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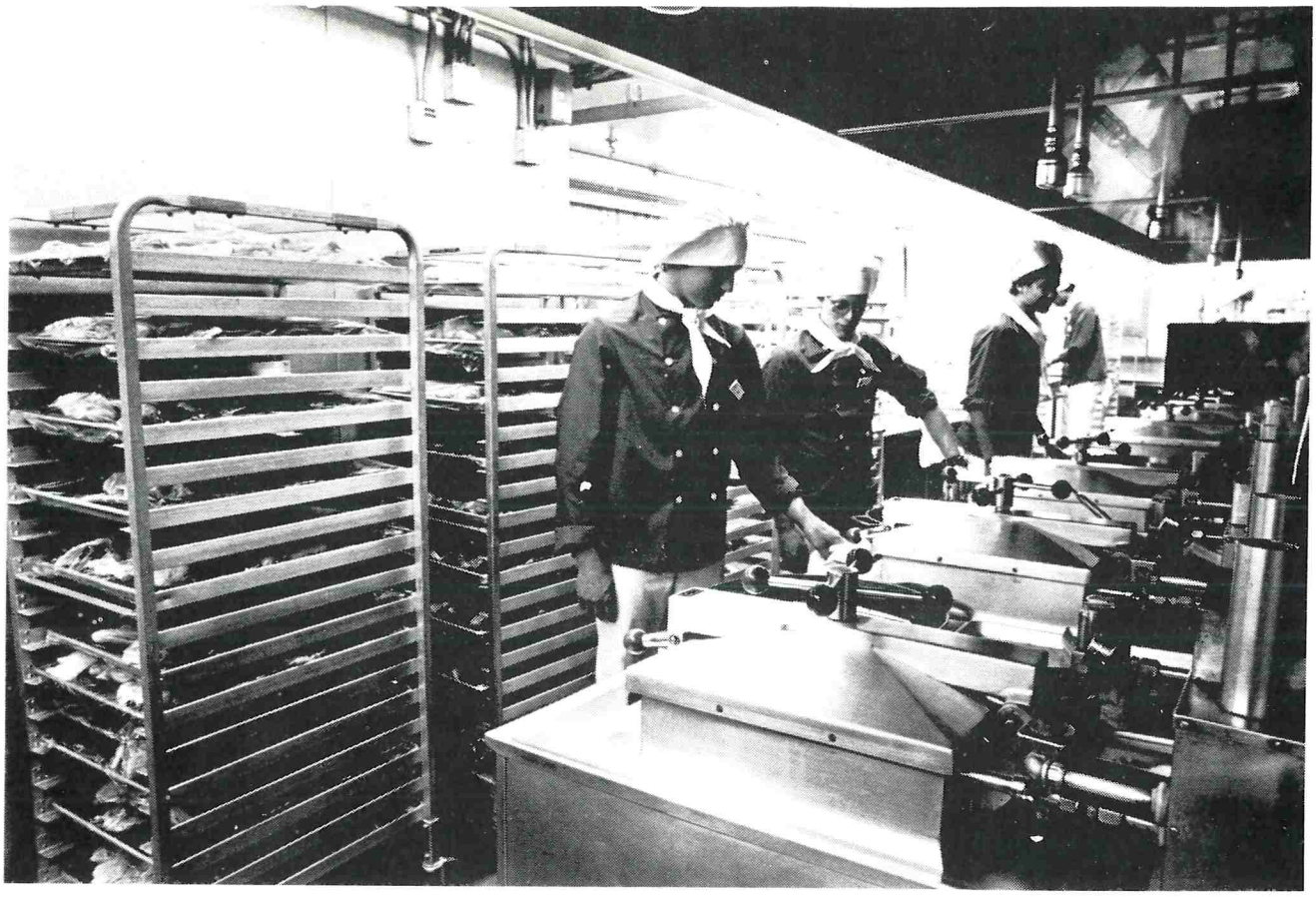

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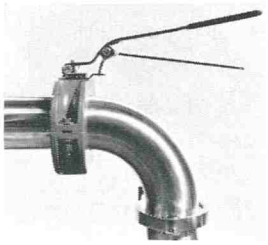

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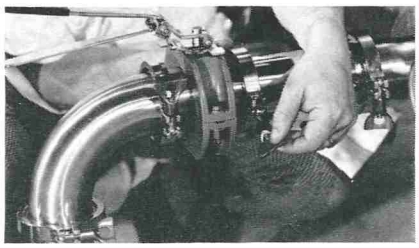

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Consumer Beliefs Concerning Intentional and Unintentional Additives in Health Food Breads: Chlorinated Hydrocarbon Pesticide Residues

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(Received for publication May 26, 1977)

ABSTRACT

The majority of one-hundred and fifty respondents to a consumer knowledge questionnaire believed breads purchased in health food stores to be free of pesticides as well as other additives. To determine the correctness of this belief, analyses were completed to compare the presence of PCBs (polychlorinated biphenyls) and 12 chlorinated hydrocarbon pesticides in five brands of bread purchased in health food stores ("health" breads) and five purchased at supermarkets ("traditional" breads). One "health" bread actually contained stoneground organic whole wheat flour. No statistical differences were noted between the breads. The average level of chlorinated hydrocarbon pesticide contamination for each "health" bread as well as for each "traditional" sample was 4.8 ppb based on the weight of a 100-g, wet sample. There was no statistical difference in the moisture content between the two bread types. The product using "organic" flour also contained residues. The most commonly occurring residue and the one present in highest concentrations was p,p′-DDT. All residues, though, were present at extremely low levels. PCBs were not detected in any sample. The data indicate that the high price of "health" breads is not justified solely by the fact that these products are pesticide free or contain residues below "traditional" samples. Some form of misrepresentation of health food breads seems evident. The consumer should be informed that it is virtually impossible to purchase any food items including "organic" products, that are totally free of unintentional food additives.

A popular health movement emphasizes "natural" and/or "organic" foods. A "natural" food is one containing no intentionally added additives such as preservatives or emulsifiers but possibly grown using pesticides and synthesized fertilizers. "Natural" food is usually displayed in its original form as produced. The term "organic" was popularized by J. Rodale to include all products, from production to consumption, untreated with pesticides, antibiotics, synthesized fertilizers and other chemicals. The term "health food" pertains to "organic" as well as to "natural" (10).

