared for drying purposes by reconstituting nonfat dry milk rather than condensing fresh milk. The dry milk was tested and found free of inhibitors. Two lots were made up, at 25 and 40% solids, respectively, on different days. Frozen concentrates of *L. acidophilus* were thawed, and the culture added directly to the concentrated milk. Aseptic conditions of handling the thawed culture were used. Temperature of milk during inoculation was 37 C. Each lot of milk was then subdivided into three sublots for drying in the Coulter/Townley pilot plant dryer of the University. This is a direct-fired, gas-heated, vertical-type dryer, using a spray nozzle and venturi inlet for atomizing the incoming milk. Though smaller than commercial dryers, it is large enough to provide generally equivalent drying conditions. Sublots were dried at a constant inlet temperature (170 C) and three different outlet air temperatures, 75, 80 and 85 C. A constant feed pump was used to pump the inoculated milk into the drying chamber and ultimately to a collector.

Samples of the inoculated milk were taken for microbiological examination before and immediately after drying. Dried samples were also stored either in air or under nitrogen. For the former, small, medium-density polyethylene pouches were used. For the latter, laminated polyester Saran, ionomer film (Champion Packages Div., Champion International, Minneapolis, MN) was used. This film has negligible moisture/vapor transmission. Stored samples were all held at 4 C, and examined for viable *L. acidophilus* after 30 days of storage.

The procedures for determining *L. acidophilus* was that given by Miles Laboratories, Inc., Marschall Division, using All Purpose agar with Tween 80 (APT agar). This is a non-selective agar which, for growth of *L. acidophilus*, requires anaerobic conditions and incubation at 37 C for 48 h. The method given by Miles Laboratories, Inc. was modified only in the way in which anaerobic conditions were created. For this work, anaerobic jars were used. Plates were placed in the jars, air removed by vacuum, and the jars flushed with carbon dioxide. Milk samples for *L. acidophilus* analyses were prepared according to *Standard Methods for the Examination of Dairy Products* (*15*).

RESULTS AND DISCUSSION

Data in Table 1 show the effect of three different exit air drying temperatures and two different MSNF concentrations on survival of *L. acidophilus*. In all instances, inlet air temperature was constant at 170 C. At both solids concentrations there was an expected (*8,9*) sharp decrease in numbers of survivors as the outlet air temperature was raised. At 40% MSNF, the numbers of viable organisms decreased from 7.0×10^8 to 2.6×10^7 colony forming units (CFU)/g of solids at 75 C and 3.6×10^6 and 1.8×10^6 CFU/g of solids at 80 and 85 C, respectively. At these three drying conditions greater survival was noted at 25% MSNF, though part of the increase no doubt was due to the slightly higher initial count. While an attempt was made to standardize the initial count, more than one can of commercial frozen concentrate of *L. acidophilus* was used as inoculum. It is possible that the counts in different cans differed to some extent. Cans had been stored three months before the investigation, and though deep frozen during that time, some loss in viable cells might have occurred. That such loss might have been different in different cans would depend to some extent on whether all cans of concentrate came from the same batch and were of similar age. A 2-h delay in doing the initial counts was also unavoidable. Samples taken from both 40% and 25% solids batches were held refrigerated during this time, but loss may well have taken place to a greater extent in the higher solids batch, mainly due to solids concentration per se. As can be noted in Table 1, pH was nearly the same for both batches. In any event, survival of *L. acidophilus* was higher in the lower solids milk concentrate. For the three outlet air temperatures (85, 80, and 75 C), numbers of survivors were 5.9×10^6, 2.9×10^7, and 9.8×10^7 CFU/g, respectively. Looking at the data in terms of log reductions in count (Log N_o/N), it may be seen that the reduction was lower at 25% solids than at 40% solids for any given exit air temperature.

Aside from milk concentration and the possible effect of osmotic pressure on cell survival, drying conditions would also be expected to vary with difference in solids concentration of incoming milk. Given similar infeed pressure and nozzle orifice size, higher solids milk would result in larger particles of spray. As others point out (*16*), larger particles are subjected to greater heat damage than smaller ones under any given set of drying conditions. Microorganisms entrapped in the particles would also be subjected to that much more heat. Several factors influence cell death or survival. First, as water activity (a_w) decreases at the surface of the particle, wet bulb temperatures are exceeded. It is at this point that bacteria may be subjected to killing temperatures. But it is also known that while the bacterial cells are in the intermediate moisture range, they are less sensitive to effects of heat (*1,7*). Furthermore, the surviving

TABLE 1. *Survival of* Lactobacillus acidophilus *after Spray Drying at Three Different Outlet Air Temperatures and Two Levels of Milk Solids Not Fat (MSNF).*

Concentration of skimmilk MSNF (%)	pH	Drying temperatures (C)		No. of viable cells		Log reduction of viable cells [Log $(N_o/N)^1$
		Air in	Air out	Before drying (CFU/g solids)	After drying (CFU/g solids)	
40	6.2	170	75	7.0×10^8	2.6×10^7	1.43
			80		3.6×10^6	2.29
			85		1.8×10^6	2.59
25	6.3	170	75	1.2×10^9	9.8×10^7	1.09
			85		2.9×10^7	1.52
			85		5.9×10^6	2.31

[1]Log (N_o/N) is taken as the number of viable cells of *L. acidophilus* in one gram of solids in the concentrated milk (N_o) divided by N, the number of viable cells of the organism in one gram of solids of skimmilk powder.

organisms, once dried, become somewhat more resistant to heat damage (*9*). Obviously, such factors for and against survival are interrelated during the drying process. But because large particles dry more slowly than small ones, heat damage to bacterial cells is likely to be more pronounced. Of course, drying conditions can be varied to some extent to produce smaller particles, thus faster drying. This is one factor that should be considered in further work, especially as drying efficiency is directly related to MSNF concentration of the product to be dried.

Though three drying temperatures constitute barely enough points to plot energy of activation (E_a) curves, it was thought worthwhile to determine whether or not the data are in reasonable agreement with such values for other organisms. In making the calculation, drying times are considered to be 2 sec. Figure 1 shows the plot of E_a at the two different MSNF concentrations. In both instances, activation energy was lower at higher temperatures, though the difference at 25% solids was slight; i.e., 17.4 Kcal/mole vs 19.9 Kcal/mole. Data on milk at 40% solids were 6.5 Kcal/mole and 23.3 Kcal/mole at higher and lower drying temperatures, respectively. Noting again that only three temperatures were used, there is evidence of a break in the curve, a point at which a new and different E_a is noted. Both the break and the E_a values are consistent with data of Elizondo and Labuza (*3*) for other organisms. Although the solids concentrations used in this study were different from those used by Elizondo and Labuza, the break in the curve comes at about the same temperature; i.e., 80 vs. 84 C, respectively. Nevertheless, further work over a broader range of temperatures is needed to determine whether or not the break in the curve is a true break or an artifact of these data.

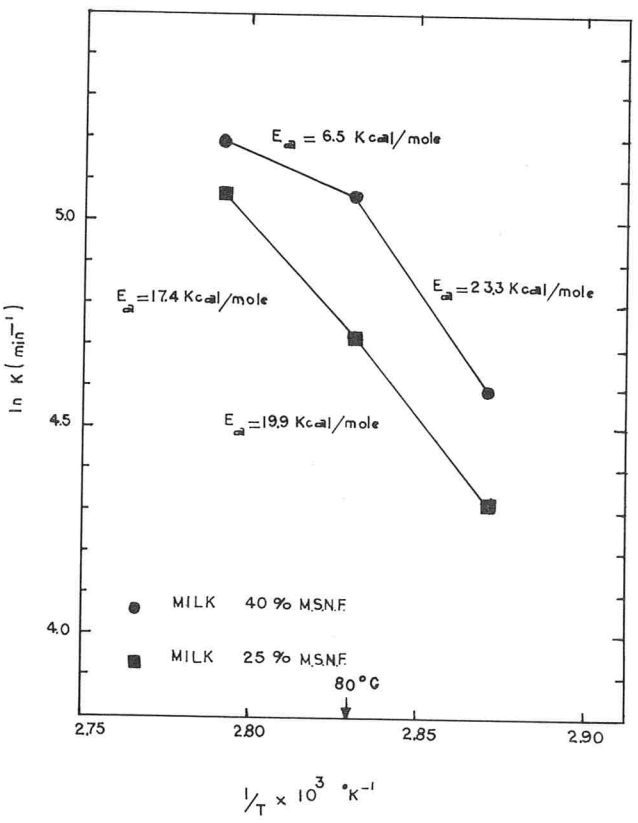

Figure 1. *Energy of activation curves for* L. acidophilus *spray dried in 25 and 40% milk solids at constant inlet air temperature.*

Data in Tables 2 and 3 show percent survival of *L. acidophilus* after 30 days of storage of the dried product at 4 C for the three different outlet air temperatures studied, and for 40 and 25% MSNF levels, respectively, Both survival in air and under nitrogen are indicated. Percent survival is expressed as percent of the original population in the concentrated milk before drying. Figures are given for percentage decrease immediately after drying and after 30 days of storage. Percent moisture in the final product, which varied because of the conditions imposed upon the drying process, ranged from higher to lower as the exit air temperature increased.

Data in both Tables 2 and 3 indicate some slight improvement in survival during storage when the product was held under nitrogen. Nonetheless, good survival, comparatively speaking, was noted in samples stored in air. To some extent, survival in air may have been improved as a result of conditions inherent in the drying system. In operation of the pilot dryer used in the study, much of the oxygen in the air is consumed. Less oxygen would be available, therefore, to cause damage to bacterial cells. Whether this effect might carry over during storage, after air has equilibrated with the powder, is another matter.

Again, major differences in total number of survivors related mainly to concentration of MSNF used in drying. While storage for 1 month resulted in about 50% loss in *L. acidophilus* organisms at any given set of drying conditions, the percentage of survivors was three to four times as great in the product dried at lower than the higher solids content. Moreover, the absolute numbers of survivors dried at 75-C outlet air and stored for 1 month were in excess of the minimal numbers necessary for achieving maintenance levels of intake; this on a dried product reconstituted to 10% solids. It remains to be determined, however, whether the organisms are still capable of growth under conditions existing in the intestinal tract. To test this potential, it would be necessary to attempt to grow the organisms under those conditions recently cited by Speck (*13*) for determining bile resistance. Obviously, much work remains to be done, both in developing the most suitable drying technology and determining the most appropriate conditions of storage. For a dried product to be of most use, potential for storage at room temperature is necessary. The work discussed herein would at least suggest that an attempt to further elucidate survival conditions for *L. acidophilus* during and after spray-drying might prove worthwhile.

TABLE 2. *Percent survival of* Lactobacillus acidophilus *spray-dried in 40% milk solids-not-fat before and after storage for 30 days at 4 C.*

Drying and storing conditions			Survival[1]			
Outlet air temp. (C)	Moisture content (%)	Atmos-phere	CFU/g of solids	After drying (%)	CFU/g of solids	After 30 days (%)
75	5.2	air	2.6×10^7	3.71	8.3×10^6	1.19
		N_2			9.0×10^6	1.29
80	4.3	air	3.6×10^6	0.51	2.5×10^6	0.36
		N_2			2.9×10^6	0.41
85	3.5	air	1.8×10^6	0.26	8.0×10^5	0.11
		N_2			9.5×10^5	0.14

TABLE 3. *Percent survival of* Lactobacillus acidophilus *spray-dried in 25% milk solids-not-fat before and after storage for 30 days.*

Drying and storage conditions			Survival[1]			
Outlet air temp. (C)	Moisture content (%)	Atmos-phere	CFU/g of solids	After drying (%)	CFU/g of solids	After 30 days (%)
75	6.1	air	9.8×10^7	8.20	4.8×10^7	4.00
		N_2			5.0×10^7	4.17
80	5.2	air	2.9×10^7	2.42	1.7×10^7	1.42
		N_2			1.9×10^7	1.58
85	4.7	air	5.9×10^6	0.49	2.5×10^6	0.21
		N_2			3.2×10^6	0.27

[1]Expressed as percent of the original population in the concentrated milk before drying.

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Preenrichment Conditions for Effective Recovery of *Salmonella* in Foods and Feed Ingredients

J. Y. D'AOUST* and C. MAISHMENT

Bureau of Microbial Hazards, Health Protection Branch, Health and Welfare Canada, Tunney's Pasture, Ottawa, Ontario, Canada, K1A 0L2.

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ABSTRACT

The efficacy of Clausen, EE, Eugon, GN, Tergitol 7, lactose and nutrient broths as *Salmonella* preenrichment media was evaluated using 165 food samples with an incident contamination level ranging from 1.5 to 460 salmonellae/100 g. Replicate food samples (100 g) were preenriched in each of seven media (900 ml) for 6 h and 24 h at 35 C; various amounts (10, 1.0 and 0.1 ml) of preenriched cultures were selectively enriched in tetrathionate brilliant green (43 C) and selenite cystine (35 C) broths and plated on bismuth sulfite and brilliant green sulfa agars. Short (6 h) and 24-h preenrichment conditions resulted in 26 (16%) and 8 (5%) false negative results, respectively. Recovery of *Salmonella* from 6-h but not 24-h preenrichment cultures also varied directly with the portion of culture inoculated into selective enrichment broths. None of the preenrichment media tested performed satisfactorily at 6 h of incubation where levels of recovery ranged from 32 to 62%; at 24 h, good recovery was obtained with all media (95 to 100%) except EE broth (74%). The incidence of competitive flora was significantly higher on selenite + brilliant green sulfa than on tetrathionate + bismuth sulfite; transfer volumes (10 and 1.0 ml) and preenrichment media did not contribute significantly to the presence of non-salmonellae on plating media. Characteristics of preenrichment media were found to be less critical than preenrichment incubation time for effective recovery of *Salmonella* in foods and feed ingredients. The use of 1.0- rather than 10-ml preenrichment transfer volume is indicated because it proved to be completely reliable under our experimental conditions and reduced the cost of analyses.

Presence of injured salmonellae with more exacting growth requirements in human and animal food products has favored use of a non-selective preenrichment step in standard cultural methods (*1,7,11,23*). In contrast to direct enrichment in tetrathionate, selenite or other selective broths, a procedure known to adversely affect growth of salmonellae (*9,27*), preenrichment in non-selective media increases recovery of *Salmonella* from products exposed to extreme environmental conditions during processing or storage (*4,5,9,18*). An optimum preenrichment incubation period for effective recovery of injured cells in foods has yet to be determined. Studies with artificial test systems have shown that metabolic lesions are repaired when stressed cells are incubated for short periods (5 to 8 h) in a suitable resuscitation medium (*3,13,17,21*); however, conflicting evidence on increased recovery of salmonellae in foods with longer incubation periods is equally noteworthy (*8,16,25*). Standard methods also differ widely in the recommended amounts of preenriched culture to be transferred into selective enrichment broths (*1,7,11,12,23*). Although the behavior of salmonellae under different selective enrichment conditions is well documented (*6,10,13,14*), comparable studies on the efficacy of preenrichment media under different time-temperature regimes are generally lacking.

The purpose of this work was to evaluate the importance of preenrichment media, incubation time and preenrichment transfer volume on recovery of *Salmonella* in naturally and artificially contaminated human and animal food products.

MATERIALS AND METHODS

Naturally contaminated food and feed products were obtained from retail outlets or as a result of monitoring or regulatory activities of Canadian federal agencies; the two lots of chocolate were obtained from different manufacturers (Table 1). Artificially contaminated skim milk powder was prepared according to the method of Edel and Kampelmacher (5). Lots of chili powder and sesame seeds previously found to contain *Salmonella* were seeded with a log-phase broth culture of *Salmonella typhimurium*, then rapidly frozen in liquid nitrogen and dried under vacuum. Egg (5.5% w/w) and non-egg containing pasta were manufactured under conditions comparable to commercial practice using a De Maco S-25 laboratory extruder. The moisture content at extrusion of durum semolina with or without added egg powder ranged from 30 to 34% (w/w). The pasta was dried at 45 C over an 18-h period during which the relative humidity of the drying chamber was lowered from 97% at the beginning to 50% at the end of the drying cycle; moisture content of the final product was approximately 12% (w/w). Contamination levels in most samples were estimated by the three-tube Most Probable Number (MPN) technique and ranged from 1.5 to 460 salmonellae/100 g.

Samples (100 g) of human and animal food products (Table 1) were preenriched 6 h and 24 h in 0.9 liter of each of seven different broth media (Fig. 1); in addition, samples of the chocolate and cocoa powder were preenriched in 10% (w/v) reconstituted skim milk powder with added brilliant green (0.002% w/v), and the artificially contaminated skim milk powder was preenriched in 0.002% (w/v) brilliant green

TABLE 1. Salmonella *in naturally and artificially contaminated products.*

Product	Serotype	Level of contamination (Cells/100 g)
Low moisture		
1. Chili powder[1]	*S. typhimurium*	10
2. Sesame seeds[1]	*S. typhimurium*	50
3. Egg powder	*S. senftenberg*	15
4. Cocoa powder	*S. tennessee*	1.5
5. Chocolate, Lot A	*S. eastbourne*	9.3
6. Chocolate, Lot B	*S. senftenberg*	2.3
7. Milk powder[1]	*S. senftenberg*	4
8. Gelatin	*S. anatum*	2.3
9. Egg pasta, Lot A[1]	*S. infantis*	15
10. Egg pasta, Lot B[1]	*S. typhimurium*	240
11. Non-egg pasta, Lot A[1]	*S. infantis*	2.3
12. Non-egg pasta, Lot B[1]	*S. typhimurium*	2.3
13. Fermented sausage	*S. panama*	93
High moisture		
14. Frog legs	*S. bredeney*	4.3
15. Liquid whole egg	*S. thompson*	460
16. Liquid egg white	*S. thompson*	93
17. Frozen whole turkey	*S. indiana*	110[2]
18. Frozen cut-up chicken	*S. schwarzengrund*	4.3[2]
19. Frozen chicken giblets	*S. nienstedten*	24
Feeds and feed ingredients		
20. Meat meal	*S. halmstad*	43
21. Meat and bone meal	*S. cerro*	7
22. Fish meal	*S. senftenberg*	23
23. Complete feed	*S. senftenberg*	93

[1]Artificially contaminated product.
[2]Contamination per 100 ml of rinse solution.

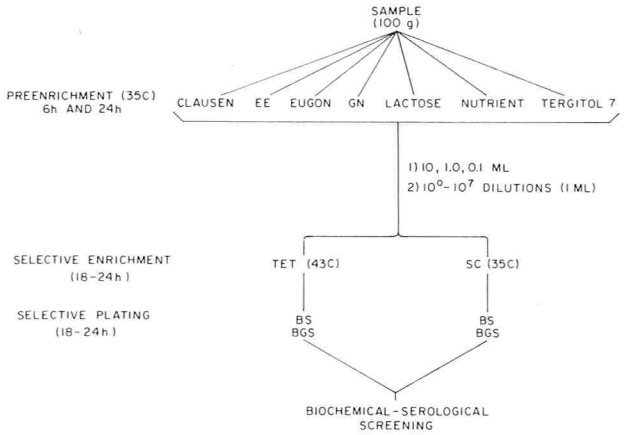

Figure 1. *Procedure for the detection of* Salmonella *in foods and feed ingredients.*

water. Selection of preenrichment broth media was based on the detergency, nutritive value or purported ability of the medium to effectively recover *Salmonella* and other enteric bacilli (Table 2). After 6 and 24 h of incubation, 10-, 1.0- and 0.1-ml portions and 1 ml of 10^{-2} - 10^{-7} serial water dilutions of each preenriched culture were transferred into tetrathionate brilliant green (Tet) and selenite cystine (SC) broths and incubated overnight at 43 and 35 C, respectively. A 1:10 preenrichment to enrichment transfer ratio was used with all samples except 0.1-ml portions which were enriched in 9 ml of selective broth. Enriched samples were streaked on brilliant green sulfa (BGS) and bismuth sulfite (BS) agars and incubated at 35 C for 18-24 h; isolation of presumptive *Salmonella* colonies on BS occasionally required an additional 24 h of incubation. Four enrichment-plating conditions were recognized: A = Tet (43 C) + BS; B = Tet (43 C) + BGS; C = SC (35 C) + BS; D = SC (35 C) + BGS. Presumptive *Salmonella* isolates were screened biochemically on triple sugar iron and lysine iron agars and confirmed serologically with somatic and flagellar antisera.

