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Vol. 36 April, 1973 No. 4

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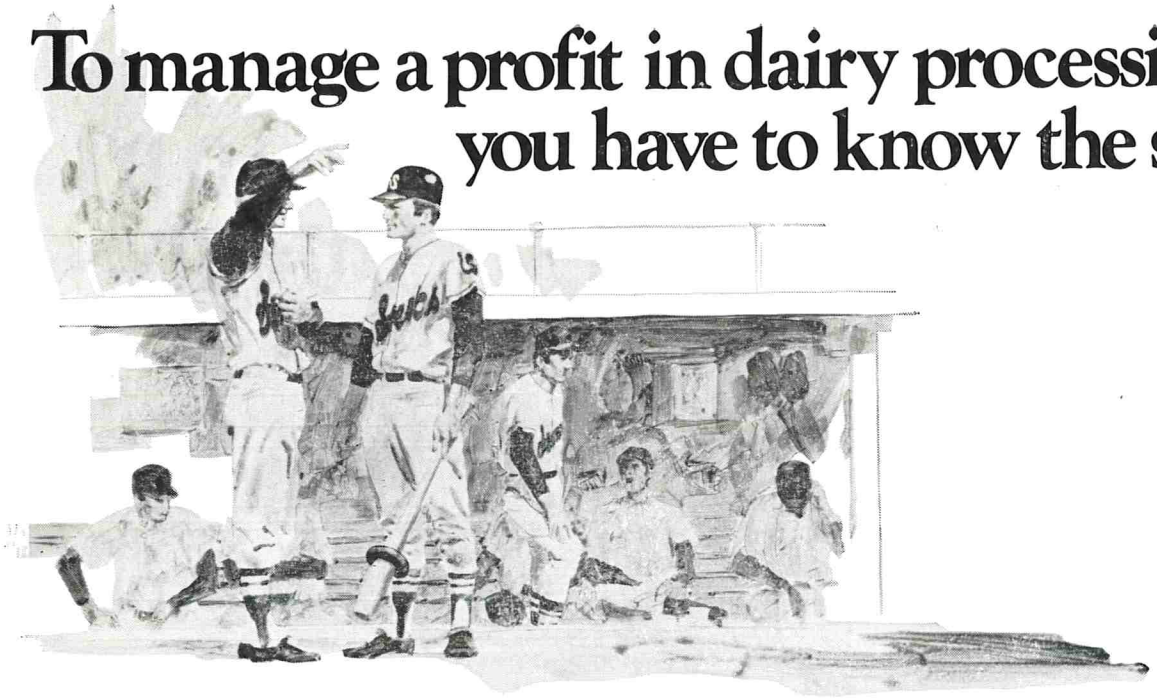

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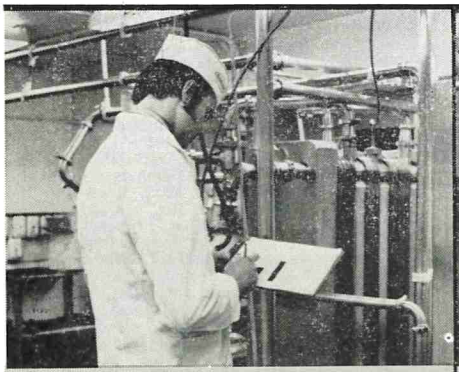

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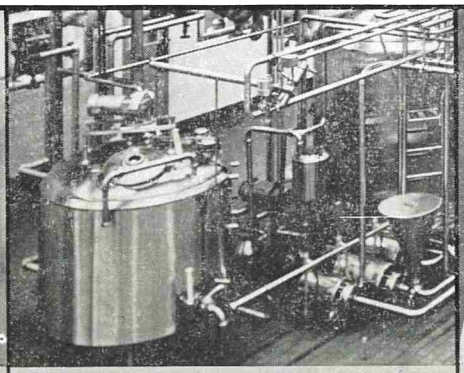

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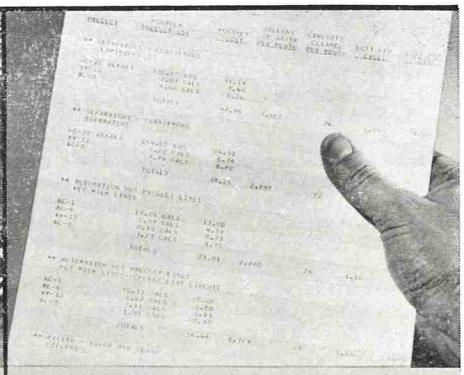

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EFFECTS OF SYNTHETIC MEAT COMPONENTS ON GROWTH OF *CLOSTRIDIUM PERFRINGENS*[1, 2]

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(Received for publication November 14, 1972)

Abstract

The 16 ingredients used to fabricate synthetic soybeef (ground beef analog) were evaluated for stimulatory effects on growth of *Clostridium perfringens*. Four ingredients—beef-like flavor, corn flour, cocoa, and vegetable oil—stimulated growth of *C. perfringens*. The remaining 12 ingredients showed no significant effect. The most stimulatory ingredients, beef-like flavor and corn flour, were optimally stimulatory over a wide range of concentrations. Thus, control of growth of *C. perfringens* may not be obtained by manipulation of the concentration of these ingredients in the absence of other precautions.

Clostridium perfringens food-borne illness is a major concern in the food industry. Mishandled cooked meats and meat products are important vehicles of transmission *(1)*. As a result of dramatic food processing advances and significant changes in consumer attitudes and marketing approaches, public acceptance of soy protein as a base in fabricated synthetic meats as well as of soy protein supplementation of meat systems appears likely in the near future *(2)*. Institutional markets, including hospitals, schools, and other institutions that must provide nutritious meals under budget restraints, are the major markets for meat analogs (synthetic meats). Certain types of restaurants are also prime initial markets for soybean protein products. The trend toward more away-from-home eating increases the potential for these products *(9)*. Since natural meats are major vehicles of *C. perfringens* food-borne illness, an evaluation of synthetic meat systems was warranted to predict the behavior of *C. perfringens* in these new products.

Earlier studies have shown that certain soy proteins used in fabricated meats stimulated growth of *C. perfringens* while other soy proteins did not *(3)*. This stimulation appeared to be related to the amount of refinement of soy protein, type of process involved in isolation, or to the addition of substances to increase functional properties. In meat loaf the growth rate of *C. perfringens* was rapid and the addition of various amounts of soy protein had little effect *(7)*. Synthetic soybeef as a liquid medium also supported rapid growth of *C. perfringens* comparable to that in natural ground beef. The growth rate of *C. perfringens* in soybeef greatly exceeded that of the soybean protein base of soybeef. Therefore, components normally added to the soy protein base in fabricating soybeef must account for this rapid growth rate.

The objective of this study was to determine which components in soybeef, when added to soy protein, have the major influence on growth rate of *C. perfringens*.

Materials and Methods

Apparatus

The apparatus and the method to determine rate of growth of *C. perfringens* in a liquid medium were outlined earlier *(3)*.

Test organism

An 18-hr culture of *C. perfringens* S-40 grown in thioglycollate medium without added dextrose was centrifuged, washed in 6.25×10^{-4} M phosphate buffer (2×), and inoculated at approximately 10^3/ml.

Effect of synthetic meat components on C. perfringens growth

Sixteen ingredients used to fabricate synthetic beef were added to the soy protein base (10 g/500 ml) in a formulation calculated by estimating the approximate concentrations in the commercial product. Sodium thioglycollate (0.3 g/500 ml) was added to lower the oxidation-reduction potential (redox). A screening fractional factorial design *(5)* was used to determine the effects of individual ingredients on growth of *C. perfringens*. The ingredients were divided into two groups of eight. Table 1 shows the fractional factorial scheme of Group 1. Vegetable oil, beef-like flavor, egg white, corn flour, sugar, caramel, cocoa, and onion powder were either absent (−) or present (+) at the concentration shown. Group 2 ingredients were held constant at the concentration shown. Group 1 ingredients were then tested as outlined by the fractional factorial design. In the second part the eight ingredients of Group 2 (sodium chloride, calcium phosphate, ferric pyrophosphate, sodium lauryl sulfate, niacin, riboflavin, vitamin B_6 and vitamin B_{12}) were either absent (−) or present (+) at the concentration shown. These eight ingredients were then tested as outlined by the fractional factorial design while the Group 1 ingredients were kept constant at (+) concentration. All samples were tested in a randomized order.

Effect of the stimulatory components (beef-like flavor, corn flour, vegetable oil, and cocoa) on the growth of C. perfringens

The design for the test to determine the effect of the four stimulatory ingredients is outlined in Table 2. In the suspension of soy protein base (10 g/500 ml H_2O), vegetable oil, beef-like flavor, corn flour, or cocoa are either absent (−) or

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[1]Presented in part at 15th Annual Meeting Canadian Institute of Food Science and Technology, June 11-15, Toronto.
[2]Paper No. 8149, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul, Minnesota.
[3]Present Address: Food Technology Section, Canada Department of Agriculture Research Station, Kentville, Nova Scotia, Canada.

TABLE 1. DESIGN FOR SCREENING OF SYNTHETIC MEAT COMPONENTS TO DETERMINE EFFECT ON GROWTH OF *Clostridium perfringens* (FRACTIONAL FACTORIAL DESIGN GROUP 1)

Run	A	B	C	D	E = ABC	F = BCD	G = ABD	H = ACD
1	−	−	−	−	−	−	−	−
2	+	−	−	−	+	−	+	+
3	−	+	−	−	+	+	+	−
4	+	+	−	−	−	+	−	+
5	−	−	+	−	+	+	−	+
6	+	−	+	−	−	+	+	−
7	−	+	+	−	−	−	+	+
8	+	+	+	−	+	−	−	−
9	−	−	−	+	−	+	+	+
10	+	−	−	+	+	+	−	−
11	−	+	−	+	+	−	−	+
12	+	+	−	+	−	−	+	−
13	−	−	+	+	+	−	+	−
14	+	−	+	+	−	−	−	+
15	−	+	+	+	−	+	−	−
16	+	+	+	+	+	+	+	+

Variables	*Level* (Concentration) −	+
A. Vegetable Oil	0	1.75 g/500 ml
B. Beef-like flavor	0	1.0
C. Egg White	0	1.0
D. Corn Flour	0	7.5
E. Sugar	0	1.0
F. Caramel	0	0.5
G. Cocoa	0	0.5
H. Onion Powder	0	0.25

Constants	*Level* (Concentration)
Sodium Chloride	1.0 g/500 ml
Calcium Phosphate	.75 g
Ferric Pyrophosphate	15 mg
Sodium Lauryl Sulfate	5 mg
Niacin	11 mg
Riboflavin	11 mg
Vitamin B_6	.32 mg
Vitamin B_{12}	.03 mg
Sodium Thioglycollate	0.3 g

TABLE 2. DESIGN TO DETERMINE EFFECT OF STIMULATIVE COMPONENTS OF SYNTHETIC BEEF ON THE GROWTH OF *Clostridium perfringens* (FACTORIAL DESIGN)

Sample	A	B	C	D
1	−	−	−	−
2	+	−	−	−
3	−	+	−	−
4	+	+	−	−
5	−	−	+	−
6	+	−	+	−
7	−	+	+	−
8	+	+	+	−
9	−	−	−	+
10	+	−	−	+
11	−	+	−	+
12	+	+	−	+
13	−	−	+	+
14	+	−	+	+
15	−	+	+	+
16	+	+	+	+

Variables	Level (Concentration) −	+
A. Vegetable Oil	0	1.75 g/500 ml
B. Beef-like flavor	0	1.0
C. Corn Flour	0	7.5
D. Cocoa	0	0.5

Constants	*Level* (Concentration)
Sodium Chloride	1.0 g
Egg White	1.0
Sugar	1.0
Caramel	0.5
Onion Powder	0.25
Calcium Phosphate	0.75
Ferric Pyrophosphate	15 mg
Niacin	11 mg
Sodium Lauryl Sulfate	5 mg
Riboflavin	.43 mg
Vitamin B_6	.32 mg
Vitamin B_{12}	.03 mg
Sodium Thioglycollate	0.3 g

present (+) at the concentration shown in Table 2. The other twelve ingredients were held constant at the concentration shown. Sodium thioglycollate (0.3 g/500 ml) was added to the medium to lower the redox potential. These four ingredients were then tested as outline by the full factorial design *(5)*. The 16 samples were tested in random order and replicated.

Determination of beef-like flavor and corn flour concentrations that produce minimal generation time during C. perfringens growth

The maximum or minimum levels of the two ingredients giving the largest response, beef-like flavor and corn flour, were determined by a sequential method for estimating response surfaces *(6)*. Table 3 shows the design layout for blocks one and two of the beef-like flavor and corn flour at the concentrations shown. Soy protein constituted the base (10 g) in 500 ml H_2O. The remaining 14 ingredients were present in calculated commercial concentrations. Sodium thioglycollate was added to lower the redox potential. All samples in each block were tested in random order.

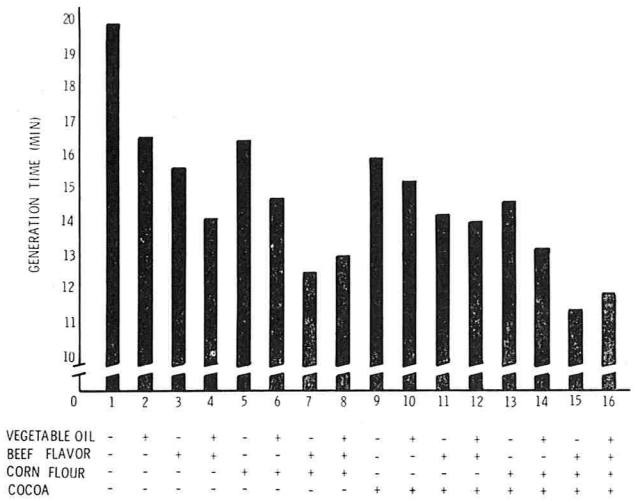

Figure 1. Effect of addition of vegetable oil, beef-like flavor, corn flour and cocoa to a synthetic soybeef medium on the generation time of *Clostridium perfringens* (factorial design). Concentrations: vegetable oil, 0 g/500 ml (−), 1.75 g/500 ml (+); beef-like flavor, 0 g/500 ml (−), 1.0 g/500 ml (+); corn flour, 0 g/500 ml (−), 7.5 g/500 ml (+); cocoa, 0 g/500 ml (−), 0.5 g/500 ml (+).

The effects of all ingredients tested in the three statistical designs were determined by comparing generation times *(3)*. Generation times were calculated from colony counts made during the logarithmic phase of growth and fitted by a linear regression. The generation time data were then evaluated by the fractional factorial, full factorial, and response surface designs utilizing appropriate computer programs provided by the Pillsbury Company, Minneapolis.

RESULTS AND DISCUSSION

Effect of synthetic meat components on C. perfringens growth

The effects of the first eight ingredients (Group 1) on the growth of *C. perfringens* are shown in Table 4. The generation time of *C. perfringens* ranged from 25 min in the absence of the first eight ingredients to 10 min in the medium containing all 16 soybeef ingredients. Beef-like flavor and corn flour showed statistically significant decreases (significant at the 1% level) in the generation time while vegetable oil and cocoa decreased the generation time less markedly (significant at 10% level). These four ingredients also shortened the lag phase and increased the extent of growth. The remaining 12 ingredients showed no significant effects. There were no significant interactions among the effects of all the 16 ingredients. The full effect of beef-like flavor, corn flour, cocoa, and vegetable oil with the elimination of confounding effects of the initial screening fractional factorial design was determined by testing these four ingredients in a full factorial design.

Effect of beef-like flavor, corn flour, cocoa and vegetable oil on the growth of C. perfringens

The average generation time values related to the four ingredients are in Fig. 1. The generation times ranged from 19.9 min for the control to 11.4 for a combination of beef-like flavor, corn flour, and cocoa. There was no significant interaction among the four ingredients. The analysis of variance shown in Table 5 indicated that beef-like flavor and corn flour exhibited the highest level of significance in increasing the growth rate (1% level) followed by cocoa at the 5% level. Vegetable oil increased the growth rate to a lesser extent but it was significant at the 10% level. In mean effects, beef-like flavor decreased the generation time by 2.4 min, corn flour by 2.2, cocoa by 1.5, and vegetable oil by 1 min.

Determination of beef-like flavor and corn flour concentrations that produce minimal generation time during C. perfringens growth

Figure 2 shows the response surface giving the concentrations of beef-like flavor and corn flour that

TABLE 3. DESIGN TO DETERMINE OPTIMAL STIMULATIVE CONCENTRATIONS OF BEEF-LIKE FLAVOR AND CORN FLOUR ON THE GROWTH OF *Clostridium perfringens*

	Beef-like flavor		Corn flour		
Experiment no.	Designation	Concentration	Designation	Concentration	Block no.
1	+1	1.0 g	+1	7.5 g	1
2	+1	1.0	−1	1.3	1
3	−1	.172	+1	7.5	1
4	−1	.172	−1	1.3	1
5	0	.586	0	4.4	1
6	0	.586	0	4.4	1
7	0	.586	0	4.4	1
8	+1.414	1.172	0	4.4	2
9	−1.414	0	0	4.4	2
10	0	.586	+1.414	8.8	2
11	0	.586	−1.414	0	2
12	0	.586	0	4.4	2
13	0	.586	0	4.4	2
14	0	.586	0	4.4	2

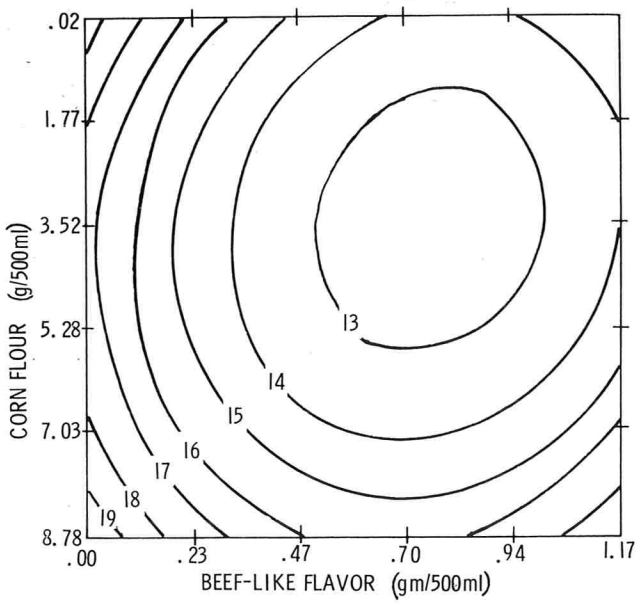

Figure 2. Response surfaces indicating generation time contours of *C. perfringens* as influenced by the concentration of beef-like flavor and corn flour. Numbers identifying contour lines are generation times in minutes. E. g. A concentration of ca. 0.5 to 1.0 g beef-like flavor plus ca. 1.3 to 5.5 g corn flour in a complete synthetic soybeef medium resulted in a *C. perfringens* generation time of 13 min or less.

support optimal growth at generation times of 13 min or less. The optimal concentrations for beef-like flavor range from 0.5 to 1.0 g/500 ml medium. The optimal concentrations of corn flour range from 1.3 to 5.5 g/500 ml medium. Therefore, a concentration of ca. 0.5 to 1.0 g beef-like flavor plus ca. 1.3 to 5.5 g corn flour in a complete synthetic soybeef medium resulted in a *C. perfringens* generation time of 13 min or less. These concentrations of beef-like flavor and corn flour are well below the concentrations added commercially. The analysis of variance of the optimal concentrations of beef-like flavor and corn flour is shown in Table 6. The analysis of variance evaluates how well the response surface model accounts for variation in the data. All the variation that was not accounted for by the model or experimental error was due to the lack of fit of the model to the data. The model was significant at the 5% level which means the model accounted for a significant amount of variation. The analysis indicates a more refined model such as a cubic model instead of the quadratic model is required. The quadratic model, however, does a reasonably good job of predicting the generation time.

The beef-like flavor consists of hydrolyzed plant protein. Studies have shown marked stimulation of *C. perfringens* by partially hydrolyzed protein substances *(8)*. Hydrolyzed plant protein may have provided readily available peptides for rapid initiation and rate of growth. These readily available peptides may also influence the growth of other food-borne pathogens such as *Staphylococcus aureus (10)*. The concentrations of beef-like flavor giving stimulation in synthetic meat are broad and lowering the concentrations does not appear to be a feasible control measure.

The stimulative effects of corn flour may be caused by certain carbohydrates present; however, no determinations were made nor was an investigation of this aspect conducted. Here again, a wide range

TABLE 4. SCREENING OF SYNTHETIC MEAT COMPONENTS ON THE GROWTH RATE (Δ LOG POPULATION/Δ HR) OF *Clostridium perfringens*: EFFECTS OF THE FIRST EIGHT INGREDIENTS (GROUP 1)

Factor	Effect	Mean effect	Mean square	F Ratio
A (Vegetable oil)	3.4950	0.4369	0.7634	5.57*
B (Beef-like flavor)	7.9196	0.9899	3.9200	28.61***
AB, CE, DG, FH	−2.7634	−0.3454	0.4773	—
C (Egg white)	−1.4194	−0.1774	0.1259	—
AC, BE, DH, FG	−2.2628	−0.2829	0.3200	—
BC, AE, DF, GH	1.4694	0.1837	0.1349	—
ABC, E (Sugar)	0.1936	0.0242	0.0023	—
D (Corn flour)	4.8572	0.6071	1.4745	10.76***
AD, BG, CH, EF	−1.7498	−0.2187	0.1914	—
BD, CF, AG, EH	−4.4200	−0.5525	1.2210	8.91**
ABD, G (Cocoa)	3.5518	0.4440	0.7855	5.75*
CD, BF, AH, EG	2.1262	0.2658	0.2825	—
ACD, H (Onion powder)	1.0448	0.1306	0.0682	—
BCD, F (Caramel)	−0.0330	−0.0041	0.0001	—
ABCD, DE, AF, CG, BH	0.6956	0.0869	0.3020	—

* 0.1 level of significance
** 0.05 level of significance
*** 0.01 level of significance

TABLE 5. ANALYSIS OF VARIANCE OF GENERATION TIME OF THE EFFECT OF THE STIMULATIVE COMPONENTS ON GROWTH OF *Clostridium perfringens* (FULL FACTORIAL DESIGN)

Source	Degrees of freedom	Sum of squares	Mean square	F Ratio
Between blocks	1	2.000	2.000	1.44
Between treatments	15	129.0388	8.6025	6.20**
Residual	15	20.8200	1.3880	
Total	31	151.8588		

	Degrees of freedom	Mean square	F Ratio	Mean effect
Vegetable oil	1	7.8012	5.62*	−0.99 ± 0.89 min
Beef-like flavor	1	47.5312	34.24***	−2.44 ± 0.89 min
Corn flour	1	38.7200	27.90***	−2.20 ± 0.89 min
Cocoa	1	18.9112	13.62**	−1.54 ± 0.89 min

* 0.1 level of significance
** 0.05 level of significance
*** 0.01 level of significance

TABLE 6. ANALYSIS OF VARIANCE OF RESPONSE SURFACE DESIGN TO DETERMINE OPTIMAL CONCENTRATION OF BEEF-LIKE FLAVOR AND CORN FLOUR ON THE GROWTH OF *Clostridium perfringens*

	Degrees of freedom	Sum of squares	Mean squares	F Ratio
Source	13	52.944	4.073	
Model	5	38.052	7.610	4.089**
Replication	5	0.440	0.088	
Lack of Fit	3	14.451	4.817	54.737***

** 0.05 level of significance
*** 0.01 level of significance

exists in the concentrations giving stimulation.

Cocoa and vegetable oil gave stimulation but to a lesser extent. The stimulatory nature of cocoa and vegetable oil is not fully known at this time. Cocoa has been shown to be inhibitory to salmonellae *(4)*.

In summary, four (beef-like flavor, corn flour, cocoa, and vegetable oil) of the 16 ingredients comprising synthetic soybeef stimulated growth of *C. perfringens*. The remaining 12 ingredients showed no significant effect. Beef-like flavor was most stimulatory followed by corn flour.

These data indicate the potential importance of a few key ingredients of a fabricated food in influencing the growth rate of a potentially pathogenic organism. Processors fabricating foods have the advantage of assessing ingredients with regard to potential hazards and substituting or modifying these ingredients to lessen the public health hazard.

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SMOKED MULLET QUALITY: AN ASSESSMENT[1]

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ABSTRACT

A survey of 12 smoked mullet processors in Florida was conducted and 33 retail samples were analyzed. Although microbiological analyses of the finished product did not indicate that any overt hazard existed, certain aspects of production, handling, and marketing could potentially generate a health hazard if not carefully controlled.

Smoked mullet is a regional delicacy common to Florida, and is primarily produced for the retail market in small (backyard) processing facilities. These producers have found that smoking is an excellent means to improve the acceptability of mullet as well as to increase the economic return on an other wise low profit item *(5)*. Because of the differences in size and scope of these operations, facilities for preparing, smoking, and storage of the product vary markedly. The most notable deficiencies observed during a preliminary survey of a number of these plants was the absence of temperature indicating equipment in the smokehouse and the lack of adequate refrigerated storage. It would seem that these deficiencies would have resulted in various health problems; however, smoked mullet has enjoyed an outstanding safety record *(2)*. Undoubtedly the inhibitory nature of the smoke components and a relatively short span between production and consumption adds a significant margin of safety.

There is little technical information available regarding the quality of smoked mullet presently marketed, and it was considered desirable to establish some background data on its production and quality. This was believed particularly necessary in that Florida's mounting tourist influx has resulted in an increased demand for this product without a concomitant increase in regulation.

MATERIALS AND METHODS

Thirty-three retail samples were obtained from 12 processors throughout the State of Florida. The method of holding the finished product, whether under refrigeration or at ambient temperature, was also recorded at the time the sample was obtained.

Smoked mullet was also prepared experimentally under pilot plant conditions by the following method. Fresh whole mullet were obtained from a local dealer. Headed fish were split along the backbone (butterflied) to remove the viscera and blood. The fish were then brined in 20% sodium chloride for 30 min. Following brining, the fish were rinsed and allowed to drain for 30 min at 34 F. The fish were then smoked for about 6 hr in an electric laboratory smoker, using Red Oak sawdust as the source of smoke. The internal temperature of the fish for the last 30 min was maintained at 180 F monitored in the thickest part of the loin by a single point potenteometric pyrometer (Mini Mite, Thermo Electric Co., Inc). Following smoking and cooling, the fish were packaged in 18 oz Whirl-Pack bags and stored at 4 C.

Microbial analyses were conducted by methods cited in the references: total aerobic count, coliform organisms, coagulase-positive *Staphylococcus*, and *Salmonella (1)*, *Clostridium perfringens (3)*, fungi *(4)*, and enterococci *(6)*. Chemical analyses for moisture, fat, protein, and sodium chloride were carried out according to A.O.A.C. *(1)*.

RESULTS AND DISCUSSION

The composition of smoked mullet from four typical plants is given in Table 1. In addition, the composition of fresh mullet and pilot plant smoked mullet is shown. None of the processors brine their fish, and only a few lightly salt the fish before smoking. With new State regulations *(2)* presently in effect, requiring 3.5% sodium chloride in the aqueous phase of the loin muscle, steps will have to be initiated by the producers to meet this requirement. The 30-min brining applied in our pilot plant studies was adequate in this respect.

Microbial analyses did not indicate that an immediate health hazard existed (Table 2). However, the presence of coagulase positive staphylococci in two of the samples at levels in excess of 100/g are undesirable, particularly since the samples were from a producer who did not refrigerate the finished product. Additional studies to determine the ability of staphylococci to grow in this product are needed. However, the widely varying manufacturing procedures, such as smoking time (1-12 hr), would make any generalization of the results difficult.

TABLE 1. COMPOSITION OF SMOKED MULLET OBTAINED FROM FLORIDA PRODUCERS

Plant	Moisture	Fat	Protein	NaCl
1	63.7	8.4	26.2	1.7
2	65.0	7.0	26.8	1.2
3	54.0	9.8	35.0	1.2
4	63.5	8.2	26.2	2.1
Average	61.5	8.4	28.6	1.6
Pilot Plant	60.5	9.2	26.5	3.8
Fresh fish	73.5	7.0	18.0	0.5

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TABLE 2. A SUMMARY OF SMOKED MULLET MICROBIOLOGICAL QUALITY FROM 12 FLORIDA PLANTS[1]

Plant	Samples	Refrig. storage	Total count	Coliform	Staph.	Enterococci	Yeasts and Molds
				(Range, No./g)			
A	5	no	160-40,000	—[2]	—	0-22,000	200-350
B	3	no	350,000-800,000	—	—	0-42,000	—
C	3	no	15-100	—	—	—	—
D	3	no	100-14,000	—	—	—	0-20
E	4	no	3,700-40,000,000	0-210	0-240	220-4,000	20-500
F	1	yes	5,000	9.1	43	20	40
G	2	yes	310,000-5,600,000	—	—	—	—
H	3	no	600-12,000	—	—	—	0-15
I	2	no	310,000-720,000	—	—	20,000-150,000	20-70
J	5	yes	10-500	—	—	—	0-30
K	1	no	150	—	—	—	—
L	1	no	45	—	—	—	—
Pilot							
6-brined-not smoked			15-7,200	0-3.6	0-0.36	—	5-80
6-freshly smoked			10-40	—	—	—	—
3-smoked, 7 days storage 4 C			10-15	—	—	—	—
3-smoked, 13 days storage 4 C			10-70	—	—	—	—

[1]Samples were negative for salmonellae and *Clostridium perfringens*
[2]Not isolated

C. perfringens and *Salmonella* were not isolated from any of the samples and coliforms were found in only three samples. The way in which the product is presently handled (open to the air with rapid product sales) would seem to preclude growth of *C. botulinum;* however, the possibility of growth by *Vibrio parahemolyticus* should be investigated.

The significance of enterococci in foods is still not clear *(7),* making interpretation of these data difficult. On the basis of the pilot plant studies which were negative for enterococci in both the raw and smoked product (Table 2), it would seem that the high enterococci counts, resulted from contamination during processing with subsequent growth of the organisms. An earlier study *(8)* indicated that mold growth was a problem on smoked mullet and in this study over half of the samples exhibited a count of 20 or more colony forming units per gram.

For many of the samples, total aerobic plate counts were excessively high and would therefore indicate improper procedures. Samples prepared in the pilot plant and handled under sanitary conditions were consistently produced with low counts, and the counts remained low through 13 days of storage (Table 2). This would indicate that the commercial samples were not heated to a temperature sufficient to destroy the original organisms present and/or that contamination and growth occurred following smoking.

The sporadic nature of most foodborne outbreaks makes results of any survey of questionable value. However, a study of this type can point out areas of weakness within a given process, and any obvious hazards can be minimized through education and regulation. While no immediate microbial hazards were uncovered in this study, certain aspects of the production and marketing of smoked mullet were sanitarily questionable.

Refrigeration of the finished product was only practiced by three of the 12 producers. Both safety and consumer acceptance of the product would be enhanced by refrigerated storage. Packaging, when used, varied from aluminum foil to wax paper; in other instances the uncovered product was merely displayed on top of a counter. A protective and appealing package would do much to improve the sale as well as the sanitary quality of this product.