According the the American Medical Association, the term "health food" is a misnomer. Any one food or any one nutrient should not be labeled a "health food" or "health nutrient." Some 50 nutrients, in varying amounts, contribute to an individuals' health (*4*). Regulatory agencies have called this term misleading because it implies conventional foods are not as healthful (*10*). Organic pertains to any compound containing carbon which includes practically everything produced by plant or animal life. No one food should be specifically called "organic" as the term pertains to all foods (*13*).

Many foods labeled as "organic" are actually not grown organically (2,3,6,7,10,11). The United Fruit and Vegetable Association has reported many instances of mislabeling by dealers who simply removed or obliterated the original trade markings from conventional produce and sold the product as "organic" and at higher prices (*5*).

Foods grown organically are not necessarily free of environmental pollutants, for certain pesticides may remain in the soil for years after their use is discontinued (*9*). Chemicals sprayed on one field or crop may drift through the air to other fields. Margolius notes in *Health Foods Facts and Fakes*, "Whether the residues in foods sold as organic occur through deliberate use to protect against blight or because they are 'in the air and soil,' as organicists tend to say, they are there. Certainly, it would be especially difficult for the health food industry to control pesticides on imported ingredients and products" (*11*). A survey on food safety reports that consumers are concerned about pesticide residues (*8*). Jukes of the University of California notes, "the extra price charged for 'health' foods is presumably paid by the purchaser because he or she thinks that the foods are free from pesticide residues. Obviously, misrepresentation is common" (*7*).

The purpose of this study was to determine consumers' interpretation of such terms as "health," "organic," and natural" foods and to ascertain if there is any benefit in purchasing such foods with respect to chlorinated hydrocarbon pesticide and PCB residues. The product

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[3]*Michigan State University.*

for study was bread, since bread is a popular item in health food stores (*14*).

MATERIALS AND METHODS

Consumer knowledge questionnarie

Two questions were designed to determine customers' interpretation of the term "health food" and whether the consumer thinks such foods to be free of pesticide residues. One hundred and fifty individuals participated in the survey. Subjects were approached randomly throughout the immediate Detroit area. Each respondent immediately completed and returned the questionnaire to the investigator. The data were statistically analyzed by the Chi square test (with Yates' correction for continuity used for all 2 × 2 contingency tables) to determine any significant difference in the distribution of responses between individuals indicating that they shopped at health food stores (sample n_1) and those noting that they have never shopped at such stores (sample n_2). In multiple foil questions with a significant difference between the distribution of responses, all incorrect foils, including the "no response" choice, were grouped under the label "incorrect answers." The resulting 2 × 2 contingency tables were then calculated, using Yates' correction for continuity, in order to significantly associate correctness with either of the two groups.

Bread sample selection and pesticide tests

Ten different brands of bread with wheat as the primary grain, all free of preservatives, were purchased throughout a 50-mile radius of Detroit. Five were from major health food stores (health food stores consisting of at least two locations within the Detroit vicinity) and five from chain supermarkets. For brevity, breads used in this study that were not considered health food products will be called "traditional." The bread was frozen to await subsequent pesticide and PCB analyses which were done at the Pesticide Research Center, Michigan State University. The following chlorinated hydrocarbons were determined: hexachlorobenzene; lindane; heptachlor; aldrin; kelthan (a commercial name for 1,1-bis (p-chlorophenyl)-2,2,2-trichloroethanol); heptachlor epoxeid;p,ṕ-DDE; dieldrin; endrin; p,ṕDDD; p,ṕ-DDT; methoxychlor and PCB (polychlorinated biphenyl). One-Hundred grams of bread was broken into small pieces and placed in a 600-ml beaker with 300 ml of redistilled hexane. The mixture was blended for 10 min using a Teckmar Tissumizer. The resultant solution, containing very fine bread particles, was filtered through a Buchner funnel. The bread filtrate was again blended 5 min with an additional 200 ml of hexane and refiltered. The combined hexane extract was air evaporated to about 10 ml, dried over anhydrous sodium sulfate and chromatographed through a 2 × 1/2 cm column of florisil using an extra 50 ml of hexane for elution. The resultant hexane solution was air evaporated to about 1 ml and transferred to a 2-ml volumetric flask and filled to the mark with hexane. A portion of the final hexane solution was then analyzed on a Beckman GC-72-5 gas chromatograph equipped with an electron capture detector, a Varian automatic sample injector, and a 6′ × 2mm I.D. glass column packed with 11% (QF-1 + OV-17) on 80/100 Gas-Chrom Q. The actual amounts of QF-1 and OV-17 in percent were, respectively: 6.1 and 4.8. The column, injector, and detector temperatures in Celsius were respectively: 210, 240, and 300. The chromatographic analog signal was fed to a PDP-8/e computer and reduced to peak area/retention time data which was then sent to a PDP-11/40 computer for final data processing (*12*). An F-test was done to determine equality of within-group variance and the data also analyzed by using pooled-t statistics.

RESULTS AND DISCUSSION

Nineteen percent or 29 of the 150 respondents reported shopping in a health food store for bread. No statistical difference was detected in the distribution of the sexes, occupations or ages as far as shopping or not shopping at health food stores. (Table 1) There was no statistical difference between the responding groups in reference to the question about the term "health food." (Question 1) The majority, of both groups, those shopping and those not shopping in health food stores, thought the term referred to both "organic" and "natural" foods.