Analysis of variance (ANOVA) in a split-split-split plot experimental design and multiple t-tests were used to identify significant differences between the selectivity of the four enrichment-plating conditions and the effects of preenrichment media and transfer volumes on the incidence of competitive flora. Growth of non-salmonellae on BS and BGS was scored using the following scale: 1 = 0-25% incidence; 2 = 26-50%; 3 = 51-75%; 4 = 76-100%. In each food category, mean scores of competitive flora for conditions A to D at given transfer volumes were computed; estimated standard errors for the means of the three transfer volumes and the 12 enrichment-plating conditions within each food category was derived from ANOVA tables. Significance between various conditions within each food category was determined by multiple t-tests at an experiment-wise error rate of p =0.05.

RESULTS

Quantitative recovery of *Salmonella* from seven preenrichment media generally increased with increased incubation time (Fig. 2). Growth differentials of 5 to 6 $\log_{10}$ units between 6-h and 24-h preenriched samples were usually obtained with all media except Eugon broth which was generally less productive. Recoveries from cocoa products preenriched in skim milk and from skim milk powder preenriched in brilliant green water did not differ markedly from that obtained with other preenrichment media (data not shown). Short (6-h) and 24-h preenrichment incubation yielded 26 (16%) and 8 (5%) false negative results, respectively. All media produced no less than two and as many as six (EE) false negative results at 6 h of incubation; EE broth accounted for six of the eight false negative results at 24 h. Good

TABLE 2. *Composition of preenrichment media.*

Broth medium	Manufacturer	Ingredients: Extract	Saccharide	Selective agent	Detergent	Recommended for
Clausen	Oxoid	Tryptone Peptone Yeast extract	Dextrose Glycerol	None	Tween 80	Sterility testing
EE (Mossel)	Difco	Tryptose	Dextrose	Brilliant green bile	None	Detection of *Enterobacteriaceae*
Eugon	BBL	Peptone	Dextrose	None	None	Sterility testing
GN	BBL	Peptone	Dextrose Mannitol	Desoxycholate citrate	None	Detection of *Salmonella-Shigella*
Nutrient	Difco	Peptone Beef extract	None	None	None	General purpose
Lactose	Difco	Peptone Beef extract	Lactose	None	None	Detection of coliforms
Tergitol 7	BBL	Peptone Yeast extract	Lactose	None	Heptadecyl sulfate	Detection of *Salmonella* and enteric bacilli

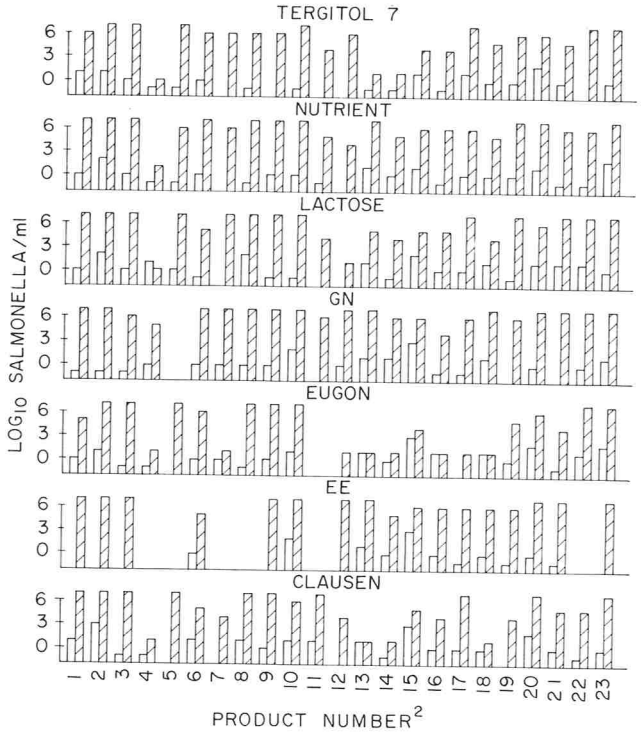

Figure 2. *Quantitative recovery of* Salmonella *with increasing incubation time*[1]. [1]*Maximum number of salmonellae per ml of preenriched culture based on four different selective enrichment-plating conditions. Open column = 6 h of incubation; shaded column = 24 h of incubation.* [2]*Numbers correspond to products listed in Table 2.*

productivity was obtained with GN, lactose and nutrient broths after 24 h of incubation. Food-specific responses were noted with cocoa powder (sample 4) which proved to be highly inhibitory in all preenrichment media except skim milk and GN broth. Recovery of *Salmonella* in non-egg containing pasta (samples 11 and 12) after 6 h of incubation was also poor and accounted for nine of the 26 false negative results.

More detailed analysis of data on preenrichment media and transfer volumes (Table 3) showed that recoveries after 6 h of incubation ranged from 32 to 62% and after 24 h of incubation from 95 to 100% except for EE broth (74%). Recovery of salmonellae from 6-h but not 24-h preenriched samples varied directly with the portion of preenriched culture inoculated into selective enrichment broth; similar findings were obtained with the skim milk and the brilliant green water preenrichment media (data not shown). All 24-h preenrichment conditions except EE were of comparable efficacy and no single condition favored greater recovery of *Salmonella* in any given food category or specific foods except for the previously noted higher quantitative recovery of *Salmonella* from GN and skim milk preenrichments of cocoa powder (Fig. 2).

The ability of enrichment-plating conditions A, B and C to detect positive samples was similar and differed considerably from condition D which produced 61 (13%) false negative results (Table 4). At given transfer volumes, the tetrathionate enrichment broth together with BS and BGS identified five samples of cocoa powder, egg white and turkey which were negative by SC enrichment; one cocoa powder sample was detected in SC alone. Low moisture foods were particularly troublesome resulting in 8% (condition A) to 18% (condition D) incidence of false negative samples. Transfer volume did not markedly affect recoverability with any of the four enrichment-plating conditions. Significant differences in the ability of conditions A to D to inhibit competitive flora were noted (Table 5). Mean scores for conditions A and D were significantly different for all food categories at all transfer volumes; in contrast, differences in the selectivity of conditions B and C were seldom significant. The 10- and 1.0-ml transfer volumes and the seven preenrichment media did not contribute significantly to the presence of competitive flora on plating media (data not shown). However, the 0.1-ml transfer volume did produce significantly lower

TABLE 3. *Recovery of* Salmonella *with different preenrichment media.*

Food sample[1]	Total *Salmonella* positive samples	Transfer volume[2] (ml)	*Salmonella* positive samples[3] Preenrichment/Incubation time: Clausen 6 h	Clausen 24 h	EE 6 h	EE 24 h	Eugon 6 h	Eugon 24 h	GN 6 h	GN 24 h	Lactose 6 h	Lactose 24 h	Nutrient 6 h	Nutrient 24 h	Tergitol 7 6 h	Tergitol 7 24 h
Low moisture	13	10	10	13	3	8	10	12	11	12	10	13	11	13	9	13
		1.0	8	13	3	8	7	12	8	12	7	13	7	13	4	13
		0.1	5	13	2	8	3	12	2	12	4	12	2	13	2	12
High moisture	6	10	5	6	5	6	5	6	5	6	6	6	6	6	6	6
		1.0	4	6	4	6	5	6	3	6	4	6	5	6	4	6
		0.1	1	6	2	6	3	6	3	6	2	6	1	6	2	6
Feeds and feed ingredients	4	10	4	4	2	3	4	4	3	4	4	4	4	4	3	4
		1.0	3	4	1	3	3	4	3	4	4	4	2	4	3	4
		0.1	1	4	0	3	3	4	0	4	2	4	2	4	1	4
	Total		41	69	22	51	43	66	38	66	43	68	40	69	34	68
	Percent		59	100	32	74	62	95	55	95	62	98	58	100	49	98

[1]See Table 1.
[2]Volume of preenriched culture transferred to selective enrichment broths.
[3]Samples found to contain *Salmonella* based on four different selective enrichment-plating conditions.

TABLE 4. *Efficacy of enrichment-plating conditions in the recovery of* Salmonella.

Food sample[1]	Total positive preenriched samples	Transfer volume[2] (ml)	*Salmonella* positive samples Enrichment/Plating media[3] A	B	C	D
Low moisture	91	10	84	81	84	79
		1.0	84	82	83	75
		0.1	81	80	81	76
High moisture	42	10	42	41	42	39
		1.0	42	41	40	37
		0.1	41	41	41	37
Feeds and feed ingredients	28	10	27	27	27	26
		1.0	27	27	27	26
		0.1	27	27	27	27
	Total		455	447	452	422
	Percent		94	93	94	87

[1]See Table 1. Each food sample was preenriched for 24 h in seven different preenrichment media.
[2]Volume of preenriched culture transferred to selective enrichment broths.
[3]A = TET (43 C) + BS; B = TET (43 C) + BGS; C = SC (35 C) + BS; D = SC (35 C) + BGS.

TABLE 5. *Variance analysis of selectivity of enrichment-plating conditions against competitive flora.*

Food sample[1]	Transfer volume[2] (ml)	Significance of selectivity of enrichment/plating media[3]
Low moisture	10	A (1.16)[4] C (1.34) B (1.74) D (2.08)
	1.0	A (1.13) C (1.38) B (1.73) D (2.26)
	0.1	A (1.15) C (1.33) B (1.35) D (2.00)
High moisture	10	A (1.26) B (1.38) C (1.86) D (2.40)
	1.0	A (1.29) B (1.45) C (1.90) D (2.24)
	0.1	A (1.07) B (1.29) C (1.62) D (1.95)
Feeds and feed ingredients	10	A (1.04) C (1.43) B (1.57) D (2.25)
	1.0	A (1.07) C (1.32) B (1.61) D (2.32)
	0.1	A (1.04) B (1.25) C (1.32) D (2.07)

[1] See Table 1. Each sample was preenriched in seven different media.
[2] Volume of preenriched culture transferred to selective enrichment broths.
[3] Combinations A to D are listed from left to right in decreasing order of efficacy and are scored by the same line if they were not found to be significantly different. Standard errors for mean enrichment-plating scores of low and high moisture foods and feed ingredients were 0.083, 0.114 and 0.121, respectively.
[4] Numbers in brackets are mean scores for competitive flora in 91, 42 and 28 samples of low and high moisture foods and feed ingredients, respectively.

levels of non-salmonellae with high moisture foods and feed ingredients.

DISCUSSION

The unacceptably high numbers of false negative samples following short (6 h) preenrichment of foods and feed ingredients (Fig. 2 and Table 3) concur with results of earlier attempts to shorten preenrichment periods without adversely affecting method sensitivity (*8,16,24,25*); however, successful isolation of salmonellae has been reported from frozen sweet corn following 8-h preenrichment in buffered peptone water or lactose broth (*22*). The high nutritive values of Clausen and Eugon broths, the detergency of Tergitol 7 and Clausen media and the adapted formulation of EE and GN broths for the selective growth of *Enterobacteriaceae* did not promote recoveries of *Salmonella* to levels greater than that obtained with nutrient and lactose broths. These findings agree with earlier reports that characteristics of preenrichment media do not appear to be critical in the recovery of *Salmonella* in foods. Nutrient and lactose broths were equally effective but inferior to trypticase soy broth for isolation of *Salmonella* in naturally and artificially contaminated gelatin (*19*). No marked differences were also noted in the ability of lactose broth and buffered peptone water to detect *Salmonella* in egg products and milk powder (*24,26*).

The transfer volume independent recovery of salmonellae from 24-h preenrichment broths (Table 3) indicates that transfers of 0.01 to 1.0% of preenriched cultures provide a sufficiently large inoculum to overcome enrichment broth toxicity (*2,24,27,28*). Similarly, no effect of transfer volume on recovery of *Salmonella* was reported for egg products, nonfat dry milk and cocoa (*20,26*). Complete agreement between results with the 10- and 1.0-ml transfer volume with all food and feed products suggests that use of a 1.0-ml inoculum does not adversely affect method sensitivity. Although the present evaluation was limited to 1-liter preenrichment cultures, the high levels of recovery with the 0.1-ml transfer volume indicate that a 1-ml inoculum would reliably detect *Salmonella* in larger preenrichment cultures. The economic advantages associated with the use of a 1.0- rather than 10-ml transfer volume would be appreciable; in addition to a reduction in operation time and incubator space requirements, substantial savings in enrichment media could be realized at the 1:10 preenrichment-enrichment ratio.

The ability of the four enrichment-plating conditions to detect *Salmonella* varied in the following decreasing order of efficacy: A<C<B<D (Table 4); a similar ranking was obtained for the selectivity against competitive flora. More positive low and high moisture food samples were detected with BS than BGS under homologous enrichment conditions. These findings agree with those

of a recent study on detection of *Salmonella* in dried foods and feed ingredients where BS in combination with different enrichment regimes consistently produced highest recovery rates (*10*); this is in contrast with an earlier report where brilliant green agar was found to be more reliable (*26*). BS effectively repressed non-salmonellae in low moisture foods (Table 5). Although rarely significantly different from conditions B and C, condition A consistently demonstrated strong inhibition of competitive flora which may in part result from the adverse effects of elevated temperatures on growth of these microorganisms (*10,13*). The poor performance of condition D may reflect the limited selectivity of BGS against non-salmonellae *Enterobacteriaceae* (*6,15,26*).

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Evaluation of Inhibitors for Rapid Enumeration of Psychrotrophic Bacteria

TERRANCE L. SMITH* and L. D. WITTER

Department of Food Science
University of Illinois at Urbana-Champaign
Urbana, Illinois 61801

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ABSTRACT

Two inhibitors of the 17 tested inhibited growth of gram positive bacteria without causing inhibition of gram negative bacteria. These were crystal violet at 2 mg/l and neotetrazolium chloride at 2 mg/l. In addition, basic fuchsin at 6 mg/l produced only marginal reduction in counts of gram negative bacteria. These inhibitors might find further use in developing a test for psychrotrophic bacteria in the presence of non-psychrotrophic bacteria.

Enumeration of psychrotrophic bacteria as a means of evaluating efficiency of the cleaning and sanitizing procedure by dairy plant clean-up crews is attractive for two reasons. First, the psychrotrophs are widespread in nature, and second they are totally destroyed by the pasteurization process in a dairy plant. Thus, any psychrotrophs found in freshly pasteurized milk must be due to post-pasteurization contamination. The most likely source of this contamination is improperly cleaned equipment, such as fillers, or recontamination of machinery surfaces by improperly sanitized water.

For such a test to be useful the results must be obtained in as short a period as possible, preferably in no more than 24 h. Detection of psychrotrophs currently requires a 10-day incubation at 7 C. Early work in reduction of the time required to obtain a psychrotrophic count was done in association with unsuccessful attempts to devise a shelf-life test. Since the psychrotrophs have their optimal growth temperature in the mesophilic range, many attempts were made to decrease the incubation time by raising the incubation temperature (*1*). However, since the non-psychrotrophs found in milk are also mesophilic their growth interfered with the counting of psychrotrophs. Only by inhibiting growth of the non-psychrotrophs can the incubation temperature be raised enough to decrease the incubation time to 24 h.

Since it has been determined that the psychrotrophs are primarily gram negative bacteria particularly of the genus *Pseudomonas* (*14*), the inhibitors of choice should have a greater action on gram positive bacteria than on gram negative bacteria. There are three classes of inhibitors that generally meet these requirements. They are surfactants, basic dyes, and antibiotics. Several surfactants were screened by Freeman et al. (*5*), including sodium desoxycholate and some fatty acids. Penicillin (*8*), erythromycin, and chloramphenicol (*9*) have been reportedly used as inhibitors with varying success. Fung and Miller (*6*) and Freeman et al. (*5*) screened a number of basic dyes for their ability to inhibit gram-positive bacteria. Crystal violet has also been used by Olson (*10*), Gyllenburg et al. (*7*) and Driessen and Stadhouders (*4*) as an inhibitor. Triphenyl tetrazolium chloride has shown both inhibitory and indicating properties. It has been used by Olson (*11*) as an inhibitor and by others as an indicator or dye reduction test (*2,3*). Diamide is a thiol oxidizing agent which Rose et al. (*12*) suggested might be used to isolate *Pseudomonas*. Dioxane was used by Rose et al. in their work on diamide and their results suggested dioxane might also be selective for *Pseudomonas*.

The purpose of this study was to reevaluate some of the previously tested inhibitors and evaluate any new inhibitors that might come to mind for their potential in the rapid enumeration of psychrotrophs.

MATERIALS AND METHODS

Organisms

The evaluation of inhibitors was done in the study using three gram negative bacteria (psychrotrophs) and three gram positive bacteria (non-psychrotrophs). The gram negatives were *Pseudomonas fluorescens* P_7, *Pseudomonas fragi* and *Pseudomonas putrefaciens*. The gram positives were *Streptococcus lactis* PC-10 and two thermoduric micrococci isolated from laboratory pasteurized milk. All six of these bacteria were from the department stock cultures.

Screening

Each organism was grown 16 h in Tryptic Soy Broth (Difco Laboratories; Detroit, Michigan) on a rotary shaker at 25 C for the psychrotrophs and 32 C for the non-psychrotrophs. Each organism was inoculated into separate flasks of melted, tempered Tryptic Soy Agar and plates poured. The inhibitors to be screened were dissolved in water to make a stock solution. The non-water soluble fatty acids were emulsified in water with a Bronson Sonicator. Sterile 12.5-mm antibiotic

assay disks were dipped into the inhibitors and placed on the surface of the hardened agar of the plates. Each organism received one disk from each of the inhibitors tested. After 24 h of incubation at 25 C for the psychrotrophs and 32 C for the non-psychrotrophs the zones of inhibition were measured. If there were no zones of inhibition the inhibitor concentration was increased and the test was done again.

Concentration determination

To determine the optimal inhibitor concentration, stock solutions of inhibitors were added to known amounts of melted and tempered Tryptic Soy Agar to obtain a series of concentrations of the inhibitor. Plates were poured and allowed to harden and dry. Sixteen-hour old cultures of the non-psychrotrophs were surface plated onto the plates and they were incubated 24 h at 32 C. The plates were then counted and the procedure repeated with different inhibitor concentrations until a concentration was found that was just sufficient to totally inhibit growth.

The psychrotrophic organisms were then grown on Tryptic Soy Agar containing the above determined inhibitor concentration and on a control plate of Tryptic Soy Agar. Colony counts of the control and test were compared by Student's paired t test to determine if there was any significant reduction in counts when grown on media containing the inhibitor.

RESULTS AND DISCUSSION

Screening

Inhibitors passed the screening test if the zones of inhibition for the psychrotrophs were smaller than for the non-psychrotrophs. This corresponds with growth of the psychrotrophs in the presence of a higher concentration of inhibitor than that tolerated by the non-psychrotrophs. The chemicals screened along with the results are given in Table 1.