As the smoked mullet industry in Florida undergoes continuing development, there appears to be a need for extensive educational programs as well as careful regulation and supervision to avoid creating a hazard due to a too rapid application of technology without accompanying safeguards. Up to this time the simplicity of the process has apparently contained sufficient natural safeguards so that a real hazard was not generated, or else if one existed it was not apparent to either consumers or health officials.

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A CASE STUDY: SYNERESIS OF COLD-PACK CHEESE FOOD RELATED TO GROWTH OF LACTIC ACID BACTERIA

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Abstract

Commercial Cold-Pack Cheese Food which exhibited marked syneresis in consumer packages had a pH of 4.60 to 4.80. Bi-weekly microbial examination of commercial Cold-Pack Cheese Food stored at 5, 10, 15, and 20 C indicated a marked growth of lactobacilli and streptococci at all temperatures above 5 C and a slight decease in staphylococci at all temperatures. It is assumed that the lactose added in the form of skim-milk or whey powders provided the carbohydrate nutrient source for these organisms so that the pH was reduced from 5.05 to 4.60. The minimum hydration of casein occurs at pH 4.60 and this lowered pH caused syneresis in the packages. Cooling the Cold-Pack Cheese Food quickly to 5 C and keeping it at or below this temperature eliminated the problem.

Cold-Pack Cheese Food[1] is the product made by comminuting one or more lots of cheese into a homogeneous plastic mass without the aid of heat. The optional ingredients may be cream, milk, solids derived from milk, and other minor ingredients. The fat content shall be not less than 23% and the product shall not contain more than 46% moisture. The U. S. Cheese Food Standards of Identity[2] are similar to the Canadian Standards.

Cheese Food is a common item on the Canadian food market. In 1971 many packages of this product were returned from supermarkets because of brittleness of the body and presence of free liquid in the package. This Cheese Food usually possesses a smooth texture and does not show any free serum. The pH of the defective samples ranged from 4.50 to 4.90 which was lower than the normal fresh product (5.00 to 5.20). Because of the decrease in pH, the nature of the food, and the optional ingredients which were high in lactose, lactic acid bacteria were believed to be involved in causing this defect. The effect of temperature and time of storage on the possible growth of lactic acid bacteria was investigated.

Materials and Methods

Manufacture of Cold-Pack Cheese Food

The cheese for this product was a blend of 6 months, and 1 and 2 years old Cheddar cheese. The cheese and other ingredients (Table 1) were thoroughly mixed in a silent cutter[3] until a smooth homogeneous mass was obtained. During the comminuting the mixture attained a temperature of 42 C. The mixture was placed in 45 kg polyethylene lined boxes and held overnight at 5 C for firming. The Cheese Food was cut into 226-g portions and were placed in preformed vacu-formed cryovac pouches (P540) of unsupported polyethylene saran, which were then evacuated and heat sealed.

Collection of samples

The samples were obtained from the manufacturing plant immediately after packaging and transferred to the laboratory in insulated boxes. The packages of Cheese Food were stored at 5, 10, 15, and 20 C. Packages were removed and analyzed at regular intervals (every 3rd and 7th day) for a period of 42 days. An unopened package was used for each set of analyses.

Analytical determinations

Eleven grams of cheese were blended for 3 min with 99 ml sterile phosphate buffer (pH 7.20) in a sterile blendor. After suitable dilutions the following microbiological analyses were carried out: *(a)* total aerobic count on Standard Plate Count Agar at 32 C for 48 hr; *(b)* coliforms on Violet Red Bile Agar at 37 C for 24 hr; *(c)* yeast and mold on Potato Dextrose Agar acidified to pH 3.5 with sterile 10% tartaric acid, incubated at 25 C for 5 days; *(d)* staphylococci on Tellurite Polymyxin Egg Yolk Agar at 37 C for 48 hr; a representative number of colonies were streaked on Plate Count Agar and incubated at 37 C for 24 hr; the cultures were then examined by gram stain and for coagulase by the slide method *(1)* using lypholized bacto-coagulase plasma; *(e)* lactobacilli on Rogosa's medium *(6)* at 37 C for 72 hr using the double layer plate method; and *(f)* streptococci on Nutrient Agar containing 0.05% thallous acetate *(3)* at 32 C for 72 hr; the double layer plate method was used. All medium ingredients and reagents were obtained from Difco.

The changes in pH during storage were determined on the Cheese Food at the same time intervals using a Fisher Accumet (Model 310) pH meter and a Beckman combination electrode.

At the end of the 42 day storage period the free liquid of unopened packages of cheese at the various temperatures was collected by gravity in tubes and the average volume and pH determined.

Results and Discussion

The ingredients, their corresponding pH, and respective microbial flora are shown in Table 1. It is interesting that coagulase-positive staphylococci were only found in trimmings from the cutting operations.

[1]Trade Information Letter No. 370, June 7, 1972, Dept. of National Health and Welfare, Ottawa, Ontario, Canada.
[2]Code of Federal Regulations, Title 21, Food and Drug 19. 787 U. S. Govt. Printing Office, Washington, D. C. 1967.
[3]Rasant cutter—Seydelmann, Stuttgart (also called Buffalo Silent cutter).

TABLE 1. MICROBIAL COUNT PER GRAM OF INGREDIENTS USED IN THE MANUFACTURE OF COLD-PACK CHEESE FOOD

Ingredients	pH	Coliforms	Lacto-bacilli	Strepto-cocci	Staphy-lococci	Total count	Molds	Yeasts
6-Months old Cheddar	5.10	4	1×10^6	6×10^6	7×10^1	5.5×10^6	6	120
1-Year old Cheddar	5.03	<2	14×10^6	10×10^6	4×10^1	16×10^6	2	<2
2-Years old Cheddar	5.23	<2	4.2×10^6	4.5×10^6	78×10^1	6.3×10^6	12	>3000
3-Years old Cheddar	5.20	<2	5.3×10^6	5×10^6	32×10^1	9.3×10^6	12	50
Butter	5.67	<2	<10	<10	20×10^1	<10	<2	110
Trimmings from cutting operation	5.35	<2	11×10^6	12×10^6	47×10^1[a]	10×10^6	200	>3000
Water	6.90	<2	1×10^1	8×10^1	<10	29×10^3	<2	<2
Sorbic acid	–	<2	<10	<10	<10	18×10^2	<2	<2
Whey powder	5.60	<2	<10	<10	<10	15×10^2	<2	<2
Skimmilk powder	6.10	2	92×10^1	2×10^2	32×10^1	28×10^2	6	120
Kling[b]	6.27	<2	50×10^2	28×10^2	6×10^1	52×10^2	24	2

[a]60% were coagulase-positive
[b]A calcium reduced skimmilk powder (Standard Spice Co., Toronto)

The cheese contributed most of the microflora. The pH of the mixture and the changes occurring during storage at different temperatures over a period of 42 days are given in Table 2. The decrease of the pH with time was more pronounced at the higher temperatures. This was also reported by Park et al. (5) on a cheese food. There was no change in pH when the product was stored at 5 C.

The degree of syneresis was related to the temperature of storage and was also accompanied with lower pH values (Table 3). The amount of exudate at the low pH is not surprising as this is close to the iso-electric point of casein or the point of minimum hydration.

The lactobacilli and streptococci constituted the predominant microflora. Their number increased slightly during storage and was affected by the higher temperatures (Tables 4-7). Once the maximum concentration was reached it was maintained at this level for the remainder of the storage period.

The total count seemed to parallel the counts of the lactobacilli. Microscopic examination and catalase reaction of isolated colonies from the total plate count showed that the majority were gram-positive and catalase-negative rods and some cocci. The pH of the cheese favors growth and survival of acid-tolerant organisms such as the lactic acid bacteria. The initial staphylococci population was low and showed no tendency to increase at any of the storage temperatures. This is in agreement with Dahiya and Speck (2) who observed inhibition of *Staphylococcus aureus* by cell-free extracts of lactobacilli and lactic streptococci. Coliforms were not detected. Yeasts were isolated only during the first 7 days of storage while molds were detected during the 42 days of storage with a tendency to decrease, probably because of the effect of sorbate in the mixture. Sorbate at the concentration used in the mixture had

TABLE 2. pH OF COLD-PACK CHEESE FOOD STORED AT 5, 10, 15 AND 20 C FOR 42 DAYS

Days of storage	Temperature of storage C			
	5	10	15	20
0	5.05	5.05	5.05	5.05
3	5.04	5.06	5.10	4.90
7	5.04	5.08	5.14	4.83
10	5.07	5.02	5.12	4.82
14	5.09	5.27	5.18	5.04
17	5.15	5.17	5.25	4.75
21	5.11	5.19	5.22	4.87
24	5.05	5.23	5.21	4.75
28	5.03	4.82	4.75	4.69
31	5.02	5.06	4.80	4.80
35	5.04	4.90	4.75	4.78
38	5.04	4.84	4.83	4.80
42	5.05	4.78	4.67	4.60

TABLE 3. THE AVERAGE VOLUME AND pH OF EXUDATE FROM 5 PACKAGES OF COLD-PACK CHEESE FOOD AFTER 42 DAYS OF STORAGE AT 5, 10, 15, AND 20 C

Temperature of storage ° C	Exudate (ml)	pH of exudate
5	0	–
10	9	4.88
15	12	4.44
20	27.2	4.45

TABLE 8. RELATIONSHIP BETWEEN pH, TYPE OF ORGANISM AND THE TEMPERATURE OF STORAGE AS EXPRESSED BY CORRELATION COEFFICIENTS

Interaction	Temperature of storage °C			
	10	15	20	
pH × lactobacilli	–.64	–.88	–.59	
pH × streptococci	.04	–.42	–.47	N.S.
pH × total count	–.66	–.86	–.62	
Streptococci × lactobacilli	.39	.67	.96	
Lactobacilli × total count	.98	.98	.97	
Streptococci × total count	.43	.70	.89	

N.S. not significant

TABLE 4. MICROBIOLOGICAL ANALYSES OF COLD-PACK CHEESE FOOD STORED AT 5 C

Days of Storage	Coliforms	Lactobacilli ($\times 10^6$)	Streptococci ($\times 10^6$)	Staphylococci ($\times 10^2$)	Total count ($\times 10^6$)	Molds ($\times 10^2$)	Yeasts
0	<10	4.1	5.1	13.0	4.4	19	250
3	<10	1.3	2.1	23.0	2.0	0.7	10
7	<10	2.5	2.4	11.0	3.8	90	<2
10	<10	2.6	2.1	9.1	4.3	35	<2
14	<10	2.7	1.6	3.0	3.4	15	<2
17	<10	3.5	1.5	1.2	5.0	60	<2
21	<10	2.4	2.4	5.4	3.1	12	<2
24	<10	1.5	2.5	1.8	2.9	27	<2
28	<10	2.3	2.6	6.4	2.4	18	<2
31	<10	2.7	0.8	1.3	4.0	35	<2
35	<10	2.4	0.5	2.2	3.2	LA[a]	LA
38	LA	LA	LA	LA	LA	LA	LA
42	<10	1.8	0.4	1.7	1.8	0.4	<2

[a]Laboratory accident

TABLE 5. MICROBIOLOGICAL ANALYSES OF COLD-PACK CHEESE FOOD STORED AT 10 C

Days of Storage	Coliforms	Lactobacilli ($\times 10^6$)	Streptococci ($\times 10^6$)	Staphylococci ($\times 10^2$)	Total count ($\times 10^6$)	Molds ($\times 10$)	Yeasts
0	<10	4.1	5.1	13.0	4.4	190.0	250
3	<10	0.6	0.9	4.5	1.0	2.0	15
7	<10	3.3	4.9	7.6	6.4	20.0	<2
10	<10	16.0	9.4	14.0	39.0	190.0	<2
14	<10	22.0	17.0	7.0	43.0	5.0	<2
17	<10	0.5	0.4	0.8	2.0	3.5	<2
21	<10	0.6	0.5	0.8	0.9	1.0	<2
24	<10	0.4	0.4	0.7	0.7	27.0	<2
28	<10	20.0	0.1	6.7	25.0	38.0	<2
31	<10	0.2	0.1	0.4	0.6	2.5	<2
35	<10	30.0	LA[a]	18.0	45.0	LA	LA
38	<10	51.0	19.0	4.3	210.0	15.0	<2
42	<10	71.0	8.7	1.6	180.0	38.0	<2

[a]Laboratory accident

little effect on the lactobacilli although yogurt bacteria have been reported to be inhibited by 0.1% potassium sorbate *(4)*.

The low pH (5.05) of the Cheese Food would inhibit growth of most organisms even at storage temperatures of 15-20 C. However, growth of lactic acid bacteria, some of which are both psychrotrophic and acid tolerant, is possible. Improper refrigeration would enhance the possibility for their growth.

The data indicate that to overcome the defect of syneresis in this type of food, the product has to be stored at 5 C. Addition of sorbate at proper concentration may provide additional protection especially at the higher storage temperatures.

The correlation coefficients indicating the relationships between pH, total count, lactobacilli, and streptococci at the various temperatures are shown in Table 8. The pH is inversely influenced by the numbers of organisms as reflected by the population of lactobacilli and the total count. It would appear that the lactobacilli have more influence on pH than streptococci. The total count is highly correlated with number of lactobacilli which might indicate that the majority of the microbial flora were lactic acid bacteria or a lack of selectivity of the lactobacillus medium. It is assumed that the added source of lactose in the whey, skimmilk powder and Kling (see Table 1) provided the carbohydrate source for the streptococci and lactobacilli. With the nutrient addition, these organisms flourished and produced lactic acid which lowered the pH to the iso-electric point of casein, producing the exudate in the packages.

The companies producing this Cheese Food had a high level of returns because of the unsightly exudate. Cooling the Cheese Food rapidly to 5 C and holding at or below this temperature eliminated the problem.

The microbiological flora does not appear to have public health significance but it should be noted that coagulase-positive staphylococci were found. In considering any public health significance Tatini et al. *(7)* found enterotoxin A production was stimulated in Swiss cheese with cooking temperatures of 50 C. The temperature of this Cheese Food attained 42 C

Table 6. Microbiological analyses of Cold-Pack Cheese Food stored at 15 C

Days of Storage	Coliforms	Lactobacilli ($\times 10^6$)	Streptococci ($\times 10^6$)	Staphylococci ($\times 10^2$)	Total count ($\times 10^6$)	Molds ($\times 10$)	Yeasts
0	<10	4.1	5.1	13.0	4.4	190.0	250
3	<10	3.7	3.7	16.0	6.5	4.0	54
7	<10	33.0	19.0	9.4	48.0	170.0	50
10	<10	<1.0	0.5	1.6	4.5	0.4	<2
14	<10	1.0	1.0	1.3	2.0	5.0	<2
17	<10	<1.0	0.2	0.6	1.5	3.0	<2
21	<10	0.6	0.5	1.5	1.0	2.0	<2
24	<10	0.4	0.4	0.4	0.8	1.0	<2
28	<10	35.0	0.1	5.6	0.3	39.0	<2
31	<10	83.0	13.0	6.4	140.0	44.0	×2
35	<10	55.0	15.0	18.0	47.0	LA[a]	LA
38	<10	150.0	57.0	3.8	280.0	4.0	<2
42	<10	94.0	12.0	15.0	230.0	39.0	<2

[a]Laboratory accident

Table 7. Microbiological analyses of Cold-Pack Cheese Food stored at 20 C

Days of Storage	Coliforms	Lactobacilli ($\times 10^8$)	Streptococci ($\times 10^8$)	Staphylococci ($\times 10^2$)	Total count ($\times 10^8$)	Molds ($\times 10$)	Yeasts
0	<10	0.04	0.05	13.0	0.04	190	250
3	<10	0.33	0.19	17.0	0.57	26	10
7	<10	1.20	0.79	8.6	1.80	95	50
10	<10	2.20	1.60	3.9	3.10	25	<2
14	<10	2.40	1.30	6.0	3.00	120	<2
17	<10	2.30	1.30	1.6	2.30	8	10
21	<10	4.20	4.00	4.8	2.30	8	<2
24	<10	3.30	3.10	1.0	3.20	17	<2
28	<10	2.40	0.83	7.8	2.30	20	<2
31	<10	3.50	1.70	4.1	5.00	9	<2
35	<10	4.20	2.00	17.0	4.20	—	—
38	<10	5.00	2.40	3.6	5.90	4	<2
42	<10	3.00	1.00	5.1	2.70	3	<2

during comminuting, without external application of heat.

It should also be pointed out that Park et al. *(5)* found viable salmonellae in an inoculated Cheese Food after 23 weeks at pH 4.70. It is therefore of utmost importance to use high quality ingredients in the manufacture of Cheese Food and to keep the temperature low during processing and storage.

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A Research Note

IMMUNOGENIC PROPERTIES OF SLIME FROM PROPIONIBACTERIA[1]

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Abstract

Slime, produced by members of the genus *Propionibacterium*, was isolated, and attempts were made with it to induce antibody formation in biological systems to demonstrate the possible use of anticapsular antibodies for serological typing. Slime produced by three different species of *Propionibacterium* did not produce detectable precipitating antibodies in rabbits. Killed whole cells of *Propionibacterium zeae* induced specific agglutinin formation. Antibodies produced against whole cell antigens of *P. freudenreichii* and *P. shermanii* exhibited substantial heterologous agglutination reactions.

The immunological properties of capsular materials are commonly known. For historical background, a review of work done before 1948 is available *(3)*. In recent years, the immunogenic properties of capsular materials have served as valuable tools to classify microorganisms *(3, 7)*. Possession of highly polymerized polysaccharide capsular antigens has permitted separation of pneumococci into approximately 75 types *(10)*. The classical work of Lancefield *(4)* demonstrated the value of polysaccharide antigens for serological classification of group-B streptococci.

The need for serological classification of *Propionibacterium* was mentioned by Malik et al. *(6)* in their taxonomic evaluation of this genus. Previously Werkman and Brown *(13)* had reported a serological study of 11 species of this genus and found extensive cross agglutination. They used preparations of 0 antigen and made no mention of capsular or slime antigens. Recently, Skogen *(9)* demonstrated capsule formation and slime production in several species of *Propionibacterium*. By analyzing slime hydrolysate using paper and thin-layer chromatography he determined that mannose and lesser amounts of glucose and galactose were present. No mention was made of the presence or absence of proteins or individual amino acids in the slime. Our investigation was undertaken to evaluate the immunogenic properties of slime from *Propionibacterium* in an attempt to exploit the possible use of anticapsular or antislime antibodies in typing members of the genus *Propionibacterium*.

Materials and Methods

Organisms used

Three strains of propionibacteria that produced copious amounts of slime *(9)* were used in this study. They were *Propionibacterium zeae* strain no. 74, *Propionibacterium shermanii* strain no. 47, and *Propionibacterium freudenreichii* strain no. 1. These strains were routinely maintained in sodium lactate broth *(12)*.

Preparation of whole cell antigens

Cultures grown in broth were killed with formalin, and cells harvested by centrifugation. The cells were resuspended in 0.05 M phosphate-buffered saline and were heated for 20 min in a water bath at 70 C. Formalin was added to a final concentration of 0.01%. The suspensions were brought to a final concentration of 1×10^9 bacteria/ml on the McFarland scale [an arbitrary optical density scale *(2)*] before injection.

Slime preparation

The method of Lindeberg *(5)* was used to isolate and purify the slime of the three slime-producing strains of propionibacteria.

Cultures were inoculated into 1 liter of glucose broth (containing 2.0% glucose, 1.0% Trypticase, 1.0% yeast extract, 0.025% K_2HPO_4, in distilled water. Incubation was at 21 C for 21 days. Cells were removed by centrifugation, and the supernatant fluid containing the loose slime was collected. Two volumes of 95% ethanol were slowly added to the clear supernatant liquid, and the fibrous mass, formed by mixing the ethanol with the supernatant fluid, was spooled onto a glass rod and removed. Slime was resuspended in distilled water and reprecipitated with ethanol. It was then collected and dried on a watch glass for 24 hr at 45 C. Dried slime was weighed and resuspended in distilled water to a final concentration of 10 mg/ml. Resuspended slime was dialyzed against cold, running, tap water for 24 hr, lyophilized, and then stored at 4 C. Lyophilized slime was used for further antigenic preparations.

Assessment of immunogenicity

Immunogenicity of propionibacterial slime was assessed by injecting New Zealand albino rabbits (2.2-2.8 kg) with either wholecell suspensions or isolated slime. When whole cells were used, increasing doses (0.5, 1.0, 2.0, and 2.0 ml) were injected intravenously on three successive days each week during a 4-week period. Animals were bled by intracardial puncture 9 days after the final injection, and sera were stored at −20 C unless used immediately.

Isolated slime was used as the antigenic dose at an initial rate of 0.1 mg/kg body weight of the individual rabbits. During the first week, rabbits were given two subcutaneous injections of 1 ml slime plus 1 ml Freund's complete adjuvant. During the second week, two 1-ml intramuscular injections of slime plus Freund's complete adjuvant, in a 1:1 ratio, were given at 3-day intervals. Freund's complete ad-

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juvant *(7)* is a suspension of killed mycobacteria in a mixture of paraffin oil and an emulsfying agent. During the third week, adjuvant was discontinued, and only the slime suspension was injected by the intraperitoneal route. Two such injections were given at 3-day intervals. One week after completion of this series of injections, rabbits were bled by intracardial puncture. Sera were stored at −20 C unless used immediately as noted.

To determine the effect of increasing doses on antibody production, isolated slime was injected intradermally at 0.85 mg/kg body weight along with adjuvant. Two such injections were given for 2 weeks. During the third week, adjuvant was discontinued, and only slime was injected by the intravenous route. Rabbits were allowed to rest 1 week and were bled by intracardial puncture. Sera were stored at −20 C.

Serological methods

Bacterial agglutination was done in 13 × 100 mm tubes. The titer was recorded by reading the antigen antibody reaction patterns after holding overnight in a refrigerator after 3-hr incubation at 50 C.

The ring test for rapid detection of precipitating antibody was carried out in tubes, using Pasteur pipettes to introduce the components. The procedure was followed as outlined by Campbell et al. *(2)*.

Capillary precipitation tests were done by the method of Swift et al. *(11)*, with reading after 12-hr refrigeration.

Agar-gel diffusion was prepared using the slide method of Schubert et al. *(8)*. Antisera were tested in a satellite pattern to determine the relationship between strains. Slides were incubated 2 days at 35 C in a moist chamber.

The Neufeld *Quellung* reaction was determined by the method outlined by Campbell et al. *(2)*.

Sheep red blood cells were sensitized for passive hemagglutination by mixing equal volumes of tannic acid-treated erythrocytes and antigen *(1)*. Following antisera addition, tubes were checked for agglutination after 3- and 12-hr incubation at room temperature.

Complement fixation was done according to the method of Campbell et al. *(2)*, employing previously titrated complement and specific hemolysin. To overcome the anticomplementary reaction of the immune serum, the test was done with an initial serum dilution of 1:128.

RESULTS AND DISCUSSION

Immunogenicity of isolated slime

Antisera from rabbits injected with isolated slime material did not contain detectable levels of precipitating antibodies when assayed by ring-test precipitation, indirect hemagglutination, gel diffusion, and complement fixation tests.

Immunogenicity of formalinized cells

Immunogenic properties of killed whole cells of the three species of *Propionibacterium* were studied by agglutination tests. Homologous antigen reacted with the three antisera that had been obtained. Presence of cross agglutination observed with the antisera of both *P. freudenreichii* and *P. shermanii* supports the earlier findings of Werkman and Brown *(13)*. Likewise, the antiserum to *P. zeae* exhibited no cross-reaction. Attempts to detect anticapsular or antislime antibodies by using the *Quellung* reaction were unsuccessful.

Results of this study indicate that slime produced by three species of *Propionibacterium* did not induce detectable precipitating antibody formation in rabbits. It is possible that slime from propionibacteria, like pneumococcal polysaccharides, may be antigenic only in certain species of mammals. Obviously it is not antigenic in rabbits and might act as a hapten. This work further indicates that subsequent attempts to induce antibody formation against propionibacterial slime should be done in species of animals other than rabbits. Since chickens are better producers of precipitating antibody than are rabbits [Wolf et al. as cited by Campbell et al. *(2)*] their use for this purpose would seem logical.

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VIBRIO PARAHAEMOLYTICUS–ISOLATION, IDENTIFICATION, CLASSIFICATION, AND ECOLOGY[1]

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ABSTRACT

Vibrio parahaemolyticus was isolated from samples of water, sediment, blue crabs, oysters, and clams collected in several areas of Chesapeake Bay. Numerical taxonomy was used to identify and classify the bacterial isolates. Deoxyribonucleic acid (DNA) base composition, serology, isozyme, gas chromatography, bacteriophage sensitivity, and DNA/DNA reassociation analyses confirmed the identification and classification of *V. parahaemolyticus* and permitted establishment of genetic relationships of the Chesapeake Bay strains with isolates from victims of food poisoning in Japan and from samples taken in geographically diverse areas of the United States. Isolates implicated in recent outbreaks of food poisoning, the first fully documented cases of *V. parahaemolyticus* food poisoning in the United States, were shown by DNA/DNA reassociation measurements to be closely related to the Japanese and other isolates collected in the United States. Fatty acid profiles of cell derivatives prepared using GLC were useful in diagnosing *Vibrio* spp., including *V. parahaemolyticus* and *Vibrio cholerae*. Bacteriophages isolated from ocean sediments collected off Cape Hatteras were found to be active against *V. cholerae* and *V. parahaemolyticus*. Distribution of *V. parahaemolyticus* appears to be restricted to coastal and estuarine regions. *V. parahaemolyticus* has been shown to be closely associated with zooplankton and a life cycle for *V. parahaemolyticus* in Chesapeake Bay is proposed.

Microbial ecology studies of Chesapeake Bay were initiated in our laboratory in 1964. Analyses of seasonal and regional differences in the microflora, specifically the bacteria, were undertaken and, in the first 3 years of the 8 years of research, two areas of Chesapeake Bay were chosen for study. Marumsco Bar, near Crisfield, Maryland, on the lower Eastern Shore and Eastern Bay, on the upper Eastern Shore of Maryland were sampled at 6-8 week intervals. Marumsco Bar suffers serious biomodal annual mortalities of the shellfish population and is no longer commercially productive for oysters. Eastern Bay remains a thriving oyster harvesting area. Thus, the two areas offer useful sites for comparative microbial ecology analyses. Research over the past 5 years has been expanded to encompass a generalized microbial ecology program with sampling sites increased in number to provide an holistic sampling of the Chesapeake Bay.

With the discoveries by Krantz et al. *(22)* in 1969 that *Vibrio parahaemolyticus* is involved in mortality of Chesapeake Bay blue crabs, *Callinectes sapidus*, and by Lovelace et al. *(24)* that *Vibrio* spp. are a dominant portion of the natural microbial flora of water, sediment, and animals, a focus of the research efforts has been on the genus *Vibrio* as a model system for heuristic approaches to the understanding of ecological interactions in Chesapeake Bay and, ultimately, bays and estuaries of the coastal regions.

V. parahaemolyticus has recently been shown to be a public health problem in the United States (*Morbidity and Mortality Weekly Report*, 20 (39):356, Week ending Oct. 2, 1971). Identification and classification of this organism, therefore, are no longer activities of academic interest but a most practical necessity. Taxonomic studies of *V. parahaemolyticus*, using both numerical taxonomy and DNA/DNA reassociation analyses, were published by Citarella and Colwell *(9, 11)* and the taxonomic aspect of the research has been extended to include application of serological, isozyme, gas chromatography, and bacteriophage typing techniques.

Japanese workers have provided extensive data on *V. parahaemolyticus (18, 19, 28, 32)*. Other papers in this symposium deal with specific aspects of *V. parahaemolyticus*, such as isolation methods *(17)*, pathogenicity *(30)*, and epidemiology *(15)*. The objectives of this report are to provide an overview of current research underway in our laboratory on the identification, classification, and ecology of *V. parahaemolyticus* and to offer some hypotheses concerning the taxonomy and distribution of this species in nature.

MATERIALS AND METHODS

Sampling methods used in the studies reported in this paper have been described elsewhere *(20, 24)*. Water, sediment, and animal samples were collected on a routine basis

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from sampling stations in Marumsco Bar, Eastern Bay, the Rhode River, Baltimore Harbor, and other sites in Chesapeake Bay. Samples were collected following aseptic technique and employing J-Z (Alpine Geophysical Associates, Inc., West Norwood, New Jersey) and Niskin (General Oceanics, Inc., Miami, Florida) samplers for water and a corer (Wildlife Supply Co., Saginaw, Michigan) and dredges for sediment.

Ship support was provided by the Smithsonian Field Station at Rhode River through courtesy of Dr. F. Williamson, Director, the Maryland Department of Natural Resources and the Johns Hopkins University Chesapeake Bay Institute (R/V RIDGELY WARFIELD) for Chesapeake Bay sampling and Duke University (R/V EASTWARD) for off-shore and ocean sampling. Samples were subjected to bacteriological analysis on board ship immediately after recovery or on return to the laboratory. Samples were plated out within a few minutes to 4 hr after collection in all instances.

Standard bacteriological methods were used in carrying out plate counts, culture, and isolations. The media employed and taxonomic tests applied, as well as the method for data coding and computer programs used in data processing by computer for the numerical taxonomy studies have been described previously *(10, 12, 14, 26)*.

Taxonomic data for all strains isolated and characterized in the course of the studies have been coded and are stored on computer for retrieval and computation as new data are accessioned. A new program has been written which permits comparison of groups of strains from a given sampling with any previous sample set (Oliver and Colwell, Abstr. Ann. Meeting, Amer. Soc. Microbiol., May, 1972; Oliver and Colwell, manuscript in preparation).

Electron microscopy of thin sections of *V. parahaemolyticus* and *Vibrio cholerae* are routinely prepared to determine ultrastructural features and morphological detail of the strains. Methods for fixing, embedding, sectioning, and staining have been described *(21)*.

DNA base compositions were determined following the method of Marmur *(25)*, using modifications described by Citarella and Colwell *(8)*.

Polyacrilamide gel electrophoresis of cell preparations was carried out according to the method of Davis *(16)*. Preparation of cell extracts, reagents, and solutions used, and procedural details are given by Wan and Colwell *(31)*.

V. parahaemolyticus strains were serotyped using commercial anti-sera provided through the courtesy of Dr. H. Zen-Yoji, Director of Public Health Laboratory, Tokyo, Japan. Agglutination reactions were used to determine the K-serotype of each isolate.

Fatty acid profiles were compiled by gas chromatographic analysis of cell preparations obtained as follows. Cells were grown in seawater broth on a shaker at 28 C for 14-16 hr. The cells were harvested, washed with physiological saline and derivatives were made according to the method described by Moore et al. *(27)*. Aliquots of 2-5 μl of the samples were injected into a Hewlett-Packard Model 7600 Gas Chromatograph equipped with EGA columns. The GLC determinations were made at 180 C for 15-20 min. (C-12) - (C-18) standards were obtained from Hewlett-Packard and 1 μl of the standard was used before and after each day's chromatographic trials.