Question 2 read: "What bread bought in a health food store is free of pesticides, chemical fertilizers, preservatives, emulsifiers, and artificial ingredients?" There was no statistical difference between the two groups answering this question with a majority of respondents answering incorrectly. It is interesting to note that, of the five baking companies producing the five brands of bread that were purchased in health food stores, two indicated that they no longer claim their product to be "organic," one used the term "natural" but admitted using bleached flour, consequently, this product cannot be listed as "natural" or "organic," one gave no reponse and only one still used the term "organic" on the label. Today, claims are not

TABLE 1. *A comparison of responses to questions about health foods between individuals who shop in health food stores (n_1) and those never having shopped in such stores (n_2). (Percentages)*

	N=150	n_1=29	n_2=121	P
My occupation is:				
Clerical and sales	35.3	41.4	33.9	
Professional, technical, managerial	26.7	41.4	23.1	
College student (non-nutrition major)	12.0	6.9	13.2	
Housewife	10.0	3.4	11.6	
Miscellaneous[a]	7.3	3.4	8.3	
Service	5.3	3.4	5.8	
Retired	3.3	0.0	4.1	NS
My age is:				
17-19	6.6	3.4	7.4	
20-24	16.6	6.9	19.0	
25-29	26.6	37.9	24.0	
30-34	15.3	17.2	14.9	
35-39	8.0	17.2	5.8	
40-44	5.3	6.9	5.0	
45-49	5.3	3.4	5.8	
50-54	5.3	3.4	5.8	
55-59	4.6	3.4	5.0	
60 and over	6.0	0.0	7.4	NS
My sex is:				
Male	57.3	58.6	57.0	
Female	42.7	41.4	43.0	NS
How often do you shop for wheat bread in health food stores?				
Never	80.7	0.0	100.0	
Sometimes	16.7	86.2	0.0	
Always	2.7	13.8	0.0	
1. What does a "health food" mean?				
a. It is organic-free of pesticides and chemical fertilizers	15.3	10.3	16.5	
b. It is natural-without preservatives, emulsifiers, or artificial ingredients	35.3	34.5	35.5	
c. Both a and b above	43.3	55.2	40.4	
d. None of the above	6.0	0.0	7.4	
e. No response[b]	0.0	0.0	0.0	NS
2. Wheat bread bought in a health food store is free of pesticides, chemical fertilizers, preservatives, emulsifiers, and artifical ingredients.				
a. True	62.7	65.5	62.0	
b. False (Correct Response)	31.3	27.6	32.2	
c. No Response	6.0	6.9	5.8	NS

[a]Misc. includes: Farming, fishing and related areas; processing: machine trade; bench work; structural work: misc., and unemployed. *Dictonary of Occupational Titles 2: Occupational Classifications,* 3rd ed., U.S. Dept. of Labor.

[b]A "No Response" category was included in the table to represent those people who didn't answer particular questions. A "Don't Know" category was not included in the questionnaire, as many respondents choose the foil simply for expediency.

TABLE 2. *Chlorinated hydrocarbon analyses of health food and traditional[a] breads (Concentration is in ppb based on the weight of a non-dried sample)*[b]

Pesticides[d]	Health food breads[c]					Traditional breads[c]				
	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10
Hexachlorobenzene	N.D.[e]	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	3
Lindane	N.D	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	1	N.D.
Heptachlor	N.D.	N.D.	N.D.	N.D.	N.D.	1	N.D.	N.D.	1	N.D.
Aldrin	N.D.	N.D.	1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Kelthane	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Heptachlor epoxide	N.D.	N.D.	N.D.	N.D.	N.D.	1	N.D.	N.D.	N.D.	N.D.
p,p′-DDE	N.D.	1	N.D.	N.D.	1	1	1	1	N.D.	1
Dieldrin	N.D.	N.D.	N.D.	N.D.	3	N.D.	N.D.	1	N.D.	N.D.
Endrin	1	N.D.	1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
p,p′-DDD	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
p,p′-DDT	3	2	2	3	6	N.D.	3	4	2	3
Methoxychlor	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

[a]Breads purchased in supermarkets.

[b]The average moisture content for health food breads was not statistically different from that of traditional products. The mean percentages ± standard deviation are, respectively: 39.1 ± 3.5 and 38.5 ± 6.0. For those wishing to convert wet weight sample data to dry weight the mositure content in percent for each bread type is given in numerical order beginning with bread number 1: 33.7, 38.8, 40.0, 43.5, 39.5, 34.2, 33.8, 38.5, 48.7, 37.2.

[c]Residues in health food products were not significantly different from those in traditional samples.

[d]PCB's (1254 standard Aroclor) were not detected at a detection limit of 50 ppb.

[e]N.D. = Not Detected (less than 0.5 ppb) A value of 0.5 ppb was rounded up to 1 ppb. Values at this level of detection could vary ± 100%. Recoveries of spiked samples at the ppb level are typically 80% ± 25%.

usually made that a product is "organic" but the consumer, when purchasing materials in health food stores, believes that the food is "natural" and/or "organic" as well as pesticide free as a majority of our respondents did. These data support the statement by Jukes, viz., "the extra price charged for 'health' foods is presumably paid by the purchaser because he or she thinks that the foods are free from pesticide residues" (*7*).

Results of the chlorinated hydrocarbon and PCB analyses are listed in Table 2. No significant difference was found between breads as purchased in health food stores and "traditional" samples. Health food products analyzed did actually contain pesticides. Perhaps the most important features of the data are that "traditional" breads do not have chlorinated hydrocarbon pesticide or PCB residues markedly above health food products and that all values are exceptionally low. These data on bread samples agree well with investigations on other food products (either "natural" or "organic") in that pesticide residues were detected (2,3,6,7,9-11).

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Estimation of Tryptophan Content by Spectrophotometric Methods: Analysis of the Interaction Between Alpha-Ketoglutaric Acid and Tryptophan

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(Received for publication August 3, 1977)

ABSTRACT

The interaction between 1-tryptophan and *a*-ketoglutaric acid (*a*-KGA), first reported by Chu and Clydesdale (*1,2*), was used as the basis for development of a method for estimation of tryptophan content. Analysis of reaction mixtures with ultraviolet spectrophotometers revealed the development of absorbance with a wavelength of maximum absorbance (λ max) at 358 nm. Four parameters were manipulated to increase the rate and amount of chromophore formed. These parameters were: concentration of HCl, concentration of *a*-KGA, concentration of sodium nitrite, and temperature.

Tryptophan is an essential amino acid. This implies that it cannot be manufactured by the human body in sufficient amounts to satisfy demand, and therefore it must be ingested to maintain positive nitrogen balance. An important function of tryptophan is its role as the precursor of niacin. If enough tryptophan is ingested in the diet, the requirement for niacin is met, and thus the disease pellagra can be prevented.