TABLE 1. *Screening test results.*

Chemical	Inhibitor concentration on disks (mg/l)	Zone difference[a] (± 1 mm)
Basic fuchsin	5000	+4
Brilliant green	5000	0
a-Bromolauric acid	3070	+5
a-Bromomyristic acid	2790	+7.5
Crystal violet	5000	+2
Diamide	500	+2.5
Dioxane	10000	0
Malachite green	5000	+3
Methylene blue	3000	0
Neotetrazolium chloride	50	+4
Roccal	50	0
Safranin O	3000	- 1.5
Sodium desoxycholate	10000	0
Sodium ricinolate	500	0
Sodium selinite	10000	- 3
Sodium taurocholate	20000	0
Triphenyltetrazolium chloride	500	+1

[a](smallest gram-negative zone) - (largest gram-positive zone), greater than 0-pass; less than or equal to 0-reject.

Concentration determination

The minimal concentrations that were inhibitory to the non-psychrotrophs are given on Table 2. The *a*-bromomyristic acid at 50 mg/l failed to totally inhibit the non-psychrotrophs. At the same time this concentration created a surface on the agar that was so greasy it was very difficult to spread the inoculum. Because of this the inhibitor *a*-bromomyristic acid was dropped from further testing.

TABLE 2. *Calculated t values.*

Inhibitor	Organism	t[a]
Basic fuchsin; 6 mg/l		
	P. fluorescens	3.244[b]
	P. fragi	- 0.215
	P. putrefaciens	- 1.339
a-Bromolauric acid; 25 mg/l		
	P. fluorescens	15.167[b]
	P. fragi	4.183[b]
	P. putrefaciens	12.936
a-Bromomyristic acid	Not suitable	
Crystal violet; 2 mg/l		
	P. fluorescens	0.226
	P. fragi	-1.411
	P. putrefaciens	- 0.431
Diamide; 55 mg/l		
	P. fluorescens	11.497[b]
	P. fragi	1.974
	P. putrefaciens	4.540[b]
Malachite green; 4 mg/l		
	P. fluorescens	30.938[b]
	P. fragi	2.921[b]
	P. putrefaciens	4.697[b]
Neotetrazolium chloride; 2 mg/l		
	P. fluorescens	0.119
	P. fragi	0.626
	P. putrefaciens	1.414

[a]One tailed test $\mu = .05$, df = 2, $t_{rej.} > 2.920$.
[b]Reject null hypothesis at 95% confidence level, one-tailed test.

The Student's paired t test, the statistical procedure used to evaluate the effect of the inhibitors on the psychrotrophs, is illustrated in Table 3. This test was done on the mean from each of the psychrotrophic organisms for each inhibitor tested. The calculated t values are in Table 2. Since we were looking for a decrease in counts of the organisms, the tests were done as a one-tailed test using the right tail. The calculated t values

TABLE 3. *Student's paired t test: sample calculation for* P. fluorescens *at 0 and 6 mg basic fuchsin/l.*

Sample: n	Log colony counts 0 mg/l	Log colony counts 6 mg/l	Difference: D
1	9.090	9.017	0.073
2	9.057	8.919	0.138
3	9.083	8.857	0.226
			$\Sigma D = 0.437$

$\overline{D} = 0.147$

$$\text{Sum of squares} = \Sigma D^2 - \frac{(\Sigma D)^2}{n}$$

$$= 0.075 - 0.064 = 0.011$$

$$\text{Variance} = S^2 = \frac{\text{Sum of squares}}{\text{Degrees of freedom}} = \frac{0.011}{2} = 0.006$$

$$\text{Pooled variance of means} = S\frac{2}{D} = \frac{S^2}{n} = \frac{0.006}{3} = 0.002$$

$$t = \frac{\overline{D}}{S_{\overline{D}}} = \frac{0.146}{.045} = 3.244$$

that were to the right of the center of the distribution curve were positive and those to the left negative. Therefore, to be significantly different the calculated t must have been greater than the tabled t of 2.920, and negative values were not considered.

The results for basic fuchsin showed *P. fluorescens* fell into the rejection area, and so basic fuchsin was not suitable to be used in enumeration of psychrotrophs. The inhibitors *a*-bromolauric acid, diamide and malachite green also were inhibitory to one or more of the psychrotrophs at the concentrations used.

Crystal violet and neotetrazolium chloride did not produce a statistically significant reduction in counts of the psychrotrophs.

CONCLUSION

Of the inhibitors tested, crystal violet at 2 mg/l and neotetrazolium chloride at 2 mg/l totally inhibited the non-psychrotrophs while not reducing the counts of the psychrotrophs a statistically significant amount. Basic fuchsin, although it did statistically reduce the counts of *P. fluorescens*, should be conditionally accepted because the effect was slight. Further testing should resolve the suitability of 6 mg of basic fuchsin/l. The *a*-bromomyristic acid was not suitable for use in solid media, but might be useful in liquid media and should be tested in such. The remainder of the tested inhibitors were not suitable for use in enumerating psychrotrophs.

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Potential Public Health Significance of Non-*Escherichia coli* Coliforms in Food

ROBERT M. TWEDT* and BRENDA K. BOUTIN

Division of Microbiology, Food and Drug Administration
1090 Tusculum Ave., Cincinnati, Ohio 45226

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ABSTRACT

Several coliform species other than *Escherichia coli* are often associated with and possibly responsible for acute and chronic diarrheal disease. Recent evidence suggests that non-*Escherichia coli* coliforms may be capable of colonizing the human intestine and producing enterotoxin(s) in high yield. Whether these organisms are newly capable of causing disease because of infestation with extrachromosomal factors mediating pathogenicity or simply because of inherent pathogenic capabilities that have gone unrecognized, they pose a potential health hazard. Food, medical, and public health microbiologists should be aware that the non-*E. coli* coliforms contaminating foods may be potential enteropathogens. This possibility may make determination of their pathogenic capabilities even more important than identification of their taxonomic characteristics.

Colonization of the small intestine by enterotoxigenic *Escherichia coli* with attendant toxin production is well recognized as being responsible for acute and chronic diarrheal diseases that have affected humans of all ages in Africa (*18*), Asia (*17*), Japan (*21*), Brazil (*4*), Mexico (*14,19*), and the United States (*20*). Adherence of *E. coli* to epithelial surfaces, which promotes colonization of the gut, is mediated by specific, heat-labile surface antigens. These antigens exhibit a fine filamentous or pilus-like structure and include the K-88 antigen of swine-specific enterotoxigenic *E. coli* (*6,25*), the bovine- and sheep-specific K-99 antigen (*16,26*), and the human-specific colonization factor antigen (*1,2*). Each of the host-specific adherence factors of *E. coli* is plasmid-mediated (*2,15,16*). Production of enterotoxins by some strains of *E. coli* is also mediated by transmissible plasmids (*5,23,24*) that are transferred between strains of the species in a manner analogous to the spread of R-factors determining antibiotic resistance (*13*).

Investigators studying R-factor-mediated antibiotic resistance related the widespread appearance of resistant bacterial strains among the healthy human population to a reservoir of R-factors (*30*). They warned that indiscriminant use of antibiotics may result in multi-resistant strains that can transfer such resistances in the human gut (*27*). The transfer of plasmids coding for toxin production (*5*) or for mucosal adhesion (*31*) among intestinal bacteria could create reservoirs of enteropathogenicity.

Recently, Wachsmuth et al. (*28*) demonstrated the plasmid mediation and transmissibility of heat-stable enterotoxin production and multiple antibiotic resistance in *E. coli* 078:K80:H12 epidemiologically incriminated in a hospital outbreak of infantile diarrhea. They showed that the conjugal transfer of the responsible plasmids into *E. coli* K-12 was signaled by concurrent transfer of resistances and enterotoxin production.

OTHER COLIFORMS

Non-*E. coli* coliforms also appear to be capable of colonizing the human gut and producing potent enterotoxins in high yield. During the last few years, strains of *Klebsiella, Enterobacter,* and *Citrobacter* (some shown to be enterotoxigenic) have been isolated from stools or the intestinal tract of children and adults in several epidemiological studies of acute and chronic diarrheal diseases (*3,4,9-12,29,30*). Reasons for induction of bacterial activities, unexpected in genera considered to have minor pathogenic significance, are as yet unclear. It is possible that a family of enterotoxins may exist among the various *Enterobacteriaceae.* Recent findings suggest that plasmids encoding for enterotoxin production may spread between related species. In fact, the intergeneric transfer of plasmids among the *Enterobacteriaceae* was one reason cited by Sanderson (*22*) for the difficulties he encountered when studying the genetic relatedness of the family.

THE TOXINS

In a recent study, Klipstein et al. (*8*) compared the enterotoxigenicity of 12 strains of coliforms (*Enterobacter cloacae, Klebsiella pneumoniae,* and *E. coli*) isolated from the gastrointestinal tract of persons ill with diarrhea with that of 13 strains of coliforms from urine cultures. They studied the effect of purified heat-labile or

heat-stable toxins from these isolates in the rat jejunal perfusion model. All 12 gastrointestinal strains, but only six of the 13 urine strains (one *E. cloacae,* two *K. pneumoniae,* and three *E. coli*), elaborated one or both forms of enterotoxin. In addition to the difference between the two groups of cultures in the proportion of enterotoxin producers, there was also a million-fold quantitative difference in the potency of the toxins produced. Toxins produced by the gastrointestinal strains had minimal effective concentrations as low as 0.1 to 10 ng/ml. In contrast, urine cultures produced toxins of weak potency.

In a companion study, Klipstein and Engert (*7*) compared the relationship of cholera toxin and the heat-labile and heat-stable toxins of enterotoxigenic *E. coli* to the toxins produced by intestinal isolates of *K. pneumoniae* and *E. cloacae*. They compared the capacity of equine anti-cholera toxin and rabbit antiserum prepared against the heat-labile toxin from each of the coliforms to neutralize homologous and heterologous toxins by the rat jejunal perfusion technique. Their results indicated that the close immunological relationship of cholera toxin and *E. coli* heat-labile toxin extends to the heat-labile toxins of *Klebsiella* and *E. cloacae* and, to a lesser extent, to the heat-stable toxins of *E. coli* and *Klebsiella.*

The immunological similarity between enterotoxins of *E. coli* and other coliforms, demonstrated by Klipstein and Engert (*7*), supports the hypothesis that these toxins are mediated by plasmid(s) transmissible between species and genera resident in the human intestine.

In an epidemiological investigation of a recent nursery outbreak of diarrheal disease, Guerrant et al. (*3*) identified nine different serotypes of three different species of enterotoxigenic organisms--*E. coli, Klebsiella,* and *Citrobacter.* To support their contention that the outbreak could have been related to the intergeneric spread of toxigenicity by a plasmid, the authors offered three main observations: (*a*) multiplicity of strains in the epidemic, (*b*) inability to demonstrate a single, common serotype, and (*c*) disappearance of toxigenicity despite persistence of identical strains in convalescence. Present evidence strongly supports the thesis that acute and chronic diarrheal disease in humans can result from colonization of the small intestine by enteropathogenic *E. coli* whose ability to adhere to mucosal epithelium and to produce potent enterotoxins is mediated by transmissible plasmids.

Food, medical, and public health microbiologists should be aware that non-*E. coli* coliforms may also develop pathogenicity as a result of acquiring a plasmid(s) while maintained in the environment or in the human host. Strains that are routinely dismissed on taxonomic grounds during microbiological examinations of suspect food may actually pose a potential public health hazard. Recognition of these organisms should therefore rely on tests for enteropathogenic capabilities. Tests for enterotoxigenicity and adherence of *E. coli* should be investigated to determine their applicability for testing other coliforms that contaminate food.

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Beneficial Interrelationships Between Certain Microorganisms and Humans: Candidate Microorganisms for Use as Dietary Adjuncts [1,2]

S. E. GILLILAND

Department of Animal Science
Oklahoma State University, Stillwater, Oklahoma 74074

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ABSTRACT

The lactobacilli most often mentioned as beneficial dietary adjuncts are *Lactobacillus acidophilus, Lactobacillus casei,* and *Lactobacillus bifidus* (*Bifidobacterium bifidum*). These organisms all possess characteristics which would permit their survival and growth in the intestinal tract. They also produce antagonistic actions toward enteric pathogens. Various products are available which contain these organisms; however, there does appear to be a need for improving the stability of these bacteria in such products.

Probably the first mention of using microorganisms as dietary adjuncts was made by Eli Metchnikoff when he suggested that man should consume milk fermented with a lactobacillus capable of living in the intestinal tract (*13*). The organism he recommended using was *Bacillus bulgaricus*; however, additional research following his initial observation identified the organism he actually used as *Bacillus acidophilus*. The name of this organism was changed in later years to *Lactobacillus acidophilus*. Since Metchnikoff's time, *L. acidophilus* has been the microorganism most often considered as being useful as a dietary adjunct, particularly for its beneficial role in the intestinal tract.

There are several characteristics of organisms which would be desirable if they are to be used as dietary adjuncts. First it is desirable that the organism be a normal inhabitant of the intestinal tract of healthy persons. If it is to function in the intestinal tract, it must survive the upper digestive tract to reach the intestines. Once there, it must be capable of surviving and growing in the intestines. Since the purpose of consuming the organisms is to have a beneficial effect, it must produce this desired effect(s) when in the intestinal tract. Furthermore, it must maintain viability and activity in the carrier food before consumption.

[1]*Part of symposium presented at the annual meeting of the American Society for Microbiology, Las Vegas, Nevada, May 1978.*
[2]*Paper number 3501 of the Journal Series of the Oklahoma Agricultural Experiment Station, Stillwater, Oklahoma.*

LACTOBACILLI IN INTESTINES

Lactobacilli are normal inhabitants of the intestinal tract of humans (*4,8,9,11,14,15,16,18*). Since the earliest work on the role of the lactobacilli in the intestinal tract, *L. acidophilus* and *Lactobacillus bifidus* have been the organisms of this group which have been most often mentioned as being the intestinal lactobacilli. However, other lactobacilli are also found in this habitat. Species of lactobacilli which have been regularly isolated from the human intestinal tract are as follows: *L. acidophilus* (*8,11,14,15,16,19,26*), *L. bifidus,* (*Bifidobacterium bifidum*) (*2,11,19,26*), *Lactobacillus casei* (*26*), *Lactobacillus fermentum* (*14,16,26*), *Lactobacillus salivarius* (*14,15,26*), *Lactobacillus plantarum* (*26*) and *Lactobacillus cellobiosus* (*26*). There have been limited reports of species other than these being isolated from the intestinal contents of man; however, because of their very limited occurrence they are considered as transitory microorganisms rather than normal inhabitants of the intestinal tract. The species which have been most often suggested as beneficial dietary adjuncts include *L. acidophilus, L. casei* and *L. bifidus* (*21,22*).

RESISTANCE OF LACTOBACILLI TO INHIBITORY SYSTEMS IN DIGESTIVE TRACT

There are a number of factors contributing to the control of bacteria in the digestive tract (*3*). The primary barrier to microorganisms in the stomach is the gastric acid with the intensity of the inhibitory action being related to pH and hydrochloric acid concentration. There are also certain enzymes in the system which are deleterious to microorganisms. One of these is lysozyme, which has the capability of lysing certain bacteria. In the intestinal tract, the microorganisms must be resistant to bile salts to establish themselves and grow. In other words, they must be able to survive at a relatively low surface tension. There are immune mechanisms in the intestinal tract which apparently aid in controlling the survival of microorganisms. This is an area in which we

don't have much information, particularly relating to microorganisms that might be used as dietary adjuncts. Another factor that has to be considered is intestinal motility or the rate at which food material passes through the intestinal tract. To be used successfully as dietary adjuncts the lactobacilli must be able to overcome these barriers, if they are to reach and survive in the intestinal tract.

Several species of lactobacilli have been compared for their relative resistance to gastric acidity (*26*). In these experiments, the lactobacilli were suspended in artificial gastric juice at pH 3 and maintained at 37 C throughout the test period. While lactobacilli are considered to be acid-tolerant bacteria, results from this study indicated that there are differences among species of lactobacilli with regard to resistance to gastric acidity. *L. casei* appeared to be the most resistant of the species studied in that it survived completely for a 3-h exposure. *L. acidophilus* and *L. plantarum* exhibited similar resistance to the acidic condition. *L. bulgaricus* did not exhibit resistance to these conditions in that it did not survive for the first 1-h exposure period.

Another factor mentioned as being important in controlling microorganisms is the action of certain enzymes, among them being lysozyme. Lactobacilli have been shown to exhibit resistance to this enzyme. Researchers who isolated DNA from the lactobacilli have encountered great difficulty in lysing the lactobacilli by classical methods which involve use of lysozyme (*12*). This difficulty encountered in lysing the organisms indicates that they are resistant to lysozyme and thus would probably have no problem in overcoming this barrier in the digestive system.

It has been known for the last 50 years that lactobacilli vary in their ability to grow at a low surface tension. An organism presumably must possess this capability if it is to survive and grow in the intestinal tract. In 1928, Albus (*1*) compared the resistance of certain species of lactobacilli to low surface tension. In these experiments the surface tension of broth media was lowered to 45.6, 42.6, and 40.4 dynes by adding sodium ricinoleate. Of the 17 strains of *L. bulgaricus* tested, none grew at a surface tension of 40.4 dynes, while 15 of the 15 strains of *L. acidophilus* included in the test grew quite well at this low surface tension. The one strain of *L. bifidus* tested also grew at the lowest surface tension while the strains of *L. casei* exhibited some variation in their resistance to the lower surface tension. All strains of *L. casei* grew at 42.6 dynes. The three species of lactobacilli most often suggested for use as dietary adjuncts all exhibited resistance to low surface tension.

Gilliland and Speck (*6*) reported an agar medium useful for enumerating bile-resistant lactobacilli. Milk or broth cultures of lactobacilli were plated on Lactobacillus Selection Agar (LBS) and Lactobacillus Selection Agar containing 0.15% oxgall (LBSO). Of the cultures tested, the only ones which did not grow equally well on the LBSO and the LBS agars were *L. bulgaricus* and *L. lactis*. The counts obtained on LBS and LBSO agars were comparable for the strains of *L. acidophilus, L. brevis, L. casei, L. plantarum,* and *L. fermentum*. This latter group of lactobacilli were all included in the list of lactobacilli considered as normal inhabitants of the intestinal tract. Thus there does seem to be some relationship between resistance to bile and the ability of the organism to inhabit the intestinal tract.

One of the mechanisms for controlling microorganisms in the intestinal tract of which we know least, especially with regard to the lactobacilli, is the immune response. There have been several articles which indicate that various strains of *L. acidophilus* exhibit a certain amount of host specificity (*14,17*). In one study, *L. acidophilus* isolated from a human intestinal tract could not be implanted in the intestinal tract of a chick (*17*). Another study indicated differences in characteristics of *L. acidophilus* isolated from man and other animals (*14*). It was suggested that this might be related to host specificity. While we don't fully understand these relationships with regard to host specificity, it is advisable at the present time to evaluate lactobacilli which are considered for use as dietary adjunct to insure that they will survive in the intestinal tract of the host for which they are intended.

ANTAGONISTIC ACTIONS PRODUCED BY LACTOBACILLI

Another desirable property of the organism to be used as a dietary adjunct is that it produce the desired effect in the host. In most instances this is the ability of the culture to exert antagonistic action toward undesirable microorganisms in the intestinal tract. The three species of lactobacilli most often suggested for use as dietary adjuncts have all been shown to exert such antagonistic actions. *L. acidophilus* has been shown to exert antagonistic actions toward such organisms as enteropathogenic *Escherichia coli, Salmonella typhimurium, Staphylococcus aureus* and *Clostridium perfringens* (*5,10,20,23,24,25*). *L. bifidus* has been shown to exert similar actions on *E. coli, S. typhimurium,* and *Shigella* species (*19*). *L. casei* has been shown to inhibit *E. coli, S. aureus, Vibrio* species and *Salmonella* species (*26*). The mechanism whereby the lactobacilli exert these antagonistic actions is not completely understood. Research has indicated that it is not entirely due to acid conditions created by the lactobacilli. Some of them have been shown to produce antibiotic-like substances (*10,20,24*). It is most likely that the antagonistic action is due to a combination of factors produced by the lactobacilli.