Bacteriophages for *V. parahaemolyticus* were isolated from sewage and also from sediment samples collected in November, 1971, off Cape Hatteras during a research cruise of the R/V EASTWARD, the Duke University research vessel. Isolation of bacteriophage was accomplished by means of enrichment experiments in which sewage or sediment samples and strains of *V. parahaemolyticus* were mixed together and allowed to incubate for 14-16 hr at 25 C. Positive results were indicated by double layering of the sample against host bacteria. Bacteriophage 7B was twice recovered from the original sample material as a check on the method and presence of bacteriophage in ocean sediment. Filtered and unfiltered sample aliquots were mixed with fresh host cultures and allowed to incubate for 18 hr, after which the contents of both flasks were centrifuged and the supernatants filtered. Filtrates were assayed by the double layering technique. Titers of the phage preparations were determined following standard methods *(1)*. Cultures of *V. parahaemolyticus* were challenged with the phage preparations for phage typing.

Extent of reassociation in heterologous DNA preparations was measured by slightly modified procedures previously published (5). Preparation of unlabeled, ^{32}P-labeled DNA, shearing of DNA, and counting procedures have been described *(7)*. The reassociation reaction consisted of unlabeled DNA fragments and ^{32}P-labeled DNA fragments in 0.14M phosphate buffer (ratio of unlabeled to labeled DNA was 400:1). The mixture was denatured at 100 C for 4 min, quickly cooled and then incubated at 60 C for 48 hr to obtain 96 Cots for the unlabeled DNA. After incubation, the duplex DNA was separated from the single-stranded DNA by adsorption of the duplexes onto hydroxyapatite by the batch procedure *(4)*. Adsorbed duplex DNA was then eluted by a thermal gradient in 0.14M phosphate buffer by 2.5 C increments up to 95 C. The thermal elution profiles thus obtained gave an estimate of percentage of unpaired bases in renatured DNA duplexes *(6)*.

RESULTS AND DISCUSSION

V. parahaemolyticus is a gram-negative, rod-shaped bacterium commonly isolated from estuarine and coastal waters and sediment. Under the electron microscope, the morphological features are that of the typical gram-negative rod. See Fig. 1a and 1b.

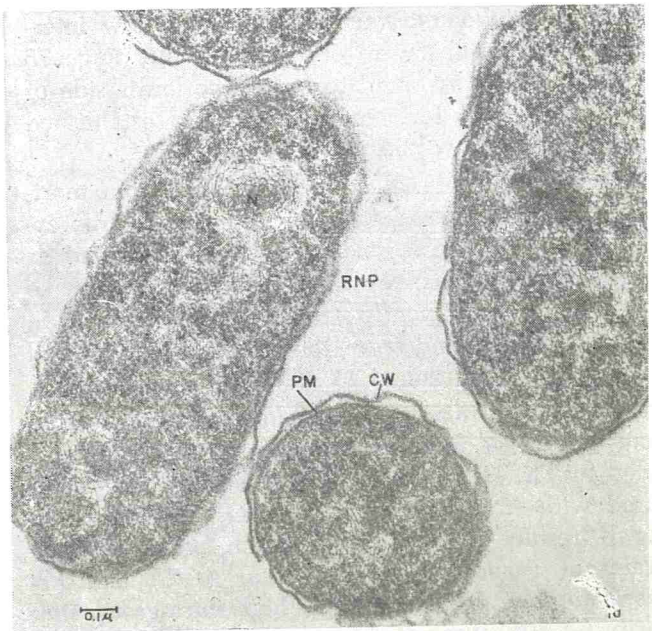

Figure 1a. Electronmicrograph of ultrathin section of *Vibrio parahaemolyticus*. Typical gram-negative structure is shown for the rod-shaped bacterium.

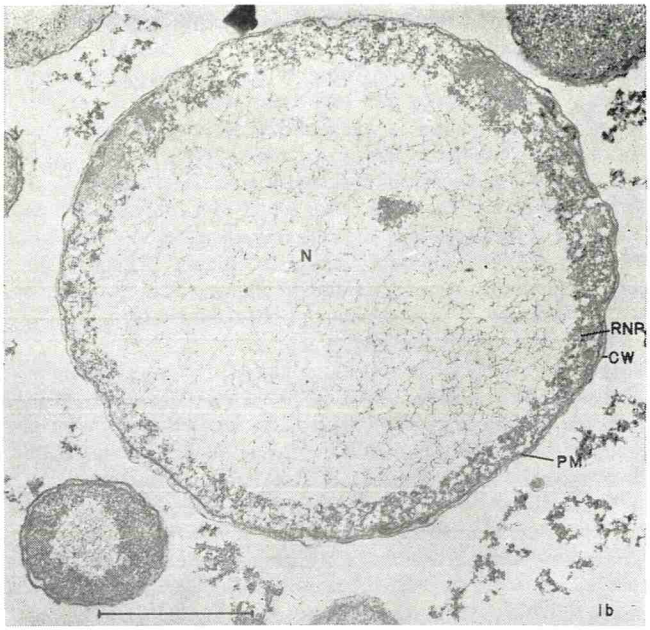

Figure 1b. Electronmicrograph of ultra thin section of *Vibrio parahaemolyticus*. Round body commonly observed in cultures of *Vibrio* spp., including *V. parahaemolyticus (21).*

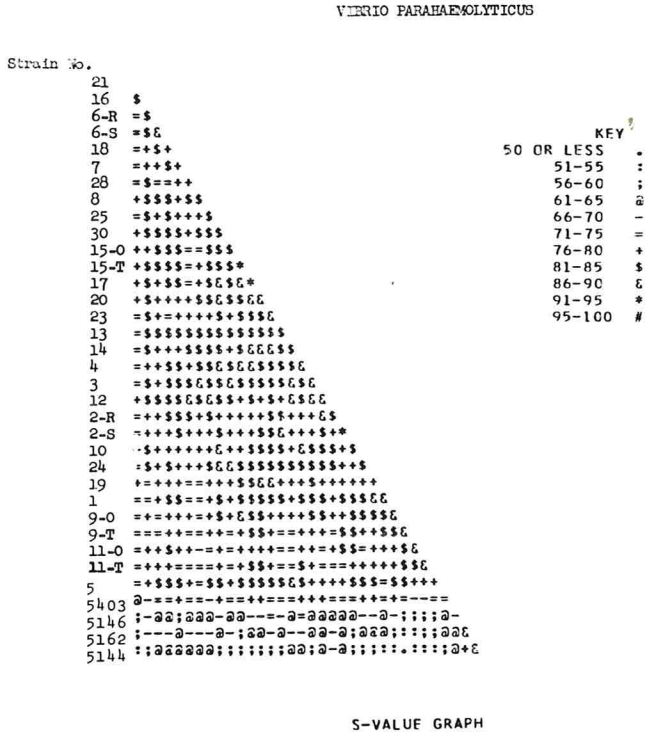

Figure 2. Total similarity value triangle output for *Vibrio parahaemolyticus*. Arrangement of strains was done by computer. Reading down the left column, strains No. 21 through 5 are *V. parahaemolyticus*. Reprinted through courtesy of the *Journal of Bacteriology (11).*

Numerical taxonomy

A set of reference strains of *V. parahaemolyticus* received from Dr. R. Sakazaki, Chief, Division of Bacteriology, National Institute of Health, Tokyo, Japan, were subjected to numerical taxonomy analysis. A total of 200 characteristics for each strain were scored, coded, and transferred to IBM cards for analysis by computer *(11).* Results of the analysis are shown in Fig. 2. Thirty-one of the 35 strains grouped in an homogeneous cluster with intra-group S-values of $\geq$ 75%. A comparison of these strains with 30 strains of *V. cholerae* was also done and low intercluster relationships were observed, in the range 75 $\pm$ 5% S. This was found to be on the lower side of the S-value level for genus relationship established in an earlier study *(12).*

A set of 28 bacterial cultures isolated from moribund and dead Chesapeake Bay blue crabs was examined and, of these isolates, 21 were identified as *V. parahaemolyticus;* see Fig. 3. The Chesapeake Bay strains of *V. parahaemolyticus* clustered at S-values of $\geq$ 75% and were found to possess over-all DNA base composition of 45 $\pm$ 1% G+C; see Table 1.

A set of 41 strains of *V. parahaemolyticus* isolated from victims of food poisoning in Japan were provided by Dr. H. Zen-Yoji. These isolates were compared with strains received from Dr. Sakazaki. In Fig. 4, results of such a comparison, with reference strains of *Pseudomonas fluorescens* (OX) and *Escherichia coli,* are given. Very high intra-group similarity ($\geq$ 80%) was noted for the *V. parahaemolyticus* strains.

Comparison of *V. parahaemolyticus* strains isolated from Chesapeake Bay blue crabs with the strains received from Dr. Zen-Yoji revealed very high similarity (Fig. 5). An analysis of representative strains of *V. parahaemolyticus* from a variety of sources is given in Fig. 6.

The high internal homogeneity of clusters of *V. parahaemolyticus* is clear from the numerical taxonomy data. Strains of *V. parahaemolyticus* isolated from diverse geographical sources and a variety of samples (blue crabs, necrotic tissues of infected swimmers, feces of patients, water, sediment, etc.) showed a remarkable phenotypic similarity.

DNA base composition analyses

Results of DNA determinations for representative strains of *V. parahaemolyticus* are given in Table 1. These data and other data, as yet unpublished, from our laboratory clearly indicate the over-all DNA base composition of *V. parahaemolyticus* to be in the range 45 $\pm$ 2% G+C. In all instances the DNA base composition determinations have provided results confirming the numerical taxonomy findings which are based on phenotypic similarities.

The DNA base composition of *V. cholerae* is higher than that of *V. parahaemolyticus,* i.e., 48$\pm$ 1% G+C, and *Vibrio marinus* and related marine vibrios lower, i.e., 40 $\pm$ 1%. See Table 1.

TABLE 1. DNA BASE COMPOSITION OF *Vibrio parahaemolyticus*, *Vibrio* SPP., AND STRAINS OF BACTERIA ISOLATED FROM OCEAN AND ESTUARY SAMPLES

Strain	No.	Tm°C	Buoyant Density	Mole % G + C
Cytophaga	2C[1]	83.3	—	34.2
Cytophaga	8F	83.5 (83.1)*	—	34.6 (33.8)*
Vibrio	15	86.1 (86.5)*	—	41.0 (42.0)*
V. parahaemolyticus	5D-Bw	87.4	—	44.2
V. parahaemolyticus	7B	87.4	—	44.2
V. parahaemolyticus	5B	87.5	—	44.4
V. parahaemolyticus	5D-Bw-Spr	87.6 (87.6)*	—	44.6 (44.6)*
V. parahaemolyticus	8E	87.6	—	44.6
V. parahaemolyticus	7Bw	87.7 (87.8)**	—	44.9 (45.2)**
V. parahaemolyticus	2B	87.8	—	45.1
V. parahaemolyticus	4A-A	87.8	—	45.1
V. parahaemolyticus	5D-Bs	87.8 (87.7)*	—	45.1 (44.8)*
V. parahaemolyticus	7D	87.8	—	45.1
V. parahaemolyticus	13A	87.8	—	45.1
V. parahaemolyticus	5D-A	88.0	—	45.6
V. parahaemolyticus	8C	88.0	—	45.6
V. parahaemolyticus	4A-B	88.1 (88.4)**	—	45.9 (46.6)**
V. parahaemolyticus	11B	88.1	—	45.9
V. parahaemolyticus	12B	88.1	—	45.9
V. parahaemolyticus	12C	88.1	—	45.9
V. parahaemolyticus	12A	88.2	—	46.1
V. parahaemolyticus	4A-Bw	88.3	—	46.4
V. parahaemolyticus	12B-Spr	88.3	—	46.4
V. parahaemolyticus	5A	88.5 (88.6)*	—	46.9 (47.0)
Unidentified strain	11A	88.6 (88.3)**	—	47.0 (46.3)**
Unidentified strain	7C-Grey	89.9	—	50.0
Unidentified strain	7C-Wh	90.0 (89.6)*	—	50.5 (49.6)*
Unidentified strain	6D	92.1 (91.8)*	—	55.5 (54.9)*
V. parahaemolyticus	CDC 4871	87.1	—	43.4
V. parahaemolyticus	CDC 4280	87.2	—	43.6
V. parahaemolyticus	CDC 4281	87.2	—	43.6
V. parahaemolyticus	CDC 3637	87.3	—	43.9
V. parahaemolyticus	CDC 3454	87.5	—	44.4
V. parahaemolyticus	CDC 5002	87.8	—	45.1
V. parahaemolyticus	CDC 6202	87.9	—	45.4
V. parahaemolyticus	CDC 7606	87.9	—	45.4
V. parahaemolyticus	CDC 8198	88.0	—	45.6
V. parahaemolyticus	CDC 8633	88.0	—	45.6
V. parahaemolyticus	CDC 1889	88.1	—	45.9
V. parahaemolyticus	CDC 8694	88.1	—	45.9
V. parahaemolyticus	CDC 6670	88.1	—	45.9
V. parahaemolyticus	CDC 1334	88.2	—	46.1
V. parahaemolyticus	CDC 6614	88.3	—	46.3
V. parahaemolyticus	CDC 5704	88.8	—	47.6
V. parahaemolyticus Sakazaki strain	1[3]	—	1.705 ± 0.001	46 ± 1
	4	—	1.705 ± 0.001	46 ± 1
	5	—	1.705 ± 0.001	46 ± 1
	13	—	1.705 ± 0.001	46 ± 1
	15	—	1.705 ± 0.001	46 ± 1
	17	—	1.705 ± 0.001	46 ± 1
	21	—	1.705 ± 0.001	46 ± 1
	23	—	1.705 ± 0.001	46 ± 1
V. cholerae (12 strains)[3]		—	1.707 ± 0.001	48 ± 1
V. marinus	MP-1	—	1.699 ± 0.001	40 ± 1
V. marinus	MP-31	—	1.700 ± 0.001	41 ± 1
V. marinus	MP-33	—	1.699 ± 0.001	40 ± 1

*One preparation, two determinations.
**Two preparations, two determinations.
[1]The first 28 strains listed were isolated from moribund and dead Chesapeake Bay blue crabs.
[2]Strains labeled CDC were isolated from localized tissue infections of individuals in the United States. Received from R. M. Twedt, U. S. Public Health Service, Cincinnati, Ohio.
[3]Data taken from Citarella and Colwell *(9)*.

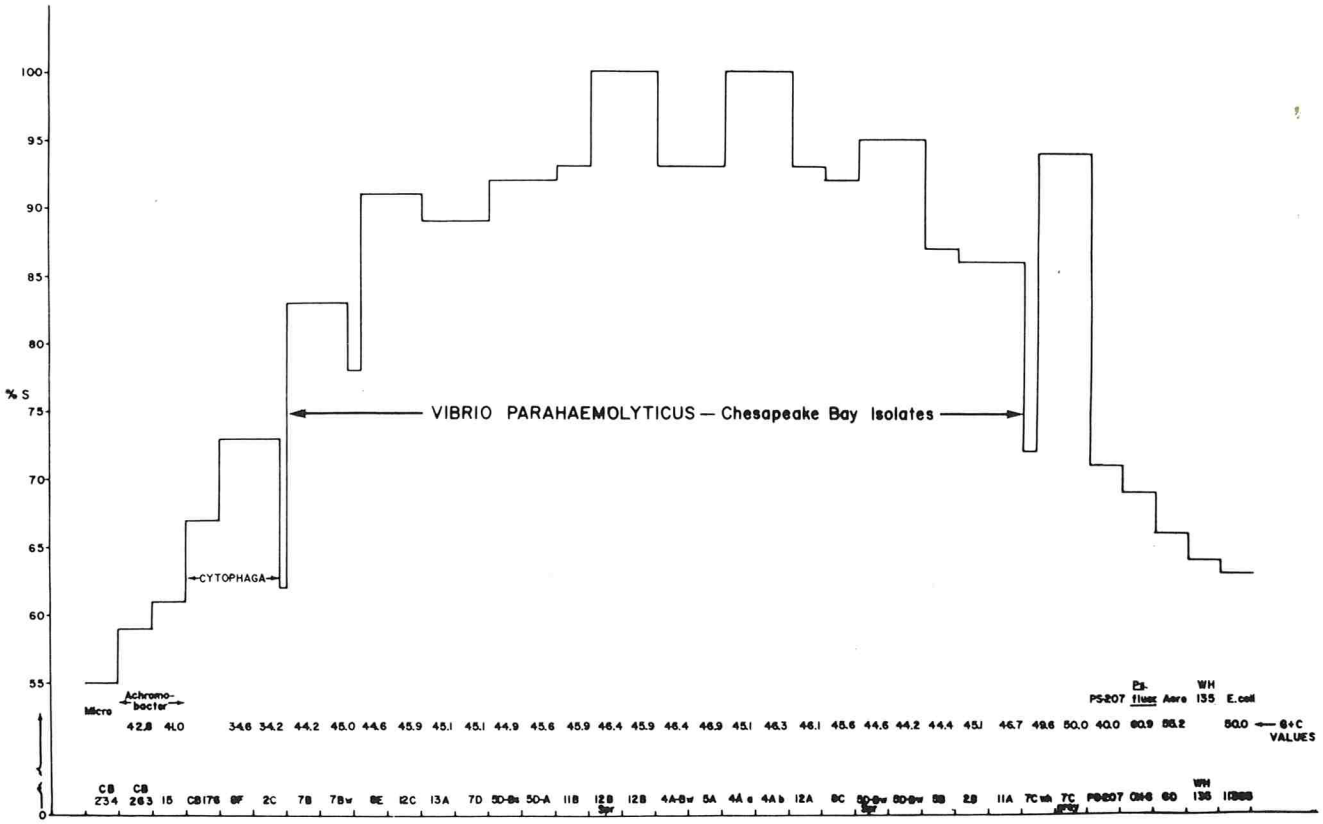

Figure 3. Numerical taxonomy analysis of 36 strains of bacteria, including five reference strains and 31 isolates from moribund or dead Chesapeake Bay blue crabs. Twenty-one strains were identified as *Vibrio parahaemolyticus,* i.e., strains 7B through 2B, reading from left to right in the figure. Strain 11A is peripheral to the main cluster but differs sufficiently from the other strains to be excluded from the species cluster.

Electrophoretic analysis

The value of isozyme analysis in identification and classification of bacteria has been amply demonstrated *(2, 13, 16, 29).* Construction of zymograms by means of polyacrilamide gel electrophoresis, followed by staining for specific enzyme activity has been shown to provide useful taxonomic comparisons at the enzyme level, since detection of minor structural differences among enzymes is permitted by this technique. Crude protein extracts and specific staining for esterase, cytochrome oxidase, and phosphatase have been done using strains of *V. parahaemolyticus.*

Typical results of esterase patterns for *V. parahaemolyticus* are given in Fig. 7. Interpretation of the banding patterns is not difficult and results of comparative analyses are shown in Fig. 8 and 9.

Esterase and cytochrome oxidase zymograms and total protein patterns (Wan and Colwell, unpublished data) appear to offer an excellent method for clinical diagnosis of *V. parahaemolyticus.*

Gas chromatography

Fatty acid profiles of more than 50 strains of *V. parahaemolyticus* were obtained using gas chromatography with the derivatives prepared from cell extracts. The major peaks observed were characteristic for the *V. parahaemolyticus* strains examined. See Fig. 10a and 10b. An interesting observation was that the fatty acid profile of *V. alginolyticus* differed very little from that of *V. parahaemolyticus* (see Fig. 10a and 10b). Gas chromatography offers a rapid diagnostic tool of considerable promise.

Bacteriophage typing

Seven bacteriophages for *V. parahaemolyticus* have been isolated from sewage and one from a sediment sample collected off Cape Hatteras in November, 1971. In isolating the phages, sewage and sediment samples were extracted with sterile seawater and the extract was added to pure cultures of host bacteria. The phages were repeatedly isolated from a given sample so that isolations of the bacteriophages were not spurious events but, in fact, a result of the presence of the bacteriophages in the source material.

Table 2 provides results of cross-reaction tests using strains of *V. parahaemolyticus* and related vibrios. An unexpected finding was cross-reaction of bacteriophage for *V. parahaemolyticus* against *V. cholerae* and *V. alginolyticus.*

DNA reassociation studies

Reassociations of ^{32}P labeled reference DNA, using

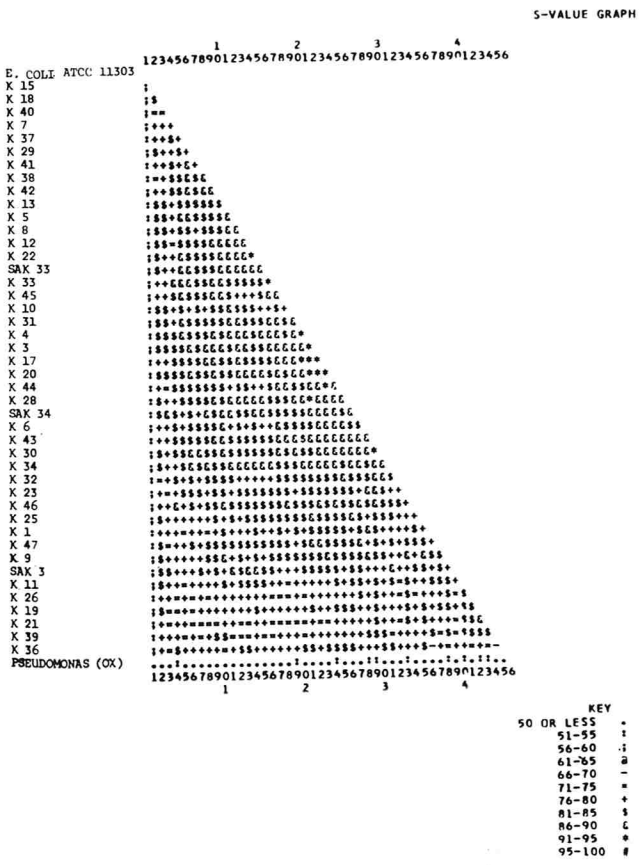

Figure 4. Results of a taxonomic analysis comparing 41 strains of *Vibrio parahaemolyticus* received from Dr. Zen-Yoji with three strains from Dr. R. Sakazaki. Two strains, *Pseudomonas fluorescens* (OX) and *Escherichia coli* served as controls.

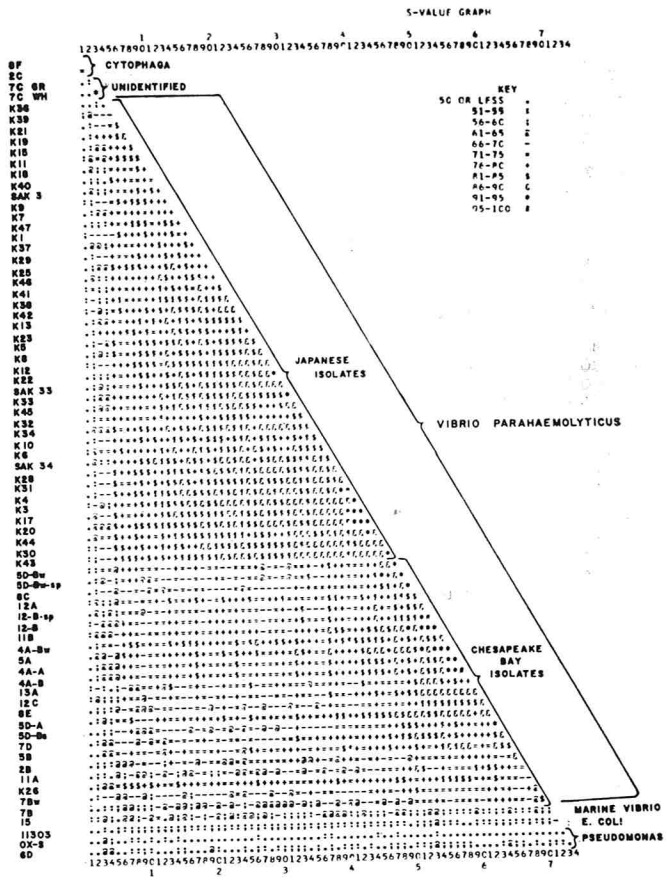

Figure 5. Comparison of *Vibrio parahaemolyticus* strains isolated from Japanese patients (labeled as K) with Chesapeake Bay blue crab isolates.

V. cholerae NIH 35A3, vaccine strain, and *V. parahaemolyticus* Sakazaki strain 4, were measured in earlier studies *(7, 9)*. Significant heterologous binding of DNA prepared from *V. cholerae* and reacted with DNA from *V. parahaemolyticus* could not be detected, although very high reassociation among strains within the species was measured.

More recent work, summarized in Table 3, indicates very high relationship among strains of *V. parahaemolyticus*, regardless of source of isolation. Confirmation of the phenetic similarities thus is provided on a molecular genetic basis.

Ecology of Vibrio parahaemolyticus

V. parahaemolyticus strains have been isolated from water, sediment, plankton, oyster, and blue crab samples. The distribution of this organism in estuaries and coastal regions appears to be widespread, very likely on a global basis. Restriction of this organism to an estuarine environment appears to be a function of salinity effect on adsorption (Kaneko and Colwell, manuscript in preparation).

A series of field trips were made to determine the distribution of *V. parahaemolyticus* from the coast to edge of the continental slope. Transects of a research cruise aboard the R/V EASTWARD in August, 1971 are shown in Fig. 11.

Total viable counts (TVC) and distribution of *Vibrio* spp. and presumptive *V. parahaemolyticus* (VLO) for water, sediment, and plankton samples are shown in Fig. 12, 13, and 14. Decrease in numbers of heterotrophic, aerobic bacteria with distance from shore was observed and is in accordance with earlier recorded observations *(33)*. *V. parahaemolyticus* could not be isolated from samples collected at distances greater than 10 miles from shore.

It is clear that *V. parahaemolyticus* is restricted in distribution to estuarine and coastal regions of the temperate zone. From observations made in our laboratory *(20)*, an association of *V. parahaemolyticus* with zooplankton has been observed in Chesapeake Bay. From the data, an annual cycle, closely bound with that of the zooplankton, has been hypothesized and is represented in Fig. 15.

V. parahaemolyticus has been shown to be present in Chesapeake Bay and is associated with the zooplankton, demonstrating rhythmic cycles of occurrence in the water column in parallel with appearance

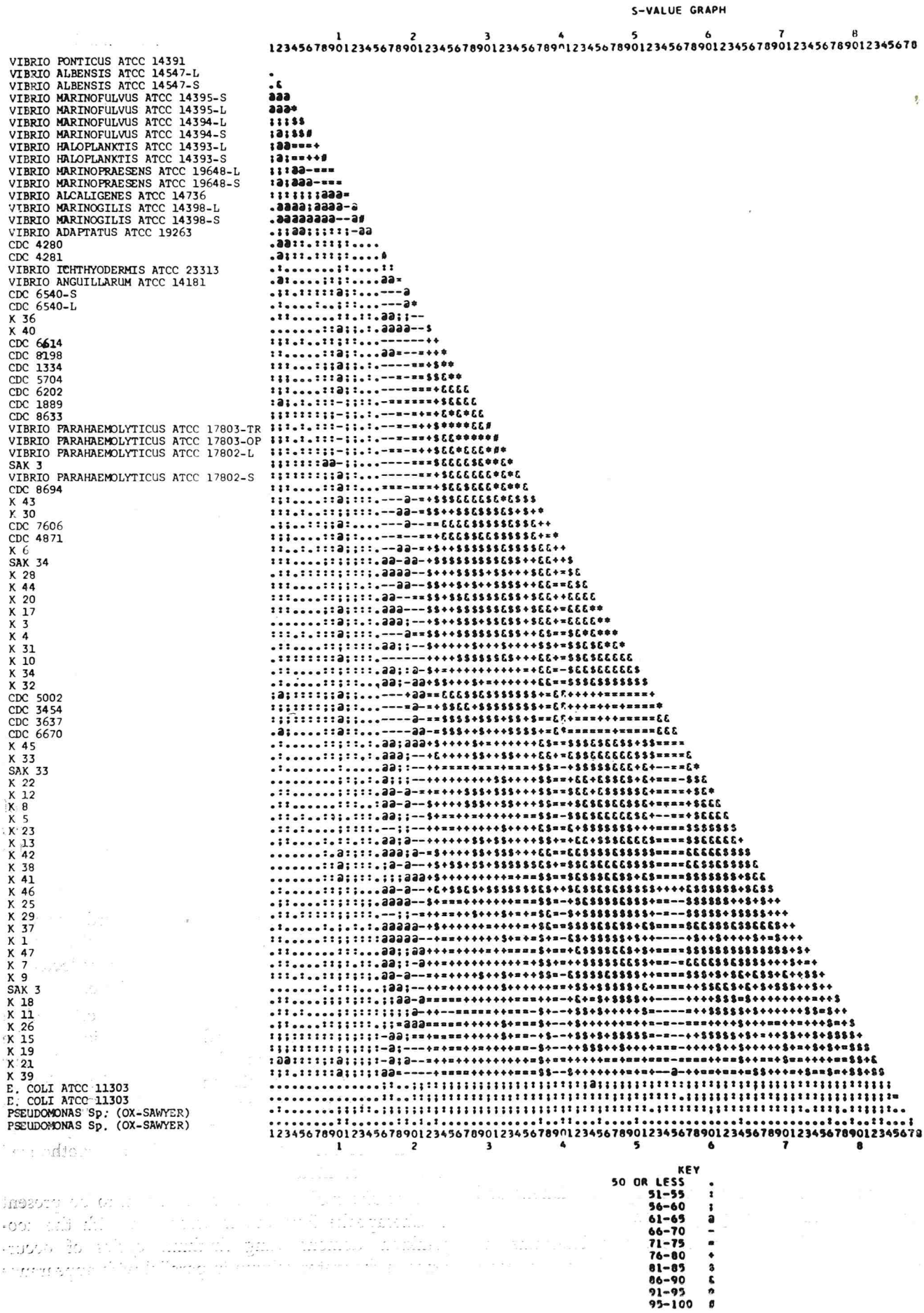

Figure 6. An analysis of *Vibrio* spp., including strains of *Vibrio parahaemolyticus* isolated from a variety of sources.