Due to the importance of tryptophan and its metabolites, various analytical methods have been developed to assay for its presence in both its free and peptide-bound forms. To date, however, most procedures have been time-consuming and/or unreliable. Friedman and Finley (*3*) wrote an excellent review article on this subject.

The purpose of this investigation was to explore the interaction between tryptophan and *a*-ketoglutaric acid (*a*-KGA). Chu and Clydesdale (*1,2*) reported that colored derivatives were produced by the interaction of tryptophan and *a*-KGA. The possibility of using this reaction as a quantitative spectrophotometric method for determination of tryptophan content was examined.

MATERIALS AND METHODS

From preliminary studies it was found that when acidic solution of *a*-KGA (Eastman Kodak Co.) were allowed to interact with 2.0-mM solutions of 1-tryptophan (Aldrich Chemical Co.), a yellow-brown color developed. On the basis of this observation certain standardized experiments were designed to individually test the effects of four parameters (concentration of HCl, concentration of *a*-KGA, concentration of sodium nitrite, and temperature) on the rate and amount chromophore produced.

Preparation of standard tryptophan solutions

A 2.0-mM solution of 1-tryptophan was prepared in a one liter volumetric flask. The flask was wrapped with aluminum foil and stored in the refrigerator (8 C) to minimize destruction of tryptophan. However, because of its known lability, new stock solutions were prepared for each set of experiments.

Measurement of color formation

In preliminary studies the increase in absorbance due to formation of chromophore was monitored by both visible and ultraviolet spectra. The General Electric Recording Spectrophotometer (GERS) was used for the visible spectrum and the Perkin Elmer Model 450 UV-VIS NIR Spectrophotometer (PE-450) was used for the ultraviolet spectrum. Since it was found that the greatest increase in absorbance occurred at 358 nm, the Hitachi-Perkin Elmer Model 139 Spectrophotometer (HPE-139) was employed to monitor the development of chromophore at the wavelength of maximum absorbance. To maintain the readings on scale the reaction mixture had to be diluted 1:5 with distilled water.

Effect of HCl concentration

Stock solutions containing 14.52 g of *a*-KGA were made up to volume in 200-ml volumetric flasks (0.5 moles *a*-KGA/liter) with each of the following: distilled water, 3 N, 6 N, 9 N, and 12 N HCl. Duplicate sample solutions were prepared in 20-ml sample bottles containing equal amounts of the *a*-KGA reagent and either stock tryptophan solution (for the test samples) or distilled water (for the blanks). All samples were incubated in an oven maintained at 40 C(± 2 C). Aliquots of the test samples and blanks were taken every 8 h for the first 80 h and then after 92, 116, and 140 total hours of incubation. These were then diluted and measured on both the PE-450 and HPE-139.

Effect of a-KGA concentration

Solutions containing 0.05, 0.10, 0.30, 0.50, and 0.70 moles *a*-KGA/liter of 6 N HCl were prepared in 200-ml volumetric flasks. As in the previous experiment duplicates were prepared for each test sample and blank and incubated at 40 C. Aliquots were taken for analysis at 12-h intervals for the first 168 h and then at 24-h intervals for the last 72 h. Thus the total reaction time was 240 h.

Effect of temperature

Test temperatures: 20, 40, and 60 C. A set of both 0.1 M and 0.5 M *a*-KGA were made in duplicate with 6 N HCl for each of the three test temperatures. Appropriate test samples and blanks were made and incubated at 20, 40, or 60 C. Aliquots from all three sets were taken for analysis every 12 h for the first 120 h and then after 144, 168, and 192 h

of total incubation.

Test temperatures: 60 and 80 C. Two sets of samples containing 0.1 M 0.5 M, and 0.01 M *a*-KGA were made up in 6 N HCl. Test samples and blanks were prepared in duplicate for each temperature and concentration of *a*-KGA used. Incubation temperatures were 60 and 80 C. Aliquots were taken for analysis every 6 h for the first 30 h and a final set was taken after 42 total hours of incubation.

Test temperature: 100 C. Duplicate sets of samples containing 0.10, 0.05 and 0.01 M *a*-KGA were made up in 6 N HCl. Test samples and blanks were prepared and incubated at 100 C. Aliquots were taken for analysis every 6 h for a total of 60 h.

Effect of oxidizing agents

Effect of $NaNO_2$ and $NaCLO_4 \cdot H_2O$. Separate solutions of 1.00, 0.50, 0.10, and 0% $NaNO_2$ and $NaClO_4 \cdot H_2O$ were prepared with distilled water. Also a 0.05-M solution of *a*-KGA in 6 N HCl was mixed with an equal amount of stock tryptophan solution and incubated at 80 C. Samples from this reaction mixture were taken after 0,3,6,12, and 24 h of incubation. To 1-ml aliquots of these samples was added 1, 2, or 4 drops of the oxidizing solutions. These samples were placed in the dark for 15 min and then analyzed for the development of chromophore.

Effect of other oxidizing agents. One percent solutions of $NaNO_2$, $NaNO_3$, KNO_2, KNO_3, $NaMnO_4$, $Mg(NO_3)_2$, and $NaClO_4 \cdot H_2O$ were prepared with distilled water. The reaction mixture used in the previous experiment was also utilized for this experiment. After 4 h of incubation at 80 C, seven 5-ml aliquots were taken and 5 drops of each of the oxidizing solutions were added. Chromophore development was then analyzed as previously outlined.

Effect of increasing concentration of $NaNO_2$. One percent, 5.0%, and 10.0% solutions of $NaNO_2$ were prepared with distilled water. Sixteen 5-ml aliquots were taken from the reaction mixture used in the previous two experiments. To these was added 1 drop, 5, 10, or 20 drops either of distilled water or the oxidizing solutions. Again chromophore development was analyzed.

RESULTS AND DISCUSSION

Measurement of color formation

Figures 1 and 2 illustrate the fact that when a 0.5 M solution of *a*-KGA dissolved in 6 N HCl was allowed to interact for 48 h at 40 C with an equivalent amount of stock tryptophan solution (2.0 mM), there is production of a chromophore which has peak absorbance at 358 nm. Both the PE-450 and HPE-139 correlated well in support of this observation. There was no absorption in the visible range.