OTHER FACTORS TO CONSIDER

Based on information thus far presented, it would appear that the three species of lactobacilli, that is, *L. acidophilus, L. casei* and *L. bifidus*, would meet the

requirements for being organisms to be used as dietary adjuncts. However, there are certain other factors which should be considered. Probably the most obvious factor related to *L. bifidus* is the fact that it is an anerobic bacterium. This characteristic would create problems in routinely producing a culture for use as a dietary adjunct. Problems could also be expected to be encountered in maintaining the organism in a viable and active state before consumption.

The Japanese have developed and are currently marketing a very successful product named "Yakult," which contains a culture of *L. casei*; furthermore, they have presented quite a lot of data indicating that this organism will produce the desired effect when consumed by humans (*26*). Both *L. casei* and *L. acidophilus* are considered to be facultative microorganisms so we would not be faced with the problem of maintaining anaerobic conditions during their production and storage before consumption. In the past *L. acidophilus* was available to consumers in the form of a fermented milk product. This product, however, had some very undesirable organoleptic characteristics. In recent years a new nonfermented product has been developed in which a massive quantity of cells of *L. acidophilus* as a concentrated culture is added to cold freshly pasteurized milk to provide a more desirable product containing this organism for the consumer. To maintain the desirable qualities of this product during storage it is important that the lactobacillus used does not produce acid during storage which would alter the flavor of the product. Since the *L. acidophilus* does not grow at temperatures as low as 15 C, this would not appear to present a problem. However, problems might be encountered if *L. casei* were to be used in this type of product since it will grow at lower temperatures than *L. acidophilus*.

STABILITY OF LACTOBACILLI IN DIETARY PREPARATIONS

One very important property of the culture to be used as a dietary adjunct is that the organism remain viable during storage in the carrier food before consumption. Gilliland and Speck (*6*) evaluated a number of products which are marketed as sources of lactobacilli as dietary adjuncts. All of the products included in the analyses were being sold as sources of lactobacilli which would survive in the intestinal tract of man. All products were obtained from either pharmacies, health food stores or from supermarket dairy cases. They were interested in evaluating these products for numbers of bile-resistant lactobacilli. Lactobacillus Selection (LBS) agar containing 0.15% oxgall as a source of bile (LBSO) was used for this purpose. Of 12 products tested, only five contained bile-resistant lactobacilli ranging in number from 1×10^6 to 1.4×10^8/g. In additional experiments on these products the predominating organisms which were present and formed colonies on LBS agar or LBSO agar were isolated and identified using methods previously described (*7*). All of the products tested were indicated to be sources of *L. acidophilus* (based on information supplied on the product labels). Only three of the products (one from a pharmacy and the two from dairy cases) contained organisms identified as *L. acidophilus*. One of these products which was supposed to obtain only *L. acidophilus* also contained *L. casei* and *L. plantarum*.

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The Nitrite/N-Nitrosamine Problem in Meats: An Update

J. I. GRAY[1]* and C. J. RANDALL[2]

Department of Food Science, University of Guelph, Guelph, Ontario NIG2W1, Canada and Poultry Division, Production and Marketing Branch, Agriculture Canada, Ottawa, Ontario

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ABSTRACT

Since certain N-nitrosamines are highly carcinogenic, formation and isolation of these compounds from food systems has received much attention in the past decade. This paper reviews the N-nitrosamine literature of the past 3 years, especially as it relates to cured meat products. This review examines occurrence of nitrites and amines in food systems, presence of N-nitrosamines in bacon, and also means of minimizing formation of these compounds. In addition, regulatory changes pertaining to nitrite usage in curing procedures as well as some areas of future research are discussed.

There has probably been no topic in the past decade that has generated as much discussion and research as the presence of N-nitrosamines in food systems. Many of these compounds are carcinogenic and, in addition, some exhibit mutagenic, embryopathic or teratogenic properties. Although there is no direct evidence that N-nitroso compounds are carcinogenic to man, indirect proof from animal studies on 12 species including monkeys, mice, rats, rabbits, guinea pigs and sheep would suggest this potential danger to man. Thus, it is understandable that the possible occurrence of these compounds in the human environment has caused considerable concern.

N-Nitrosamines are formed principally from the reaction of naturally occurring amines with nitrites that may be added to foods or produced by the bacterial reduction of nitrates. These compounds have been reported in various foods including wheat products, mushrooms, alcoholic beverages, cheese, milk and soybean oil as well as in meat and fish products. Many reviews on N-nitrosamines have been published in the past few years, dealing with their formation and occurrence in foods and their toxicology and human health hazards (*12,13,22,39,57,76,77,96,99,109,124,125*).

The food items of major concern are the cured meat products, especially bacon. Literature before 1975 on presence of N-nitrosopyrrolidine (N-Pyr) and other N-nitrosamines in these products has been adequately reviewed (*12,22,96*). The purpose of this paper is to review the literature of the past 3 years regarding N-nitrosamines in cured meats, with particular reference to formation of N-Pyr in cooked bacon. In addition, regulatory changes pertaining to nitrite usage, plus some areas of future research will be discussed.

OCCURRENCE OF AMINES IN MEAT PRODUCTS

N-Nitrosamines may be formed in the environment, particularly food and water, whenever secondary or even primary or tertiary amines are exposed to nitrite (*96*). In addition, ingestion and exposure of a combination of foods, water, drugs, and medical preparations containing both reactants to the acid environment of the stomach could also promote formation of N-nitrosamines. Although sources of nitrite are well documented in the literature, information on occurrence of individual amines in meat is sparse. Recent papers (*66,86,112,114*) would indicate that methodology to determine amines in meat is now available.

Spinelli et al. (*114*) determined the effects of processing on the amine content of pork bellies by extracting the amines with perchloric acid and forming the dansyl chloride derivatives. These fluorescent derivatives were separated by thin-layer chromatography, extracted and quantitated spectrofluorometrically. The monoamines (histamine, tryptamine, tyramine, and ethanolamine) and polyamines (spermine, spermidine, putrescine, and cadaverine) were identified in fresh pork bellies at concentrations ranging from 0.03 mg for cadaverine to 8.1 mg for spermine per 100 g of tissue. Processing into bacon did not significantly alter the levels of the free amines. Similar amines were identified in fresh hams with concentrations ranging from 0.5 mg for tyramine to 189 mg for putrescine per 100 g of fresh tissue (*66*). This latter study demonstrated that cooking decreased the concentration of amines and that

[1]*University of Guelph. Present address: Department of Food Science and Human Nutrition, Michigan State University, East Lansing, Michigan 48824.*
[2]*Agriculture Canada.*

significant increases in spermine, spermidine, putrescine and cadaverine occur during putrefaction. Commercial curing and smoking of hams had no discernible effect on the concentration of individual amines.

Singer and Lijinsky (*112*) described a procedure for analysis of naturally occurring secondary amines in foodstuffs, based on formation of the p-toluenesulfonamide derivatives. They reported the presence of dimethylamine, di-n-propylamine, pyrrolidine, morpholine, and piperidine in baked ham at concentrations of 2 μg/kg or less. Patterson and Mottram (*86*), using a gas chromatographic technique, determined the concentrations of volatile amines in pork carcass meat at several stages of curing. The highest concentration detected was 1900 μg of methylamine per kg of fresh meat, which decreased during the curing process. Smaller amounts of dimethylamine, trimethylamine, ethylamine, n-propylamine and isopropylamine were reported. Various amines (histamine, putrescine, tyramine, cadaverine and 2-phenylethylamine) have also been reported in dry and semi-dry sausages (*91,118*).

These results indicate that amines are naturally present and cannot be avoided in meats. However, the question arises as to whether the amounts found in meat are of much importance with respect to N-nitrosamine formation.

NITRATE/NITRITE LEVELS IN FOODS

There are numerous sources of nitrate and nitrites in the diet besides that attributable to the curing of meats. Several recent articles examine nitrate and nitrite levels in foods (*5,119,123*) and as components of the normal environment (*60*).

Nitrates are natural components of the environment and constitute the primary source of fixed nitrogen for green plants. Although nitrite is presently the chemical of concern because of its involvement in N-nitrosamine formation, nitrate is also important since it is readily reduced to nitrite under physiological conditions. From United States data (*123*), it has been estimated that the average daily consumption of nitrate is 99.3 mg, 86.3% of which comes from vegetables and 9.4% from cured meats (Table 1). The amount contributed by cured meat is probably even lower today. In the period from 1970 to 1974, the percentage of processors not using nitrates in their cures increased from 50 to 100% for shelf-stable meats, 33 to 78% for refrigerated canned meats and other perishable cured meats, and for fermented and semi-dry sausage, nitrate levels decreased from 1328 to 625 mg/kg (*5*). Walker (*119*) concluded that in the United Kingdom, vegetables and water supplies make a greater contribution to the mean weekly nitrate intake than do cured meats. In Canada, elimination of nitrate from most cures probably means that cured meats are also a minor contributor to our nitrate intake. Thus, the simplest way to reduce dietary nitrate intake is to eat less celery, lettuce, spinach and beets since these contribute 42% of the nitrate from all food sources (*60*).

There are two main sources of nitrite in the human diet (*123*). Of the average daily intake of 11.22 mg, 76.8% is derived from saliva and 21.2% from cured meats (Table 1). The source of the salivary nitrite is derived from the ready conversion of dietary nitrate to nitrite by the action of normal bacteria and other microorganisms in the mouth. Consumption of vegetables and vegetable juices containing high levels of nitrate have resulted in increases of salivary nitrite to hundreds of parts per million, many times higher than that permitted in any food product (*113*). A complementary study by Tannenbaum et al. (*116*) also revealed that the extent of salivary nitrite formation is related to the quantity of nitrate, to the concentration of the nitrate source, and to the oral microflora. Because of this salivary nitrite, reductions in nitrite usage and residual nitrite levels in cured meats will not drastically reduce the ingestion of nitrite.

TABLE 1. *Estimated average daily ingestion for U.S. resident* (123).

Source	Nitrate		Nitrite	
	(mg)	(%)	(mg)	(%)
Vegetables	86.1	86.3	0.20	1.8
Fruits, juices	1.4	1.4	0.00	0.0
Milk and products	0.2	0.2	0.00	0.0
Bread	2.0	2.0	0.02	0.2
Water	0.7	0.7	0.00	0.0
Cured meats	9.4	9.4	2.38	21.2
Saliva	30.0[a]		8.62	76.8
Total	99.8	100.0	11.22	100.0

[a]Not included in total.

In the light of these findings on natural sources of nitrates and nitrites, the suggestion of some groups that elimination of nitrates and nitrites from cured meats will solve the N-nitrosamine problem appears illusary.

Studies on amounts of nitrate and nitrite in foods require accurate methodology. A recent review (*117*) critically compares the various published methods for determination of nitrate and nitrite levels in foodstuffs, especially meats. Published methods show wide variations in techniques for extraction, clean-up of extracts, and final determinative steps. The most widely used method for nitrate determination involves nitrate reduction by spongy cadmium, and nitrite determination by formation of a diazo compound. Problems with these techniques are critical for determination at the 10-20-mg/kg levels and are outlined in the review. At these concentrations, another significant source of error could be the filter paper which has been shown to contain up to 18.4 mg of nitrite/kg (*21*) and 20-30 mg of nitrate/kg (*20*).

Various new techniques including automatic methods and ion selective electrode methods have been proposed and need to be tested using meat systems. A nitrate-sensitive electrode method was found to be rapid (15-20 min) and accurate for amounts above 20 mg/kg (*71*). Results comparable to those obtained with the AOAC method (*2*) were obtained using a nitrogen oxide

electrode method for measuring the nitrite content of fish (*111*). A fluorometric determination using fluorescamine has been found to be a useful alternative to the AOAC method (*10*). It is just as lengthy as the latter method, but is free from interference due to cloudy or colored solutions. Hilsheimer and Harwig (*54*) modified the Kamm extraction procedure (*61*) by increasing the time of nitrite extraction from 10 to 30 min followed by reaction with a sulfanilic-5-amino-2-naphthalene sulfonic acid mixture. The authors claim that this provides a suitable and convenient method for the determination of nitrite in various foods.

FATE OF NITRITE IN CURED MEAT PRODUCTS

Before 1974, with the exception of the cured meat pigment, nitric oxide myoglobin, and the residual nitrite, knowledge of the fate of added nitrite in cured meats was minimal. Earlier studies of nitrite depletion in either model systems or commercially processed meat products all showed that the nitrite level fell rapidly with time (*51,84,90*). However, these investigations failed to account for most of the nitrite lost. Determination of the fate of the unknown portion of the depleted nitrite is necessary, since it may provide information in helping to evaluate potential hazards as well as in clarifying the role nitrite plays in other functions such as flavor or bacterial inhibition (*98*).

Using a model system composed of myoglobin, nitrite and ascorbate, Japanese researchers (*32*) recovered all of the nitrogen in nitrite as residual nitrite, nitrate, denatured nitric oxide myoglobin and gaseous nitrogen compounds (nitric oxide, dinitric oxide and nitrogen gas). In a later study with meat systems (*16*), recoveries of 66-99% of added nitrite were obtained, which are similar to the results of University of Wisconsin investigations (*98*). Both studies indicate that nitrite is a very reactive compound in a meat product and besides the pigment fraction, it is involved with the water-soluble and salt-soluble meat fractions. It has been postulated that nitrite can react with the sulfhydryl groups of muscle proteins to form nitrosothiols (*33*), but little of the nitrite-nitrogen has been found in this form (*16,65*). This would suggest that only a small proportion of the total nitrite lost during the curing process can be accounted for by the direct reaction between nitrite and sulfhydryl groups.

It has been recently demonstrated that bovine serum albumin and myosin have the ability to bind appreciable amounts of nitrite with resulting modifications of the protein (*128*). These included production of 3-nitrotyrosine, 3,4-dihydroxyphenylalanine, and possibly N-nitrosotryptophan. Such results indicate that C-nitrosation reactions involving protein must be considered as one of the major pathways for loss of nitrite in cured meats. With liquid and conventionally smoked cured meat products, C-nitrosation of phenols can also occur (*63*).

Woolford and Cassens (*127*) attempted to trace the reaction pathways of nitrite in bacon using ^{15}N-labelled sodium nitrite-cured pork bellies, with and without addition of sodium ascorbate. Depletion of nitrite, originally at 156 mg/kg, was followed during the processing operation and it was shown that ^{15}N was incorporated into both the protein and lipid portions. Mass spectral measurements showed that between 73 and 87% of the added ^{15}N remained in the bacon lean portion. A subsequent paper by the same research group (*38*) confirmed the reaction of nitrite with components in the adipose tissue of bacon and indicated that distribution of free nitrite in injected whole adipose tissue was variable. When whole adipose tissue was divided into lipid, connective tissue, and water phases, free nitrite was detected in each.

In another study, Lee et al. (*70*) determined the effect of the chemical state of myoglobin and presence of ascorbate on conversion of nitrite to nitrate. In model systems, nitrate was not formed when nitrite was incubated in the presence of either metmyoglobin or ascorbate. However, when both ascorbate and metmyoglobin were present, most of the added nitrite was converted to nitrate. The authors suggested that metmyoglobin was reduced to myoglobin by ascorbate, and then the myoglobin was oxidized simultaneously with nitrite to form nitrate. Nitrate was also detected in cured meat products, both in the absence and presence of ascorbate.

The fate of nitrite in cured meat products can therefore be generalized as follows, where the values are a percentage of the nitrite originally added (*8*): nitrite, 5-20%; nitrate, 1-10%; gases, 1-5%; sulfhydryl, 5-15%; lipid, 1-5%; protein, 20-30%; and myoglobin, 10-20%. It is interesting to note that heated myoglobin binds approximately twice as much nitrite as non-heated myoglobin, suggesting that both the free coordination positions of iron are occupied by nitric oxide in the presence of nitrite in heated samples (*69*).

OCCURRENCE OF N-NITROSAMINES IN CURED MEATS

In 1976, the U.S. Food and Drug Administration surveyed 121 foods for 14 volatile N-nitrosamines (*50*). The classes of food products included baby foods containing cured meats, imported cheeses, lard, Icelandic foods, spice-cure mixes, six different total diet samples and various meat products (hams, liver, bacon). Besides bacon, N-nitrosamines were found only in spice-cure mixes, and included dimethylnitrosamine (DMN), N-Pyr and N-nitrosopiperidine (N-Pip) at levels of 50-2000 μg/kg. Studies in the United Kingdom (*34*) also indicated large amounts of N-Pyr and N-Pip in spice-cure mixes. Such results support Canadian and United States regulations requiring that curing agents and spices be packaged separately (*39*).

The product of concern is still bacon; the bacon familiar in North America and Wiltshire bacon in the

United Kingdom. N-Pyr is commonly detected in cooked bacon samples, whereas DMN is only sporadically found. Interestingly, the amounts being detected in the cooked bacon or the rendered fat constitute only a portion of the total quantity of N-nitrosamines being formed. During frying, a substantial portion of the N-nitrosamines is volatilized in the fumes. This phenomenon has been investigated by several workers who reported a wide range of values for the percentage of N-nitrosamines found in the vapor (Table 2). Obviously, the mode of cooking as well as the moisture content of the bacon samples influences the amounts of N-nitrosamines in the vapor.

TABLE 2. *N-Nitrosamines in the fumes produced during the frying of bacon or ham.*

Investigators	N-Nitrosamine (μg/kg) N-Pyr	DMN	Sample
Gough et al. (*35*)	60-95	75-100	bacon
Hwang and Rosen (*56*)	14-37	-	bacon
Warthesen et al. (*121*)	20-40	-	pork belly[a]
Sen et al. (*107*)	28-82	28- 92	bacon
Eisenbrand et al. (*15*)	45-52	74- 83	ham[b]
Gray and Collins (*41*)	27-49	-	pork belly[a]
Mottram et al. (*81*)	57-75	73- 80	bacon
Gray et al. (*43*)	-	56- 80	pork belly[a]

[a]Contained added nitrite.
[b]Contained added N-nitrosoamino acids (40 μ mole 50/g of tissue).

The major factors which influence formation of N-Pyr in bacon have been well documented (*39*) and include the method of cooking, nitrite concentration, sodium chloride concentration, and presence of ascorbic acid. Since the rate of N-nitrosation of secondary amines is directly proportional to the square of the nitrite concentration (*76*), it is not surprising that the amount of nitrite permitted in bacon has received considerable attention. Since N-nitrosamine formation in bacon is directly related to the initial nitrite concentration, an amendment (April, 1975) to the Canadian Food and Drug Regulations regarding use of nitrites and nitrates in bacon has been made (B.16.000, Items P_1 and P_2, Table xi, Part 1). The amount of nitrite to be used in preparation of side bacon has been reduced to 150 mg/kg, calculated before any smoking, cooking, or fermentation. Use of nitrate in pumping pickle has also been prohibited.

At the end of 1975, about 6 months after promulgation of the new law, the Health Protection Branch, Health and Welfare Canada, carried out a survey of a limited number of bacon samples for nitrite, nitrate and N-nitrosamine contents (*103*). Results of this survey, when compared to those of previous surveys (Table 3), indicate that the amounts of N-Pyr in cooked bacon have decreased considerably during the past few years. However, it is not clear whether this is due to the lowering of permissible levels of nitrite or nitrate, or to a combination of other factors such as greater control of the input of these additives by the meat industry, sampling variations, storage conditions, addition of ascorbates or variations in cooking conditions.

TABLE 3. *Levels of nitrite and N-nitrosopyrrolidine in Canadian bacon* (103).