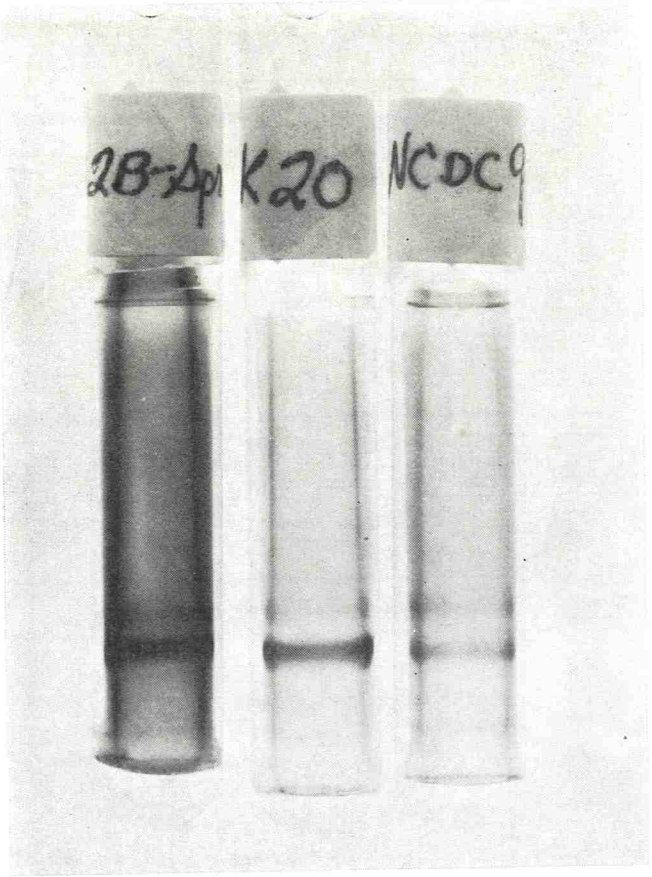

Figure 7. Typical esterase patterns for three strains of *Vibrio parahaemolyticus*. From left to right: Chesapeake Bay isolate, Zen-Yoji strain, and a strain of *V. parahaemolyticus* isolated from a U.S. tourist returning to the United States from a tour of the Far East (culture provided by the Center for Disease Control, Atlanta, Georgia). Details of procedures have been published *(31)*.

of plankton blooms.

The causative agent of the food poisoning outbreak occurring in Bainbridge, Maryland, in late summer, 1971, by all the methods applied, including DNA/DNA reassociation measurements with reference strains, has been diagnosed as *V. parahaemolyticus*.

The taxonomy of the genus *Vibrio*, at present, includes a good description of the type species *V. cholerae* and, from recently accumulated data, of *V. parahaemolyticus*. The relationship between *V. cholerae* and *V. parahaemolyticus* remains unclear. However, it would appear premature to separate these species into different genera, as proposed by other workers *(3)*. A study of the *Enterobacteriaceae* presently being undertaken in our laboratory in collaboration with Drs. Sakazaki and Brenner should provide insight for saltation of species within genera and between genera.

It is clear, from our experience, that microbial ecologists have only just begun to attack the highly complex problem of speciation of bacteria in nature. *V. parahaemolyticus* provides a good example of the

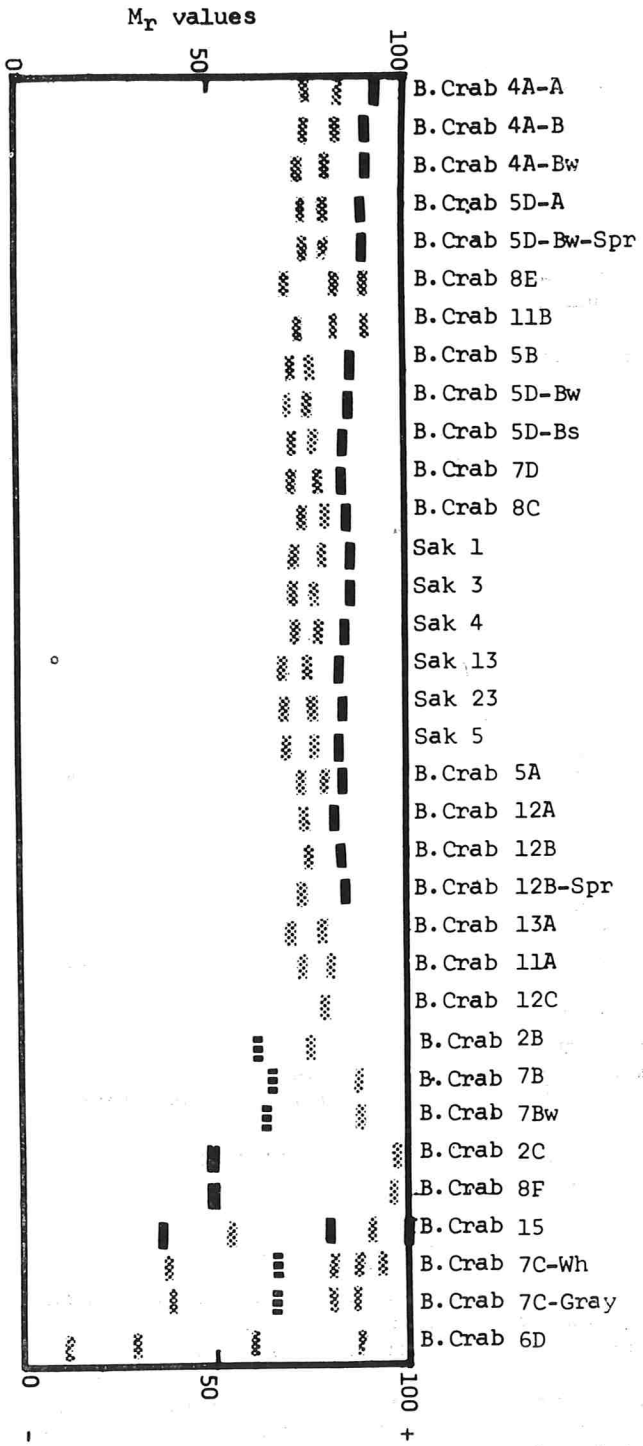

Figure 8. Esterase zymogram for *Vibrio parahaemolyticus* isolated from Chesapeake Bay blue crabs. Strains B-crab 2C, 8F, 15, 7C-Wh, 7C-Gray, and 6D are not *V. parahaemolyticus*. (See Fig. 2 and Table 1). Shading indicates intensity of staining.

difficulty in recognizing "core strains" representative of a species. Many vibrios have been isolated from the estuary and ocean environment in the course of our work, including luminescent vibrios, which appear to be strains "intermediate" between species. The problem of the strains related to *V. parahaemolyticus*

TABLE 2. HOST SPECIFICITY OF *Vibrio parahaemolyticus* BACTERIOPHAGES

Organism, strain	øKI	ø2G	ø7B	øID$_B$L	øID$_B$S	ø2BL	ø2D$_B$S	øFC291
Vibrio alginolyticus								
Sakazaki 157-70(85)	–	–	–	–	–	–	–	–
165-70(86)	–	–	–	–	–	–	–	–
157-70(87)	–	–	–	–	–	–	–	–
156-70(88)	–	–	–	–	–	–	–	–
166-70(89)	+	–	–	+	+	+	+	+
163-70(90)	–	–	–	–	–	–	–	–
Vibrio anguillarum								
Bulloch 8.3	–	–	–	–	–	–	–	–
8.9	–	–	–	–	–	–	–	–
Vibrio cholerae el tor								
ATCC 14033	–	+	–	–	–	–	–	–

TABLE 3. POLYNUCLEOTIDE SEQUENCE RELATIONSHIPS BETWEEN *Vibrio parahaemolyticus* SAK 4, *V. parahaemolyticus* FDA 8657 AND OTHER MARINE VIBRIOS. (STALEY AND COLWELL, PRELIMINARY DATA)

			Reference strains			
			SAK 4		FDH 8657	
Strain	Source	Mole % (G + C)	% Relative binding	ΔT_m(e)	% Relative binding	ΔT_m(e)
Vibrio parahaemolyticus						
SAK 4	human	46	100	0.0	93	0.0
8657	human	46	93	0.0	100	0.0
FC1011	crab	46	90	0.0	91	0.0
2RR236	plankton	46	90	0.0	91	0.0
12RR82	water	46	90	0.0	92	0.0
crab	crab	45	90	0.0	91	0.0
V. alginolyticus						
166-70	–[c]	46	59	9.0	60	9.5
Vibrio spp.						
9RR212	plankton	47	29	18.5	29	15.5
V. cholerae						
35A3[a]	–	48	18	20.5	20	17.5
E. coli						
K12[b]	–	50	4	20.5	6	20.0

[a]35A3 (*V. cholerae* NIH 35A3)
[b]K12 (*Escherichia coli* K12, W1485)
[c]received from Dr. R. Sakazaki as *Vibrio alginolyticus*.

must be faced and there, very likely, lies a decade of research.

ACKNOWLEDGMENTS

Dr. G. B. Chapman and Mrs. S. Zane were most helpful in preparation of electron micrographs. The excellent laboratory assistance of Mrs. J. Robinson is acknowledged.

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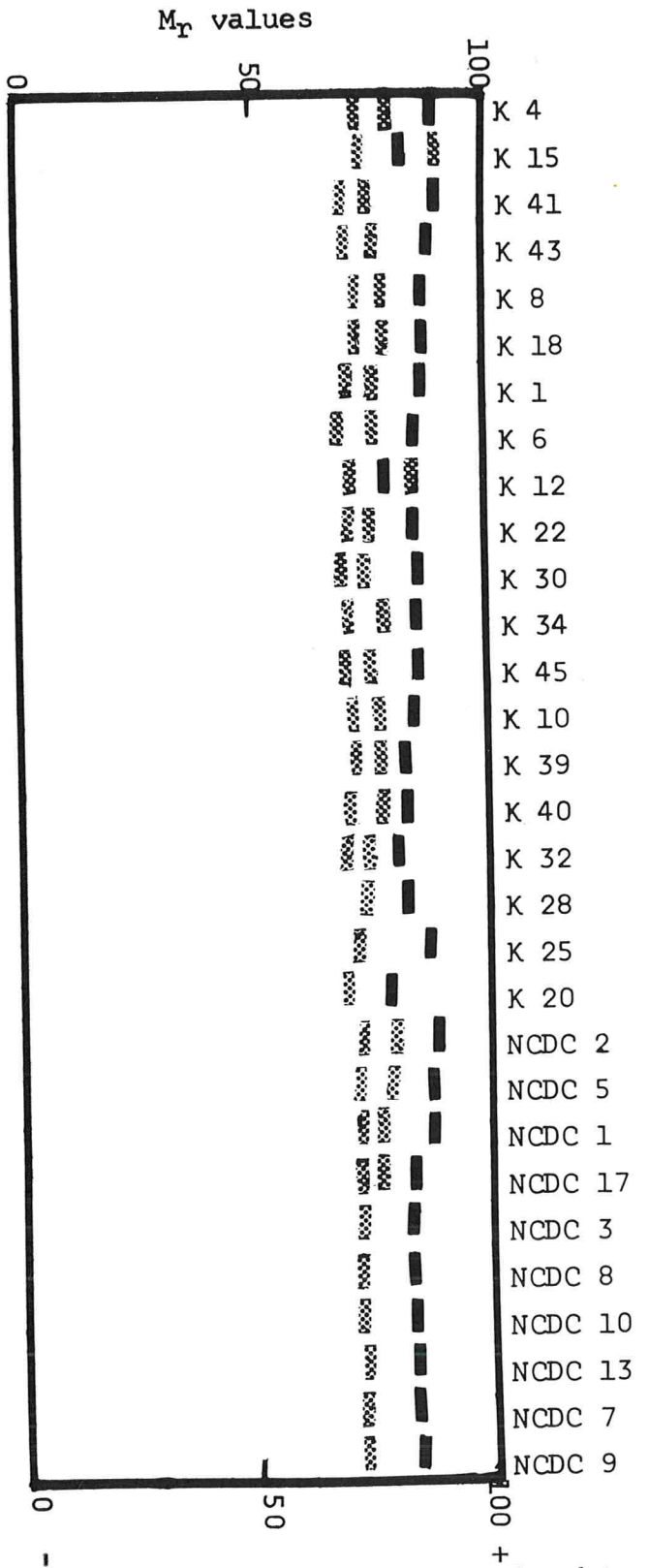

Figure 9. Esterase zymograms for *Vibrio parahaemolyticus*. Strains labeled K received from Dr. Zen-Yoji and NCDC, from Dr. Twedt. Shading indicates intensity of straining.

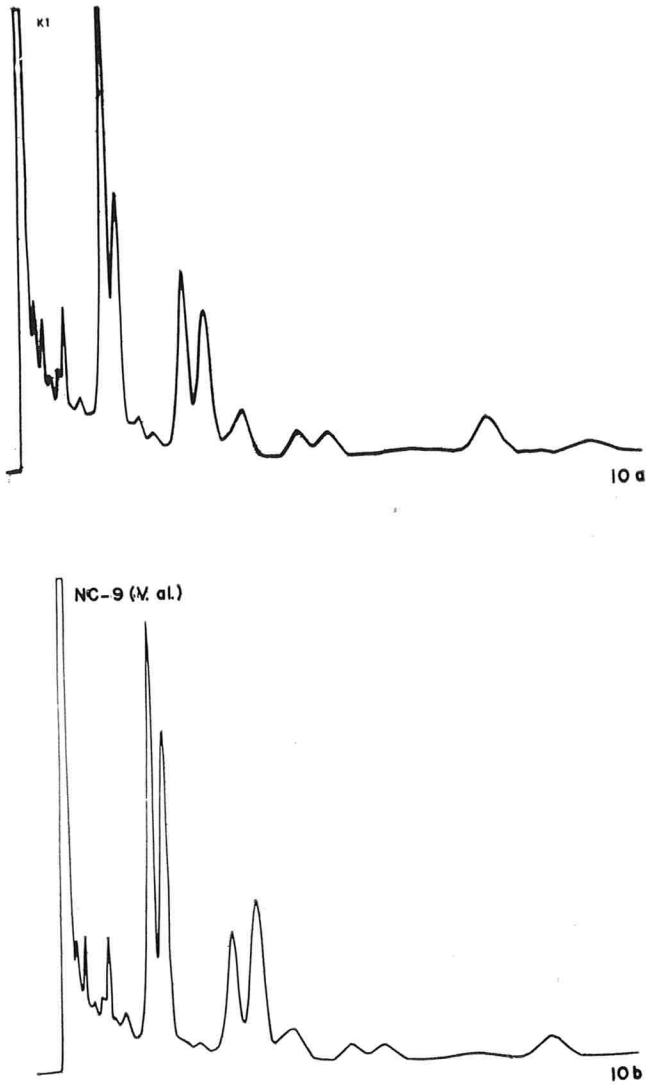

Figure 10. Fatty acid profiles of *Vibrio parahaemolyticus* (a) and *Vibrio alginolyticus* (b) prepared by gas chromatography analysis of cell extracts. See Materials and Methods.

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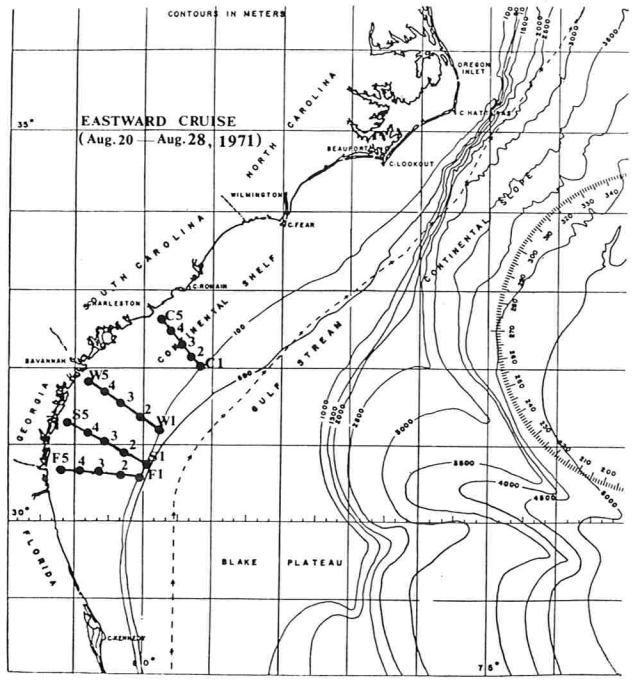

Figure 11. Sampling stations for R/V EASTWARD Cruise, August 20-28, 1971.

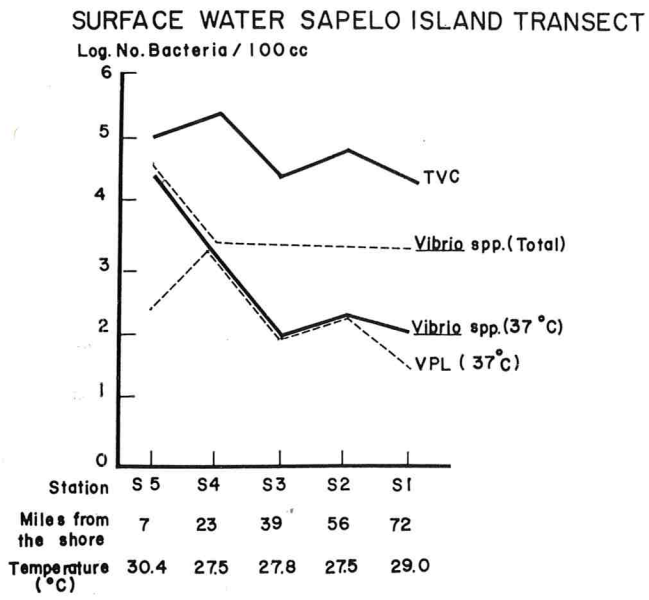

Figure 12. Distribution of aerobic, heterotrophic bacteria in surface water off the southeastern coast of the United States. VPL = Presumptive *Vibrio parahaemolyticus*.

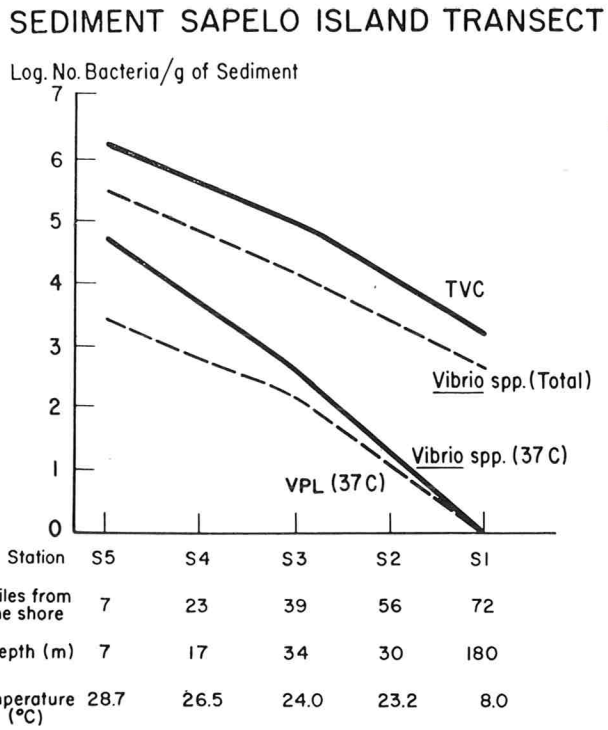

Figure 13. Distribution of aerobic, heterotrophic bacteria in sediment collected off the southeastern coast of the United States.

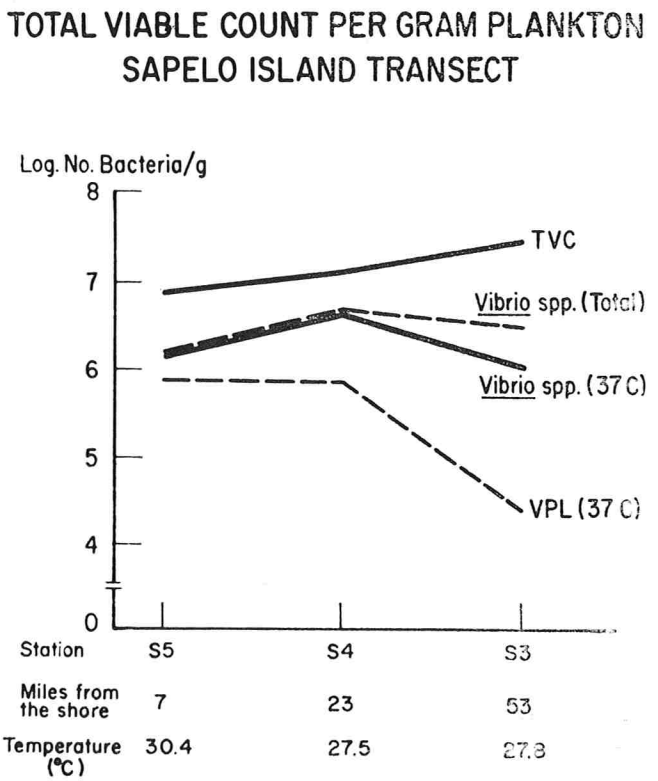

Figure 14. Distribution of aerobic, heterotrophic bacteria associated with plankton collected off the southeastern coast of the United States.

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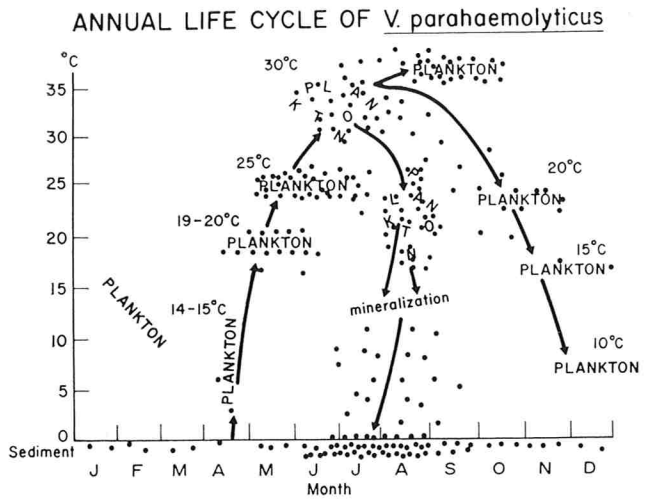

Figure 15. Annual cycle of *Vibrio parahaemolyticus* in Chesapeake Bay.

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WEIGOLD JOINS DFISA STAFF

George W. Weigold, who has been actively engaged in the dairy industry all his life, has joined the staff of Dairy and Food Industries Supply Association on a part-time basis to handle special projects, announced John H. Vogt, executive vice president. He will work jointly on DFISA technical activities, government liaison, the Collegiate Dairy Products Evaluation Contest and related programs.

This arrangement will enable continuation of membership services, govermental relations and overseas market development activities of the Dairy Society International (DSI) program. Mr. Weigold has served as Managing Director of DSI since 1960.

Following graduation from the University of Connecticut with major in dairy manufacturing, Mr. Weigold was for 20 years plant superintendent of his family owned business, Torrington Creamery, Inc., Torrington, Conn., until being selected as General Manager for a newly established by-products plant in Puerto Rico. This two-year assignment involved selection and installation of all equipment, training new employees, developing and introducing new products to the market place and in general providing efficient utilization of all available milk in excess of that needed for fluid demands.

He joined the staff of DSI in 1957 and greatly expanded the overseas market development program. Over the years, regional offices were established in Colombia, Thailand, Beirut, Lebanon, and Santiago, Chile. Dairy industry participation was coordinated by DSI in 46 International Trade Fairs and surveys of more than 30 countries were conducted.

Mr. Weigold has participated in numerous industry activities, including the Collegiate Dairy Products Evaluation Contest, representing Connecticut; speaker at conventions of the Milk Industry Foundation-International Association of Ice Cream Manufacturers; membership on the Board of Directors of the Connecticut affiliate of the Milk, Food and Environmental Sanitarians, and associate director of the American Dairy Association. He was the dairy industry representative for four years on the Food for Peace Council at the White House. In 1972, he was recipient of the Distinguished Service Award of the American Dairy Science Association.

PHENOTYPIC CHARACTERIZATION OF *BENECKEA PARAHAEMOLYTICA*: A PRELIMINARY REPORT[1]

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(Received for publication August 7, 1972)

ABSTRACT

Eighty-six strains which were isolated from cases of gastroenteritis and had the general properties of the genus *Beneckea* were submitted to an extensive nutritional, physiological, and morphological characterization. The results indicated that this collection of strains, which included the type strain of *Beneckea parahaemolytica*, was phenotypically homogeneous and distinguishable from the other known species of *Beneckea* by multiple, unrelated, phenotypic traits. When grown in liquid medium, strains of *B. parahaemolytica* had single, sheathed, polar flagella; when grown on solid medium, these strains had unsheathed, peritrichous flagella in addition to the sheathed, polar flagellum. Additional traits of use for differentiation of this species from the remaining species of the genus *Beneckea* were the ability of *B. parahaemolytica* to grow at 40 C, utilize D-galactose, L-leucine, L-histidine, and putrescine and the inability to utilize sucrose, DL-β-hydroxybutyrate or give a positive Voges-Proskauer reaction. The validity of some of the traits previously used to identify *B. parahaemolytica* as well as the possible difficulties encountered in the identification of this organism from marine sources are considered.

About three years ago, we began a study of facultatively anaerobic, gram-negative, flagellated eubacteria of marine origin *(2, 7, 8)*. Our study involved 159 strains, most of which were isolated from samples of surface and subsurface sea water as well as from sea fish obtained off the coast of Oahu, Hawaii. These strains were subjected to an extensive nutritional, physiological, and morphological characterization and the data submitted to a numerical analysis. On the basis of phenotypic similarity, the strains were sorted into clusters which could readily be distinguished from one another by multiple, unrelated, phenotypic traits. The majority of these clusters were given species designations and assigned to a redefined genus *Beneckea (7)*. At this time we were aware of the existence of a marine enteropathogen (*Vibrio parahaemolyticus*) which appeared to have properties characteristic of the redefined genus *Beneckea (14)*. Not being medical microbiologists, we were reluctant to include this organism in our initial screening. However, we were forced to do so when our studies indicated that the criteria used to identify this organism failed, in most instances, to differentiate it from several common species of the genus *Beneckea (1, 4, 5, 6, 12, 14, 17, 18)*. The previously described criteria *(14)* were adequate for the identification of *V. parahaemolyticus* when it was isolated from the feces of patients suffering from gastroenteritis, since a selection for the pathogen by the host reduced or completely eliminated other similar marine bacteria *(19)*. These diagnostic criteria were inadequate, however, when the source was sea water or sea fish since the tests used were not valid for distinguishing this organism from other common marine bacteria. The literature purporting to isolate *V. parahaemolyticus* from non-human sources reflects the lack of agreement concerning the phenotype of this enteropathogenic organism as well as the almost complete absence of knowledge concerning the taxonomy of related marine bacteria *(1, 4, 5, 6, 12, 14, 17, 18)*. At the time of our initial study, we were able to screen only five authentic enteropathogenic marine bacteria which had been isolated from stools of patients suffering from gastroenteritis. Our results indicated that these organisms were phenotypically similar and were readily differentiated from species of the genus *Beneckea* by unrelated phenotypic traits *(7)*. The general properties of these strains suggested their reassignment into this genus under the name *Beneckea parahaemolytica*.

Recently, our taxonomic conclusions were confirmed and extended by the elegant work of Anderson and Ordal *(3)*. Using *in vitro* DNA/DNA hybridization, these authors demonstrated that a collection of 80 strains, most of which appear to have properties characteristic of the genus *Beneckea*, could be subdivided into five groups on the basis of DNA homology. The group comprising strains of *B. parahaemolytica* (all of which were from enteric sources) had an average homology of 67% with the group containing strains of *B. alginolytica*, indicating a high degree of genetic similarity. Hybridization with the remaining groups was on the order of 30%. An additional important finding was that only 3 of 12 strains isolated from marine sources (and designated *V. parahaemolyticus*) hybridized with the group containing strains isolated from human sources. These findings support our conclusions as to the inadequacy

[1]Presented at the 72nd Annual Meeting of the American Society for Microbiology, Philadelphia, Pennsylvania, April 23-28, 1972.

TABLE 1. MORPHOLOGICAL, PHYSIOLOGICAL, AND NUTRITIONAL PROPERTIES OF *B. parahaemolytica* AND *B. alginolytica*[a]

	B. parahaemolytica	*B. alginolytica*
Number of strains	86	18
Straight rods	+	+
Peritrichous[b]	+	+
Motility	+	+
PHB[c]-Accumulation	−	−
Fermentation of glucose[d]	+	+
Arginine dihydrolase[e]	−	−
Voges-Proskauer reaction	−	+
Oxidase	+	+
Denitrification	−	−
$NO_3^- \rightarrow NO_2^-$	+	+
Growth at 4 C	−	−
Growth at 40 C	+	+
Amylase	+	+
Gelatinase	+	+
Lipase	+	+
Alginase	−	−
Chitinase	+	+
D-Ribose	+	+
D-Xylose	−	−
L-Arabinose	53	−
L-Rhamnose	−	−
D-Glucose	+	+
D-Mannose	+	8
D-Galactose	+	2
D-Fructose	+	+
Sucrose	−	+
Trehalose	+	+
Maltose	+	+
Cellobiose	7	−
Melibiose	8	−
Lactose	−	−
Salicin	−	−
Gluconate	+	+
Glucuronate	54	−
N-Acetylglucosamine	+	+
Acetate	+	+
Propionate	+	+
Butyrate	76	16
Isobutyrate	−	7
Valerate	3	13
Isovalerate	70	12
Caproate	25	−
Heptanoate	82	17
Caprylate	+	16
Pelargonate	+	17
Caprate	+	17
Malonate	−	−
Succinate	+	+
Fumarate	+	+
Glutarate	−	−
DL-Malate	81	16
D-Tartrate	−	−
L-Tartrate	−	−
meso-Tartrate	−	−
DL-β-Hydroxybutyrate	−	−
DL-Lactate	+	+
DL-Glycerate	+	+
Citrate	+	+
α-Ketoglutarate	+	+
Pyruvate	+	+
Aconitate	85	+
Mannitol	+	+
Sorbitol	−	−
Inositol	−	−
Glycerol	+	+
Ethanol	81	12
Propanol	82	12
Butanol	−	−
Benzoate	−	−
p-Hydroxybenzoate	−	−
Phenylacetate	−	−
Quinate	−	−
Glycine	68	16
L-α-Alanine	84	16
D-α-Alanine	+	+
β-Alanine	−	−
L-Serine	82	17
L-Threonine	85	+
L-Leucine	+	17
L-Aspartate	82	8
L-Glutamate	+	+
L-Arginine	84	15
L-Ornithine	−	−
L-Citrulline	−	−
γ-Aminobutyrate	−	−
δ-Aminovalerate	−	−
L-Histidine	85	+
L-Proline	+	+
L-Tyrosine	85	17
Putrescine	71	15
Spermine	−	−
Betaine	−	−
Sarcosine	−	−
Hippurate	−	−
Erythritol	−	−

[a] + = All strains positive; − = all strains negative; numbers indicate number of positive strains; boldface numbers indicate that the number represents 80% or more of the strains.
[b] Peritrichous when grown on solid medium; single polar flagellum when grown on liquid medium.
[c] PHB = poly-β-hydroxybutyric acid.
[d] Acid but no gas is produced.
[e] Tested for 15 representative strains of each species

of the traits used by most investigators to differentiate *B. parahaemolytica* from other species of common marine bacteria. The following is a preliminary report of work involving an extensive characterization of 86 enteropathogenic strains. The results of this study confirm and extend our previous observations.