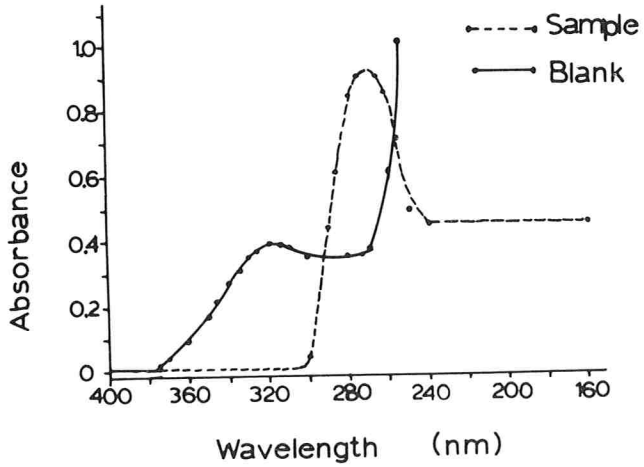

Figure 1. *Spectral curves of a sample* (a-KGA + *tryptophan*) *and a blank* (a-KGA + H_2O) *at time O obtained using the PE-450.*

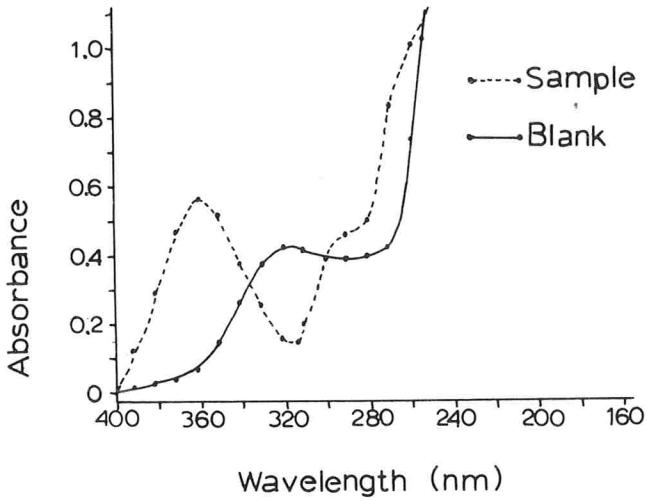

Figure 2. *Spectral curves of a sample* (a-KGA + *tryptophan*) *and a blank* (a-KGA + H_2O) *after 48 h of incubation at 40 C obtained using the PE-450.*

Effect of HCl concentration

The production of chromophore with λ max at 358 nm was found to be catalyzed by acid. Figure 3 clearly illustrates that when 0.5 moles of *a*-KGA were dissolved per liter of 3, 6, 9, or 12 N HCL rather than in distilled water, greater amounts of chromophore were produced when allowed to react with stock solutions of tryptophan at 40 C.

When the *a*-KGA was dissolved in 6 N HCl the yield of chromophore and the rate of its production were at a maximum. Production of chromophore leveled off after 48 h and was stable for an additional 40 h. Thus 6 N HCl was used in subsequent experimentation.

Effect of a-KGA concentration

Figure 4 shows that the lower (0.05 and 0.10 M) concentrations of *a*-KGA produced the greatest increase in absorbance at 358 nm. However, the reaction took over 200 h to complete. As the concentration of *a*-KGA was increased, the initial reaction rate was greatly increased. The sample containing 0.70 M *a*-KGA exhibited the fastest rate of production but this ceased

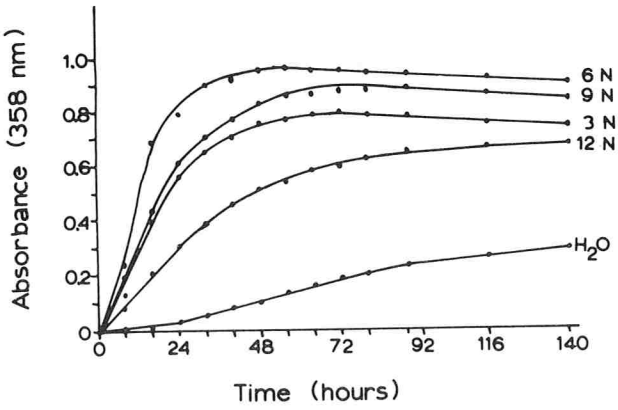

Figure 3. *Production of increased absorbance with time when a-KGA was dissolved in distilled H_2O or 3,6,9 or 12 N HCl and reacted at 40 C or with a stock tryptophan solution.*

after 34 h, yielding only approximately two-thirds as much chromophore as did the sample containing 0.10 M *a*-KGA.

The sample containing 0.50 M*a*-KGA produced somewhat more chromophore than did the sample containing 0.70 M*a*-KGA. In both instances the chromophore production was stable for almost 60 h. Inasmuch as the two reactions were similar with respect to the rate, amount, and stability of chromophore produced, the lower concentration (0.5 M) was selected for further testing to conserve reagent.

Effect of temperature

Test temperatures: 20, 40, and 60 C. Analysis of chromophore production revealed that at the higher temperature, 60 C, samples containing 0.1 M*a*-KGA produced more chromophore and at a faster rate than did the sample containing the 0.5 M*a*-KGA solution (See Fig. 5 and 6). In both instances chromophore production peaked after approximately 24 h and started to decrease within a few hours thereafter.

At 40 C the sample containing the lower concentrations of *a*-KGA (0.1 M) yielded higher amounts of chromophore but the production had not leveled off even after 192 h of incubation. Again, the sample containing 0.5 M *a*-KGA leveled off after 48 h of incubation and maintained a stable level for 60 additional hours. At 20 C all samples failed to cease chromophore production even after 192 h of incubation.

Test Temperatures: 60 and 80 C. The results of the previous experiment indicated that at higher temperatures, samples containing relatively low concentrations of *a*-KGA produced the greatest amount of chromophore. Therefore 0.10, 0.05, and 0.01 M solutions of *a*-KGA dissolved in 6 N HCl were used in these experiments. Explanations for this behavior were not attempted.