	Sodium nitrite in uncooked bacon[a] (mg/kg)			μg total N-Pyr produced by cooking 1 kg raw bacon			
	1972	1974	1975	1970	1972	1974	1975
	30	64	131	20	20	22	28
	30	69	30	25	40	15	12
	76	30	68	10	18	14	16
	44	25	32	25	75	35	11
	32	101	110	10	25	30	22
	54	25	72	4	21	25	12
	12	30	52	trace	15	15	14
	32	86	95	10	30	44	2
	94	59	56		21	11	10
	20	90	2		25	20	1
	20	10	45		30	21	9
	54	32	54		31	30	10
		35				40	
		76				10	
Mean	41	52	62	13	29	24	12

[a]No data available for 1970.

Greenberg (*46*) also reported a gradual reduction in amounts of N-Pyr detected in U.S. bacon. A study by the Food and Drug Administration's Washington Laboratory has shown that the mean N-Pyr content of nine brands of bacon picked up at local retail stores decreased from 67 μg/kg for the period, December, 1971 to April, 1974, to 17 μg/kg for the period, October, 1975 to May, 1976. This reduction has taken place without any changes in U.S. Government regulation of bacon curing or processing. It is apparent that many commercial processors have taken steps that have somehow improved their bacon manufacturing process. This steady decrease in N-Pyr content is again evident from results of surveys conducted in 1973-1975 by the USDA Eastern Regional Laboratory (Table 4). These samples from Philadelphia

TABLE 4. *N-Nitrosopyrrolidine content of United States fried bacon taken from retail markets in Philadelphia* (46).

Year	Number of samples	N-Pyr (μg/kg)
1973	8	13
1974	17	10
1975	13	5

supermarkets, after frying for 6 min. at 171 C, averaged 13 μg/kg in 1973, 10 μg/kg in 1974, and 5 μg/kg in 1975. Birdsall (*6*) also investigated the N-Pyr content in bacon obtained from 10 commercial bacon production plants, two of which used ascorbate and the others, erythorbate. None of these bacon samples prepared with different amounts of added nitrite were found to be positive for N-Pyr at a level of 10 μg/kg or more. Lowering the ingoing amount of sodium nitrite from 120 to 80 mg/kg did not appear to reduce the occurrence of very low levels (less than 10 μg/kg) of N-Pyr.

Wasserman et al. (*122*) recently conducted a series of experiments in which randomized slices of bacon were cooked at home by 25 consumers and then analyzed for N-nitrosamines. Of these samples, five contained DMN and five contained N-Pyr in concentrations greater than

10 μg/kg. Only one sample contained both N-nitrosamines in concentrations greater than 10 μg/kg. Although the association between the extent of N-nitrosamine formation, time and amount of heat applied was rather inconclusive, it appeared that frying bacon at low or medium heat for less than 10 min resulted in less than 10 μg/kg DMN or N-Pyr.

PRECURSORS OF N-NITROSAMINES IN BACON

Various precursors of N-Pyr in bacon have been suggested from model system studies and include proline (and N-nitrosoproline), collagen, putrescine, spermidine, pyrrolidine, glycyl-L-proline, and L-prolyglycine (*39*). Although putrescine may make a contribution to the amount of N-Pyr during the cooking of bacon, free proline is the most likely precursor. Gray and Collins (*41*) investigated the role of proline and putrescine as precursors of N-Pyr in model and pork systems containing 150 and 1000 mg of sodium nitrite/kg. Their results indicated that, although the contribution of putrescine to N-Pyr formation cannot be disregarded, it appeared that free proline is the most important precursor of N-Pyr in fried bacon. A similar study by Warthesen et al. (*121*) also showed that when 0.1% putrescine was added to ground pork before heating, increased amounts of N-Pyr were produced. However, addition of an equal amount of proline resulted in production of even more N-Pyr. These results were also confirmed by Hwang and Rosen (*56*) who showed, using ^{14}C-labelled amines, that proline is more likely to be the precursor of N-Pyr than either putrescine or spermidine in fried bacon.

How N-Pyr is formed from proline has, as yet, not been firmly established. Several pathways have been reported. Sen et al. (*102*) proposed that free proline in bacon is converted to N-nitrosoproline (N-Pro) which, when subjected to thermal stresses is decarboxylated to N-Pyr. These findings are consistent with the data of Hwang and Rosen (*56*). Nakamura et al. (*83*) reported that the mechanism involved depends on the cooking temperature. In the temperature range of 100-150 C, amounts of N-Pyr formed from free proline via pyrrolidine were almost similar to those formed via N-Pro. At temperatures above 175 C, the yield of N-Pyr via pyrrolidine was greater than that formed via the N-Pro pathway.

The free proline contents of pork bellies have been quantitated. During storage of green pork bellies for 1 week (a reasonable holding period before curing), the free proline content increased 52% in the intact bellies, and 50 and 90% in the separated lean and adipose tissues, respectively (*67*). Similar increases were reported in a Canadian study (*40*), thus indicating that sufficient free proline is available for production of N-Pyr during frying of bacon.

Presence of N-Pro in raw bacon has been investigated by numerous workers and a large variation in amounts has been reported (Table 5). Kushnir et al. (*64*) were first to report the isolation and identification of N-Pro in uncooked bacon by forming the methyl ester and subjecting it to gas chromatographic-mass spectrometric analysis. Other methods that have been developed for quantitation of N-Pro in bacon include (*a*) denitrosation, followed by derivatization of the amino product with 7-chloro-4-nitro-benzo-2-oxa-1,3-diazole (*126*); (*b*) conversion of N-Pro to its trimethylsilyl ester followed by gas chromatography-thermal energy analyzer (TEA) analysis (*49*); (*c*) high pressure liquid chromatography with a specific photohydrolysis system (*49*); and (*d*) photolytic decomposition, followed by the Griess reaction (*14*).

TABLE 5. *N-Nitrosoproline in uncooked bacon.*

Investigators	N-Nitrosoproline (μg/kg)	Methods of analysis
Kushnir et al. (*64*)	810-1,180	GLC, methyl ester
Ivey (*58*)	13-62	GLC, methyl ester
Nakamura et al. (*83*)	N.D.[a]	GLC, methyl ester
Dhont (*14*)	400-1,500	Photolysis
Sen et al. (*105*)	24-44	GLC, methyl ester
Hansen et al. (*49*)	70	GLC-TEA, trimethylsilyl ester

[a]N.D. - Not detectable.

The more recent studies have indicated that amounts of N-Pro in raw bacon generally are less than 100 μg/kg. Therefore, if N-Pro was to be considered the principal precursor of N-Pyr in cooked bacon, then at least 75% of the N-Pro in raw bacon must be converted to N-Pyr during cooking. This has been shown not to happen. For example, Gray and Collins (*42*) showed that when bacon slices containing 1mM N-Pro were cooked at 180 C for 12 min, only a 1.48% yield of N-Pyr was obtained. Similar results were obtained by Hwang and Rosen (*56*) who observed that the rate of decarboxylation of the initial N-Pro in uncooked bacon is not great enough to account for the N-Pyr isolated from cooked bacon.

While most of the recent N-nitrosamine research has centered on N-Pyr and its precursors in bacon, information on formation and precursors of other volatile N-nitrosamines is still lacking. Although DMN has been reported in fried bacon, there have been very few reports as to the actual precursors of this N-nitrosamine. Model system studies in the past have implicated several compounds including dimethylamine and trimethylamine (*17,23,97*), quaternary ammonium compounds (*23*), sarcosine (*15,17*) and lecithin (*78,87*). Gray et al. (*43*) investigated various compounds, all of which were endogenous to bacon systems, as possible precursors of DMN in bacon. Under conditions normally encountered in the pan-frying of bacon, choline-containing compounds and sarcosine produced measurable quantities of this N-nitrosamine. Because of the extreme carcinogenicity of DMN, additional research is required to elucidate its mode of formation in bacon and other cured meat products.

Diethylnitrosamine (DEN) has not often been reported in cured meat products. This is not surprising since to the present time most investigations have concentrated on N-Pyr and DMN. Panalaks et al. (*85*) reported DEN in a number of meat products including fried bacon, liver sausage and meat. Groenen et al. (*47*) also reported the presence of DEN in bacon and showed that during "mild" frying of two bacon samples, the DEN concentration increased from 4 and 13 to 16 and 43 μg/kg, respectively. It was also present in two smoked meat samples in amounts of 7 and 91 μg/kg, respectively. In view of these apparently large amounts, presence of this N-nitrosamine as well as its potential precursors in cured meats should be more thoroughly investigated.

While much research has fully established the presence of N-Pyr and, to a lesser extent DMN, in cooked bacon, much work remains to be done on "unusual" N-nitroso compounds. Belonging to such a group is the N-nitrosamine, 3-butenyl-(2-propenyl) nitrosamine, which is the major N-nitroso compound formed from N-nitrosation of spermidine or spermine (*52*). The same research group also reported formation of two hydroxylated dialkyl-N-nitrosamines, 3-butenyl-(3-hydroxypropyl) nitrosamine and 4-hydroxybutyl-(2-propenyl) nitrosamine when sodium nitrite and spermidine-3HCl were reacted at pH 3.5 and 80 C (*53*). A further study revealed formation of bis (hydroxyalkyl)-N-nitrosamines as products of the N-nitrosation of spermidine (*56*). As yet, none of these compounds have been identified in cooked bacon (*48*).

NON-VOLATILE N-NITROSAMINES IN CURED MEATS

As well as the volatile N-nitrosamines, non-volatile N-nitroso compounds may be found in cured meats. Presence of these compounds in foods has not been widely reported, since their non-volatile character does not facilitate their isolation from foodstuffs. However, there have been several recent reports regarding formation of 3-hydroxy-1-nitrosopyrrolidine (HN-Pyr) in model and cured meat systems. This compound had not been previously isolated and identified, probably because the solvent systems (dichloromethane or diethyl ether) normally employed for N-nitrosamine extraction were not adequate for the more polar HN-Pyr.

Sen et al. (*106*) described a highly specific gas chromatographic-mass spectrometric technique, whereby HN-Pyr could be determined at nanogram quantities after conversion to a stable, volatile derivative, namely 3-methoxy-1-nitrosopyrrolidine. This method was used to determine the HN-Pyr content in cooked bacon (*101*) and included extraction of the sample with acetonitrile followed by removal of fats by liquid-liquid extraction with n-heptane. The extract was cleaned up by acidic alumina column chromatography, followed by derivatization of HN-Pyr to its methyl ester. Of 13 cooked bacon samples analyzed, only two contained measurable (8 and 12 μg/kg) amounts of HN-Pyr. The limit of detection was 2 μg/kg. Eisenbrand et al. (*15*) also reported presence of HN-Pyr in 7 of 11 different cured meat products that had been fried for 8 min at 180-190 C. Values ranged from traces to 9 μg/kg.

Two other groups (*44,68*) have also isolated HN-Pyr from model systems using methanol as the solvent. Gray et al. (*44*) blended aqueous solutions containing HN-Pyr with carboxymethylcellulose which was freeze-dried and then extracted with methanol. This procedure yielded a 20% recovery of the HN-Pyr. However, since the primary objective of the study was to demonstrate that 4-hydroxyproline was indeed a precursor of HN-Pyr, improvement in the procedure to increase the recovery was not considered. Lee et al. (*68*) demonstrated that decarboxylation of 4-hydroxy-N-nitrosoproline readily occurs in a model system simulating conditions under which bacon is fried at a pan temperature of 170 C. They also reported isolation and identification of HN-Pyr in fried bacon and fried-out fat and that efforts to quantitate the N-nitrosamine were being continued.

ANALYSIS OF N-NITROSAMINES IN CURED MEATS

Determination of N-nitrosamines in food products requires analytical methods having adequate sensitivity and selectivity. Much of the literature on this topic has been recently presented in several reviews (*12,13,31,96*). The complexity of analyzing for N-nitrosamines is well documented and, as stated by Foreman and Goodhead (*31*), "the main problem in estimating N-nitrosamines in foodstuffs is to isolate the particular N-nitrosamine in a form in which it can be unequivocally identified, bearing in mind that it will be most likely present, if at all, at a concentration of about 1 part/10^9 in a complex matrix, many components of which will contain nitrogen and react chemically in a similar manner to the N-nitrosamine." In addition, it is a widely held opinion that the only unequivocal confirmation of a N-nitrosamine in a food matrix is achieved using high resolution mass spectrometry (MS).

In the past 3 years, development of the thermal energy analyzer (TEA) has resulted in a very sensitive and specific method for N-nitrosamine analysis. This technique is uniquely selective to the N-nitroso functional group and is sensitive to picogram quantities (*29*). When the TEA was interfaced directly with a gas chromatograph (GC), GC-TEA and GC-MS results were in excellent agreement when analyzing for trace amounts of volatile N-nitrosamines in foodstuffs (*26*). In addition, comparable results were obtained by GC-TEA analyses of crude aqueous distillates and final methylene chloride extracts following extensive clean-up (*28*). When combined with high pressure liquid chromatography, the TEA is capable of quantitatively detecting μg/kg levels of non-ionic nonvolatile N-nitroso compounds in complex foodstuffs (*24,25*).

The TEA system which detects N-nitrosamines as nitric oxide after catalytic cleavage of the N - N = 0 bond is simple in concept but is much more expensive than a conventional GC detector. Gough and co-workers (*36,37*) have stated that, although it has a much better detection limit than existing detectors for N-nitrosamines, it cannot be regarded as a replacement for the relatively cheap but unsatisfactory detectors for screening, nor at the present time at least, as an alternative to MS for confirmation. These authors have described the construction of an inexpensive detector also based on the principle of chemiluminescence and reported that it will respond selectively to trace amounts of N-nitrosamines. The detection limit was the same as that obtained using GC-MS and it was claimed that the detector was suitable for screening extracts of foodstuffs and other complex mixtures before confirmation of the presence of N-nitrosamines by MS.

PREVENTION OF N-NITROSAMINE FORMATION

To prevent N-nitrosamine formation, the ideal situation would be to eliminate the precursors, nitrite and amines from the diet. However, both nitrites and amines are natural components of our environment, making total elimination impossible. To date, sodium ascorbate has been the preferred compound in attempts to reduce N-nitrosamine formation in meat products. Additional studies have demonstrated that with Wiltshire bacon fortified with dimethylamine, addition of sodium ascorbate resulted in an 80% suppression of DMN formation (*79*). Recently more attention has focused on the lipophilic derivatives of ascorbic acid since it has been reported that sodium ascorbate has no effect on N-nitrosamine formation in the lipid phase (*88*). Propyl gallate and L-ascorbyl palmitate were found to be more effective than sodium ascorbate in reducing N-Pyr formation during the cooking of bacon (*104*).

Mottram and co-workers (*79,80*) also studied the effect of ascorbate reductants on N-nitrosamine formation in a model system resembling bacon fat. The N-nitrosation of dipropylamine and pyrrolidine was examined in a two-phase system comprising an aqueous buffer and a non-polar solvent. Contrary to previous observations with purely aqueous systems, sodium ascorbate increased amine N-nitrosation in this two-phase simulated fat system by between five and 25 times compared with ascorbate-free controls. Lipophilic ascorbyl palmitate, however, generally reduced N-nitrosamine formation. Walters et al. (*120*) reported that, while both *a*-tocopherol and ascorbyl palmitate had similar actions in reducing or eliminating N-nitrosamine production in model systems simulating bacon frying, there was no noticeable synergistic effect when the two antioxidants were used in combination. Reduction of N-nitrosamine formation with *a*-tocopherol had been previously reported by Gray and Dugan (*45*). Pensabene et al. (*89*) also investigated the effect of novel water-dispersible *a*-tocopherol mixtures on the N-nitrosation of pyrrolidine in an oil-aqueous-protein model system consisting of oil, water, protein, sodium chloride and sodium tripolyphosphate. They showed that *a*-tocopherol, when dissolved in the emulsier Polysorbate 20 in the ratios of 1:6, 1:1, 1:0.4 and 1:0.2, inhibited N-Pyr formation. Further work is currently in progress to evaluate the distribution of *a*-tocopherol in bacon prepared from pork bellies cured with a solution containing *a*-tocopherol, Polysorbate 20, salt, sugar and sodium tripolyphosphate, and the resulting effect on N-nitrosamine formation.

Rubin and Bharucha (*95*) investigated the effects of long-chain acetals of ascorbic acid as potential anti-N-nitrosamine agents. They demonstrated that the acetal prepared from dodecanal, when used at the 500 and 1000 mg/kg levels, was capable of reducing N-nitrosamine formation in the cooked-out fat of bacon by 92 and 97%, respectively. Acetals prepared from higher molecular weight aldehydes (C_{16} or C_{18}) again showed excellent inhibition of N-nitrosamine formation (approximately 96%) in the cooked-out fat and moreover, did not impart the objectionable soapy aftertaste normally associated with the C_{12} or C_{14} acetals.

The patent literature also contains proposed methods of reducing N-nitrosamine formation in bacon. Many of these, before 1974, have been summarized by Kelly (*62*). More recent proposals involve replacement of sodium nitrite with butyl nitrite (*3*) in the curing of bacon and a two-step curing process, involving use of sodium nitrite in the first step and sodium metabisulfite in the second step (*9*).

TOXICOLOGY OF NITRATE, NITRITE AND N-NITROSO COMPOUNDS

The current data on the toxicology of these compounds have been discussed in several reviews (*109,115*). Nitrate is essentially non-toxic to mammals, whereas nitrite presents a toxic hazard because of the direct toxicity of nitrite and by formation of carcinogenic N-nitroso compounds by reaction with amine compounds. N-Nitroso compounds are known to be toxic, teratogenic, mutagenic and carcinogenic (*75*) but how this relates to the etiology of cancer in man requires elucidation.

There are two opposite opinions on the latter matter. Jones and Grendon (*59*) contend that continuous dietary exposure of humans to cured meats, including bacon, controlled within current levels of nitrites and nitrates, would not produce cancers within the lifespan of humans. These estimations were supported by demographic trends in the U.S. population. On the other hand, Lijinsky (*72*), as a result of rat studies, concluded that the levels of N-Pyr found in cooked bacon posed a definite health hazard. In addition, he stated that the effect of carcinogens can be considered to be cumulative, so that any amount of a carcinogen can add its effect to those of others to which the organism was exposed, resulting at some stage in development of a tumor.

The problem of nitrite and N-nitrosamines in our diet, particularly as it relates to cured meats, revolves around

the question of toxicology (*94*). What is the significance of 10 μg of N-nitrosamine/kg in one small part of the human diet? The relative risk of the population from ingesting preformed N-nitrosamines in foods must be evaluated in light of the exposure to these compounds from other known sources. It has been reported that a 30-g portion of bacon containing N-Pyr at a concentration of 10 μg/kg contributes less N-nitrosamine than 20 commercial filter-cigarettes (*7*). These investigators have also shown that non-smokers in an indoor environment polluted with cigarette smoke can expect to inhale DMN in amounts from 5×10^{-9} g per hour in a bank to 2×10^{-7} g per hour in a bar. N-nitrosamines have also been reported in samples of urban air (*27*) and, in large amounts in certain cutting oils, agricultural chemicals and cosmetics (*18,19,93*).

At present our knowledge of the total exposure of the population to N-nitroso compounds is still incomplete. Most studies have centered on the volatile N-nitrosamines and it is possible that these compounds represent only a portion of our total exposure (*57*). Studies of more complex non-volatile N-nitroso compounds will be required to permit accurate assessment of the total exposure of the human population to these potential human carcinogens for reference in future epidemiological studies.