MATERIALS AND METHODS

Methods used in this study have been previously described *(7, 15)*. The 86 strains, which were assigned to *B. parahaemolytica*, were isolated from fecal specimens obtained from patients suffering from gastroenteritis. Five of these strains have been previously characterized *(7)*. Seventy strains were isolated in Japan and donated by Dr. R. Sakazaki (20 strains) and Dr. Y. Miyamoto (50 strains). The remaining 11 strains were donated by Drs. R. M. Twedt and M. Fishbein and were isolated during a gastroenteritis outbreak which occurred in Maryland in August, 1971 *(9)*. Of the 18 strains assigned to *B. alginolytica*, 12 have been previously described *(7)*; the remaining 8 were donated by Dr. R. E. Weaver. The media used to test for salt tolerance consisted of the basal medium (BM) previously described *(7)* containing 0.5% (w/v) YE, 0.2% (v/v) glycerol, and NaCl added to a final concentration of 7 or 10%. Five-milliliter aliquots of the media were dispensed into test tubes. The cultures were incubated at 30 C with agitation and were observed for 2 days.

RESULTS AND DISCUSSION

The strains which we assigned to *B. parahaemolytica* were found to be very similar with respect to their phenotype. All were gram-negative, straight rods which when grown in liquid medium had a single polar flagellum (Fig. 1). When grown on solid medium, all had peritrichous flagella (Fig. 2-16). Examination of representative strains by means of the electron microscope showed that strains grown in liquid medium had a sheathed, polar flagellum (Fig. 17). Strains grown on solid medium had unsheathed, peritrichous flagella in addition to the sheathed, polar flagellum (Fig. 18). The morphological, physiological, and nutritional properties of the strains comprising the species *B. parahaemolytica* are given in Table 1. All strains were oxidase-positive, facultative anaerobes which fermented glucose with the production of acid but no gas. Sodium ion but no organic growth factors were required for growth. The moles percent guanine plus cytosine in the DNA of *B. parahaemolytica* has been previously determined to be about 46% *(7, 10)*.

As seen from data in Table 1, there is a great deal of phenotypic similarity between strains of *B. parahaemolytica* and *B. alginolytica*. This similarity is reflected in the high degree of genetic homology between these two species *(3)*. The traits of use in differentiating *B. parahaemolytica* from *B. alginolytica* are given in Table 2. With the exception of D-galactose and glucuronate, all of these traits have been reported to be of diagnostic value *(14)*. Two additional traits previously used to differentiate these two species are the ability of *B. alginolytica* to swarm on solid media and grow in the presence of 10% NaCl. Use of swarming as a differential trait presents some problems since it is influenced by a number of factors. Swarming occurs only on complex media and is enhanced by addition of a wetting agent (Tween-80) or by use of freshly prepared rather than dried media. Our results indicated that on freshly prepared Difco Marine Agar, all strains of *B. alginolytica* were able to swarm. Some strains of *B. parahaemolytica* were, however, capable of a limited degree of swarming which usually was considerably less than that of *B. alginolytica*. Since a clear-cut distinction between *B. parahaemolytica* and *B. alginolytica* is not always possible on the basis of this property, it is our opinion that this trait should be used with caution. The ability to grow in a medium containing 10% NaCl likewise failed to unequivocally differentiate these two species, since all strains of *B. alginolytica* and 53% of the strains of *B. parahaemolytica* were able to grow in this medium. Furthermore, growth in the presence of 7% NaCl was of no use in differentiating *B. parahaemolytica* from other species of the genus *Beneckea*. A screening of representative strains indicated that several species contained strains able to grow in the presence of 7 and 10% NaCl. These

TABLE 2. TRAITS OF USE IN DIFFERENTIATING *B. parahaemolytica* FROM *B. alginolytica*[a]

	B. parahaemolytica	*B. alginolytica*
Number of strains	86	18
L-Arabinose	53	−
D-Galactose	+	**12**
Sucrose	−	+
Glucuronate	54	−
Voges-Proskauer reaction	−	+

[a] + = All strains positive; − = all strains negative; numbers indicate number of positive strains.

Figures 1-16. Leifson flagella stains of *B. parachaemolytica*. Marker in Fig. 16 represents 5 μm. Fig. 1, This photomicrograph is representative of all strains of *B. parahaemolytica* in exponential phase of growth in liquid medium. The actual strain is 9373 of Dr. Y. Miyamoto. Fig. 2-16, Flagellation of strains grown on solid medium. Fig. 2-6, strains 125, 126, 132, 138, and 143, respectively, of Dr. R. Sakazaki. Fig. 7-13, strains 8181, 8192, 8279, 8455, 8911, 8961, and 9373, respectively, of Dr. Y. Miyamoto. Fig. 14-16, strains 8659, 10734, and 12042, respectively, of Drs. R. M. Twedt and M. Fishbein.

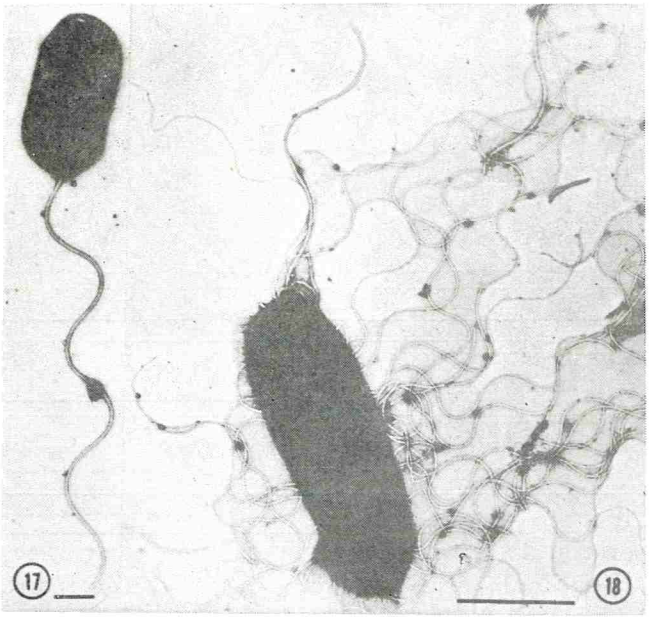

Figure 17. *B. parahaemolytica,* strain 116, in exponential phase of growth in liquid medium, having a single sheathed polar flagellum. Negatively stained. Marker represents 0.5 μm. Figure 18. *B. parahaemolytica,* strain 115, grown on solid medium. Note the sheathed polar flagellum and the unsheathed peritrichous flagella. Negatively stained. Marker represents 1 μm.

findings are consistent with those of other investigators *(11, 16).*

Table 3 lists selected traits useful in distinguishing *B. parahaemolytica* and *B. alginolytica* from the other species of the genus *Beneckea* and group E-3 (a group unassigned with respect to genus). The traits have been selected so that these two species are separable from other species of the genus *Beneckea* and group E-3 by at least four traits. Properties diagnostic for *B. parahaemolytica* are: peritrichous flagellation when grown on solid media, inability to accumulate poly-β-hydroxybutyrate or give a positive Voges-Proskauer reaction, ability to grow at 40 C, ability to utilize D-galactose, L-leucine, L-histidine, and putrescine as sole sources of carbon and energy, and the inability to utilize sucrose, DL-β-hydroxybutyrate, or *p*-hydroxybenzoate. In addition, two distinctive traits which are present in about 60% of the strains are the ability to utilize L-arabinose and glucuronate. A property which has been reported to be diagnostic for *B. parahaemolytica* is the ability to hemolyze human red blood cells when tested on a special medium [Wagatsuma's medium *(13)*]. In our initial work on the genus *Beneckea (7),* several species besides *B. parahaemolytica* were found to be hemolytic on Wagatsuma's medium, indicating that this test is of no diagnostic value.

Organisms isolated from marine sources and having the properties of the genus *Beneckea* as well as the above phenotypic attributes can be presumed to be *B. parahaemolytica.* This statement should be qualified for the following reasons: *(a)* The taxonomy of the facultatively anaerobic eubacteria of marine origin is just beginning. It is probable that other species having the general properties of the genus

TABLE 3. SELECTED TRAITS OF USE IN DIFFERENTIATING *B. parahaemolytica* AND *B. alginolytica* FROM OTHER SPECIES OF THE GENUS *Beneckea*[a]

	B. parahaemolytica	*B. alginolytica*	*B. campbellii*	*B. neptuna*	*B. nereida*	*B. pelagia*	*B. natriegens*	*B. nigrapulchrituda*	Group E-3[d]
Number of strains	86	18	60	27	8	11	6	14	2
Peritrichous[b]	+	+	**51**	**26**	−	−	−	−	−
PHB[c]-Accumulation	−	−	−	−	+	−	+	7	+
Voges-Proskauer reaction	−	+	−	−	−	−	−	−	−
Growth at 40 C	+	+	−	−	**7**	−	**5**	−	1
Lipase	+	+	+	+	−	+	**5**	+	+
D-Galactose	+	2	−	**24**	2	+	+	+	−
Sucrose	−	+	−	+	+	+	+	−	+
DL-β-Hydroxybutyrate	−	−	−	−	+	−	+	+	+
p-Hydroxybenzoate	−	−	−	−	−	−	+	−	−
L-Leucine	+	**17**	−	2	6	−	**5**	−	1
L-Histidine	**85**	+	6	−	6	7	+	**13**	+
Putrescine	**71**	**15**	−	−	6	+	+	−	+

[a] + = All strains are positive; − = all strains are negative; numbers indicate number of positive strains; boldface numbers indicate that the number represents 80% or more of the strains.
[b] Peritrichous when grown on solid medium. All other strains have a single polar flagellum.
[c] PHB = poly-β-hydroxybutyric acid.
[d] Group of facultative anaerobes undesignated with respect to genus *(7).*

Beneckea will be described, necessitating use of additional diagnostic traits. In addition, most of our isolates from non-human sources were obtained from a geographically restricted area (Oahu, Hawaii) and may not be representative of the total bacterial flora of the ocean. *(b)* It would be desirable to determine, by means of a reliable pathogenicity test, whether isolates from the ocean having the diagnostic traits of *B. parahaemolytica* are in fact pathogenic. *(c)* Strains designated *V. parahaemolyticus* have been isolated from wounds and infections of the eye, ear, and other human, non-enteric sources. The relationship of these strains to the enteropathogenic *B. parahaemolytica* is not yet clear and is currently under investigation.

ACKNOWLEDGMENTS

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THE PENN. STATE UNIVERSITY FIELDMEN'S CONFERENCE

The 31st Annual Dairy Fieldmen's Conference will be held on June 12 and 13, 1973 at the J. O. Keller Building on the campus of The Pennsylvania State University. This year's program will include talks on Pennsylvania Department of Agriculture activities, trends in milk supplies and quality control problems in large dairy cooperatives. One panel will discuss automated milk testing, and other problems of environmental pollution control of concern to dairy farmers. Other topics will cover treatment of farm water supplies for bacterial control and potential contaminants of public health concern in milk. On Monday evening, June 11, the group will inspect the new milking facilities at the dairy center of the University.

FOOD DISTRIBUTION IN TODAY'S CONSUMER CLIMATE[1]

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ABSTRACT

"The old order changeth and yieldeth place to new." Today's consumer climate is not just one of change, but of revolutionary change. The supermarket is on the front line of the consumer battleground for full disclosure labeling, food purity, and weights and measures. Desires under these headings include unit pricing, open-date coding, nutrition information, ingredient disclosure, chemical additives, grade labeling, protection and safety, visibility of contents, and container fill. In addition we are where the battle is on inflation, pollution, recycling, and the sale of biodegradable materials. Some food distribution practices, once unwanted or regarded as unsuccessful, have now become opportunities. To meet the public needs is our first order of business.

We have as common interest the pursuit of modern, protective procedures in the purchase, manufacture, distribution, and retail sale of clean, wholesome foods to our respective publics. In those locations where Safeway operates, our interests merge; and it is with considerable satisfaction that on such matters as sanitation, good manufacturing practices, and good distribution practices, I find that each of us, from his own standpoint, pretty much merges in his beliefs upon the same bridge of understanding and responsibility. My purpose is to identify some of the new developments in our society as they pertain to foods and food distribution. This paper will discuss retail distribution's position in today's consumer climate.

SOCIAL RESPONSIBILITY

For many years Safeway has taken strong corporate leadership in what we have identified as community relations, institutional relations, and public services. Time does not allow a detailed report of our activities in these areas.

Last March the *Chain Store Age*, a trade publication, featured an article of 6 pages, entitled, "Safeway Meets Social Needs with Action." The article tells about some of the more urgent domestic programs we had participated in even before "social responsibility" became household words.

When the National Alliance of Businessmen organized to ease social tension and establish job training for the underprivileged, Safeway donated—and continues to donate—full-time executives to this work.

Through direct Company encouragement many of our executives with whom I work are advisors to the Jr. Achievement Program for High School Students.

Last May, Safeway joined with the League of Women Voters and Prentice-Hall Publishers in the cooperative publication of a book, *You and Election '72*, to encourage informed and active citizen participation in the key issues of this election year.

Some of my associates and I are actively assisting an ambitious black employee from one of our Retail Divisions to develop several precooked menu items which we hope will result in the establishment of her own business. Thus it goes. Throughout our Company, we have been alert to the needs of the community for many years.

The necessary understanding to all such community service is that these are not necessarily altruistic ventures on our part, or because "social responsibility" is a popular slogan. We do them primarily because meeting the public needs is good business. We live here too. For the good of our communities and for our society, corporate profits and social responsibilities go hand in hand. We believe we can have both profit and progress together.

INDUSTRY RESPONSE

On August 5, 1971, President Nixon, by Executive Order, established the National Business Council For Consumer Affairs. This Council is comprised of over 100 chief executive officers, including Safeway's, of business enterprises of all sizes. The Council encourages and assists in the establishment of voluntary programs for industry action to anticipate and resolve consumer problems. It advises the President and various Government Agencies, through the Secretary of Commerce, and, when appropriate, makes recommendations concerning legislation or Executive action.

Three months ago, this Council issued *Guiding Principles for Packaging and Labeling* wherein it recommended 14 principles for industry to observe to assist in protecting consumers. It is a practical, progressive set of guidelines. It includes most of the suggestions that have been made from the market place, consumer surveys, and professional groups within the bounds of practicability. While preparing this paper, I have reviewed each of its guidelines.

I find we fully comply with all except three; and for two of these three, our compliance is consider-

[1]Presented at the 59th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Milwaukee, Wisconsin, August 21-24, 1972.

ably more than token, and we are among the very few in industry's forefront of their compliance. I shall be mentioning these later on. The third is a generalization, not truly a guideline. It has to do with conservation of natural resources, recycling packaging materials, and minimizing pollution. I would view with considerable skepticism any business or even any group who today laid claim to full compliance with this recommendation.

DISCLOSURE LABELING

A label can furnish information. It cannot provide understanding. The records show that Safeway has played a missionary role in providing consumers with the kind of information they are demanding today, on what we call "Disclosure Labeling." It has several facets which I will describe briefly.

Open dating

The first is Open Dating. What we mean by this expression is the placement of an uncoded, legible date on the package or label, a date beyond which the product should not be sold—if the product is perishable (3 months storage life or less), or semi-perishable (3 to 6 months). A normal home life is included in this open dating system. Surveys have indicated that 89% of retail shoppers want open dating as a guarantee of product freshness.

In the past, many of our labels showed this date by symbols that had to be decoded. This often resulted in improper rotation of stocks because of the difficulty of deciphering the code by our own store personnel. Now that we have increased the size and type of an uncoded "open date" on 2,000 food items packed under Safeway brand names, proper rotation on these items is no longer a problem.

Unit pricing

A second facet of disclosure labeling is unit pricing, sometimes referred to as dual pricing. In addition to the package price, the price per ounce, pint, pound, or square foot is also posted.

We undertook this project so the consumer can make easy comparison of quantity values. We placed computer-printed price tags on the shelves where the products are displayed. We find that customers appreciate unit price information.

We also find there are operating efficiencies resulting from the system. They are improved inventory control, less labor from reordering, less labor for producing and posting the traditional price tags, and more accurate pricing.

Grade labeling

Quality is as important to price as is quantity. But you cannot quantify a quality; it is an individual thing that does not come in standardized sizes. So it is only in the rarest of instances that unit pricing alone permits a complete value comparison. (We quality experts are limited only to a handful of pure chemical items, like salt, sugar, and bleaches.) To overcome this, many consumerists call for grade labeling on food packaging.

There are numerous reasons why grade labeling for consumers will not work. They range from the philosophical to the very practical of "how do you grade for flavor?" In fact some consumerists themselves innocently point out one of the greatest invalidities. The magazine of one consumer organization expresses this in the language of the absolute,

> PACKAGERS OF FOODS AND OTHER GOODS FOR CONSUMERS KNOW WHAT QUALITY GOES INTO THEIR PACKAGE. THEY SHOULD BE REQUIRED TO REVEAL THIS QUALITY, IN TERMS OF STANDARDIZED GRADING, ON THE LABEL.

Before pointing out the invalidity of this statement, let me explain that the food technologists in my Quality Control Department evaluate lots containing 3/4 billion retail food packages yearly. They do not grade a single package. They accept or reject a lot because the product either meets our specifications, which are dynamic, or it fails. This is what branded merchandise is all about.

In making these evaluations, we normally test against five properties of the food, including flavor. Suppose we assign two possible qualities, A and B, to each of these five properties. We could then have five-squared, or 25 combinations of quality differences. If we assigned three quality differences, A, B, and C, to each of these five properties, we could then have five-cubed, or 125 different quality combinations.

Without belaboring this illustration, it should now be apparent that the packager of a food product does *not* know the quality he put there. He knows *the very numerous qualities.* And it is doubtful that he will ever be able to communicate them to consumers in any better manner than by placing his brand name on his package. Brand labeling has always been an important facet of disclosure labeling.

Nutrition information

There are several formidable obstacles to overcome in devising a practical way to declare nutrition information on a label. These are: a confused legal status, the uncertainties associated with forthcoming guideline regulations, normal variations of nutrients plus the greater demands of processing and quality controls to keep them in line, and the cost. We were first motivated to declare nutrition information by the findings of the White House Conference on Food Nutrition and Health, then by our own customer surveys.

Those economic conditions which permit an afflu-

ent population to suffer from malnutrition should not be tolerated. If food products which will not survive nutritional labeling continue to be manufactured and promoted, then the public is entitled to some means for recognizing this.

Safeway continues to push ahead in declaring nutritional information on its own branded merchandise and this information now appears on about 200 of our items. We also began a program last year of providing leaflets to our customers in some areas describing the nutritional characteristics of some 26 common foods. These leaflets are a joint project of Safeway and the National Council for Consumers, a non-profit organization based at the University of Iowa.

Ingredient disclosure

On the matter of ingredient disclosures, we followed the recommendations of the White House Conference on Food and Nutrition's Panel on Food Safety. This means that Safeway is now committed to listing all ingredients and additives on its labels whether or not this is required by regulations.

However, we have gone farther than was called for by these recommendations. We are adding explanations or descriptions of ingredients and additives to make them more understandable. For example, in soft drinks stannous chloride prevents color change, erythorbic acid stabilizes the solution, and benzoate prevents microbial growth from breaking down both the product and its nutrients.

Ingredient percentages

A second extension to the ingredient disclosure concept is to detail the percent occurrence of important ingredients, particularly those which are a significant factor of cost, such as the percent pepperoni on a pizza, or drained weight of canned fruits and vegetables. There are numerous problems pertaining to definitions and accuracy here which are still under our study before we feel ready to act.

However, there are no really significant problems of consumer protection involved. Minimum weights for the pepperoni on the pizza, and for drained weights in the canned foods are established by government regulation, just as other protective regulations are established for dairy products and other foods.

Food Additives

We fear what we do not understand. Dr. Richard Hall, Immediate Past President of the Institute of Food Technologists explains, "Today's food industry is being asked to protect the consumer against unknown hazards and the unknown effects of known hazards." Recently there has been such an array of partial truths and conflicting testimony about food additives, that perhaps the only certainty is that we food technologists are truly the "mad scientists."

True, you and I have no fear whatever about food additives. We eat and drink whatever we please without giving them second thoughts. We monitor their safe use in the formulations that come under our vocational jurisdictions; and we personally have a much greater concern for other higher public health risks, such as the microbiological, that are of almost no concern to the public.

There is no question about the need for chemical food additives as one of the essential, interlocking systems for all methods of preserving foods.

The question remains whether we can regain the consumer's confidence in the purity and wholesomeness of our food supply, and prove that we are not somehow slowly and insidiously, poisoning her and her family. The fact that in modern times normal use of chemical additives has never been the cause of a single death is either unimpressive or obscure. The understanding that foods are chemicals and all chemicals, without exception, are harmful if used to excess, has never reached the public. When we talk of there being no harmless chemicals, only harmless ways of using them, we have been talking only to ourselves.

The Environment

A Compusamp Survey released last March showed that 84% of supermarket customers thought that food stores should not carry products potentially harmful to the environment. We have made some beginnings in the ecological area of recycling paper wastes. We aid in its collection and we sometimes advertise our use of the recycled product. During 1971 waste paper balers became standard equipment in those Safeway Stores having average volume sales or higher. At this point we can only observe that necessity is the mother of invention, and today's necessary concerns often provide the basis of tomorrow's profits.

Naturalism

There is currently great interest in "natural" foods and "health" foods and the nature cult frequently condemns technology as the cause of man's more complicated ills. This blame of technology is a contradiction. Every great cultural advance in history has been accompanied by technological advance, and accomplished by a card-carrying creature of nature, man. Professor Leakey has proved that man's ancestors, the hominids who lived in the great African Gorge 5 million years ago, not only discovered the use of tools, but also stored them for their future use. The great significance of these discoveries is that technology has always been as natural for man as

flight is for birds.

The paradox of technology is that from it flows both our affluence and our effluent. Pollution is not the cost of technology, nor is it the abuse of technology. It is the result of a shift in the benefits of technology from the privileged few to the right of all. There is no smog in Moscow or Calcutta. We find it in our cities because it is our belief that one man has as much right to own an automobile as another.

We have not yet learned how to live with plenty. It makes little sense to talk about the quality of life unless we have in mind a choice among alternative satisfactions. The Reformation ethic was based upon scarcity and mankind's living with famine for so many centuries. We need a new ethic.

As yet science is the only method we have found for turning human knowledge into rational action, the name given to technology. The solutions to our pollution and environmental problems are, therefore, cultural and technological.

FOOD PURITY

The most important requirement for sound legislation and for consumer recommendations is that they be based upon truthful information. FDA's releasing early this year its filth tolerances for some 80 processed foods is the best thing that happened to the food industry in decades. In one simple stroke, it put to rest the erroneous supposition that foods are "pure" in the absolute sense.

These published levels are the factual recognition that microbes, insects, and animals compete for our food supply, often infesting it before harvest. They leave their evidence, often including their remains, not all of which can always be removed.

QUALITY CONTROL

You might appreciate a brief discussion about the quality control systems in Safeway which rank with the most modern of food manufacturing quality control systems.

We have five separate quality control departments, each serving distinctly different product organizations. On the manufacturing lines ours is the total approach of quality assurance, from ingredient procurement through the final inspection of finished product, and the use of statistical techniques wherever required. For multiple plant operations such as dairy, we have a central quality control laboratory as well as individual plant quality control. Ingredients and products all come under closely maintained specifications.

In procurement quality control, where we procure over 1,000 canned, frozen, and dried food items for our Safeway labels under our own written specifications, we are organized into 7 geographical areas. Each area is under control of a Quality Control Supervisor, who is a graduate food technologist and whose minimum experience in the food business is, at present, fourteen years.

Several of our Q. C. organizations have separate sections for product development and research, an activity that may also include packaging research.

The heads of our five Q. C. Departments, often accompanied by their staff assistants and others such as the corporate legal staff, meet together periodically to comprise what is known in our Company as the Technical Committee. As such we are looked upon as an important Company resource for technical matters pertaining to food science, food law, and labeling.

WEIGHTS AND MEASURES

It is obvious that part of a food product's integrity is the accuracy of its measure. The guiding principle of weights and measures officials everywhere is that equity shall prevail. A considerable body of law and regulation has evolved to protect both the packager and the consumer.

Net fill is an *average* concept. The equity principle entitles some packages to be below the declared net so long as others are above, and so long as "unreasonable" fill is not included as part of the consideration.

Here is another of those facts of life that is rarely explained to the public. In fact, the public often believes that "net" means "minimum," although of course, it does not.

To a statistician no package can ever be filled to an exact weight or volume. The only exact pound in existence resides at the National Bureau of Standards in Washington, D. C. Therefore, all fills will be either over or under the declared net by some finite amount depending upon how many decimal places we may talk about.

The technology of filling containers on a statistically controlled packaging line calls for fill targets to be at least one standard deviation above the declared net. The statistics of one standard deviation are such that if five random packages are checked for fill, they will average greater than the declared contents, and thus they will pass regulation requirements in 99% of all instances. While this may seem like more protection than the packager needs, there are many sound, practical reasons why he should regard this as minimum protection.

The interesting statistic, however, is what this minimum protection means to Mrs. Housewife. Of course, the plus-one standard deviation means that one container in six will contain something less than the declared fill, but five out of six will be overfilled. If we extend the computation to her pur-

chasing the national average of sixteen packages during her weekly trip to the supermarket, the probability of her receiving less than the declared total net of the sixteen packages is so remote that it probably never has, or never will, happen once. It is less than one chance in several trillions.

The Future Is Us

It is apparent to us that in the 70's accelerating demands will be made to commit financial resources and executives' time toward solving social problems in the community. For the good of our society as a whole, corporate profits and social responsibilities must go hand-in-hand. Corporations structured to generate profit must also generate "social profit."

The complexities of our problems reflect the level of our ignorance. The many problems of our society including those identified as consumerism, are not going away soon. They are still complex!

Regulations should be synonymous with liberty; they should protect, not penalize. They should be based upon honest information, not contrived.

We must be careful that our enthusiasm for important causes does not carry us beyond the limits of our competence. We need to know, more than ever before, whether our programs are working, and whether there are better alternatives. The best, and often the only way to study this is through a series of measurements, and their analysis—the scientific method.

Both government, the public and the academic community must come to terms with the fact that there exist in industry a great many scientists who have not compromised their ethics, either as scientists or as citizens, just because they have left the university. Most practical problems will not be solved without the cooperation of these scientists.

No discipline or agency alone is equipped to resolve the problems of the individual consumer within our society today. We must work as a team, not adversaries. The future dictates that government, the business community, and the consumer must work together toward eliminating deficiencies in the marketplace.

NEW PUBLICATION ON FDA REGULATIONS BEGUN BY MIF/IAICM

A new specialized publication which will report and analyze new and proposed Federal government regulations on dairy product definitions and standards, packaging and labeling has been launched by the Milk Industry Foundation and the International Association of Ice Cream Manufacturers.

Thrust will be sent exclusively to members of MIF & IAICM to provide them with fast and accurate information on the stepped-up regulatory programs of the Food and Drug Administration and other agencies. The first issue of the new publication was January 19, the same day FDA published a record number of 12 labeling regulations and proposed regulations and standards.

Three additional issues of *Thrust* were published by the dairy processor associations within the same week. Included in the four editions were reprinted copies of the regulations themselves, interpretations and examples relating to their many provisions, and sample labeling schemes which will be required or optional under the new regulations.

Thrust joins the group of approximately half a dozen specialized publications issued separately by MIF or IAICM, or published jointly, as the situation demands.

PUBLICATIONS RECOMMENDED BY THE DAIRY FARM METHODS COMMITTEE

1. *Modern Mastitis Management.* Cost: Free; Write to: UpJohn Co. Kalamazoo, Michigan 49001.

2. *The Modern Way To Efficient Milking.* Cost: 1st copy, $1.00, Additional copies 30 cents; Published by The Milking Machine Manufacturers Council of The Industrial Equipment Institute; Write to: Farm and Industrial Institute, 410 N. Michigan Ave., Chicago, Illinois 60611.

3. *Bulk Hauler's Guidelines For Proper Collection of Milk,* Bulletin No. 26, By: Prof. R. P. March Cornell University; Cost: 25 cents per copy; Write to: College of Agriculture, Cornell University, Ithaca, New York.

3. *Milkhouse and Parlor Cleaning and Sanitizing.* 70 colored slides (No trade names used in slides); Cost: Free for use; Write to: George W. Towers, Branch Manager West Agro-Chemical Inc., 501 Santa Fe St., Kansas City, Mo. 64105.

4. *Milk Quality Tests.* 60 colored slides with 20 min Script; Cost: $25.00 per set; Write to: Sidney E. Barnard, 213 Borland Laboratory, Pennsylvania University, Pennsylvania Park, Pennsylvania 16802.

5. *Cleaning and Sanitizing Farm Milking Equipment.* 65 colored slides with 20 min script; Cost: $25.00 per set; Write to: Sidney E. Barnard, 213 Borland Laboratory, Pennsylvania State University, Pennsylvania Park, Pennsylvania 16802.

MILK ALLERGY[1]

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ABSTRACT

A general review of milk allergy and a summary of current research on milk at Dairy Products Laboratory (DPL) is presented. Milk allergy occurs primarily in infants and children under 2 years of age. It became more prevalent in the U.S. as breast feeding declined and feeding of cow's milk increased. Milk allergy (atopic and anaphylactic) has an immunological basis as distinguished from such diseases as lactose intolerance and galactosemia. The reported incidence of milk allergy varies widely from 30% in allergic children to 0.1 to 7% in nonallergic children. Symptoms of milk allergy are asthma, rhinitis, vomiting, abdominal pain, diarrhea, urticaria, and anaphylaxis. Crib deaths have been attributed to milk allergy. Prognosis is that milk allergy usually disappears by age 2. Milk proteins are the etiological agents in milk allergy. Milk contains from 12-14 immunologically distinguishable proteins, all of which are potential allergens. DPL is doing basic research on milk allergens to elucidate the mechanism of the allergic response to ingested milk. Demonstration of new antigens (potential allergens) generated by brief pepsin hydrolysis of four milk proteins-casein, α-lactalbumin, β-lactoglobulin and bovine serum albumin, is the basis for a new concept of the role of digestion products in immediate type milk and food allergy.

A distinguished allergist at Massachusetts General Hospital stated in 1950 that, "There is perhaps no field of medicine in which more divergent views are held than in that of allergy to foods" *(34)*. A search of the literature on milk allergy reveals that, although much research has been done since that time, it appears that this opinion is still valid. The literature on milk and food allergy has been amply reviewed *(5, 8, 9, 10, 13, 14, 20, 26, 32, 33)*.

The term "allergy" was first used by von Pirquet *(51)* in 1906 to denote an altered capacity of a human to react to a second injection of horse serum. Since that time allergy generally has been used to describe all forms of hypersensitivity in man. An allergen may be defined as an ordinarily harmless substance present in the diet or environment, able to produce such human diseases as asthma, hay fever, eczema, and gastrointestinal upsets upon contact with a previously sensitized person.

Similarly, Portier and Richet *(38)* in 1902 first used the term "anaphylaxis" to describe sensitization and later shock when a foreign protein (antigen) was injected into an animal.

[1]Presented at the 59th Annual Meeting of International Association of Milk, Food, and Environmental Sanitarians, Milwaukee, Wisconsin, August 21-24, 1972 under the title: "Dairy Food Allergens and Allergy."

Studies of allergy and anaphylaxis have been closely linked because there is a basic relationship between them although they do have important distinguishing differences. Both involve release of histamine or histamine-like substances when allergen or antigen comes in contact with specific antibodies (immunoglobulins) in various shock tissues thereby producing characteristic reactions. By definition milk allergy is the body response to an allergen-antibody reaction. There are three types of allergy, namely, atopic immediate type in which symptoms appear in a few up to 60 min; atopic delayed type in which symptoms appear in 1 to 36 hr although delays of up to several days have been reported; anaphylactic type in which symptoms are violent, sometimes fatal, and which may occur in seconds to a few minutes.