At both temperatures, 60 and 80 C, samples containing 0.01 M *a*-KGA yielded the least amount of chromophore at the slowest rate (See Fig. 7 and 8). Since production of chromophore had not leveled off by the time the experiment was halted, it may be implied that

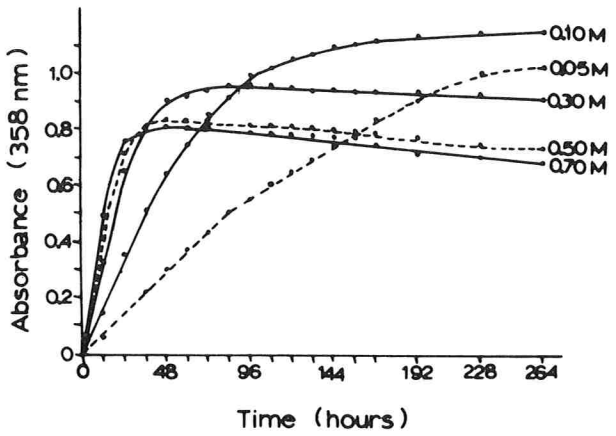

Figure 4. *Production of increased absorbance with time at 40 C for various molarities of a-KGA; 0.05 M, 0.30 M, 0.50 M, and 0.70 M.*

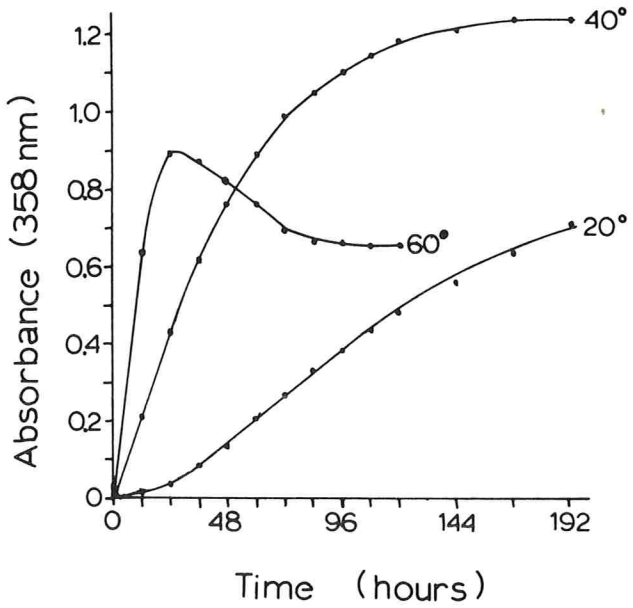

Figure 5. *Development of increased absorbance with time for reagents containing 0.10 mole a-KGA per liter of 6 N HCl when incubated at 20, 40, and 60 C.*

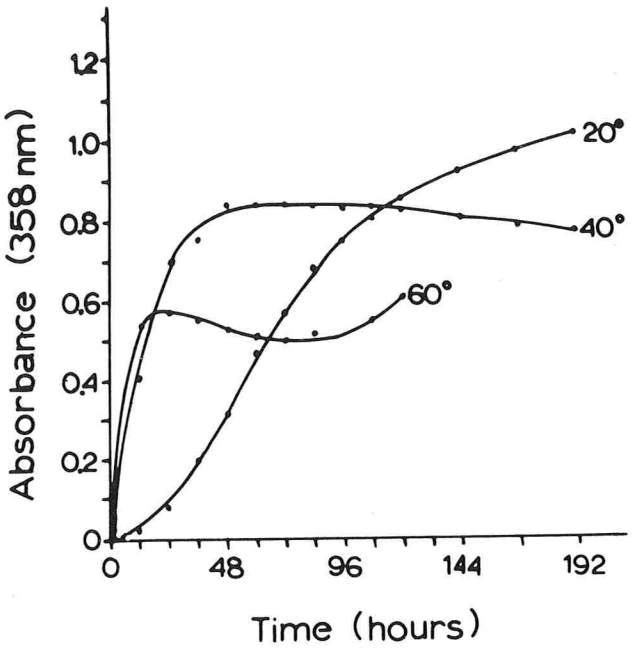

Figure 6. *Development of increased absorbance with time for reagents containing 0.5 mole a-KGA per liter 6 N HCl incubated at 20, 40, and 60 C.*

these lower concentrations of *a*-KGA would eventually produce the greatest amount of chromophore. This hypothesis was based on the earlier results.

The samples containing 0.05 M *a*-KGA produced almost equivalent amounts of chromophore at approximately the same rate at both temperatures. Since the sample incubated at 80 C showed more of a plateau region after 22 to 30 h of incubation, those conditions were chosen for further experimentation.

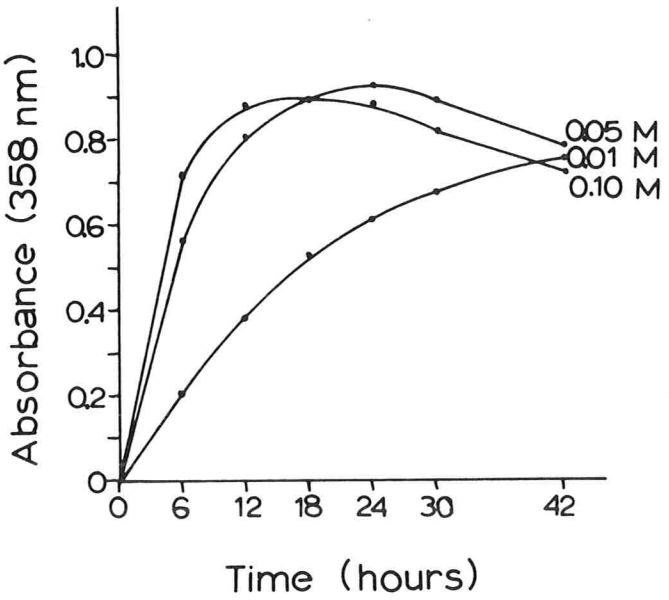

Figure 7. *Development of increased absorbance with time at 60 C for various molarities of a-KGA per liter of 6 N HCl; 00.1 M, 0.05 M, and 0.10 Ma-KGA.*