In recent years, many toxicological experiments have been conducted on N-nitrosamines or their precursors. Several interesting studies on the concurrent feeding of an amine and nitrite to guinea pigs are worth noting. This species is very suitable for such tests since it resembles man in requiring an exogenous source of ascorbic acid and its stomach pH is similar to that of the human stomach. On the basis of studies using this species, it can be concluded that ingestion of low levels of strongly basic secondary amines, such as dimethylamine or diethylamine, and nitrite is unlikely to lead to formation of significant levels of N-nitrosamines in the stomach (*108*). At levels which caused tumors in mice, feeding of morpholine and nitrite together with ascorbic acid did not cause any of the toxic responses seen with N-nitrosomorpholine (*1*). These authors speculated that man and other species requiring dietary sources of ascorbic acid may have a built-in protection against in vivo N-nitrosation. Shank and Newberne (*110*) also fed various amounts of mixtures of morpholine and nitrite with up to 1000 mg/kg of each to rats and hamsters. Tumors were readily produced in rats, but not in hamsters, illustrating an interesting species difference. While the relation of these experiments to real-life situations is not entirely obvious, these investigators pointed out that nitrates, nitrites and amines have been widespread in the environment for many years, without any apparent increase in chemically related cancer of the types produced in experimental animals.

One of the most promising of recent toxicological studies is the bacon-feeding study conducted by Bio Research Laboratories in Montreal for the Health Protection Branch, Health and Welfare Canada (*4,94*). In this study, the test rats were fed a diet of 25% commercial bacon cured with 150 mg of nitrite/kg. Preliminary results showed that the rats fed nitrite-cured bacon containing the suspected N-nitrosamines did not have a significantly higher incidence of tumors than did the rats fed nitrite-free bacon (*4*). While these preliminary results are encouraging, the meat industry and governmental agencies must withhold judgement until the experimental results are fully analyzed.

REGULATORY CHANGES AFFECTING NITRITE/NITRATE USAGE

Various changes in the Canadian Food and Drug Regulations for nitrite and nitrate usage in preserved meat and meat by-products were implemented as of April 8, 1975 (*39*). Nitrate (potassium or sodium) was eliminated from all but dry and semi-dry sausages and certain preserved meat and meat by-products prepared by slow-cure procedures. The level permitted was 200 mg/kg, based on total batch weight. Nitrite (potassium or sodium) will be controlled at an input level of 200 mg/kg, based on total batch weight before any smoking, cooking or fermentation. The only exception is bacon for which the input level was reduced to 150 mg/kg. Previously, these amounts referred to that permitted in the final product, that is, residual amounts. In addition, the same regulations apply to preserved poultry meat by-products.

In the United States, the Department of Agriculture, on a recommendation by an Expert Panel on Nitrites, Nitrates and N-Nitrosamines, published proposed regulatory changes for nitrite and nitrate usage (40 Fed. Reg. 5264, Nov. 1975). These included: (*a*) discontinuing use of nitrate in processed meat and poultry with the exception of dry-cured and fermented sausage products and country ham; (*b*) limiting the amount of ingoing nitrite in bacon to 125 mg/kg in the presence of 500 mg of sodium erythorbate or ascorbate/kg; (*c*) restricting the ingoing level of nitrite to 156 mg/kg in cooked cured sausages; (*d*) limiting the ingoing level of nitrite in processed product except for bacon and dry-cured products; and (*e*) reducing the residual levels of nitrite in the finished product from 200 mg/kg to (*i*) 50 mg/kg in canned, cured, sterile products such as corn beef hash and deviled ham, (*ii*) 100 mg/kg in cooked sausage products such as frankfurters, bologna and luncheon meats, and (*iii*) 125 mg/kg in pickled cured products such as hams, picnics, shoulders, canned cured shelf-stable products such as canned, chopped meat and ham salad spreads, and canned perishable products such as canned ham. As indicated in the Cast Report (*11*), the cured meat industry began voluntarily to adopt the 1975 recommendations, even though the latter have not yet been adopted as regulations.

IMPACT OF LOSS OF NITRITE ON THE PORK INDUSTRY

The ramifications of discontinuing the use of nitrite in meat processing have been discussed at length in recent years (*4,11,74*). Without nitrite, a large class of traditional foods would be eliminated from our diet. As pointed out by MacDougall et al. (*73*), cured meats are distinctly attractive in their color, texture and flavor, and are popular because they combine this variety in a meat with the convenience of high storage stability.

More important, however, than the loss of traditional food items are the economic implications. Domestic production of cured meats in the United States is more than 9 billion pounds per year. Consumption is even higher because the United States is an importer of cured meats, and it has been calculated that the retail value of cured meats sold each year exceeds $12 billion (*4*). A nitrite ban in bacon would result in loss of farm income, fewer hog farmers and less employment in meat packing, distribution and retail establishments, loss of export markets for pork, financial losses from the closing of facilities, and loss of cash and future markets. The magnitude of the losses to consumers, farmers and the food industry from the loss of bacon would be multiplied several times if use of nitrite were discontinued in all processed meats. Madsen (*74*) has summarized the costs and benefits from the elimination of nitrites in cured meats (Table 6).

TABLE 6. *Summary of the costs and benefits from the elimination of nitrites in cured meats* (74).

Costs	Benefits
1. Possible botulism	1. Possibility of a reduction in the risk of cancer
2. Less farm income	
3. Less employment in farming	
4. Less employment in meat packing, distribution and retailing	
5. Loss of export markets for pork	
6. Depressed trimmings market	
7. Losses from closing of facilities	
8. Less choice for consumers at the meat counter; fewer convenience foods	
9. Loss of cash and future markets	

The pork industry is committed to use of nitrites in preparation of cured meat products, simply because there is, as yet, no suitable alternative. The danger of botulism is regarded as very real and there are regular outbreaks of botulism from home-cured meats in France and Spain, where nitrite and /or nitrate are not used, or used under poor control. In constrast, no botulism has resulted from the commercially produced cured meats where use of these salts is controlled, and this despite the demonstrable presence of *Clostridium botulinum* (*92*). Consequently, proposals to alter curing practices must take this into account if the enviable record of bacon in relation to food poisoning is to be maintained. As pointed out by Madsen (*74*), the risk of botulism is real whereas the risk of cancer is only theoretical.

CONCLUSIONS

The large number of research publications over the past several years has answered a number of questions about the functions of nitrite and the formation, chemistry, precursors and toxicity of N-nitrosamines in cured meats. However, even with our increased knowledge in this area, a number of questions remain unresolved. Some of these include:

1. DMN has been sporadically detected in cured meats. What is the mechanism of its formation and why is it not found more consistently?
2. Trace amounts of DEN and other volatile dialkyl nitrosamines have sometimes been reported in cured meats and other foods. What are the origins of these N-nitrosamines?
3. Cooking of bacon under normal conditions of frying results primarily in N-Pyr formation. Would the frying of other cured meat products - wieners, sliced ham, luncheon meats, bologna - give similar results?
4. Recent studies indicate that a large percentage of the N-Pyr and DMN being formed is volatilized during the cooking process. Of what toxicological significance are these N-nitrosamines in our environment?
5. Until recently, little research has been conducted on the non-volatile N-nitrosamines. How extensive are these in our foods, of what significance are they toxicologically, and are they precursors of the more volatile N-nitrosamines?
6. Are N-nitrosamines being formed in the stomach and if so, to what extent?
7. Although known to be carcinogenic, how serious a health hazard is the presence of N-nitrosamines in our foods, especially bacon?
8. Unless being conducted by industry and not being reported, research on alternative curing procedures is lacking.
9. Are there alternative ways in which pork bellies could be utilized?

These are just some research areas which require further extensive investigation. The development of more rapid, sensitive and specific methods of analysis will facilitate some of these studies, while further toxicological experiments will hopefully enable us to make a reasoned judgement as to the risks involved in using nitrite.

NOTES ADDED IN PROOF

1. Since the preparation of this manuscript, the U.S. Department of Agriculture has amended the Federal meat inspection regulations regarding the use of nitrates, nitrites, and ascorbates in bacon (*Federal*

Register 43 (95):20992, May 16, 1978). Regulations now prohibit use of sodium and potassium nitrate in bacon, require that 120 mg/kg ingoing sodium nitrite, or an equivalent amount of potassium nitrite (148 mg/kg) be added to bacon, and require that specified levels of sodium ascorbate or sodium erythorbate (isoascorbate) be used in the preparation of bacon. Additionally, bacon containing confirmable levels of carcinogenic N-nitrosamines after preparation for eating is deemed to be adulterated.

2. The information in Table 1 is taken from the correction printed in *J. Agr. Food Chem.* 24:202 (1976).

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Principles of Food Inspection

BOYD T. MARSH

Environmental Health Services
Cleveland Department of Public Health and Welfare
1925 St. Clair Avenue, Cleveland, Ohio 44114

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ABSTRACT

A brief review of the history of food-caused illness in humans and those categories or areas in a foodservice operations that are considered critical by public health personnel are discussed. The role of the foodservice manager is examined with respect to foodborne outbreaks. Some issues divergent from basic sanitation, but nevertheless, important to the foodservice manager and to public health personnel, are presented.

When the average consumer enters a foodservice establishment and purchases anything from a snack to a complete meal, that person is most immediately concerned with appearance and taste of the food, "atmosphere" of the eating place, and cost of the food. Rarely does the consumer give thought to the safety of the food. Most of those consumers simply assume the food to be safe just as they assume that pasteurized milk is safe. Usually their assumption is correct, but sometimes an unpleasant surprise awaits them 3 or more hours after they consumed food in a foodservice establishment (*6*).

The recognition that food may cause sickness and death goes back to the earliest period of recorded history. Experience taught man that certain naturally occurring plants always produced illness when eaten. These were long ago eliminated from the eating habits of mankind. They also learned that some animal foods produced illness during certain seasons. These they also guarded themselves against. That certain naturally occurring acceptable food sometimes caused illness was also known early in man's history, but the reasons why this happened did not come to light until the germ theory of disease was demonstrated and became an accepted fact. This was scarcely more than three quarters of a century ago (*4*).

Most of the precise knowledge about foodborne poisons and foodborne infections is of relatively recent discovery, and even today much is yet to be known about these subjects. The field at present recognizes only a few fundamental causes for the spoiling of food, although the circumstances in which these fundamentals operate are innumerable, most of which relate to the handling of food. For example, one fundamental cause of spoiling is bacterial contamination, but such contamination may take place in any number of circumstances associated with handling of food in the process of production, preservation, storage, distribution, and preparation for consumption (*4*).

Preparing and serving wholesome food to the public is a very important obligation of the foodservice industry. It is an obligation that can only be fulfilled if everyone in every establishment understands what sanitation is, appreciates its importance, and practices it in whatever task performed. Practicing sanitation means applying sanitary measures at every stage of the operation for the sake of cleanliness and for the sake of protecting the health of the public served (*5*).

SANITARY PRACTICES

Sanitary practice, then, is concerned with the purchase of a sound food supply and its sanitary storage; with the adequacy of the physical plant and its maintenance regarding repairs and cleanliness; with the adequacy and cleanliness of storage facilities, equipment, utensils; with sanitary dishwashing operations; with the good health, good personal hygiene, and good working habits of the food handler; with the sanitary manipulation of food and effective time-temperature control throughout preparation and service; and finally with the education of foodservice employees in the various aspects of sanitation in a foodservice operation (*5*).

Many of the factors covered in the inspection of eating establishments are inferentially related to health. For example, it might be difficult to show that a restaurant floor had to be constructed of smooth and non-absorbent material to protect patrons (*1*). Additionally no valid proof exists, nor is there necessarily a direct relationship, that if dust or dirt gets on food, the person who eats the food will have his health automatically impaired. Also, there may be no overwhelming evidence that a person who coughs on food will produce illness in another person who eats the food. Yet each item checked in an

inspection contributes to the overall image of good, safe operating practices and the public should expect, and is entitled to, sanitary and safe food when dining in a public eating establishment.

The basic, bottom line reason for public health inspection of foodservice operations is to prevent, or at least to reduce the probability of food-borne illnesses. Every step in the handling of food may be a point at which contamination may occur.

CAUSES OF FOODBORNE ILLNESS

It cannot be too strongly emphasized that in the vast majority of outbreaks of food-borne sickness, the food affected is not noticeably altered in appearance, taste and/or smell. The principal causes of food-borne disease outbreaks are:

1. Failure to refrigerate perishable foods.
2. Sick or diseased employees.
3. Poor employee personal hygiene.
4. Failure to thoroughly cook foods that might be contaminated.
5. Obtaining food from unsafe sources.
6. Failure to clean and sanitize kitchen utensils and equipment.
7. Poor food storage practices leading to contamination by rodents, sewage, customers and/or with toxic materials.

Reduced to the simplest terms. Protecting people from illness due to contaminated food does appear to be a pretty straightforward matter. It is! The stumbling block comes in awakening people, foodservice personnel, to the existence of the problem and getting them to act on it - by habit. Key factors in the protections of the public are:

1. Informing employees on the nature of disease. They should not come to work when they have a communicable disease.
2. Safe water supply.
3. Proper toilet and lavatory facilities.
4. Approved methods of waste disposal.
5. Proper refrigeration and food storage.
6. Proper bactericidal treatment.

Some formal training in basic principles of foodservice should be required of everyone who handles food, and sanitation must be an important part of such training (*5*). The foodservice manager has a clear-cut single objective: to protect people against illness from contamination of food by harmful organisms and their toxins, and by other poisonous materials (*7*). To do this the foodservice manager must be committed to one or both of two courses of action:

1. To keep food free of bacterial contaminants in the first place; and/or
2. To prevent growth of bacteria that may invade food during storage, preparation, and service.

In the final analysis, the integrity of the management and the quality of the employees are the true keys to sanitary and safe food in foodservice operations.

CHANGES IN FOODSERVICE OPERATIONS

Protection of food from unwanted microbial contamination and unwanted microbial growth is a present, and will be a future, concern of the foodservice industry. Even with this in mind, we must remember that the foodservice industry is different now than it was years ago.

There are fewer full-service food operations which means less food preparations and, consequently, fewer opportunities for contamination. Foodservice operations are purchasing more pre-portioned and pre-prepared foods that are more safely stored and need little handling or preparation. Any reduction in food handling also reduces the probability of contamination and less opportunity for bacterial growth. There are more chain and/or franchised operations with limited menu items allowing for reduced and specialized food handling.

Additionally, there are issues upon us that are different than protecting food from contamination and/or reducing the probability of a food-borne outbreak. The foodservice industry and public health personnel are also concerned with:

1. Truth-in-menu criteria.
2. The use of analogue foods.
3. Nutritional labeling on menus
4. The use of consumer give-aways.
5. Public disclosure of foodservice inspection records.

Truth-in-menu guidelines

There appears to be a need for development of truth-in-menu guidelines that are directed toward protection of the consumer's health. For example, "low calorie", "salt-free" or "low sugar" term usage on a menu must be supportable by specific data.

The use of generally accepted industry and regulatory terms with regard to definition of standards of identity, quality, grade or portion must not be violated. Here is where consumers, consumer groups, or health departments acting as advocates for the consumer find instances that approach outright consumer fraud (*8*).

The Truth-In-Menu Guidelines recently published by the Chicago and Illinois Restaurant Association point out four areas where deception might take place (*2*):

1. Incorrect or deceptive geographic points of origin should not be used.

Examples of the type of menu items which might be violating this guideline if their point of geographic origin could not be substantiated by observing the labels or invoices are: Lake Superior Whitefish, Maine Lobster, Colorado Trout, Idaho Potatoes.

2. Merchandising terms relating to the method of preparation or characteristics of a food product which cannot be verified by the owner should be avoided. This area of menu misrepresentation is a difficult one to define and yet deals with the very heart of the truth-in-menu problem.

Included in this category would be such statements as: "our own special sauce", "fresh daily", "home-made", "best blend", etc. An effective operating rule in this

rather gray area would seem to be that if a restaurant chooses to use descriptive words or phrases, the words should be accurate and the description verifiable.

3. The menu description or quality should accurately represent that item that is actually being served.

Any product brand that is advertised must be the one that is actually being served. Examples of possible violations would be serving other colas for "Coca-Cola" or other gelatine products for "Jello". Some easy examples of what to avoid with regard to dairy products in this area of prohibited misrepresentations are: margarine cannot be represented to be butter, whipped topping should not be called whipped cream, or non-dairy creamers should not be served as cream. Other common violations in this area are the substitution of halibut for turbot, sharkmeat for whitefish and small chickens for Rock Cornish game hens.

4. Menu items represented to be of specific sizes or weights must be no less than what is advertised.

Any representation of size whether it is in terms of pounds or inches should be accurate and must be verifiable. For example, what is presented in the menu as a 12-oz. steak or a ½-lb hamburger must weigh that amount before being cooked.

Analogue foods

Only analogue foods that have been "approved" by U.S.D.A. and/or F.D.A. should be used as ingredients in foods sold, prepared, or served by foodservice operations. When an analogue is used as a substitute for a valuable constituent in whole or in part, for example, textured vegetable protein is substituted for chicken in chicken salad, the menu listing of the food item should disclose to the customer such a substitution (*8*).

Nutritional labeling

If a nutritional claim is made, then it must be supported by documentation. Such documentation must be available to the consumer upon request and the menu listing must accurately reveal the nutritional information (*8*).

Consumer give-aways

The foodservice industry should take proper measures to protect itself and the consumer in the use of "give-aways" by having appropriate testing done and using adequate labeling as to use and/or function of the item (*8*).

The above-mentioned areas of Truth-in-Menu, Analogue Foods, Nutritional Labeling and Consumer Give-Aways are becoming of more and more concern to the aware consumer. Advocates of the consumer are beginning to ask those questions not previously asked and are asking that the foodservice operator prove what is being stated.

Many of these same consumer groups are encouraging and/or lobbying for legislation against consumer fraud in the foodservice industry. Enforcing such regulations are not just governmental consumer agencies but health departments.

In the State of California health departments are responsible for this consumer-related legislation in the foodservice industry. One could expect that this might establish a trend that would spread to other political jurisdiction. There could be a good bit of discussion, not only as to need of such legislation, but also as to what agency should be responsible.

PUBLIC DISCLOSURE OF FOODSERVICE INSPECTION RECORDS

This is a rather sensitive issue both to the foodservice manager and to health department personel. Most local law departments have ruled that the foodservice inspection reports should be considered as public records and made available upon request.

Recently in a newspaper article in the *Cleveland Plain Dealer* (*3*) a Denver, Colorado assistant city attorney ruled that the foodservice inspection records would no longer be available to the public. He indicated that they were investigatory files for law enforcement purposes and that their publicaton in the newspaper was contrary to the public interest. The news article went on to indicate that the local newspaper didn't agree and would take legal action, if necessary, to gain access to the reports. This will be an interesting case to follow!

This brief overview is a way of looking at the evolution of the foodservice inspection. Why it was necessary in the first place, what changes have occurred and are occurring in the foodservice industry that may modify that original intent and a look into the future to see what else we should be concerned about.

The "principles" of food inspection may need to stray away from the words in the original law. I don't suggest that we lose sight of those basic health concerns of cleanliness, sanitation, refrigeration, dishwashing, etc., but that we also become interested in and concerned about those consumer-related issues that are also important. The foodservice industry and public health officials all have a stake in ensuring that each foodservice is a safe and honest place to eat.