Not all symptoms which arise on ingestion of milk are caused by milk allergy. There are several nonimmunological reactions to ingestion to milk which resemble allergy such as lactose intolerance *(39)*, galactosemia, and bacterial contamination. Also several foreign substances in milk cause immunological reactions. Among those reported are ragweed tops, linseed, cottonseed, wheat, peanut, and penicillin *(9)*. Penicillin became important enough to require legal restriction on the use of milk for a period of time after injection of penicillin into the udder. It was generally assumed that there is enough penicillin in milk from treated cows to cause allergic reactions in sensitive persons but probably not enough to sensitize persons not already sensitive *(9)*.

INCIDENCE OF MILK ALLERGY

The reported incidence of bovine milk allergy ranges from < 0.1% to 30% *(9)*. The incidence depends on the group selected and other criteria. The highest value was 30% in a group of allergic children. In a group designated "nonallergic children," the incidence was 0.3%. There is, as to be expected, considerable variation in incidences in similarly designated groups reported by different workers. Thus in four groups designated as "all children," the incidences were from < 0.1 to 7%. In a group designated "well babies" the incidence was 1%. This wide variation reflects diagnostic difficulties in selection of the groups. At present there is no objective laboratory test available that has more than contributory value in diagnosis. Diagnosis is made by clinical evalu-

ation which is difficult because of the multiplicity of symptoms and that many of these symptoms result from other causes. The provocative test, to be described below, at best, involves some subjective evaluation by the physician as well as the ever-present possibility that patients do not follow elimination diets either accidentally or wilfully.

Diagnosis of Milk Allergy

Diagnosis of milk allergy is difficult not only because of the multiplicity of symptoms which are duplicated in other diseases but also because of the lack of clear-cut objective tests. Another complicating factor is the widely varying degrees of sensitivity ranging from a drop to a glass of milk required to trigger a response. Time of onset of symptoms also varies from seconds to hours or even days.

The scratch or skin test, which is quite useful in diagnosis of inhalant allergies such as those caused by pollens, molds, animal danders, as well as some ingestants such as seeds and nuts, is unreliable in diagnosis of milk allergy. Many who are clinically sensitive to milk do not give a positive skin test and some who do give a positive test are not clinically sensitive, although a strongly positive skin test is regarded as suggestive and worthy of special attention. Diagnosis of milk allergy can only be made by evaluating several criteria. The most important test is the provocative test which consists of inducing symptoms on ingestion of milk followed by remission of symptoms on elimination of milk from the diet. This should be repeated to increase the certainty of the diagnosis. In using the provocative test nonallergic causes of symptoms such as lactose and galactose must be ruled out. This can be done by using lactose-free milk preparations for the challenge. Also, sensitivity to contaminants in the milk such as penicillin must be ruled out.

Personal and family histories, response to antihistaminics, and the passive transfer test can provide important results contributory to diagnosis.

Symptoms of Milk Allergy

The multiplicity of symptoms of milk allery and the proportions of these symptoms exhibited by patients was determined in a careful study by Goldman and associates *(18, 19, 41)* in 1963. They studied 700 pediatric patients suspected of milk allergy. A diagnosis of milk allergy was made on the basis of several criteria including principally the thrice repeated provocative test. Milk allergy was diagnosed in 89 or 13% of the patients. The patients with milk allergy were divided into two groups for the provocative tests to determine percentages of symptoms. Group A was challenged orally with 100 ml of skim milk in the form of 9 g of Starlac in 100 ml of water and with four purified milk proteins in the amounts normally found in 100 ml of milk, namely, with casein, β-lactoglobulin, α-lactalbumin, and bovine serum albumin (BSA). Group B was challenged with milk alone. The two groups reacted similarly. Multiple symptoms were produced by the challenges in 77% of the patients of both groups. The challenge symptoms and their proportions were as follows: vomiting, 33%; diarrhea, 37%; abdominal pain or colic, 28%; rhinitis, 35%; asthma, 27%; atopic dermatitis, 35%; urticaria, 11%; anaphylaxis, 9%; and central nervous system and other symptoms, 18%. When challenged orally, every patient in Group A gave an allergic reaction to one or more of the purified proteins. The incidence of reactions to these proteins was: casein, 60%; β-lactoglobulin, 62%; BSA, 52%; and α-lactalbumin, 53%. Nineteen reacted to one protein; 12 to two proteins; 7 to three proteins; and 6 to all four proteins. The median time of onset of symptoms was 1 hr with a range of 10 sec to 25 hr. The median duration of reaction was 24 hr and the range was from 40 min to 96 hr.

Symptoms attributed to cow's milk allergy are tabulated and discussed by Collins-Williams *(9)*.

Breast Milk Versus Cow Milk in Infant Feeding as Related to Milk Allergy

Milk allergy occurs primarily in infants and children up to 2 years of age. Several reasons for the allergic sensitization of infants to cow milk appear throughout the literature. From birth until the third month, the gastrointestinal mucosa is more permeable to undigested food proteins than in older children and adults. Hence feeding milk during this period exposes the sites of antibody formation to sensitizing doses of foreign protein which in some instances produces allergic antibodies, particularly in infants from families with an allergic background. It has been suggested that the infant immune system may respond more vigorously to small quantities of foreign protein than that of older children and adults. Glaser *(16)* pointed out the enormous amount of milk protein which an infant may ingest compared to an adult on a body weight basis. A 9-kg child may easily take a liter of milk daily which corresponds to about 8 liters daily for a 72-kg adult on the same body weight basis. The digestive tract is more permeable than in normal infants in certain diseases involving diarrhea which increases the sensitizing potential of ingested foods *(21)*. Likewise enzyme insufficiencies in the digestive tract can allow more undigested protein to be absorbed than would occur under normal conditions.

The question of breast feeding versus cow milk feeding of infants in the first months of life with

regard to development of milk allergy has been given much attention. Since milk allergy occurs primarily in infants and children under 2 years of age, and since milk antibodies are known to appear in infants within a month after ingestion of milk, it has seemed reasonable that breast feeding should be encouraged in the first months of life. Glaser *(16)* and others have advocated breast feeding from birth into the early months of life, and if that is not possible, a milk-free supplement or substitute should be used especially in families where an older child has milk allergy or the family has an allergic background. The views of some others have been to go ahead with a milk diet and treat milk allergy if and when it appears.

Regardless of the views of those recommending breast feeding of infants, the practice has declined markedly in the past 2 to 3 decades *(3)*. In 1948, Bain *(1)* reported that 38% of infants were entirely breast fed and 27% partially so at the time of discharge from the hospital in about 1 week. Ten years later, Meyer *(35)* reported that only 21% were breast fed and 16% partially so at the end of the fifth day. In 1963, in the Boston area, Salber and Feinleib *(40)* reported that only 22% of mothers even attempted breast feeding and nearly one-half of these discontinued in the first month.

Pertinent to this problem is the 1970 report of Gery *(15)* in which he states, "Infants fed on breast milk for different periods, before being switched to cow's milk, reacted with significantly lower levels of antibody than those fed on pasteurized or powdered milk right after birth, with the degree of reduction in response being directly related to the period of breast feeding."

FORMATION OF MILK ANTIBODIES

Formation of antibodies to milk proteins in the blood of infants and children following ingestion of milk is regarded as a normal immunological response *(22, 37, 41)* although there have been several reports of their association with various pathological conditions such as chronic respiratory diseases *(28, 29, 30)* and gastrointestinal illnesses *(28, 47, 48)* other than milk allergy. Milk antibodies are detectable within less than a month following ingestion of milk. The shortest reported time for appearance of milk antibodies following ingestion is 5 days *(23)*. Antibody concentration reaches a peak in about 3 months after their first appearance and gradually decreases after that *(15)*.

There are four main types of antibodies in the blood serum, I_gG, I_gA, I_gM, and I_gE which are induced by ingestion of milk. I_gG is frequently associated with anaphylactic type of reaction and I_gE is associated with the atopic allergic reactions *(31)*.

Gery *(15)* has reported that although I_gE was usually found in the serum of most cases of elevated antibody levels, including cases without allergic symptoms, in some cases of milk allergy no I_gE was found.

Although the relationship of milk antibodies to milk allergy has been studied extensively, further discussion of the subject is beyond the scope of this paper. The subject has been reviewed recently by Hanson and Johansson *(26)*.

CRIB DEATHS OR SUDDEN INFANT DEATH SYNDROME

Sensitivity to milk has been suggested as one of many possible causes of sudden and unexpected death in infants, commonly known as crib deaths in the United States and cot deaths in England. The common-usage term "crib death" will be used in this paper instead of the scientific term "sudden infant death syndrone" *(49)*. This subject has been excellently reviewed by Valdes-Dapena *(50)*.

Crib deaths in the United States account for 15,000 to 25,000 human lives annually *(50)*. Parish and associates *(36)* reported in 1955 that there were 1432 crib deaths in England and Wales. Carpenter and Shaddick *(7)* estimated that these deaths accounted for 16% of all infants deaths in England and Wales in 1960. Emery *(11)* suggested that the number of crib deaths is increasing in Great Britain each year. Fontaine in 1962 reported that in France crib deaths accounted for 10% of all infant deaths in 1954 *(12)*. It is apparent that crib death is a major health problem. It is generally agreed that the greatest number of crib deaths occurs in infants under 6 months of age with the peak incidence between 2 to 4 months.

The usual history of crib death is that a well child or a child with a minor illness is fed and put to bed. Within the next 3 to 4 hr the infant dies without struggle or noise. The psychological trauma of this on parents is devastating. And, in the United States, there are two organizations composed of parents who have experienced this tragedy to help them cope with its aftermath *(49)*.

Explanation of the cause of crib death is controversial. Some believe that a single mechanism is involved in most cases while others believe there are many causes. Only 15% of the deaths can be satisfactorily explained on autopsy. The possible causes of the deaths are discussed in detail by Valdes-Dapena *(50)*.

A few of these causes are suffocation by overlay or bedclothes, fulminating viral or bacterial infections, and hypersensitivity to cow milk. It is of interest to describe briefly the development of the milk allergy hypothesis and some of the experimental evidence supporting it.

In 1954, Barrett *(2)* in England, suggested that crib deaths might be caused by inhalation of an amount

of food too small to cause asphyxiation *per se* but which might cause an inflammatory reaction with edema which could cause death. In 1960, Parish and associates *(36)* hypothesized that since milk antibodies can usually be detected in infants that at least in some cases, crib deaths may result from anaphylactic-type shock on inhalation of milk proteins in vomitus. These authors simulated crib death in model experiments using normal guinea pigs and those sensitized with cow milk protein. Introduction of up to 2 ml of soluble milk proteins into the respiratory tract of an unsensitized guinea pig produced negligible effects, whereas introduction of this amount into sensitized guinea pigs produced severe, typical anaphylactic reactions often terminating in death. If, however, only 0.25 ml of milk or gastric contents from an infant who died of "cot death" were introduced into the upper respiratory tract of a slightly anaesthetized, milk-sensitized guinea pig to simulate sleep, many animals died rapidly without struggle similarly to crib death in infants.

Experiments of Parish and others aroused great interest and research has been continuing, especially in England. As usual there are investigators who challenge the foregoing explanation of crib deaths *(17)*. It is evident that because crib deaths are a major health problem and because milk has been implicated, that further research should be done to clarify the role of milk in this connection.

Milk Allergens

Milk proteins are the etiological agents in milk allergy. Almost all proteins are antigenic and, while not all antigens have been characterized as allergens, nevertheless, they must be considered as potential allergens until definite information is available.

Milk is a very complex mixture of proteins consisting of the caseins and the whey proteins. Among the known caseins are the genetic forms of α_{s1}-casein, β-casein, k-casein, and γ-casein. The whey proteins consist of the genetic forms of β-lactoglobulin and α-lactalbumin. BSA, the immunoglobulins, and several enzymes are also present in whey. The allergenic activity of β-lactoglobulin, α-lactalbumin, BSA, and casein have mainly been studied. Allergenic activity has been attributed to all of these proteins. Although, principal allergenic activity has been ascribed to β-lactoglobulin and α-lactalbumin, the question is controversial. One difficulty in assessing allergenic activity of so-called pure proteins is that almost all preparations are contaminated with other milk proteins and allergenic activity can be triggered by minute amounts of a protein.

The immunoelectrophoretic technique has been very useful in demonstrating at least a minimum number of separate antigens in milk. Hanson *(24)* and Hanson and Johansson *(25)* have shown at least 12 to 14 separate antigens in mature bovine milk with many more in colostrum which come from blood serum.

Berrens *(4)* has postulated and presented considerable though not conclusive, evidence that a carbohydrate-lysine linkage (lysyl-[ε-amino-(1)]-1-deoxy-2-ketose) formed in the browning or Maillard reaction is a grouping responsible for atopic allergic reactions. Bleumink and Young *(6)* reported over a 100-fold increase in skin reactivity on milk-sensitive persons to relatively inactive β-lactoglobulin after prolonged heating with lactose at 50 C at pH 7.0. They attributed this increased skin-reactivity to a browning reaction condensation product of lactose and the ε-amino group of lysine in the β-lactoglobulin. These authors did not use a control test for non-dialyzable antigens in the lactose. This point was tested by Spies *(42)* who determined that two lactose samples contained small amounts of 4 new antigens in the retentate which were not identifiable with known milk proteins. Therefore the increased skin reactivity attributed to the browning reaction product of β-lactoglobulin and lactose may be due to these new antigens in lactose. The point requires clarification.

Prognosis

Milk allergy, which starts in the first months of life, fortunately disappears relatively early in life. Clein *(8)*, who reported 6% incidence of milk allergy in his pediatric practice, stated, "Somewhat less than 80% of infants lose their allergy to cow's milk before they become a year old. Somewhat less than 15% more lose their allergy to cow's milk by the time they are two years old. About 2% of the infants who initially had allergies to cow's milk continue to be allergic to this food after they reach the age of six years." However, Clein stated that about 80% of the infants who had cow milk allergy develop major allergies to other things before the age of puberty. Several authors believe that if milk allergy can preferably be prevented or at least treated promptly, it would tend to minimize the chances of milk allergic infants developing other allergies in later life *(16, 32)*.

Current Work on Milk Allergens at the Dairy Products Laboratory

The broad objective of the allergens investigations of the Dairy Products Laboratory of the Department of Agriculture is the control or inactivation of the allergens of milk so that this nutritious food will be acceptable to those who cannot now tolerate it because of allergic response to ingestion of milk. The relatively low incidence of milk allergy in the total

population (<0.1 to 7%) might seem too insignificant to justify research on milk allergy by Dairy Products Laboratory. However, the significance of this work is broader than these figures indicate at first glance. Firstly, it is pediatric practice in many cases in families with a history of any allergy to eliminate milk from the diet of infants and children under 2 years of age whether they are allergic or not to minimize their chances of acquiring milk allergy. Secondly, as pointed out above, it is believed that prevention of milk allergy in infants tends to lessen the chances of infants acquiring other allergies in later childhood, after milk allergy, should they have acquired it, had disappeared. And thirdly, milk contains well-characterized proteins which are ideally suited as model substances for studying all food allergies.

It must be evident from the foregoing brief review of the subject of milk allergy that the present state of knowledge requires, primarily, fundamental scientific investigations to elucidate the mechanism of the allergic response to ingestion of milk before we can elaborate feasible procedures for the control of milk allergens.

For the past 5 years we have been studying the immunological significance of pepsin hydrolytic products of milk proteins. The immunologic significance of enzyme hydrolytic products of ingested allergenic proteins long has been the subject of speculation and sporadic investigations. The consensus of the clinical significance of digestive products has been that they may be the cause of delayed clinical reactions of from 1 to 36 hr or even days in some instances. Our work suggests that digestive products of milk may be the cause of immediate type allergic response possibly in addition to delayed responses.

We first demonstrated *(46)* generation of a new antigen in the dialysate of the 8-min pepsin hydrolysate of each of 4 major milk proteins, namely, β-lactoglobulin, α-lactalbumin, casein, and BSA. The endo fraction of BSA contained a second new antigen. In our studies the term "new antigen" is defined as an antigen with a specificity distinct from that of the protein from which it was generated.

The objective of later studies *(43, 44)* was to determine whether one or several new antigens are generated by a simulated stomach digestion of β-lactoglobulin. In this study β-lactoglobulin was hydrolyzed six, successive, 8-min periods during which approximately 90% of the protein was split into fragments with a molecular weight of 12,000 or less. Six dialysate (D1-D6) and six endofractions (E1-E6) were separated and analyzed for the presence of new antigen using the Schultz-Dale and gel diffusion analysis, respectively. All of the dialysates contained common, nonprecipitating new antigens. The first dialysate (D1) did not contain all of the new antigens common to the other five, D2-D6, indicating at least two new antigens in the dialysates. Six precipitating new antigens were demonstrated in the endofractions. Now by anology to β-lactoglobulin, if pepsin hydrolysis generated at least eight new antigens from each of the 12 to 14 antigenic proteins in milk, the body immune system would be exposed to about 100 new antigens, all of which are potential allergens, on ingestion of milk. These results may explain why milk and other foods, in many instances do not give skin reactions on persons who give an immediate allergic response on ingestion of the food. Such persons may be sensitive to these new antigens formed by pepsin in the stomach during digestion.

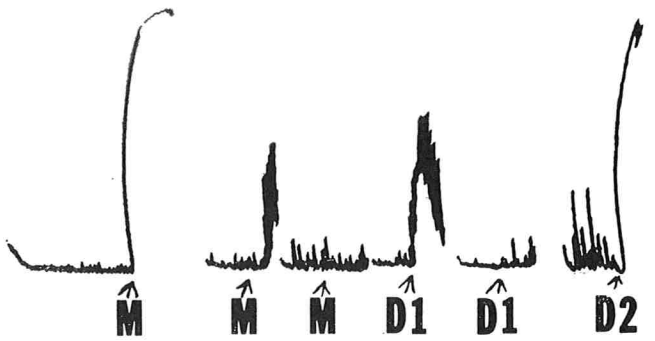

Figure 1. Demonstration of common new antigen in dialysate fractions, D1 and D2, and that D2 contains new antigen other than that present in D1 by Schultz-Dale technique. Sensitizing antigen: D2. Challenge doses in μg total nitrogen of fractions: M(β-lactoglobulin, pepsin, PEPD), each component, 10: D1, 10: D2, 10.

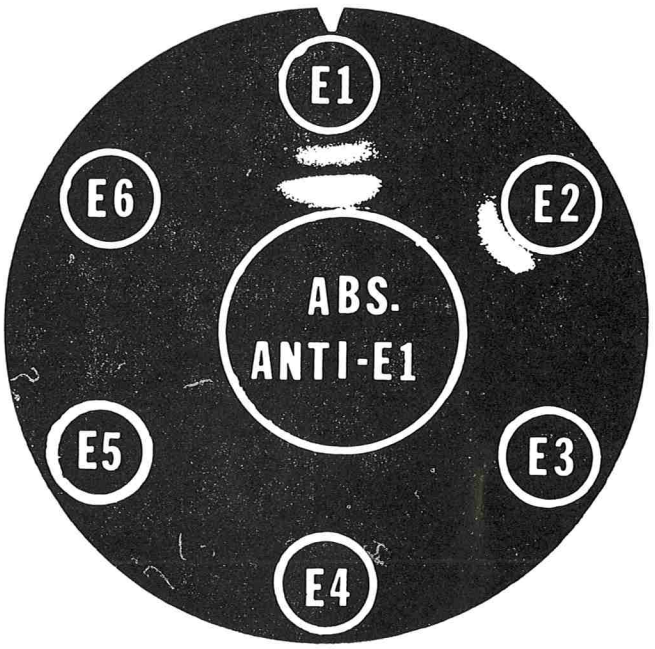

Figure 2. Demonstration of precipitating new antigens in endofractions, E1 and E2, by gel double diffusion analysis. Center well, 0.5 ml of absorbed anti-E1 (rabbit) (absorbed with β-lactoglobulin and PEPE); peripheral wells, 0.07 ml of indicated endofraction, 0.5 mg endofraction nitrogen per ml.

Although the sensitizing properties of these new antigens are unknown as yet, it seems likely that some of them, at least, might act as allergic sensitizers for food digestion products in a manner similar to that of other low-molecular weight substances such as drugs. It was demonstrated *(46)* that new antigen could be detected after only 1, 2, and 4 min pepsin hydrolysis of total milk protein. Later *(43)* it was apparent that common new antigens continue to be generated over a period of 48 min. Since absorption of immunologically significant amounts of allergens are known to occur in a few minutes *(27, 45, 52, 53)*, this continuous production of new antigens in the dialysates tends to enhance their sensitizing potential.

The Schultz-Dale technique, was used to demonstrate the two, nonprecipitating new antigens in dialysates, D1 and D2, as shown in Fig. 1. Figure 2 illustrates use of the gel double diffusion technique in demonstrating two precipitating new antigens in endofraction E1. Details of these studies are described in reference *43*.

Current studies are in progress on isolation and chemical and immunological characterization of these new antigens. Following this the purified fractions will be evaluated clinically to determine their allergenicity.

Demonstration of this multiplicity of new antigens (potential allergens) generated by pepsin hydrolysis of milk proteins, as a simulated first step in digestion, opens up a new area of study which should clarify an important aspect of the many perplexing aspects of food allergy in general and milk allergy in particular.

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REPORT OF EXECUTIVE SECRETARY AND MANAGING EDITOR, 1971-1972

Again I am proud and happy to report to you that this has been the biggest year we ever had, both as to total income and net income; over $73,000 in total income as compared to $61,000 last year and $7,000 in net income as compared to $3,000.00 in 1971. This record is exceptional because the period from July 1 to Dec. 31 represented an interval during which we sustained over 20% increase in costs but were unable to realize any increase in income for subscriptions and advertising because most subscriptions expire December 31 and advertising was covered by contracts of 6 months or more. I am gratified for the way the membership has responded to the dues increase. We had anticipated we might suffer a much greater loss than occurred. A net loss of less than 16 direct members and 150 affiliate members was encountered. Many of these have been reinstated since June 30. Two important steps have been taken with respect to future membership and advertising activities. One is the appointment of Harold Heiskell as IAMFES Membership Chairman, and the second, George Willits has been made advertising solicitor. Harold has been organizing his committee and has plans for an effective membership campaign. George has also planned and organized an advertising program and has begun to put it into effect. I am sure we can expect an excellent report from them at our next annual meetinf.

Again the Journal is the largest we have ever published-500 pages to date in 1972 as compared with 422 last year. We also have a new look for the cover beginning with the March issue. We have received only favorable comments regarding this change. Our food coverage continues to expand and as a result our membership and subscriptions are growing in this area. *Foodborne Investigations, and 3 A Sanitary Standards* continue to sell very well and we have added a new one this year, *Methods for Production of High-Quality Raw Milk*, which was done by our Dairy Farm Methods Committee. This booklet contains excellent information very valuable to the farm sanitarian, fieldman, and milk producer.

The third payment has been made on my retirement which leaves only one more to be paid in January. The future of IAMFES still looks bright and again for the 21st time, it has been a pleasure to serve you.

H. L. THOMASSON
Executive-Secretary
and Managing Editor

FLAVOR QUALITY OF VARIOUS GRAIN FLOURS AND OILSEED, MILK, AND MARINE PROTEIN SUPPLEMENTS[1]

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Abstract

Comparative sensory and analytical flavor evaluations of whole wheat flour (WWF), non-fat dry milk (NFDM), sodium caseinate (Na Cas), buttermilk solids (BMS), corn flour (CF), defatted soy flour (DSF), full-fat soy flour (FFSF), isolated soy proteinate (ISP), rice flour (RF), fish protein concentrate (FPC), peanut flour (PF) and cottonseed flour (CTF) were performed. The odor of CF, FFSF, RF, and PF was judged to be more bland in the liquid-suspended state than in the dry state. The flavor intensity of all products except CF and CTF was judged to be less bland than their liquid-state odors. Sensory panel results indicated that NFDM, BMS, and RF were the most bland, while FPC and PF were the least bland. Total GLC peak areas were lowest in Na Cas and highest in ISP. Total carbonyl content ranged from 4 ppm in BMS and ISP to 230 ppm in FFSF.

Although numerous food flavor investigations have been reported, few deal directly with the flavor properties of whole grains and flours even though these products are consumed on a worldwide basis.

In a study involving the volatiles associated with cooked rice grain, Yasumatsu et al. *(17)* identified acetaldehyde, n-caproaldehyde, methyl ethyl ketone, n-valeraldehyde, and either propionaldehyde or acetone. Yasumatsu et al. *(18)* also reported that the stale flavor of rice is partially due to the presence of n-caproaldehyde.

McWilliams and Mackey *(9)* identified acetaldehyde; isobutyraldehyde; butyraldehyde; hexanal; heptanal; octanal; crotonaldehyde; 3-methyl-2-butanone; 2, 2-dimethyl -3- pentanone; diacetyl; and ethyl acetate in lightly milled whole grain soft wheat. They also tentatively identified butanone, valeraldehyde, isovaleraldehyde, cyclopentanone, phenylacetaldehyde, amyl alcohol, and isoamyl alcohol. Citing the review of Johnson et al. *(7)*, McWilliams and Mackey *(9)* believe that the flavor components they isolated from wheat contribute directly to bread flavor.

Recently Hougen et al. *(5)* compared the headspace vapors of maize, rye, rapeseed, wheat, barley, oats, flax, soybean, and sunflower seed. Compounds identified included methanol, ethanol, ethanal, propanal, propanone, 2-methyl propanal, butanal, butanone, 3-methyl butanal or 2-methyl butanal, pentanal, hexanal, and heptanal. Hougen and co-workers *(5)* concluded that the different species and varieties evaluated contained the same volatile components but varied in the proportions of these components.

Okada *(12)* used steam distillation and ether extraction to concentrate volatile compounds from wheat gluten. Through sensory tests he concluded that the aroma of wheat gluten was composed mainly of carbonyl compounds with n-caproaldehyde found in the highest concentration.

Several recent studies have investigated the flavor associated with roasted barley. Shimizu et al. *(13)* used an alcoholic extract of roasted barley and found 5-hydroxymaltol, maltol, 5-methylcyclopent-2-en-2-ol-1-one, phenol, m-cresol, pyrocatechol, and resorcinol. Using a gas-liquid chromatography headspace technique, Collins *(4)* identified ethanal; propanal; 2-methylpropanal; 2 methylbutanal; 3-methylbutanal; pentanal; hexanal; furfural; propanone; butanone; 2, 3-butanedione; 2-3-pentanedione; ethanol; and pentanol. The basic fraction in this study *(4)* contained nine pyrazine compounds.

Intensified efforts are currently under way to supplement the major grains of the world (rice, wheat, corn) with animal, vegetable, and marine protein sources. An earlier study *(10)* has compared the flavor properties of various protein supplements. Therefore, this study was designed to compare flavor properties of these typical grain flours and compatible supplements by using flavor indicators such as sensory panels, gas-liquid chromatography headspace patterns, and carbonyl content. By performing direct comparisons by various methods it is hoped to show the flavor assets and limitations of the grains and supplements investigated.

Materials and Methods

Products

A total of 12 commercial products were evaluated. Fresh samples were requested directly from the manufacturers. Products included whole wheat flour, low-heat non-fat dry milk (NFDM), sodium caseinate, buttermilk solids, defatted soy flour, full-fat soy flour, isolated soy proteinate, rice flour, fish protein concentrate, peanut flour, and cottonseed flour.

Proximate composition

Standard AOAC procedures *(1)* were used to determine

[1]Published with the approval of the Director of the Colorado State University Experiment Station as Scientific Series Paper No. 1712.

moisture, protein, fat, ash, and fiber content of all products. All results are averages of triplicate samples.

Sensory panel

Odor intensity and flavor evaluations were randomly repeated three times at room temperature by 20 college-age female students. The panel was instructed to use a scale of from 1 to 10 to evaluate product blandness. A product with a "completely bland" character (one having no detectable odor or flavor) received a score of 10 and a "strong" material was scored 1. The odor intensities of both powder and 3% liquid suspensions were evaluated. Odor intensities of powders were judged by sniffing three times 5 g of each respective powder in an odor-free 35 ml capacity screw cap vial. Odor intensities of liquids were rated by sniffing 20 ml of a 3% suspension of product in an odor free 50 ml glass beaker. Suspension was with freshly distilled and demineralized water. Samples were permitted to equilibrate at room temperature for 0.5 hr before evaluation. Approximately 5-ml amounts of thoroughly stirred liquid suspension were used to rate flavor intensities. Swallowing of samples was not permitted and rinse water was available at all times.

In an effort to determine overall sensory blandness of the products, values for odor intensity and flavor intensity of liquids were combined.

Statistical analysis

All sensory data were evaluated by analysis of variance and the honest significant difference intervals *(14)* calculated at $\alpha = 0.05$.

Headspace analysis

GLC direct headspace analysis has been reported to be an effective means of determining food acceptability *(2, 3, 6, 15)*. However, heating samples at 120 C for 2 hr *(5)* or steam distilling volatile material in preparation for GLC analysis *(9, 12)* could produce artifacts. Therefore, a milder method for concentration of volatile componuds was employed *(2)*. One-half gram of product along with 1.2 of anhydrous sodium sulfate and 2 ml of freshly distiled and demineralized water were heated in a rubber septum sealed 5 g serum vial for 10 min at 60 C. At the end of the heating period 1 ml of vapor was withdrawn from the sample and injected onto the GLC unit.

A model 600 Varian-Aerograph GLC unit was used. The 12 ft × one-eighth inch stainless steel column was packed with 20% Carbowax 20M and 60/80 mesh Chromosorb P (AW-HMDS). Temperatures used were 100 C isothermal for the column, and 150 C and 170 C, respectively, for the injection port and hydrogen flame detector. Nitrogen at a flow rate of 15 ml per minute was used as the carrier.

No attempt was made to identify each peak since this was one phase of a comparative study. Wilkens et al. *(15)* stated that GLC volatile profiles, even if designated by unidentified peaks, can be invaluable if related to taste panel results.

Carbonyl content

Total carbonyl content of all samples was determined by the colorimetric method reported by Lappin and Clark *(8)*. The carbonyls from 5 g of each product were extracted with 50 ml of carbonyl-free chloroform. A standard curve based on butanal was prepared and the sample optical density values converted to parts per million from this curve.

RESULTS AND DISCUSSION

Proximate composition

The approximate composition data for all samples can be seen in Table 1. These data are presented to provide the reader background information on the composition of the specific products evaluated.