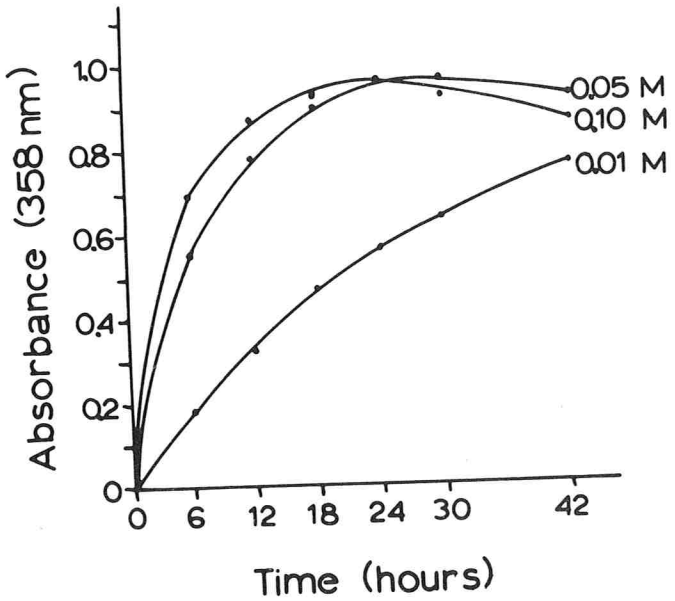

Figure 8. *Development of increased absorbance with time at 80 C for various molarities of a-KGA per liter of 6 N HCl; 0.01 M, 0.05 M, and 0.10 Ma-KGA.*

The samples containing 0.10 M *a*-KGA produced less chromophore than did the samples containing 0.05 M *a*-KGA at both temperatures. The rate of chromophore production was faster for the 0.10 M *a*-KGA. This supports the observation that at higher temperatures, samples containing more *a*-KGA produced lower yields of chromophore but the rate of production was faster and there was a long time period when production was stable.

Test temperature: 100 C. When samples containing 0.10, 0.05, and 0.01-M solutions of *a*-KGA dissolved in 6 N HCl were incubated at 100 C, production of chromophore was limited (Fig. 9). All three levels showed

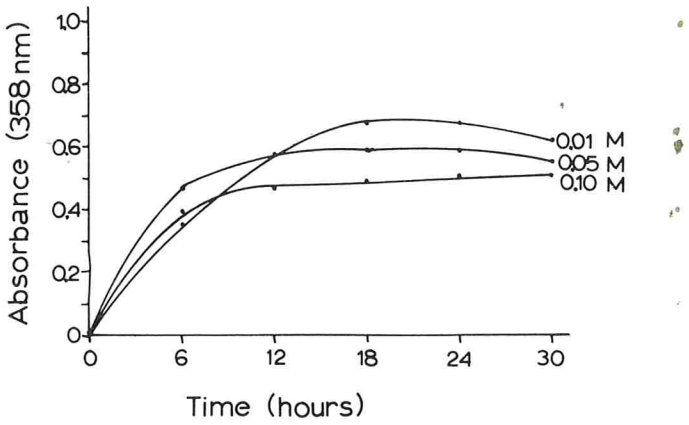

Figure 9. *Development of increased absorbance with time at 100 C for various molarities of a-KGA per liter of 6 N HCl; o.01 M,0.05 M, and 0.10 M a-KGA.*

a fast initial rate of chromophore production with the sample containing the least amount of *a*-KGA (0.01 M), producing the most chromophore.

At all concentrations tested, at least 18 h were required for production of chromophore to level off. Since the total amount of chromophore produced at 100 C was significantly lower than the amounts produced at lower temperatures, no further work was done at this temperature.

Selection of parameters for further analysis

The purpose of this investigation was to investigate the possibility of utilizing *a*-KGA in a quantitative analysis of tryptophan. In view of the objective, two sets of reaction conditions, named RC-1 and RC-2, were chosen because they produced a measurable amount of chromophore within a reasonable time. Also of prime importance was the fact that chromophore production was stable for a relatively long period and the absorbance produced was reproducible.

RC-1 was defined as a 0.5 M solution of *a*-KGA made up in 6 N HCl which was reacted with equal amounts of solutions containing free tryptophan at 40 C for 48 h.

RC-2 was defined as a 0.05 M solution of *a*-KGA made up in 6 N HCl which was reacted with equal amounts of solutions containing free tryptophan at 80 C for 24 h.

Effect of oxidizing agents

Many of the early methods developed for analysis of tryptophan involve the reaction between tryptophan and various aliphatic or aromatic aldehydes in acid media. One of the most successful reactions utilizes p-dimethylaminobenzaldehyde (p-DMB). It has been theorized that the reaction involves two separate reactions. The first one is a fast condensation reaction between tryptophan and p-DMB. The second reaction is a slow oxidation of this colorless condensation product which forms the chromophore. This reaction has been accelerated through the addition of oxidizing agents, $NaNO_2$ or light (*4-9*).

Since *a*-KGA contains a carbonyl group and the interaction between it and tryptophan is a slow one which

is catalyzed by acid, the possibility existed that this reaction was similar to the p-DMB reaction. Therefore, various oxidizing agents were tested to determine their possible use in speeding up the reaction time.

Effect of $NaNO_2$ and $NaClO_4 \cdot H_2O$. Figure 10 illustrates the effects that different concentrations of $NaNO_2$ and $NaClO_4 \cdot H_2O$ have on the reaction involving RC-2 and typtophan. $NaClO_4 \cdot H_2O$ had no effect as can be seen by the fact that despite the various amounts and concentrations of $NaClO_4 \cdot H_2O$ added, the amount of chromophore production was identical to that produced when water alone was added.