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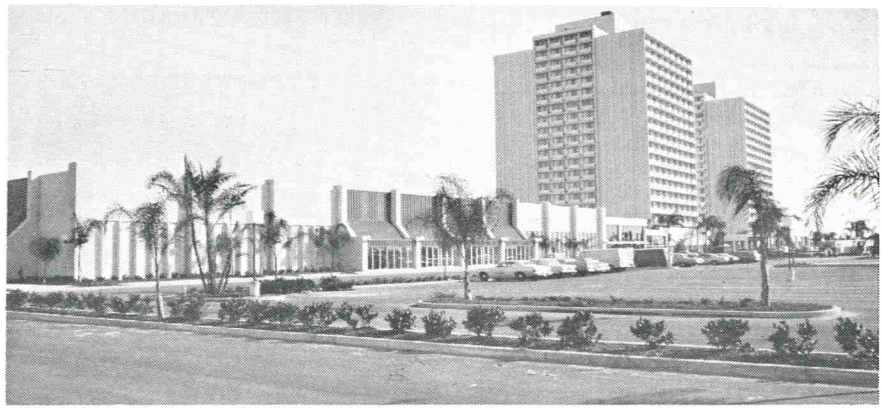

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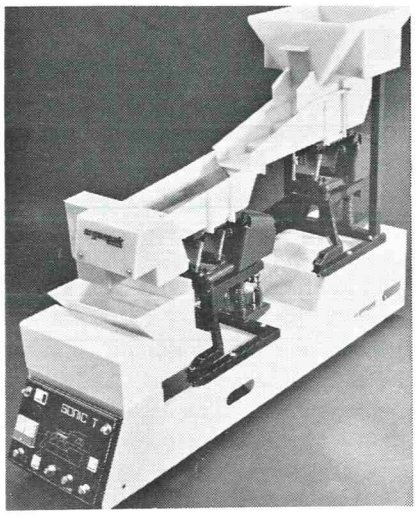
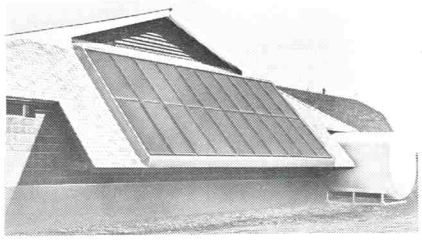


• *A British-made automatic weighing machine* has an advanced weighing mechanism based on an electro-magnetic vibrating wire, to provide simple and flexible operation. The Sonic System is manufactured primarily in non-toxic polypropylene; all contact parts — including trays and hopper — can be removed for cleaning in liquids up to 400° F. Accurate and rapid weighing is ensured by a mechanism based on a short piece of wire which is vibrated by an electro magnet at a set frequency when unloaded. As weight is added to the support platform the frequency changes, the degree of change is detected and amplified to control the system. Two models can be supplied; one weighs loads up to 1kg (2.2 lb), the other up to 5kg (11 lb). Interchangeable parts allow one model to be changed to the other within seconds. Inquiries from the US should be directed to the US subsidiary. British co.: Organpak Ltd., Pipers Roads, Park Farm, Redditch, Worcestershire B98 OHU England. Telephone: (0527) 23103. Telex:337804. US subsidiary: Weigh Count Packaging Inc., 1810 Auger Drive, Tucker, GA 30084. Telephone: (404) 939-2426.

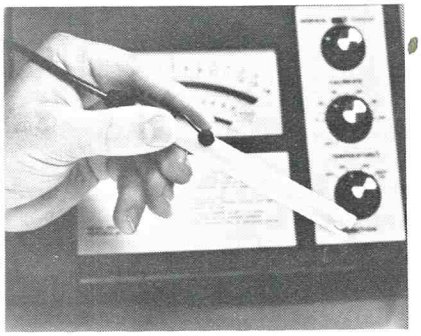
• *Bindicator Co.* has announced it will manufacture and market a new American-made milk and milk products analyzer in both the U.S. and Europe, and has formed a new division, Analytic Equipment, to market the product line worldwide, according to H. J. McBride, president of Bindicator. The Multispec M Infrared Dairy Products Analyzer is a technically improved instrument developed by Dr. Jack Shields of Shields Instruments, Ltd., York, England, which has been acquired by Bindicator. Multispec M analyzes and identifies up to four basic component parts of milk and milk products including fat, protein and lactose. Designed to speed-up testing, the Multispec M completes a four-component analysis in 20 seconds or one-component analysis in less than 10 seconds. Contact Philip Romeo, Bindicator Co., Suite 486, 30100 Telegraph Road, Birmingham, MI 48010, phone 313-644-2861.

• *Groen Division/Dover Corportion* is offering new, affordable, continuous whey concentration systems for use at the cheese plant level. Designed for both natural and cottage cheese plants, the efficient system will concentrate raw whey up to 30% solids in a single pass. The Groen Model 6E-372-R3 Six Tube Rising Film Concentrator, with Central Cyclone Separator, is a continuous whey concentration process which operates at low temperatures, and does not denature the whey protein. Groen has designed systems which are capable of handling almost any size cheese plant. Vapor recompression is available as an option. Contact Groen Division, the Dover Corporation, 1900 Pratt Boulevard, Elk Grove, Village, Illinois 60007.



• *The Surge Solar System* is designed with flat plate collectors to gather both direct and diffused solar radiation. Between 300 and 1,000 BTU's per square foot of collector surface per day are provided by these solar collectors. The hot air produced by the panels is drawn by fan through existing or new duct work to provide space heating. The number of collectors required depends on the size of the space to be heated. Water heating cost can be dramatically reduced when solar space heating is adapted to help heat water. The water heating system is available as a separate modular system just for water heating, if desired. The Surge Solar System works completely with air as the collecting medium so there is no problem with freezing, corrosion, boiling or leakage. It also means virtually no maintenance on the system and greater life expectancy. For additional details on cost or design, contact a Surge dealer or write Babson Bros. Co., 2100 South York Road, Oak Brook, IL 60521.

• *Early detection* of milkstone deposits can now be accomplished in minutes with the B-100A Blak-Ray ®Lamp, manufactured by Ultra-Violet Products, Inc. Milkstone is a calcium build-up which allows bacteria to breed. The B-100A is a powerful lamp, capable of fluorescing the back of a 6,000 gallon tank from the entrance port. The lamp head rotates a full 360° and is removable from the transformer base for more portable hand use. For additional information and free brochure, write: Ultra-Violet Products, Inc., 5100 Walnut Grove Ave., San Gabriel, Calif. 91778.



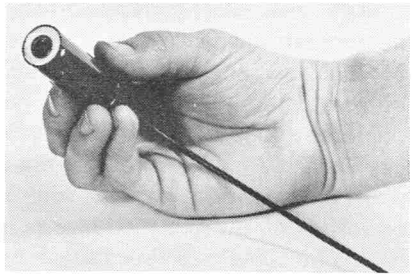
• *Corning Science Product's* low-cost plastic-barrel electrode provides rugged, reliable performance in such general purpose use as checking waste water or water treatment processes, or afield in checking streams and rivers. The electrode uses an Ag/AgCl internal reference and its flowing KCl junction provides a stable reference to insure low drift and fast response from 10 to 80° C. Additional information is available from Corning Science Products, Corning Glass Works, Corning, N.Y. 14830.

• *New Disc-O-Seal Butterfly valve,* specifically designed for sanitary service in the food, beverage and pharmaceutical industries or any other industry where sanitation control is of primary importance, has been announced by Bay State Controls Corporation, Worcester, Massachusetts. The Disc-O-Seal can be welded directly to stainless piping without the need for dis-assembly. Integral pipe ends are supplied to match schedule 5S, 10S or OD Tube. It is designed for straight line flow with no pockets or crevasses to trap media. For more information and prices contact: Mr. Wayne Day, Bay State Controls Corporation, 51 Union Street, Worcester, MA 01608. Telephone (617) 799-0335.

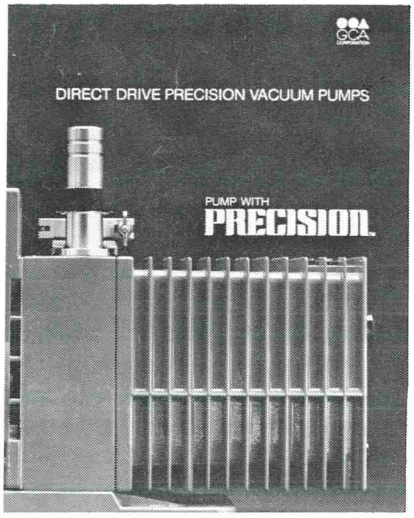
• *An industrial coatings,* Teflon[R] products, films and fabrications brochure is available from American Durafilm Co., Inc. of Newton, Massachusetts. The illustrated pamphlet features a coatings chart which details the materials and chief characteristics of lubrication and release coatings, electrical insulation coatings and anti-corrosion coatings. The brochure is available free from American Durafilm Co., Inc., P.O. Box 625, Newton, Lower Falls, MA 02162.

• *A new sanitary designed dust collector* for the food processing and chemical industries has been developed by Seneca Environmental Products, Inc. of Tiffin, Ohio. The system includes a top bag removal collector without the customary unsanitary hardware which normally contributes to product contamination. The collectors are designed to reduce particulate emissions by as much as 99.99%. For new brochures or additional information contact Seneca Environmental Products, Inc. at 82 North Washington Street, Tiffin, Ohio 44883. 419-447-1282.

• *Now available* from Walker Stainless Equipment Company, Inc., New Lisbon, Wisconsin, is a new 12 page brochure, covering their line of Stainless Steel Processors. The brochure is illustrated with various models, built for the dairy, food and chemical industries. Described in detail are specifications, dimensions and options for all Walker models. Copies may be obtained from Walker Stainless Equipment Company.



• *Soiltest now offers the new LT-250 Planar Dura-Probe,* which makes possible quick determination of the surface pH of soils, paper, thin liquid films, cheese, meat, and other products requiring surface-only contact. Typical uses for the LT-250 are in soil research, water treatment and monitoring, food processing, and culture media work. The range of the LT-250 Planar Dura-Probe is from 0 to 14 pH at temperatures from 32° to 176° F. (0° to 80° C). The probe has a 30-inch (76-cm) insulated wire and connector for use with the L-185 and L-180 Mini pH Meters. For further information, write to Soiltest, Inc., 2205 Lee Street, Evanston, Illinois 60202, U.S.A.



• *GCA/Precision Scientific Group* has published a new catalog covering its expanded line of rotary vane direct drive vacuum pumps. The 12-page catalog covers the complete line of 10 Precision direct drive vacuum pumps. It contains sizing information to enable the user to properly select a pump according to criteria of volume, pumpdown time and pressure. There are also sections on installation and maintenance tips, and accessories. The catalog can be obtained by writing to: GCA/Precision Scientific Group, 3737 West Cortland Street, Chicago, Illinois 60647.

• *Environeering's marble bed Hydro-Filter*[R] and some of its applications in particulate collection, absorption, and odor, fume, and smoke removal are described and illustrated in a recently issued product bulletin. The new literature shows how the device uses smooth glass marbles in a constantly flooded state to form a highly turbulent zone directly above the bed of marbles. Air laden with dust, dirt and/or fumes passes upward through the small bubbles in this turbulent layer. Complimentary copies of the new brochure, Bulletin 120, are available from: Environeering, Inc., 4233 N. United Parkway, Schiller Park, IL 60176, (312) 671-6300.

• *American Can Company* has installed a new Rexham pilot sterilizer for retortable pouch study at the Neenah Technical Center, Neenah, WI. Automatic controls in a self-contained panel board can be set for operating the unit as a steam processor, with a steam/air mixture or as water immersion/flow with overriding air pressure. An automatically controlled pump can transfer either hot or cold water from the holding tanks to the simulator in one minute or less. For more information, write: American Can Company, Food Packaging or Special Products Packaging, American Lane, Greenwich, Connecticut 06830.

• Nasco's sampling equipment catalog 533 is now available. The publication includes information on Whirl-Pak sampling bags and accessories, as well as equipment for bulk milk sampling, sampling in dairy plants and laboratories, as well as veterinary, food, medical, water, and soil sampling. For more information, contact Nasco, 901 Janesville Ave., Fort Atkinson, WI 53538, 414-563-2446, or Nasco West, 1524 Princeton Ave., Modesto, CA 95352, 209-529-6857.

• A complete line of starter media for cheese making is now available in newly revised packaging from Galloway-West Company. The cheese media, known as bulk starter media, are available for American, Cottage, and Italian cheese varieties. Each media offers the cheese maker the nutrient support necessary for proper bacterial growth. The product line consists of Actilac for Cottage cheese, a whey based medium, Actilac for American cheese, a whey based medium, Actilac for Italian cheese, a whey based medium, Certilac for American cheese, a whey based medium, and three varieties of Culture Mate for American cheese, all non-fat dry milk based. Each media is packed in 50-lb. bags. For more information, contact Galloway-West Co., P.O. Box 987, Fond du Lac, WI 54935.

• *Derustit SS-3 stainless steel cleaner* has been approved by the USDA for removing scale from stainless steel food processing equipment after fabrication or repair. Derustit SS-3 removes scale from weld beads fast and economically, eliminating the need for grinding or sand blasting. Derustit SS-3 is available in liquid or paste for immersible or stationary applications. For complete information on Derustit SS-3 stainless steel cleaner, write: Bradford Derustit Corporation, Box 151, Clifton Park, New York, 12065.

• *Babson Bros. Co.,* builders of Surge dairy farm equipment, is helping take the guesswork out of planning a new milk parlor by offering dairymen a free Dairy Planning Guide. The eight page booklet points out many questions to be considered in planning a new facility. It also gives basic information on planning the new parlor in relationship to the overall dairy facility. Additionally, some of the nation's top parlor planners have developed a program to provide the dairyman a computerized recommendation for his particular operation. This recommendation and floor plans for the parlor or parlors recommended will be returned to the dairyman after he fills out the questionnaire included in the planning guide. To receive the guide, send your name and address to Surge Dairy Planning Guide, c/o Babson Bros. Co., 2100 South York Road, Oak Brook, Illinois 60521.

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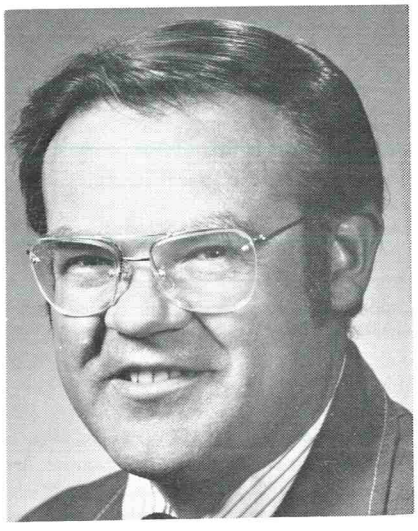
Floyd Bodyfelt and Robert Marshall



Floyd W. Bodyfelt
Professor, Food Science
Oregon State University

Floyd W. Bodyfelt is Professor of Food Science and Extension Dairy Processing Specialist, Department of Food Science and Technology, Oregon State University, Corvallis, Oregon. Floyd was born in Seaside, Oregon on May 9, 1937 and raised on a dairy farm in Tillamook County, Oregon.

Floyd received a B.S. in dairy manufacturing in 1963 and an M.S. in food science from Oregon State University in 1967. He served in the U.S. Army Medical Services as a medical laboratory technologist at Brooke Army Medical Center, San Antonio, Texas, 1959-1962. His dairy processing experience was gained through employment with Mayflower Farms and the Tillamook County Creamery Ass'n. in Oregon.

His first assignment with Oregon State University began in 1964 as an instructor for dairy processing and as Extension Dairy Processing Specialist. From 1965-1969, Floyd also managed the OSU Dairy Products Laboratory. Since 1969, he has been responsible for activities in extension (75%), research (15%), and teaching (10%), and coaching the OSU dairy products judging team.

Floyd's applied research activities have included the flavor chemistry of Cheddar cheese, buttermilk, sour cream, and yogurt, utilization of cheese whey for wine production, relative absorption of chemical contaminants by multiuse milk containers, temperature control of perishable foods in production and distribution and consumer acceptance studies of dairy products.

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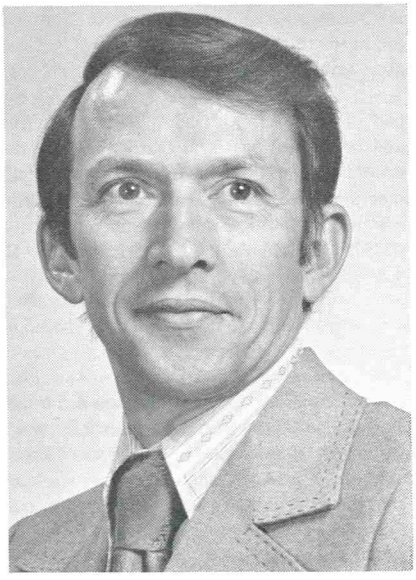
Robert T. Marshall, Professor
Food Science and Nutrition
University of Missouri-Columbia



Robert T. Marshall is Professor, Food Science and Nutrition, University of Missouri-Columbia. He was born near Springfield, Missouri on July 27, 1932. He received the B.S. degree (Dairy Manufacturers) in 1954 from the University of Missouri. He served three years as a pilot in the U.S. Air Force in Europe. His M.S. and Ph.D. degrees were granted from the University of Missouri-Columbia in 1958 and 1960, respectively.

Bob joined the faculty of the Department of Dairy Husbandry, University of Missouri-Columbia in 1960. He transferred to the Department of Food Science and Nutrition in 1967 and became Professor in that department in 1970. He has taught virtually every course related to milk and milk products at UMC and currently teaches two courses in dairy technology, two in food microbiology, one in food quality and sanitation and coaches the Dairy Products Evaluation team. His research concerns psychrotrophic microorganisms and their enzymes in foods, cleaning and sanitizing and automated processing. He is co-operator with USDA researchers. He has authored or co-authored more than 75 scientific papers and is co-author of the dairy science text book *The Science of Providing Milk for Man*.

He has served IAMFES as its representative on the Intersociety Council on *Standard Methods for the Examination of Dairy Products*, 14th ed; the Editorial Board, *Journal of Food Protection*; and on subcommittees of the Applied Laboratory Methods and the Farm Methods committees. In the American Dairy Science Association he is currently vice-chairman of the Dairy Foods Research Section. Previously he was chairman of the Industry and Busi-

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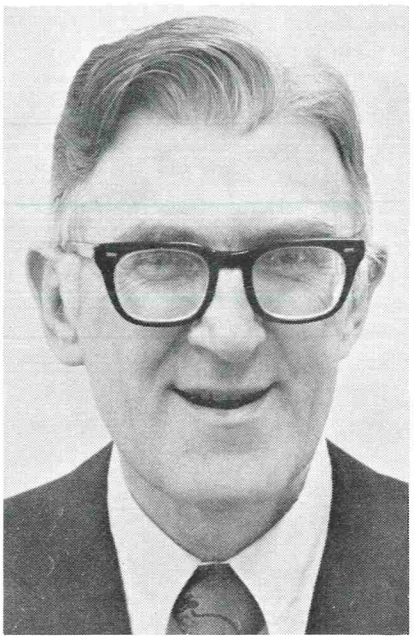
Secretary-Treasurer

A. Richard Brazis and Harold Wainess



A. Richard Brazis
Corporate Microbiologist
Fairmont Foods

Dr. A. Richard Brazis is Corporate Microbiologist, Research and Development Laboratory, Fairmont Foods Company, Omaha, Nebraska. Dick has responsibility for product quality control and laboratory services for operating divisions at all locations.

Dick was born in Bridgeport, Connecticut, November 14, 1926. He received a B.S. from Norwich University in Vermont and M.S. and Ph.D. degrees from the University of Missouri in 1951 and 1954, respectively. Dick served as a tank driver and gunner in a tank company in the first Calvary Division in the Pacific Theater during World War II. He also served in the Vermont National Guard following return to college.

His public health sanitation and laboratory experience began as a dairy technologist with the City Health Department in Columbia, Missouri in 1951. He was an assistant instructor at the University of Missouri between 1952 and 1954. After he joined the USPHS (active duty) in 1954, his first assignment was as a microbiologist with the Water Supply and Pollution Control Program in Cincinnati, until 1959 when he transferred to the Milk Sanitation Research Unit at the same station. From 1959 to 1965, Dick conducted research and assisted in the PHS program for standardization and approval of State and Territorial milk laboratories participating in the NCIMS. In 1966 he became Chief of the Laboratory Development Section of the Milk and Food Branch and served in that capacity until his retirement from the USPHS/FDA in February, 1978.