TABLE 1. PROXIMATE COMPOSITION[1] OF PRODUCTS EVALUATED

Product	Moisture	Protein	Fat	Ash	Fiber
Whole wheat flour	11.8	13.1	2.1	1.6	2.2
Non-fat dry milk	3.0	35.9	0.8	8.0	0.0
Sodium caseinate	3.6	90.3	1.0	4.1	0.0
Buttermilk solids	2.8	34.3	5.3	7.6	0.0
Corn flour	12.1	7.7	2.7	0.7	0.7
Defatted soy flour	6.8	51.9	0.8	7.1	2.3
Full-fat soy flour	8.2	38.8	19.6	4.7	4.0
Isolated soy proteinate	4.5	92.5	0.1	4.0	0.2
Rice flour	11.9	6.6	0.5	0.5	0.4
Fish protein concentrate	2.2	78.1	0.4	19.1	0.0
Peanut flour	7.2	48.1	9.4	4.0	2.9
Cottonseed flour	6.2	48.0	6.5	6.1	2.1

[1]All values expressed as percent on an "as is" basis and represent the averages of three samples each.

Sensory evaluation

Honest significant difference intervals of the sensory data are summarized in Fig. 1-4. Data on the odor intensity of powders (Fig. 1) revealed that statistically all products except fish protein concentrate were equivalent to whole wheat flour. Besides fish protein concentrate, the odor intensities of peanut flour and full-fat soy flour were judged to be on the strong side while the odor intensities of cottonseed flour, NFDM, and isolated soy proteinate were considered to be bland. Among the grain flours, whole wheat and rice had essentially the same degree of odor blandness while that of corn flour was found slightly more intense.

If product odor intensities in the liquid state are considered (Fig. 2), the same general trends appeared. Again, if whole wheat flour is considered to be the comparative standard, all products were statistically the same in odor intensities except that again fish protein concentrate was statistically stronger and NFDM was more bland.

When flavor intensities of liquids were considered (Fig. 3), no statistical differences among products was observed with whole wheat flour being the comparative standard. However, if corn flour served as the comparative standard, the two soy flours, isolated soy proteinate, peanut flour, and fish protein concentrate were statistically stronger in flavor intensity. Thus, it becomes apparent that certain protein supplements have odor and flavor intensities more closely related to certain grain flours than others.

In Fig. 4 both odor and flavor intensities of liquids are considered. Again, with whole wheat flour serving as the comparative standard, all products except

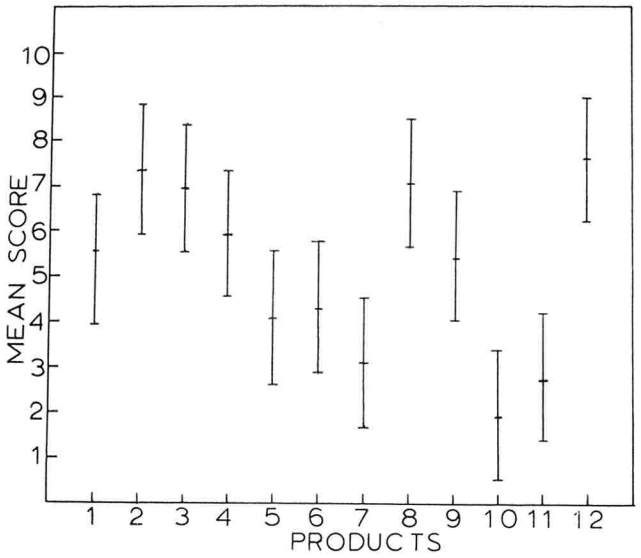

Figure 1. Honest significant difference intervals ($\alpha = 0.05$) of sensory powder odor intensities. Products: 1. whole wheat flour; 2. non-fat dry milk; 3. sodium caseinate; 4. buttermilk solids; 5. corn flour; 6. defatted soy flour; 7. full-fat soy flour; 8. isolated soy proteinate; 9. rice flour; 10. fish protein concentrate; 11. peanut flour; 12. cottonseed flour.

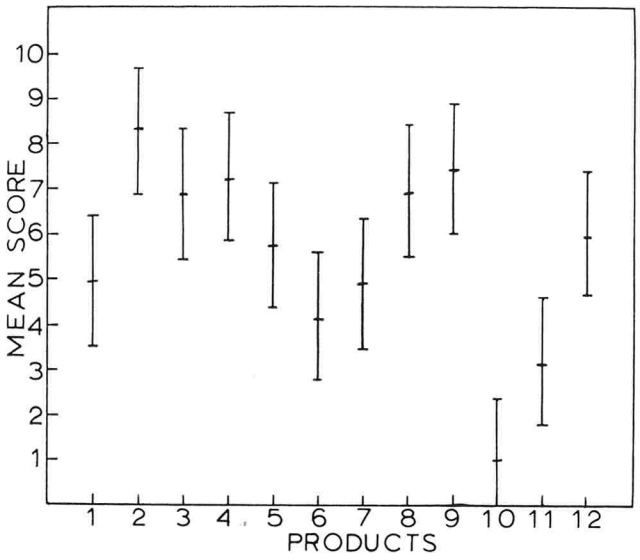

Figure 2. Honest significant difference intervals ($\alpha = 0.05$) of sensory liquid odor intensities. Products: Same as Fig. 1.

fish protein concentrate were statistically equivalent in overall flavor intensity. Although NFDM was statistically more bland in overall flavor intensity than whole wheat flour, no statistical difference was found when NFDM was compared for overall flavor intensity with corn and rice flour. Corn and rice flours were statistically more bland in overall flavor intensity than the two soy flours, peanut flour, and fish protein concentrate.

All of the sensory data indicated that as a class, milk protein supplements were the most bland and uniform product supplements. Within the vegetable and marine supplement group, fish protein concentrate was consistently inferior in odor and flavor intensity while defatted cottonseed flour proved to be relatively bland in odor and flavor intensity.

Headspace investigations

Since this was a comparative study, only one set of GLC conditions was employed and no effort was made to achieve more complete separation through temperature programming. Under the conditions employed, separation of compounds similar to those observed by sniffing were effectively detected. Sample size and conditions were the same for all products. As reported by Hougen et al. *(5)*, this study also found compounds having similar retention times in all products with the main difference among products being quantity of compound.

In an effort to numerically compare these headspace data, total peak areas of attenuated scans were calculated (peak height times peak width at half height). These data are in Fig. 5. Although full-fat soy flour was not judged particularly bland by the taste panel, the headspace technique indicated that it contained relatively few detectable volatile compounds. A possible explanation for this discrepancy would be that a majority of the flavor constituents associated with full-fat soy flour are fat soluble and thus were not released for detection under the GLC conditions employed. Another discrepancy between taste panel and GLC results occurred with corn flour. Taste panel members regarded it as one of the mildest products yet GLC total peak area of corn flour showed it to be high in volatile material, mainly because of a large, later occurring peak.

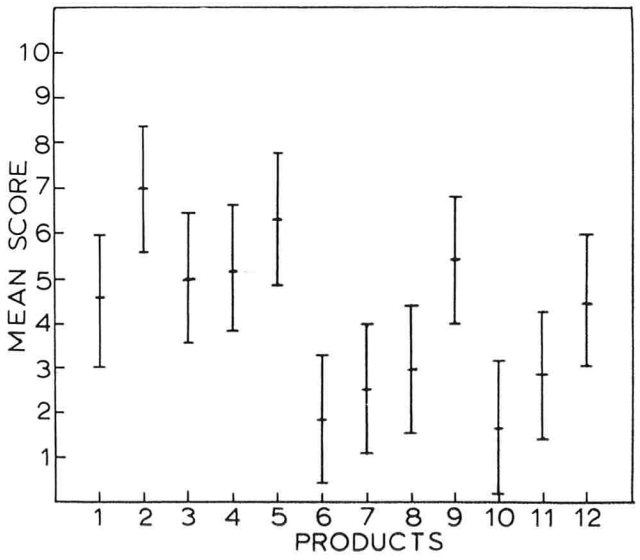

Figure 3. Honest significant difference intervals ($\alpha = 0.05$) of sensory liquid flavor intensities. Products: Same as Fig. 1.

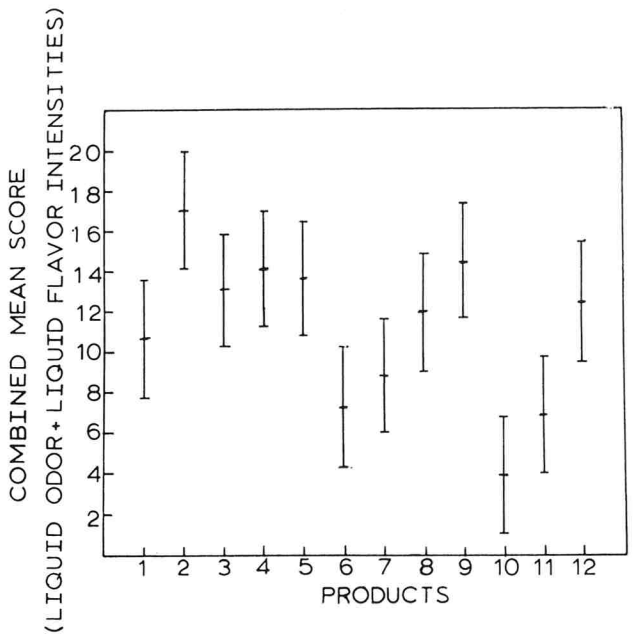

Figure 4. Honest significant difference intervals (α = 0.05) of overall flavor intensities. Products: Same as Fig. 1.

An inherent problem with this type of analysis is that the actual flavor thresholds of compounds separated by this method vary greatly. Thus, although a compound may appear as a small peak, its contribution to total flavor may be extremely important because of its low threshold. Likewise, a major peak may actually contribute very little to total flavor due to its high threshold. Therefore, it becomes apparent that caution is required in attempting to correlate total peak area as determined by direct GLC headspace techniques and flavor quality or blandness characteristics among various products.

Carbonyl content

The importance of carbonyl compounds in bread flavor has been reported *(7, 11, 16)*. Therefore, total carbonyl content was determined and reported in Fig. 6. Among the three grain flours, carbonyl levels were in agreement with panel blandness scores since the milder the product, the lower the carbonyl level. However, it should be noted that bread made solely from wheat flour has a characteristic flavor which is indicative of a vast number of flavorful compounds, including carbonyls.

The panel scores for blandness and carbonyl content of the milk-based supplements were in direct agreement. However, one notable exception between blandness scores by the panel and carbonyl values occurred for fish protein concentrate. Carbonyl level is not a good indicator for fish protein concentrate flavor quality since the predominant flavor in this product is of an amine nature.

Perhaps carbonyl levels would be a more valid test to follow processing and storage changes. An example of carbonyl changes with processing can be seen within the soy products evaluated. There was a dramatic decrease in carbonyl content in processing full-fat soy flour into defatted soy flour and finally isolated soy proteinate.

ACKNOWLEDGMENTS

This study was supported in part by a grant from the Quaker Oats Co. The authors also thank Dr. Thomas Boardmann, Statistical Laboratory, Colorado State University, for his assistance.

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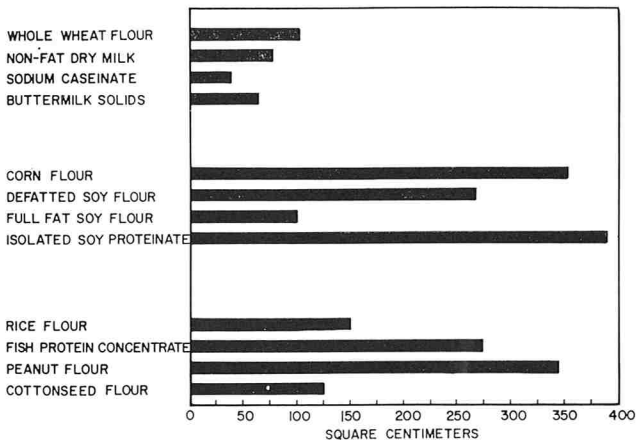

Figure 5. Total GLC peak areas calculated from headspace scans.

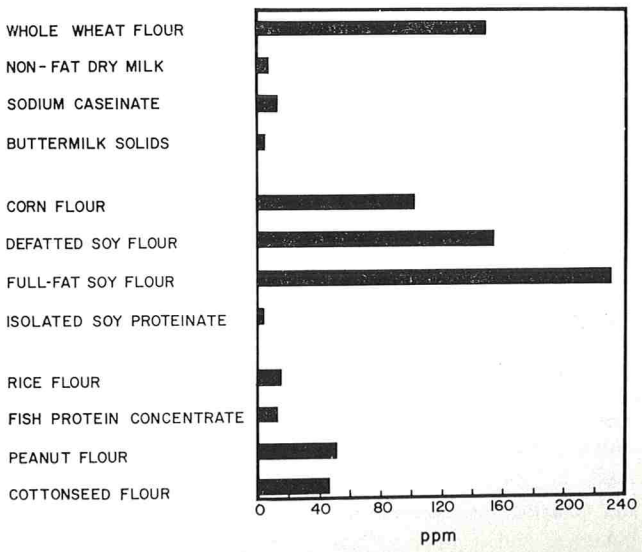

Figure 6. Total carbonyl content of products evaluated.

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REPORT OF THE 3-A SANITARY STANDARDS SYMBOL ADMINISTRATIVE COUNCIL, 1971-1972

The President of the IAMFES appointed Pat Dolan, Regional Administrator, Bureau of Dairy Service, California Department of Agriculture, Sacramento, California, to replace James Meany of Chicago. Fred Uetz was reappointed to the Council by the Dairy Industry Committee and D. G. Colony was reappointed by the Dairy and Food Industries Supply Association, Inc. Members of the Council are appointed for a period of 2 years. Other members of the Council are Paul K. Girton, Dr. W. S. Clark, Jr., Dr. K. G. Weckel, D. C. Cleveland, and E. O. Wright.

The Executive Council has worked hard this past year to secure tax exempt status from the Internal Revenue Service. This was achieved on August 2, 1972. A complete audit was taken for 1971 by a C.P.A. and everything was found to be in order.

There were two meetings of the trustees held during this period. They were at the Sir Francis Drake Hotel, San Francisco, California, on November 3, 1971, and at the Marriott Motor Hotel, Chicago, Illinois, on June 13, 1972. Complaints registered with the Secretary by sanitarians were dealt with and actions are being taken to overcome the problems. A systematic complaint form is being developed by a committee to aid in gathering the right type of information when it is felt that equipment doesn't comply with the 3-A Standards.

During this period there were 21 new authorizations issued and seven authorizations relinquished. (The comparative numbers of authorizations covering each type of equipment are found in Table 1.) An increase from 146 to 161 authorizations is indicated.

We have received inquiries from Denmark, Japan, France, and Canada, for information concerning use of the 3-A Symbol.

Through the fine cooperation of sanitarians and fieldmen and the excellent cooperation of the industry, the 3-A Symbol program is working successfully.

TABLE 1. 3-A SYMBOL COUNCIL AUTHORIZATIONS

Types of equipment	Sanitary standards serial numbers	Authorizations in effect on July 31, 1971	Authorizations in effect on July 31, 1972
Storage tanks	0102	15	16
Pumps	0204	16	17
Homogenizers & pumps	0402	3	4
Auto. milk tanks	0506	17	18
Fittings (Piping)	0808	18	20
Fittings (Thermo)	0902	2	2
Filters	1002	1	1
Heat exchange (Plate)	1102	7	7
Heat exchange (Tube)	1202	4	6
Farm milk tanks	1303	17	17
Leak protector valves	1400	4	4
Evaporators	1603	6	5
Fillers & sealers	1702	8	7
Ice cream freezers	1901	2	2
Silo-type tanks	2201	8	9
Package fillers	2300	3	4
Batch pasteurizers	2400	5	5
Batch processors	2500	5	6
Dry milk sifters	2600	5	7
Dry milk packaging equipment	2700	0	0
Flow meters	2800	0	4
TOTAL		146	161

E. O. WRIGHT
Secretary-Treasurer
Department of Food Technology
Iowa State University
Ames, Iowa

SANITARY CONDITIONS IN TWELVE FLUID MILK PROCESSING PLANTS AS DETERMINED BY USE OF THE RINSE FILTER METHOD[1]

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(Received for publication December 20, 1972)

Abstract

Experiments were done in 12 fluid milk processing plants to determine their sanitary condition. Tap water was sterilized by membrane filtration and sodium thiosulfate was added to inactivate residual sanitizer. This water was used to rinse the pasteurized milk storage tank, filler bowls and filled containers. Samples of rinse water were filtered through membrane filters which were incubated on media to produce colonies. Total and coliform counts were made. Storage tanks contributed fewer bacteria to the water than other equipment. About 60% of the first cartons taken from fillers were contaminated with at least 1 bacterium per 100 ml of rinse water and 37% contained at least 1 coliform per 100 ml. The method appears useful to evaluate the sanitary conditions of equipment.

Current industry marketing practices for fluid milk products make it practically imperative that the shelf life of these products be at least 10 days at 7.2 C (45 F). Therefore, the extent of post-pasteurization contamination with psychrotrophic bacteria must be limited to a few, perhaps none, per carton. This point is illustrated by Fig. 1 which was constructed based on the assumptions that the count would be 1/ml on the fifth day of storage, and that the milk would be spoiled when the count reached 17,000,000/ml. Based on this simplified example, shelf life of at least 10 days could be expected only if there was a lag time of approximately 3 days, the generation time exceeded 6 hr and the initial count was 1/half pint. Counts may be higher initially, and lag times may be shorter if the generation time is made longer by lowering the temperature of storage, or if by chance the contaminants are of a slow growing type.

A dairy's quality control department is faced with the problem of evaluating operations to insure that contamination is minimized, but the tools at its disposal are inadequate for the task. The agar plate method can accommodate only a small sample and does not differentiate the psychrotrophic bacteria. Results are delayed by 48 hr incubation. The psychrotrophic plate count suffers even more from the latter criticism, requiring a 10-day incubation.

Shelf life tests, obtained by incubating finished product at 7 C for 5 days, are excellent to evaluate an operation, providing sufficient samples are taken. However, it is usually too late to act with regard to the product represented once defects are discovered.

An indirect technique, the rinse/filter method, to evaluate the post-pasteurization contamination level within a plant was developed in our laboratory. Large quantities of tap water are sterilized by membrane filtration, residual sanitizer is inactivated with sodium thiosulfate, the water is passed over milk contact surfaces, representative samples are taken at strategic points, and large quantities of this rinse water are tested by the membrane filter technique for numbers of bacteria. Results are available within 24 hr.

With the cooperation of 12 processors a study was made during the summer of 1972 with the rinse/filter method to ascertain the amounts of post-pasteurization contamination which can be expected in today's grade A fluid milk processing plants.

Material and Methods

Water from the municipal or private supply was prefiltered through a wound, cotton string filter (type 12531, Gelman Instrument Co., Ann Arbor, Michigan) and then filtered through a membrane filter (Millitube cartridge, Millipore Filter Corp., Bedford, Massachusetts) with a pore size of 0.45 μm (Fig. 2). This water (300 to 400 gal) was introduced into a pasteurized milk storage tank which had been cleaned and sanitized. Approximately 1500 ml of 10% sodium thiosulfate were added with the water to inactivate residual sanitizer, except in plants where hot water was used for sanitization.

Water was pumped or allowed to flow by gravity to filler bowls which had been sanitized and drained. This water was then packaged in cartons in the same manner as milk.

Samples of the water were taken aseptically at each of the following locations: filtrate from filter apparatus; pasteurized storage tank; filler bowls; and cartons, jugs or plastic bags.

Counts of coliform bacteria in the samples were made according to the standard method for membrane filter analyses *(1)*. Sample volume was 200, 250, or 300 ml. Total counts, herein known as filter counts, were done in a similar manner except that double strength Tryptic soy broth was the culture medium and the sample size was 100 ml. Frequently, small numbers of samples of 10 ml quantity were analyzed to allow determination of counts which exceeded about 1000 per 100 ml.

Filters were incubated at near 100% relative humidity for 24 hr at 32 C before counting. A binocular microscope with

[1]Contribution from the University of Missouri Agricultural Experiment Station. Journal Series Number 6577.
[2]Present address: Carnation Company, Oconomowoc, Wisconsin.

TABLE 1. CONDITION OF FILTRATE FROM FILTER APPARATUS AS DETERMINED BY FILTER COUNTS AND COLIFORM COUNTS

Range of counts/100 ml	Filter count/100 ml		Coliform count/100 ml	
	All samples	Avg. no. per plant	All samples	Avg. no. per plant
<1	8	3	11	9
1-10	14	8	2	1
11-100	2	0	0	0
110-1000	1*	1*	0	0
>1000	0	0	0	0
No. of samples	25	12	13	10

*Defective filter

variable magnification from 7 to 30 × was used in counting.

Samples, taken at each location, contained all the contaminants introduced up to the point of sampling. Therefore, the following protocol was used to determine the count at any particular sampling point:

Sampling point	*Method to determine count*
Filtrate (from apparatus)	Count of filtrate
Pasteurized storage tank	Count at tank minus count of filtrate
Filler bowl	Count at bowl minus count at storage tank
Container	Count at container minus count at filler bowl

Samples were taken once from each of 12 commercial fluid milk plants in the following states: Missouri 6; Illinois, 3; Kansas, 2; and North Carolina, 1. The method of sanitization and the general state of repair of the equipment were noted for each of the plants.

RESULTS AND DISCUSSION

Capability of the filtration system

In 22 of 25 samples taken, the filtration apparatus produced water with a total bacteria count of < 10/100 ml (Table 1). In one plant the filter membrane was ruptured, producing a count of 270/100 ml. A simple test is available to detect faulty filters, but the test was not applied in this plant. Disregarding the one high count, the average total count for the remaining 10 plants was 1.7/100 ml. It is probable that limited contamination occurred during sampling. Therefore, it is considered that the filters performed satisfactorily from the standpoint of removal of microorganisms.

Coliform bacteria were found in the filtrate of only one of 10 plants, and the count was only 1/100 ml. A coliform analysis was not done on filtrate in two plants.

Rate of flow of water through the filtration system varied because of differences in water pressure but, more importantly, to undissolved substances in the water. Failure to completely flush water lines before connecting the filters sometimes resulted in rapid plugging of filters. Large amounts of rust and/or extraneous matter were observed in water from more than 50% of the plants. About 1 hr was required in most plants to produce sufficient water to rinse the equipment.

The normal rate of filtration was 7 gal/min when filters were clean and pressure at the pre-filter was 40 psi. A pressure drop of about 18 psi occurred across the pre-filter when it operated properly. As the pre-filter began to plug, water pressure increased at the inlet. As the membrane filter began to plug, the difference decreased between pressure at the inlet to the pre-filter and pressure at the inlet to the membrane filter.

It was possible to reuse clogged filters, both pre-filters and the membrane filters, after soaking them about 4 hr in a hot solution of 10% acid-type detergent.

Condition of pasteurized storage tanks

A relatively low number of contaminating microorganisms entered the system from the pasteurized milk storage tanks (Table 2). One plant used a closed system which precluded sampling directly from this tank, and data were not included in Table 2. However, in this plant counts at two of the filler bowls were <1/100 ml, indicating practically sterile conditions in the storage tank. High counts of 1000/100 ml or more were observed in tanks of 2 plants. In one of these plants there were two possible sources of external contamination, viz., *(a)* a leaking air supply line to the interior of the tank, and *(b)* a leaking indicating thermometer which was replaced by the operator after sanitization without adequate precautions.

Coliform counts were <1/100 ml in pasteurized storage tanks of 8 of 10 plants. Counts of 7 and 82/100 ml were observed in the two plants which had high filter (total) counts in their storage tanks.

Condition of milk lines and filler bowls

A relatively large amount of contamination occurred between the pasteurized milk storage tanks and the filler bowls. In 10 of the 12 plants, average counts for all filler bowls exceeded 10/100 ml and 4 of these were greater than 100/100 ml (Table 3). The sampling method precluded determination of whether

TABLE 2. CONTRIBUTION OF MICROORGANISMS BY PASTEURIZED STORAGE TANKS AS DETERMINED BY FILTER COUNTS AND COLIFORM COUNTS

Range of counts/100 ml	Filter count/100 ml		Coliform count/100 ml	
	All samples	Avg. no. per plant	All samples	Avg. no. per plant
<1	4	2	8	8
1-10	4	3	3	1
11-100	5	4	1	1
110-1000	0	0	0	0
>1000	3	2	0	0
No. of samples	16	11	12	10

contamination occurred in the milk lines (including valves) or in the filler bowls.

Coliform counts were exceptionally high (more than 100/100 ml) in 5 samples of the 43 tested.

Condition of filler valves and containers

On an average count per plant basis the data suggested that filler valves and containers were the greatest contributors to microbial contamination. In eight of the plants, filter counts of water in the sealed milk containers averaged 110/100 ml or higher (Table 4). This may be somewhat misleading in that only 32% of the total samples were in this category. These counts represent only the organisms picked up in the filling operation since the count of the samples from the filler bowl was subtracted from that of the container for the respective samples.

Coliform contamination occurred in the process of filling in 7 of 11 plants, but in only 33% of all of the filling operations of all plants. Numbers exceeded 100/100 ml in about 3% of all the samples.

General observations

Contamination was generally least in plants which used hot water for sanitization. There were two of these plants, numbers 6 and 11, and 14% and 45% of their samples, respectively, contained one or more bacteria per 100 ml (Table 5). In one other plant, number 5, only 33% of the samples produced colonies. No samples from plant 11 contained coliforms, and only 13% of the samples from plants 6, 8, and 10 produced coliform counts.

Excluding samples of the filtrate, 60% of the samples taken from all plants contained one or more bacteria per 100 ml, and 37% contained one or more coliforms per 100 ml.

Average counts only serve to give a general idea of the sanitary status within the plant. One high count, when averaged with several of <1/100 ml, may produce a misleading average. Nevertheless, data in Table 6 indicate that plants with lowest average counts tended to be those with fewest positive samples. Plant number 10 had the lowest overall level of contamination.

The following plants were able to package water with counts of <1/100 ml in the percentage of samples shown in parentheses: 11 (57%), 10 (25%), 3 (20%),

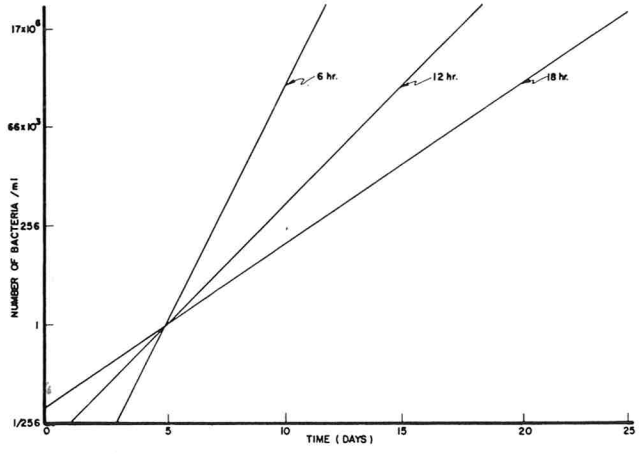

Figure 1. Time to reach a count of 17,000,000/ml with generation times of 6, 12, and 18 hr and a count of 1/ml after 5 days of storage.

TABLE 3. CONTRIBUTION OF MICROORGANISMS BY MILK LINES AND FILLER BOWLS AS DETERMINED BY FILTER COUNTS AND COLIFORM COUNTS

	Filter count/100 ml		Coliform count/100 ml	
Range of counts/100 ml	All samples	Avg. no. per plant	All samples	Avg. no. per plant
<1	16	1	26	5
1-10	10	1	11	2
11-100	16	6	1	2
110-1000	7	2	3	1
>1000	4	2	2	1
No. of samples	53	12	43	11

TABLE 4. CONTRIBUTION OF MICROORGANISMS BY FILLING APPARATUS AND CARTONS AS DETERMINED BY FILTER COUNTS AND COLIFORM COUNTS[1]

	Filter count/100 ml		Coliform count/100 ml	
Range of counts/100 ml	All samples	Avg. no. per plant	All samples	Avg. no. per plant
<1	80	1	99	4
1-10	21	0	27	4
11-100	28	3	23	3
110-1000	35	6	3	0
>1000	26	2	1	0
No. of samples	190	12	153	11

TABLE 5. PERCENT OF SAMPLES[1] FROM INDIVIDUAL PLANTS PRODUCING COLONIES IN TESTS FOR TOTAL COUNT[2] AND COLIFORM COUNT[3]

Plant no.	Filter count	Coliform count
1	86	30
2	100	64
3	63	37
4	90	60
5	83	80
6	14	13
7	63	37
8	57	13
9	79	70
10	60	13
11	45	0
12	60	—
Overall	60	37

[1]Excludes samples taken directly from the water filtration apparatus.
[2]100 ml samples.
[3]200 to 300 ml samples.

TABLE 6. MEAN FILTER COUNTS AND COLIFORM COUNTS PER 100 ML FOR ALL SAMPLES TAKEN IN INDIVIDUAL PLANTS

	Source of samples					
	Storage tank		Line & filler bowl		Filler & container	
Plant no.	FC/100 ml	CC/100 ml	FC/100 ml	CC/100 ml	FC/100 ml	CC/100 ml
1	1	<1	5000	2500	440	7
2	8	*	2000	1000	90	2
3	83	<1	95	1	70	2
4	1300	7	50	<1	5000	1
5	1000	82	<1	<1	**	50
6	<1	<1	490	70	<1	<1
7	22	<1	32	40	260	28
8	7	<1	17	<1	890	<1
9	20	<1	80	3	1600	20
10	<1	<1	1	<1	22	<1
11	<1	*	160	<1	210	<1
12	27	*	65	*	250	*

*No test
**Data unreliable for calculation of an average

6 (20%), and 8 (10%). All samples of the other plants contained bacteria.

The following plants were able to package water with coliform counts of <1/100 ml in the percentage of samples shown in parentheses: 11 (100%), 10 (90%), 8 (90%), 6 (60%), 1 (60%), 3 (55%), 7 (33%), and 9 (4%).

The logistics of the operations were such as to preclude repeated, day-after-day, sampling in the same plant. Controls were limited so that one could not always be sure that the bacteria found were not introduced by the experimenters. High levels of contamination in the pasteurized storage tank may have precluded accurate assessment of numbers of bacteria added downstream. Ideally, the method should be used in repeated sampling under the same conditions until definite trends are observed. Nevertheless, it is believed that these data are representative of existing situations in modern fluid milk plants, and that they generally demonstrate both the applicability of the rinse/filter method, including its limitations, and the sanitary status of the plants under actual conditions of operation.

Conclusions

The rinse/filter technique provides a readily useable method to determine the efficiency of cleaning and sanitization. It makes possible the approximate location of relatively low levels of contamination.

The probability of obtaining water with a count of <1/100 ml by membrane filtration under normal conditions of operation in dairy plants is about 30%. However, average counts of <2/100 ml, as obtained in this study, should be satisfactory, especially since 9 of 10 coliform counts were <1/100 ml.

Storage tanks are the least likely source of contamination of pasteurized milk. Relatively large numbers of bacteria are introduced from lines, valves, and filling apparatus. About 60% of the first few cartons of milk would be expected to become contaminated with at least 1 bacterium per 100 ml, and

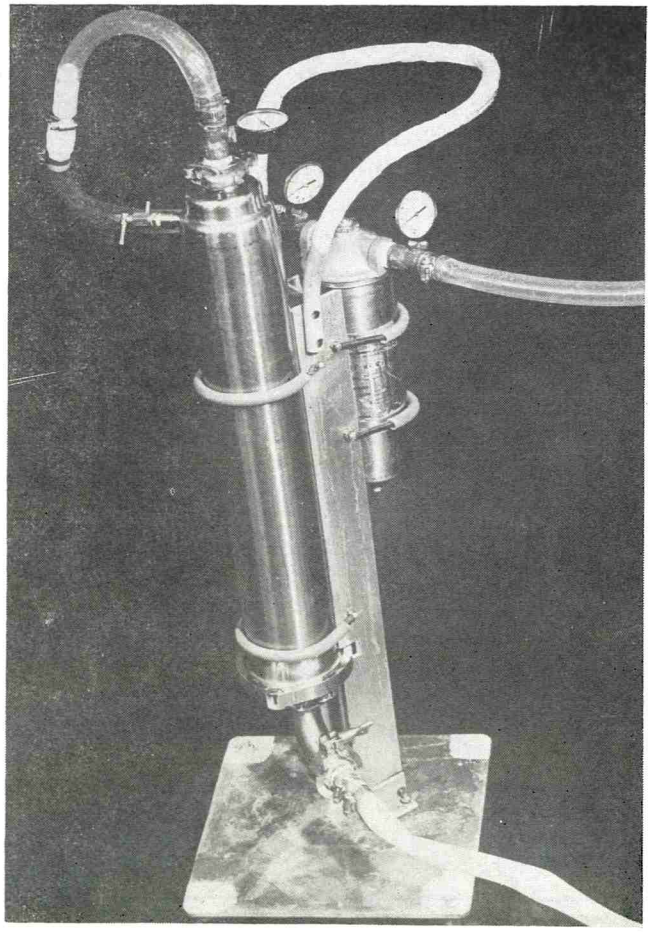

Figure 2. Apparatus used to sterilize tap water. Short cylinder contains the prefilter and long cylinder contains the membrane filter (0.45 μm).

37% would be expected to pick up at least 1 coliform per 100 ml in the modern fluid milk plant.

It is possible to package milk with a total count of <1/100 ml in today's equipment, but the probability is low. It is much more likely that products can be packaged with fewer coliforms than 1/100 ml. The plant which had the best overall performance was equipped with many special devices to assist in control of post-pasteurization contamination.

ACKNOWLEDGMENT

Appreciation is expressed to the following for providing the facilities for these studies and for assistance in collection of samples: A & P Dairy Center, High Point, North Carolina; Central Dairy, Jefferson City, Missouri; Dean Foods Co., Chemung and Huntley, Illinois; Fairmont Foods Co., Kansas City, Missouri; Foremost Foods Co., Springfield, Missouri; Hiland Dairy Co., Springfield, Missouri; Hill Farm Dairy, Melrose Park, Illinois; Kroger Co., St. Louis, Missouri; Meyer Dairy, Basehor, Kansas; Sealtest Foods, St. Louis, Missouri; and Safeway Stores, Inc., Kansas City, Kansas. (The order of listing here does not correspond to numbers listed in the paper.)

REFERENCE

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REPORT OF THE JOURNAL MANAGEMENT COMMITTEE—1972

We would like to compliment the Editor and the Managing Editor for the improvement of the Journal during the past year, not only in content and make-up, but also in considerably improved page lay-out. We would like to especially comment on the page heading showing volume, page, and date at the beginning of each article. This is exceptionally helpful. We would like to make several comments and suggestions for the year ahead:

(a) Page 1 should be used for advertising only and a study should be made as to the practicability of inserts for meeting notices, hotel reservations, subscriptions, publications, order forms, membership forms, etc.

(b) In general, we object to the placement of advertising throughout the Journal and suggest it be confined to either the front or back pages of the Journal.

(c) The News and Events Section should be better identified by using larger headings and, where possible, start at the beginning of a page. Succeeding page headings in this section would be better identified with larger type.

(d) A special effort should be made to get our meeting notice in the "coming events" sections of trade magazines, recognizing that 4 to 5 months lead time is needed for most publications.

(e) The Managing Editor should make an effort to establish a section discussing new equipment, new organizations being formed, and publications of great importance that are not readily available—for example, Ohio State's Study for EPA with information on the pollution situation in the current dairy industry. This type of information is currently provided on a hit-or-miss basis and should be more formalized.

(f) We suggest that a regular feature be established listing new publications, bulletins, etc., of interest to the membership. A small committee of people, who read a cross section of Journals rather extensively, should be established with responsibility to submit on a regular basis finished copy for publication of this type of information. There is a large number of new publications, particularly in the food service and environmental fields, appearing on a regular basis. Our membership could benefit by becoming acquainted with this material.

(g) In the increased activities relating to advertising, companies should be encouraged to use an innovated type of advertising program in our *Journal.* Since our membership is made up of people who normally do not do extensive purchasing, it might be more desirable for companies to get "a message" of what they are attempting to do for the industry into their ads—rather than using a canned picture, month after month, of equipment for sale. A good example of innovative advertising is that currently being used by Babson Bros., on the back page of the *Journal.*

W. C. LAWTON, *Chairman;* J. C. OLSON, JR., K. G. WECKEL, AND C. K. JOHNS.

LETTER TO EDITOR

March 15, 1973
International Assn. of Milk, Food & Sanitarians, Inc.
Box 437
Shelbyville, Indiana 46176
Gentlemen:

Recently I had occasion to visit the Tampa Independent Dairy Farmers Association, Inc., and was so impressed with the efficiency of the lab there.

Also, one young man impressed me so with his courtesy to the dairymen and those contacting the lab, I wish to mention it to your group.

I don't do these things often, but I was so impressed with his sincerity, his willingness to assist people and his attitude of concern that I was prompted to inquire of an association I could mention this to.

Wish more people were as pleasant to others as this young man named Kenneth J. Crothers, lab technician, of Tampa, Florida.

Sincerely,
Mrs. E. E. Snyder
1602 E. Kirby St.
Tampa, Florida 33604

HEALTH FOODS VERSUS TRADITIONAL FOODS: A COMPARISON[1]

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(Received for publication January 8, 1973)

Abstract

Health and traditional foods (24 of each) were compared for proximate composition, cost, microbial content, pesticide levels, and polychlorinated biphenyl (PCB) contamination. Only minor differences were detected for proximate composition and microbial content. No pesticides were detected but 7 samples of health and 3 of traditional foods contained PCBs. The major difference between the samples was the greater cost of the health foods.

Today the safety and nutritional quality of our national food supply is being questioned. Advocates of health and organic foods are proclaiming that their products are safer and more nutritious than traditional foods *(5)*. While trained scientists can detect many of the fallacies claimed, numerous Americans are confused and accept the arguments of the health food proponents *(1)*. In view of the widespread current interest in health and organic foods *(8)*, a survey was conducted to establish whether there are any gross differences in nutritional quality, microbial contamination, and pesticide residue content between foods obtained from health food stores and corresponding conventional foods obtained at supermarkets.

Materials and Methods

Twenty four food samples were obtained from health food stores in the Gainesville, Florida area. Comparable foods were obtained from a chain store supermarket and designated as traditional foods. Unit price of each was noted at time of purchase.

Microbial analyses were by APHA methods *(2)* and proximate composition by AOAC *(3)*. Samples for pesticides and polychlorinated biphenyls (PCB's) were dried and ground through a 20-mesh screen. Liquid samples were mixed with anhydrous sodium sulfate until a free-flowing powder was obtained. Samples were then extracted for 12 hr with petroleum ether in a Soxhlet apparatus. Extract purification, detection, and quantification were conducted according to Burchfield and Johnson *(6)*. This method will detect PCBs, chlorinated pesticides, and many of the organophosphate pesticides.

When PCB's were detected in samples, extracts were subjected to silica gel chromatography described by Snyder and Reinert *(7)*. This technique allows the separation of PCBs and other pesticides present.

The identities of PCBs were confirmed in a few samples by preparing the perchloro-derivative *(4)* and subjecting it to gas chromatography and mass spectrometry.

Results and Discussion

Proximate composition and unit price are given in Table 1. The results show a similarity in chemical composition between the two groups of foods. This similarity can be quite marked, particularly when the samples are from the same processor, as was true of the honey samples. Occasional differences in composition, observed in products such as coconut, ice cream, and brazil nuts, probably result from formulation and processing, rather than from inherent differences between the two samples, although the effects of biological variation cannot be completely discounted. Cost ratio data, however, show that the health foods are expensive, averaging 1.7 times higher than the traditional products. Traditional powdered milk, coconut, dates, nuts, and one cereal product are the exceptions. The higher cost of these conventional commodities probably results from the increased processing involved in preparing the traditional product (i.e., pitting, roasting, instantizing).

The microbial analyses are typical of foods in normal channels and do not necessarily indicate that one group of foods was of better microbial quality than the other; although, coliform analysis, a sanitary index, was positive for 3 of the traditional and 6 of the health foods. With the health foods, much of the contamination can be accounted for by the lack of adequate packaging. The high total aerobic counts for the cheese samples is not unusual in a fermented product, and the high count in the traditional wheat cereal was found to be due to sporeforming bacilli.

Pesticides were not present in any of the samples in excess of 0.01 ppm (well below most Federal tolerances), the lower limit of method sensitivity, although 7 of the health and 3 of the traditional foods were found to contain PCBs. This contamination was considered unusual and points out the widespread distribution of these environmental contaminants in the food chain.

The data illustrate that foods whether labeled "health" or not may be contaminated with a variety of environmental constituents and that purchase of foods from a specialty store does not guarantee purity.

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[1]Florida Agricultural Experiment Stations Journal Series No. 4626.

TABLE 1. PERCENTAGE PROXIMATE COMPOSITION AND UNIT COST OF HEALTH (H) AND TRADITIONAL (T) FOODS

	Moisture		Protein		Fat		Ash		Carbohydrate		Cents/oz		Ratio
Products	H	T	H	T	H	T	H	T	H	T	H	T	H/T
Bread, whole wheat	36.8	35.4	11.9	12.2	1.9	2.2	2.6	2.2	46.9	48.0	3.4	2.1	1.62
Cheese, Cheddar	38.2	37.7	22.6	24.0	30.0	29.9	3.3	3.4	5.9	5.0	11.6	6.6	1.76
Milk, dry	4.3	5.3	34.3	34.1	0.1	0.1	7.9	7.6	53.3	52.9	4.3	5.1	0.84
Cashews	3.8	2.5	20.1	18.7	45.2	47.7	2.2	6.1	28.8	25.1	10.6	10.7	0.99
Peanut butter	0.2	0.5	28.9	28.9	47.7	48.4	2.7	3.4	20.6	18.8	5.6	4.2	1.33
Rice	10.8	11.8	8.1	8.4	0.6	0.5	1.4	0.5	79.0	78.8	1.8	1.2	1.50
Honey, Tupelo	15.1	15.1	0.3	0.3	0.2	0.2	0.1	0.1	84.4	84.4	8.1	3.6	2.25
Cereal, whole wheat	11.9	9.2	11.8	12.6	0.6	0.7	2.3	1.5	73.4	75.9	4.4	3.0	1.47
Preserves, blackberry	32.9	26.9	0.9	0.8	0.1	3.4	0.2	0.2	65.9	68.7	6.8	3.7	1.84
Ice cream, peach	56.3	53.4	4.2	4.4	7.4	14.7	0.7	0.7	31.4	26.9	4,3	0.9	4.78
Pecans	3.3	3.1	13.2	12.0	67.1	68.9	1.8	1.6	14.6	14.5	15.3	16.5	0.93
Pancake mix	8.4	8.0	14.7	10.9	2.9	9.3	4.6	4.3	69.5	67.5	3.7	1.9	1.95
Mayonnaise	8.7	9.4	2.5	2.4	80.9	79.8	1.3	1.2	6.6	7.2	4.7	4.0	1.18
Tomato juice	95.6	91.0	0.8	0.9	0.0	2.3	0.7	1.0	2.9	4.8	2.9	1.0	2.90
Almonds	4.1	3.6	26.0	25.5	46.3	47.3	3.0	3.0	20.7	20.6	10.4	10.9	0.95
Rice cereal	16.9	9.1	5.8	5.8	0.7	0.5	0.8	0.3	75.8	84.2	6.5	7.0	0.93
Apples, dried	13.8	16.6	1.0	0.8	0.2	0.2	1.5	2.7	83.5	79.7	8.4	5.6	1.50
Fig bars	16.6	14.3	5.4	5.7	4.3	5.9	5.4	1.6	68.3	72.5	4.2	3.0	1.40
Brazil nuts	2.4	2.2	18.7	19.1	60.8	24.8	3.5	3.4	14.6	50.5	7.6	13.0	0.58
Apple juice	88.6	87.5	0.4	0.1	0.3	0.1	0.2	0.2	10.5	12.1	2.9	0.9	3.22
Apple butter	50.9	53.2	0.5	0.4	0.0	0.0	1.7	0.3	46.9	46.1	5.9	1.3	4.54
Corn snacks	6.7	2.0	9.4	5.5	28.2	28.8	4.6	2.7	51.1	61.0	14.0	7.5	1.87
Dates	20.8	20.5	2.0	1.4	0.1	0.1	1.4	1.6	75.7	76.4	3.3	4.1	0.80
Coconut	2.6	16.1	8.6	4.8	63.0	21.7	1.8	0.9	24.0	56.5	3.0	4.9	0.61

TABLE 2. AEROBIC PLATE COUNT, NUMBER OF COLIFORM ORGANISMS AND POLYCHLORINATED BIPHENYL (PCB) LEVELS IN HEALTH (H) AND TRADITIONAL (T) FOODS

	Aerobic plate count[1]		Coliforms[1]		Polychlorinated biphenyls[2]	
Products	H	T	H	T	H	T
Bread, whole wheat	200	<10	0	0	0.00	0.00
Cheese, cheddar	9,600,000	14,000	0	3	0.00	0.00
Milk, dry	990	240	0	0	0.00	0.00
Cashews	650	<10	9	0	5 (Arochlor 1254)	0.00
Peanut butter	280	50	0	0	0.00	0.00
Rice	4,800	220	23	0	0.00	0.00
Honey, Tupelo	55	90	0	0	0.00	0.00
Cereal, whole wheat	<10	51,000	0	0	1.5 (Arochlor 1242)	0.00
Preserves, blackberry	<10	<10	0	0	0.00	0.00
Ice cream, peach	58,000	75	23	0	0.00	0.00
Pecans	200	40	0	4	4 (Arochlor 1254)	0.00
Pancake mix	1,900	4,000	4	29	5 (Arochlor 1242)	5 (Arochlor 1242)
Mayonnaise	<10	<10	0	0	0.00	0.00
Tomato juice	90	<10	0	0	0.00	0.00
Almonds	450	910	0	0	5 (Arochlor 1254)	4 (Arochlor 1254)
Rice cereal	30	35	0	0	4 (Arochlor 1254)	4 (Arochlor 1254)
Apples, dried	70	<10	0	0	0.00	0.00
Fig bars	300	4,000	0	0	0.00	0.00
Brazil nuts	350	850	4	0	2.5 (Arochlor 1254)	0.00
Apple juice	<10	<10	0	0	0.00	0.00
Apple butter	<10	<10	0	0	0.00	0.00
Corn snacks	12,000	15	0	0	0.00	0.00
Dates	390,000	25	0	0	0.00	0.00
Coconut	4,200	90	9	0	0.00	0.00

[1]Number organisms per gram of product.
[2]Parts per million based on weight received.

Furthermore, while few differences were found in proximate composition of these foods, this does not imply that other more subtle differences in flavor, vitamins, or functionality might not exist. However, the question must be raised as to whether these factors, if indeed they exist, justify the rather substantial price difference between these two groups of foods.

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(Notice to membership—ballots can only be mailed to paid up members as of May 15, 1973)

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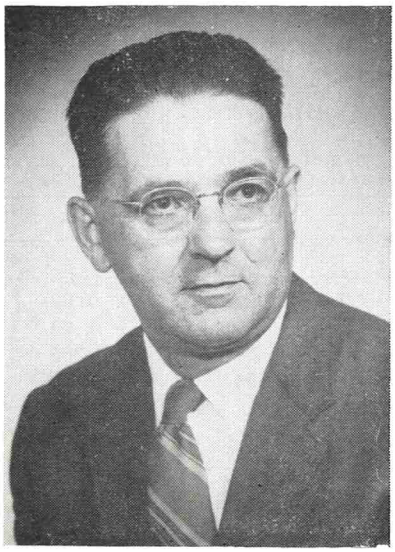


HENRY V. ATHERTON

Henry V. Atherton is Professor of Dairy Industry and Dairy Bacteriologist in the Animal Sciences Department at the University of Vermont. He is a Vermonter by birth and received his B.S. and M.S. Degrees at the University of Vermont as a Dairy Manufacturing major. He obtained is Ph.D. at Penn State in 1953 and then returned to Vermont to the position he now holds.

His chief research interests are in the area of milk quality control as influenced by bulk cooling on farms, farm water supplies, and dairy sanitation. He is author or co-author of a number of papers which have appeared in technical and trade journals in the dairy field and is co-author of the textbook "The Chemistry and Testing of Dairy Products."

He is an active participant in the Farm Methods Committee of the International Association of Milk, Food, and Environmental Sanitarians and is on the Executive Board of the Northeast Dairy Practices Committee. He is Secretary of the Vermont Dairy Industry Association and a member of the New York State Association of Milk and Food Sanitarians as well as other sanitarian's groups in the Northeast.

JOHN C. BRUHN

John C. Bruhn is an extension food technologist with the University of California Agricultural Extension and works out of the Department of Food Science and Technology located on the Davis campus and is a member of the food science graduate research group. He also holds a lecturer title in the Department of Food Science and Technology. At present extension programs account for 100% of his time.

He was raised in Ohio and attended Michigan State University, receiving a B.S. degree in Food Science in 1962. He then entered the graduate school at the University of California, Davis, and was awarded the Ph.D. degree in Microbiology in 1969. Immediately following graduation, Dr. Bruhn was hired as extension food technologist and given the re-

JOHN C. BRUHN

sponsibility of developing research and educational programs for the California dairy foods industries.

Both his research and educational programs have concerned themselves with the quality of milk and dairy foods, with principal emphasis on flavor and composition.

He is active in a number of state organizations, including the California Dairy Industries Association where he has held several offices, the California Association of Milk and Dairy Sanitarians, the California Creamery Operators Association and the Northern California Section of the Institute of Food Technologists. In addition, he has served on committees of the American Dairy Science Association and holds memberships in the Institute of Food Technologists, and International Association of Milk, Food and Environmental Sanitarians.

Recently married, Dr. Bruhn and his wife, Christine, live in Davis, California.

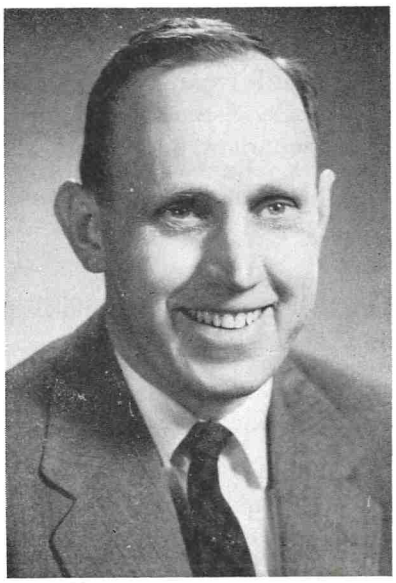
SECRETARY-TREASURER

Richard P. March is a professor in the Department of Food Science at the New York State College of Agriculture, Cornell University, Ithaca, New York. Until 1965, he devoted 75% of his time to extension work as a specialist in milk quality and fluid milk handling and processing, and the balance of his time in research and teaching courses in fluid milk processing and quality control. At present, extension accounts for 90% of his time with 10% for research activities.

He was raised in Massachusetts, majored in dairy industry at the University of Massachusetts, receiving a B.S. degree in 1944. After a tour in the U. S. Marine Corps, he entered the Graduate School at Cornell University to major in dairy industry, receiving an M. S. degree in 1948.

Professor March taught a one-year program in dairy manufacturing until its termination in 1951, at which time he was promoted from instructor to assistant professor. He became an associate professor in 1955, and full professor in 1965. In 1965 he also became department extension leader and is still serving in this capacity.

He is active in the New York State Association of Milk and Food Sanitarians, serving as secretary-treasurer from 1957 and executive secretary since 1967, secretary of the Dairy Industry Equipment



RICHARD PELL MARCH

Committee from 1952-57, secretary of the Farm Practices Committee from 1955-62, and secretary of the Council of Affiliates since 1952. He is a member of the International Association of Milk, Food, and Environmental Sanitarians, a member of their Farm Methods Committee from 1959-65, secretary of their Council of Affiliates in 1961, and chairman in 1962 and 1963.

In both the State and International Associations he has served as chairman of a number of subcommittees including the Uniform Milkhouse Plans for the Northeast, Milk Transfer Systems, Sediment Testing, and Training Programs for Bulk Tank Truck Operators, and co-chairman of the Northeast Committee on Uniform Guidelines for Loose Housing Systems. In 1963 he was the recipient of the New York State Association's Dr. Paul B. Brooks Memorial Award for outstanding contributions to the organization.

KENTUCKY ASSOCIATION OF MILK, FOOD AND ENVIRONMENTAL SANITARIANS, INC. 1973 EDUCATIONAL CONFERENCE

The 1973 Educational Conference for Fieldmen and Sanitarians was held February 27-28, 1973 at the Executive Inn Motor Hotel, Louisville, Kentucky.

A total of 280 (county and state health department sanitarians, milk and food industry fieldmen, plant managers and related service companies and University officials) were registered. States other than Kentucky represented were as follows: Georgia, Indiana, Missouri, Ohio, Tennessee, Wisconsin, Alabama, Mississippi and Minnesota.

The program was broken into general sessions, food and environmental sanitarians sections and milk sanitarians sections. Fourteen papers were presented during the conference.

Te following awards were given at the awards banquet by the Kentucky Association of Milk, Food and Environmental Sanitarians, Inc.

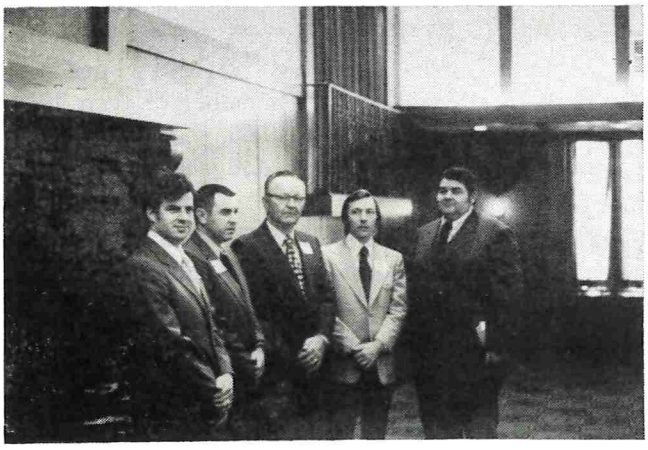
Outstanding Sanitarian Award: Harold Walden, Christian County Health Department, Hopkinsville, Kentucky.

Outstanding Fieldman Award: William McCormick, Dairymen Inc., Owensboro, Kentucky.

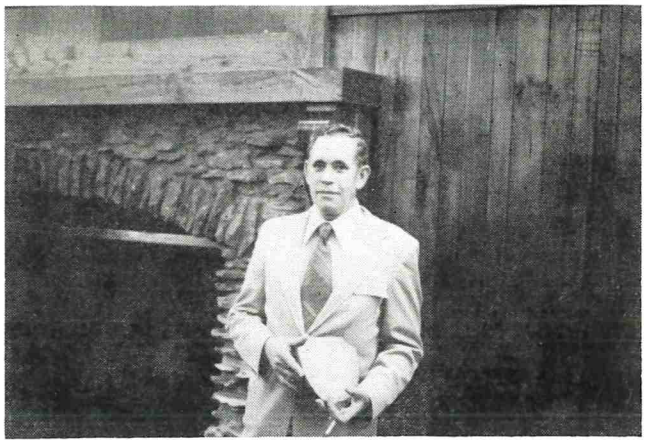
Outstanding Service Award: Roy L. Scott, Avalon Cheese Company, London, Ky.

A past presidents plaque was also presented to out going president, Donald Colgan, Fleming County Health Department, Flemingsburg, Kentucky.

New Officers and Directors elected at the business meeting were as follows: President, Dudley J. Conner, Kentucky State Health Dept., Frankfort. President-elect, Dr. James C. Hartley, University of Kentucky, Lexington. Vice President, Bruce K. Lane, Louisville-Jefferson County Health Department, Louisville, Kentucky. Secretary-Treasurer, Leon Townsend, Kentucky State Health Dept., Frankfort. Directors: Midwestern Region: Doug Perkins, Dean Foods, Glasgow; Hubert D. Edds, Daviess Co. Health Dept., Owensboro. North Central Region: A. P. Bell, Louisville-Jefferson Co., Health Dept., Louisville, Kentucky. Floyd Gritton, Kraft Foods, Owenton, Kentucky. Paul Devine, Cudahy Company, Harrodsburg, Ky.



1973 Officers–KAMFES. Left to right: Pres. Elect, Dr. James Hartley; Sec.-Treas., Leon Townsend; Director, Hubert D. Edds; Pres., Dudley J. Conner and Past Pres., Donald L. Colgan.



1973 Outstanding Sanitarian Award. Harold "Shine" Walden, Christian County Health Dept., Hopkinsville, Ky.

The conference was sponsored by the Kentucky Association of Milk, Food and Environmental Sanitarians, Inc.

NOTICE TO MEMBERSHIP

After many years of hopes and frustrations toward a possible merger of International Association of Milk, Food and Environmental Sanitarians and the National Environmental Health Association (Formerly N.A.S.) some preliminary considerations have again been made by their respective Boards of Directors. The one step agreed to at this time is to poll the memberships on their views.

The poll is being conducted by IAMFES in connection with the election of Officers for 1973-74. May we please ask that you all respond on this important matter. We trust that this will also increase the response for the Officer's election which is always an important function of any organization.

The identical wording of the questions will also be sent out by N.E.H.A. to their membership. When each has completed the survey, there will be further talks between Charles Gillham, President of N.E.H.A. and myself.

Walter F. Wilson,
President

SEMINAR NOTICE

The Department of Food Science and Technology at the University of Massachusetts will sponsor a seminar on "Establishing Thermal Process Requirements for Food Sterilization." The seminar will be held in the Campus Center on June 25 to June 28, 1973.

Seminar registrants will be limited to fifty in number. University faculty and industry experts will comprise the course instruction staff.

For details contact Prof. Kirby M. Hayes or Prof. Charles R. Stumbo, Department of Food Science and Technology, University of Massachusetts, Amherst, Massachusetts 01002.

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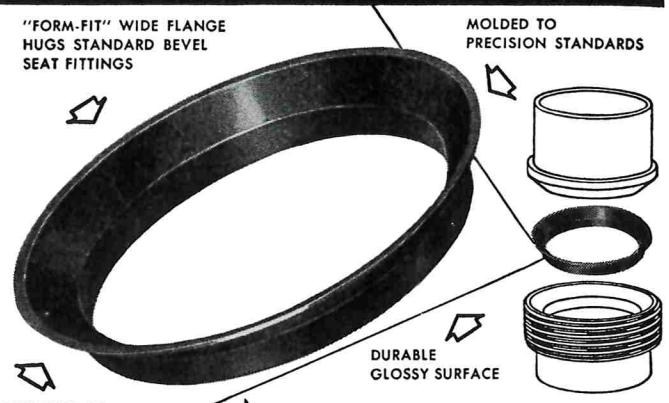

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Dairy authorities speak out
on better cow milking.

The importance of individual quarter milking

George D. Marx / University of Minnesota / Crookston

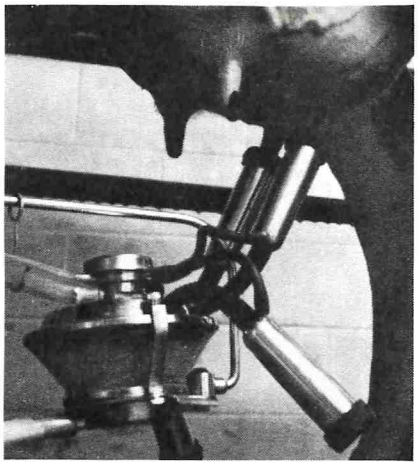
Characteristics of individual quarters of a cow's udder were studied using special equipment for milking 60 cows at the University of Minnesota, Northwest Experiment Station, Crookston. Sixteen of these cows were milked with a quarter milking machine at every milking during the entire lactation.

Factors such as milking time, machine stripping, amount and percent of complementary milk, incidence of mastitis and treatment, loss of milk on quarters with mastitis, bacterial organisms and abnormal physical condition of the udder and teat were studied.

The average milk yield of each quarter in percentage of total yield of the entire udder was 20.4% for the RF, 30.2 for the RR, 30.7 for the LR and 18.7 for the LF quarter. Similar percentages were obtained when comparing morning and evening milkings. Rear quarters produced 61% of the total milk and front quarters 39% for the total lactation; however, during the first and second month of lactation, rear quarters produced 58% and front quarters 42% of the total production and, by the ninth and tenth month of lactation, rear quarters were producing 66.5% and front quarters only 33.5% of the milk. Average daily production was 48.2 pounds.

Average milking time including machine stripping for the RF was 3.86 minutes, RR 4.42, LR 4.57, and LF 4.04 minutes. There were distinct differences between the milking rate of the higher-yielding rear and lower-yielding front quarters. Rear quarters produced an average of 0.54 pounds more milk per minute than front quarters, took 9% longer to milk and took 36% longer to machine strip.

Front quarters milk out faster than rear quarters, so dairymen should be prepared to take the machine off those quarters when they are milked out to avoid mastitis. Most herds have just as much mastitis on the front quarters as rear quarters, despite the lower amount of milk produced and less stress on front quarters. This high incidence of mastitis is likely caused by over-milking.



Complementary milk is the milk remaining in the cow's udder after normal milking is completed and can be obtained by giving oxytocin injections. Monthly checks of the cows in the experiment showed the percent complementary milk on the front two quarters averaged 17.8% and rear quarters averaged 14.4%. Cows with a high percentage of complementary milk will decrease in milk production faster during the lactation cycle than those with a low percentage. Individual quarter milking is likely to keep complementary milk to a minimum and still avoid the hazards of over-milking individual quarters.

MILKING PROCEDURE

Quarter milking is important but only one part of proper milking procedure. The program used at the Northwest Experiment Station at the University of Minnesota is keeping mastitis under control. The herd of 60 cows once had mastitis infections in 30% of the quarters but this has been reduced to below 1% at the present time, using the following type of milking management:

1. The strip cup is used at every milking to detect clinical mastitis.
2. Routine use of the CMT test to detect subclinical mastitis.
3. Organisms causing mastitis are identified by laboratory culturing of milk samples on every quarter four times a year.
4. Milking equipment is maintained and kept in good repair. Our policy has been to follow the manufacturer's recommendations.
5. A teat dip is used immediately following each milking.
6. We treat all persistent infections during the dry period.
7. Continual attention is given to proper milking procedures.

The ultimate goal for this research project and our general recommendations are to help dairymen get top production and a respectable margin of profit from dairying.