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Harold Wainess
Consultant
Harold Wainess & Assoc.

Harold Wainess is a Food Sanitation Consultant with a background of over 39 years in the dairy and food industries. He has an MS in Dairy Bacteriology from Purdue and has operated a dairy plant, established control laboratories, and was a trouble shooter and equipment installer for the York Corporation, specializing in HTST pasteurization.

He spent eleven years as a milk and food consultant with the U.S. Public Health Service, and was the Chief Sanitary Officer of the Chicago Health Department.

In 1953 he inaugurated his own consulting services, headquartered in Northfield, Illinois. He is a consultant to many of the world's leading manufacturers of food and dairy equipment, food and dairy processors, chemical processors, manufacturers of vending machines, fabricators of plastic and paper food containers, and manufacturers of aseptic food packaging systems.

As a participating member of IAMFES Harold has been actively involved in a number of committees including 3-A Sanitary Standards, Food Equipment, Food Protection and Baking Industry Sanitary Standards. He was chairman of the membership committee and is currently the IAMFES representative to the International Dairy Federation.

Harold is also very active with the Illinois Affiliate and served as its president for two years. He was co-chairman of the 63rd Annual Meeting of the IAMFES in 1976 at Arlington Heights, Illinois.

For many years, he has presented subjects of topical interest at dairy and food short courses and affiliate meetings in 16 states. During his tenure with the Public Health Service he conducted courses in dairy farm sanitation, HTST pasteurization and food handling throughout the country. As a survey officer he made milk and food sanitation surveys in many states and local communities.

News and Events

Foodservice for 1980 Olympics Featured at N.Y. Meeting

Food service for the 1980 Olympics was one of the featured topics at the annual meeting Sept. 20-22 of the New York State Association of Milk and Food Sanitarians. Nat Gliserman, consultant for Olympic food operations spoke to a large audience on the planning, design, and operation of the multiple food facilities planned for the world sporting event.

The meeting was the association's 55th and it took place at the Stevensville Country Club at Swan Lake near Liberty, N.Y., with over 400 persons in attendance. Speakers at the general session were J. Roger Barber, a commissioner of the New York State Department of Agriculture and Markets, and Eugene J. Cahalan of the State Health Department.

Electronic cell counters were the subject of a presentation by Douglas P. Collins of the New York Dairy Herd Improvement Cooperative and Roger Natske of the Animal Science Dept. at Cornell University. Dr. T. W. Downes of Cornell's Food Science Dept. presented a plastics updating, highlighting those used for food packaging. Dr. Robert Zall, also of Cornell's Food Science Dept., addressed a session on sales strategy versus technical ability in the marketing of dairy products.

Annual awards recipients and Honorary Life Members for the New York State Association were named at the meeting. L. George Texter, a fieldman for Upstate Milk Cooperatives, was presented the Theodore H. Reigh Memorial Award for fieldmen. Derwood H. Marlatt received the Howard B. Marlatt Memorial Award, an award made each year in honor of his father. Derwood and his mother, Mrs. Howard Marlatt, manage the Orange County Dairy Laboratory in Middletown, NY, founded by his father. John G. Burke, president of NYAMFS in 1974-75, was presented the Dr. Paul B. Brooks Memorial Award for outstanding contribution to the welfare and progress of Association affiliates. Burke is District Manager for Borden, Inc. in Watertown, NY.

New York State Association of Milk and Food Sanitarians' Executive Board, left to right: William Y. Perez, 1976-1977 President; Richard P. March, Executive Secretary; Albert J. Lahr, Past President; Alfred R. Place, Board Member; Charlotte W. Hinz, President-Elect; Howard I. Cobb, Board Member; William K. Jordan, President; and Gaylord B. Smith, Chairman, Council of Affiliates.

Joseph A. Salvato, Jr., was selected to receive the Emmet R. Gauhn Memorial Award which recognizes outstanding service and leadership in the dairy and food industry. Salvato is recently retired from the position of Assistant Commissioner, Division of Sanitary Engineering, New York State Health Department.

Members presented Honorary Life Memberships during the year include Bernard J. Scheib, Thomas J. Mahoney, Anthony J. Skurski, Duane Dewan, William G. Gale, and Franklin B. Hicks.

Ontario Affiliate Sponsors Workshop

"Sanitation Motivation," a workshop jointly sponsored by the Ontario Milk and Food Sanitarians Association and the Canadian Institute of Food Science and Technology, Guelph Section, was held Nov. 22, 1978 at the Arboretum Centre of the University of Guelph.

Featured presentations at the workshop included "Sanitation Philosophy," by Dr. John E. Sterns of the Veterinary Public Health Service of the Ontario Ministry of Health, "Sanitation Success for Processors," and "Sanitation Success for Retailers," presented, respectively, by Robert Tiffin of J. M. Schneider, Inc., and R. A. Cauley, of Kentucky Fried Chicken. "Sanitation Motivation" was a presentation made by Paul Baker of the Development Section, Human Resources Branch, Ontario Ministry of Health.

Following the day's sessions, a general discussion session and a wine and cheese buffet and cheese auction were held.

William S. Hickey, 1907-1978

William S. Hickey, President of IAMFES in 1960, died in October in Salt Lake City, Utah, of an apparent heart attack. He was 71.

Bill had lived in Salt Lake City since retirement from the Single Service Institute in 1971.

He began his career in pest control in a job which brought him in constant contact with the Salt Lake City Health Department. Bill decided one day to take the exam conducted by the health department to recruit new employees. He'd all but forgotten the test when he saw his name in the paper a few weeks later, listed as a city milk inspector. From then on, public health was his business. His career included positions of Davis County (Utah) sanitarian; Head, Division of Sanitary Engineering, Salt Lake City; Field Representative, Public Health Committee of the Paper Cup and Container Institute (PCCI); Secretary of the PCCI Public Health Committee, editor of PCCI's *Health Officers News Digest*; executive editor, *Environment News Digest*, and finally, following retirement from the PCCI, consultant to PCCI.

Committees and organizations with which Bill worked included IAMFES, American Public Health Association, National Health Council, Interstate Milk Shipments Conference, the National Sanitation Foundation, and numerous other regional and local organizations. He was also one of the founding members and first presidents of the Utah Association of Sanitarians.

Bill's widow, Bernice, three children, and several grandchildren survive him.

Sauerkraut Report Now Available

The "1978 Sauerkraut Seminar" report, Special Report No. 30, is now available upon request from D. L. Downing, NYS Agricultural Experiment Station, Geneva, NY 14456.

The report is the result of a meeting jointly sponsored by the National Kraut Packers Association, Inc., New York State Agricultural Experiment Station, and the New York State Cooperative Extension, which was held in July, 1978.

Book Review

Modern Food Microbiology - Second Edition. James M. Jay, D. VanNostrand Co. New York, 479 pages, $14.95.

As one would expect, the second edition of *Modern Food Microbiology* is an updated version of the first edition. Author Jay has done an exceptional job of drawing information and data from many sources and weaving it into a framework useful not only as a source book but also as a textbook. There is a lot of information between the covers of this book. It should be useful as a textbook in both a first and second course in food microbiology.

Two new chapters have been added to the contents, Chapter 4, "Determining Microorganisms and Their Products in Foods" details some of the most recent developments in enumerating microbes and microbial toxins and gives the traditional methods. It should be a useful chapter in an advanced course. The second new chapter, 14, "Fermented Foods and Related Products of Fermentation," in addition to discussing traditional fermentations, this is a limited discussion on the fermentation of single cell protein.

Other chapter titles are essentially the same as in the first edition and include: History of Microorganisms in Food, Intrinsic and Extrinsic Parameters of Foods that Affect Microbial Growth, the Incidence and Types of Microorganisms in Food, three chapters on Food Spoilage by Microorganisms, five chapters on Food Preservation, three on Foodborne Disease and several other chapters dealing with specific types of microorganisms that are common problems in food.

The nomenclature and classification of the microorganisms given in the test is in conformity with the 8th edition of *Bergy's Manual of Determinative Bacteriology.* This should be useful to those of us concerned about correct usage of nomenclature. The book is well written and will be useful in the teaching of food microbiology. I recommend its use.

Edmund A. Zottola

Dept. Food Science and Nutrition
University of Minnesota, St. Paul 55108

"New Horizons in Health" Theme for Indiana Association

The 28th Annual Indiana Association of Sanitarians' Educational Conference was held at the Holiday Inn, Merrillville, ID, Sept. 26-28, 1978.

Approximately 160 persons attended the conference, whose theme was, "New Horizons in Health."

The Outstanding Sanitarian of the Year Award was given to Floyd Bosley. Robert L. Hackett of Indianapolis was presented the Tim Sullivan Memorial Award. The Southern Chapter won the Harry E. Werkowski Memorial Award.

Presentations at the meeting included the keynote address by E. Frank Ellis, M.D., M.P.H., who is Regional Health Administrator for HEW Region V. Other presentations available to conference attendees included "Stress and Stress Management," by Douglas Mosel of United Health Services, "Resource Conservation and Single Service," by Charles Felix of the Single Service Institute, "Getting the Other Guy to Do Your Work Through Food Service Manager Sanitation Training," by C. Dee Clingman of the National Institute for the Food Service Industry, and "Low Temperature Ware Washing and Energy," by Syl Williams and Hugo Nordin, both of Economics Laboratory, Inc.

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Coming Events

Feb. 14-15--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. 19-22--ICE CREAM TECHNOLOGY SHORT COURSE. Ramada-Crabtree Inn, 3926 Arrow Drive, Raleigh, NC 27612. Contact: W. S. Arbuckle or R. B. Redfern, Ice Cream Technology, 3413 Blue Ridge Rd., Raleigh, NC 27612.

Feb. 25-Mar. 2--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--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. 27-28--KENTUCKY ASSOCIATION OF MILK, FOOD AND ENVIRONMENTAL SANITARIANS, INC., Annual Meeting. Contact: Dale Marcum, Milk Control Branch, Frankfort, KY.

Feb. 27-Mar. 1--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-Mar. 2--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--27th FOOD TECHNOLOGY CONFERENCE, Food Ingredients--Sweeteners and Preservatives. Co-sponsored by IFT sections of St. Louis and Kansas City and by the Department of Food Science and Nutrition, University of Missouri-Columbia. Advance registration, $35. Contact: R. T. Marshall, 203 Eckles Hall, Columbia, MO 65211.

Mar. 1-5--BISSC AND BEMA WINTER MEETINGS. Hyatt Regency Chicago Hotel, River and Michigan Avenue, Chicago, IL. Contact: Raymond J. Walter, Executive Secretary, Bakery Equipment Manufacturers Association, 521 Fifth Avenue, New York, NY 10017, 212-687-9071.

Mar. 6-7--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. 9-10--SEVENTH ANNUAL FOOD INDUSTRY ASSOCIATION OF SOUTH CAROLINA CONFERENCE. Hilton Head, SC. Contact: T. C. Titus, Food Industry Association of SC, Box 708, Clemson, SC 29631.

Mar. 19-21--KULTURES AND KURDS KLINIC. Sponsored by the American Cultured Dairy Products Institute. Hilton Inn, Columbus, OH. For additional information and/or advance registration forms, contact: Dr. C. Bronson Lane, DAFNC, P.O. Box 7813, Orlando, FL 32854 or Margie Franck, ACDPI, 910 17th Street, N.W., Washington, D.C. 20006.

Mar. 19-23--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. 20-21--NATIONAL SANITATION FOUNDATION SEMINARS, Dallas, TX. For more information, contact: Education Service, National Sanitation Foundation, NSF Building, P.O. Box 1468, Ann Arbor, MI 48106, 313-769-8010.

Mar. 26--IOWA ASSOCIATION OF MILK, FOOD AND ENVIRONMENTAL SANITARIANS, Annual Meeting. Gateway Center Motel, Ames. Contact: Hale Hansen, Dept. of Health, Lucas Building, Des Moines, IA 50319.

Mar. 26-28--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. 26-28--MICHIGAN ENVIRONMENTAL HEALTH ASSOCIATION, Annual Educational Conference. Sheraton Inn, Kalamazoo, MI. Contact: James Szejda, 414 Washington, St., Grand Haven, MI 49417.

Mar. 27--DAIRY INDUSTRY CONFERENCE. Scheman Building, Iowa State University Center, Contact: Verner Nielsen, Food Technology, ISU, Ames, IA 50011.

Mar. 27-28--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. 28--ONTARIO MILK AND FOOD SANITARIANS ASSOCIATION, Annual Meeting. Holiday Inn, Airport Rd., Toronto. Contact: Roger Wray, 32 Windsor St., Guelph Ont., N1E 3N2, Canada.

April 10-11--PRESENT AND FUTURE TRENDS IN FOOD AND BEVERAGE PACKAGING, Short Course. Holiday Inn, Clemson, SC. Contact: T. C. Titus, Food Science Dept., Clemson University, Clemson, SC 29631.

April 19--IOWA FOOD INDUSTRY CONFERENCE. Stouffer's Hotel, Cedar Rapids, IA. Contact: Bill LaGrange, Food Technology, ISU, Ames, IA 50011.

April 23-24--NSF SEMINARS, Washington, D.C. For more information, see entry for Mar. 20-21.

May 13-16--BRITISH NATIONAL MEAT TRADES FAIR. Exhibition Centre, Harrogate, England. Contact: British Information Services, 845 Third Avenue, New York, NY 10022, 212-752-8400.

May 14-17--DELEX, Delicatessen International Exhibition. Royal Horticultural Society's New Hall, London, England. Contact: British Information Services, 845 Third Avenue, New York, NY 10022, 212-752-8400.

May 21-22--NSF SEMINARS, Seattle, WA. For more information, see entry for Mar. 20-21.

May 21-24--NALVEX, National Licensed Victuallers and Caterers Exhibition. National Exhibition Centre, Birmingham, England. Contact: British Information Services, 845 Third Avenue, New York, NY 10022, 212-752-8400.

May 22-27--INTERNATIONAL FOOD FAIR. Copenhagen, Denmark. Contact: Bella Center A/S, Center Boulevard, DK-2300 Copenhagen S., Denmark.

June 4-5--NSF SEMINARS, Memphis, TN. For more information, see entry for Mar. 20-21.

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Coming Events, con't. 191

June 5--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--INSTITUTE OF FOOD TECHNOLOGISTS 39th ANNUAL MEETING AND FOOD EXPO. Alfonso J. Cervantes Convention and Exhibition Center, St. Louis, MO. Contact: C. L. Willey, Institute of Food Technologists, Suite 2120, 221 N. LaSalle St., Chicago, IL 60601.

June 24-27--AMERICAN SOCIETY OF AGRICULTURAL ENGINEERS, Summer Meeting. Winnipeg, Manitoba, Canada. Contact: Roger R. Castenson, ASAE, 2950 Niles Road, Box 410, St. Joseph, MI 49085, 616-429-0300

Aug. 13-17--WORKSHOP ON EDUCATIVE PROCESSES IN FOOD MICROBIOLOGY. Sponsored by the Joint American Society for Microbiology/Institute for Food Technologists Committee on Food Microbiology Education. Quadna Resort, Hill City, MN. Contact: E. A. Zottola, Dept. of Food Science and Nutrition, 1334 Eckles Ave., University of Minnesota, St. Paul, MN 55108.

Aug. 29-31--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--WESTPACK. Convention Center, Anaheim, CA.

Nov. 20-23--FIE FOODPACK, International Food Industries Exhibition, Olympia, London, England. Contact: British Information Services, 845 Third Avenue, New York, NY 10022, 212-752-8400.

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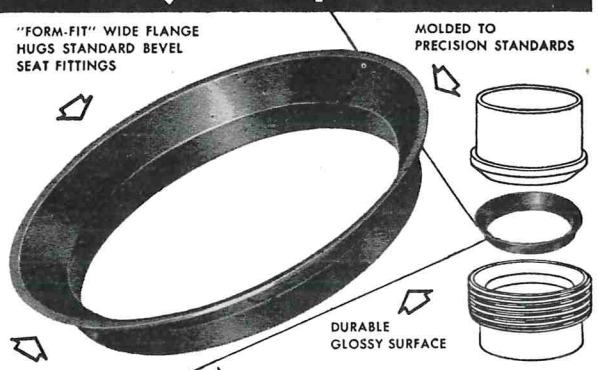

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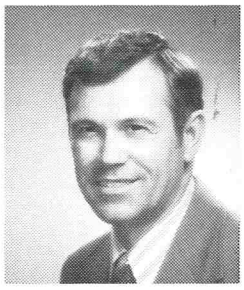
University of Missouri—Columbia



For the corn producer, the most important harvest he makes occurs only once a year—when he goes into the fields with his corn picker. But, for the dairyman, the most important harvest takes place two, and in some cases, three times a day, every day of the year. And, the use of good milking practices helps dairymen to have a good harvest every time they milk their cows. Additionally, a complete milk harvest today will help the cow produce more milk tomorrow.

Milk-making Cells Work Harder With Use

The milk-making (epithelial) cells work the hardest immediately following milking because that is when intramammary pressure is the lowest. At each milking a hormone called prolactin or lactogen is released and its effect is to cause the milk-making cells to go back to work. But, if through poor milking practices, some of the milk is left in the udder, intramammary pressure mounts faster and this, in turn, slows down milk secretion. Research indicates that milk secretion each hour following milking is approximately 90 to 95 percent of that of the preceding hour. But as the udder fills, this percentage decreases. Naturally, milk left in the udder following milking will shorten the period of time that the milk-making cells work at maximal capacity.

Repeated failure to remove milk from mammary glands causes the milk-making cells to become inactive. Thus, for maximal milk production, the milk secreting cells must be challenged... and that means removing all of the milk possible at each and every milking. Although incomplete milking will not have a big detrimental effect in one or two milkings, it sure will over a period of several days. Not only will the milk left in the udder not be harvested and, therefore, not be sold, it will, in addition, accelerate the cow's decline in level of production and, thereby contribute to unprofitable dairying.

Persistency: A Slower Decline Means More Profit

A cow reaches her peak production about two months into the lactation. After this, a natural, gradual decline in milk production occurs. The relationship between milk given one month compared to that produced the next is known as persistency. Persistency can be improved if good milking practices are used to assure a full harvest of the milk crop at each milking.

Eight Steps Toward Getting a Full Milk Harvest

Good cow milking practices include eight steps which, when done properly, will achieve the fullest possible harvest of your valuable milk crop.

1. Environment: Provide a comfortable, stress-free environment.
2. Proper Stimulation: A vigorous massage of the mammary glands will help insure complete letdown of milk.
3. Strip Foremilk: Stripping acts to further stimulate the cow and, at the same time, eliminates much of the bacteria-laden first milk.
4. Timely Application of Milking Machine: This should be done one minute after starting stimulation to take advantage of maximal letdown.
5. Adjust Machine: Proper forward/downward adjustment is important for complete milking.
6. Remove Teat Cups as Quarters Milk Out: Avoid over-milking which can lead to tissue irritation and mastitis.
7. Dip Teats: Teat dipping gives the teat end the protection it needs against mastitis-causing bacteria while the streak canal regains its full constriction.
8. Maintain Equipment Properly: Properly adjusted and maintained equipment is an essential step toward better milking.

The amount of milk a cow produces in a lactation from the time she freshens until she stops lactating is determined by a number of factors; some can be controlled—others cannot. However, good milking practices will go a long way toward helping you achieve more nearly the full potential of your cows' production. In other words, good milking will give a more complete harvest of your most important crop—and that means more profit.