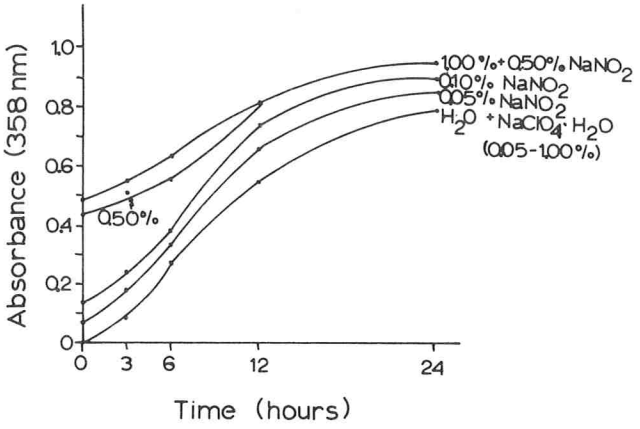

Figure 10. *Development of increased absorbance with time after addition of four drops of 0, 0.05, 0.10, 0.50, and 1.0% $NaNO_2$ and $NaClO_4 \cdot H_2O$ and incubation at room temperature in the dark for 15 min.*

When various concentrations of $NaNO_2$ were added, there was an increase in absorbance which was almost proportional to the amount of $NaNO_2$ added.

Since the $NaClO_4 \cdot H_2O$ produced no change in absorbance, it was hypothesized that the reaction involving *a*-KGA and tryptophan was dissimilar to the p-DMB tryptophan reaction. The $NaNO_2$ was thought to have some other effect on the reaction which was not due to its oxidizing ability.

Effect of other oxidizing agents. Several other oxidizing agents were tested for their effect on chromophore production. Also, nitrate ions and KNO_2 were tested to determine if the increased absorbance was due to the action of the nitrite ions.

Table 1 reveals that production of increased absorbance is due to the nitrite ion. Both $NaNO_2$ and KNO_2 elicit the same behavior whereas $NaNO_3$, KNO_3, and other oxidizing agents have no effect.

Effect of Increasing Concentrations of $NaNO_2$. Figure 10 suggests that there might be an upper limit to the amount of $NaNO_2$ added that will effect any further change in absorbance. This hypothesis was tested by adding more concentrated solutions of $NaNO_2$ to a reaction mixture. Table 2 illustrates that no upper limit was found. In fact the absorbance was found to proceed off-scale when increased amounts of $NaNO_2$ were added. Therefore, nitrite ions were considered to be an interfering substance and were not a means to speed up the reaction time.

TABLE 1. *Effect of various oxidants on the interaction between RC-2 and tryptophan*

Solution	Absorbance (358 nm)[a]
$NaNO_2$	616
KNO_2	609
$NaNO_3$	200
KNO_3	202
$NaMnO_4$	191
$Mg(NO_3)_2$	199
$NaClO_4 \cdot H_2O$	206

[a]Samples were taken after 6 h of interaction with RC-2 and the standard tryptophan solution. Readings were taken after addition of 5 drops of 1% solutions of the oxidants and incubation for 15 min.

This investigation showed that by the appropriate manipulation of pH, temperature, time and concentration of *a*-KGA, reproducible amounts of a stable chromophore could be produced. These results indicate that a procedure for quantitative analysis of tryptophan might be developed on this basis in future work.

TABLE 2. *Effect of various concentrations and amounts of $NaNO_2$ on the development of increased absorbance*

	Absorbance at 358 nm (x1000)			
	Number of drops added			
% $NaNO_2$	1	5	10	20
0[a]	231	229	224	214
1	466	624	664	789
5	646	834	1166	1859
10	690	1123	1954	2000

[a]0 = addition of H_2O

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The Automated Pyruvate Method as a Quality Test for Grade A Milk

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ABSTRACT

Concentrations of pyruvate in Grade A raw and pasteurized milks were determined by an automated procedure. The method was sensitive to 0.1 mg of pyruvate per liter and the coefficient of variation of the method was 1.6% for a sample of milk containing 3 mg/l. Since about twice as much pyruvate was produced in samples incubated 24 h at 20 C compared with 15 C, the higher temperature is suggested for the pyruvate difference test (ΔP); however, psychrotrophs would more likely be detected with an incubation temperature of 15 C. Pyruvate difference tests of pure cultures in steamed milk distinguished between concentrations approximating 10^2 and 10^3 *Pseudomonas fragi,* 10^3 and 10^4 *Pseudomonas fluorescens,* and between 10^4 and 10^5 *Escherichia coli, Streptococcus faecalis, Lactobacillus acidophilus* and *Micrococcus luteus*, but did not distinguish between 10^4 and 10^5 initial concentrations of six other gram-positive bacteria and *Salmonella typhi.* Both the initial pyruvate (IP) concentration and the ΔP in raw milk were correlated with the logarithm of the Wisconsin Mastitis Test score, suggesting that somatic cells contribute to pyruvate content. Correlations of IP and ΔP with Standard Plate Counts of raw and pasteurized milks were low or insignificant and only with raw milk from normal quarters was there a significant correlation of IP and ΔP with initial Psychrotrophic Plate Counts (PPC). Rates of pyruvate production by pure cultures in steamed milk and by the mixed natural flora of pasteurized milk were consistent with rates of growth. However, certain psychrotrophic bacteria reduced the concentration of pyruvate to undetectable levels after producing 10 or more mg/l. Suggestions are made regarding applicability of the test in controlling keeping quality of pasteurized milk and in receiving of bulk raw milk.

Testing of milk for bacteriological quality is a major activity of dairy firms and health departments. The Standard Plate Count (*1*) has been the major bacteriological test for Grade A milk for many years. However, much dissatisfaction with this method has been voiced, and users have pointed to its lack of applicability to the present situation. Not only is the Standard Plate Count method subject to relatively large error, but the population of microorganisms it measures is not necessarily related to the quality of milk.

Microbiological tests done on milk have two main purposes, viz. (*a*) to determine whether sanitary practices were adequate and (*b*) to gain information that will aid in predicting keeping quality. Good sanitary practices are required to limit chances for disease transmission through milk and to minimize its rate of spoilage. And spoilage is our major problem, not disease transmission. Application of the provisions of the Grade A Pasteurized Milk Ordinance provides strong safeguards against the latter.

Gram-positive cocci usually predominate in bulk tank milk. Jackson and Clegg (*3*) found that gram-positive cocci predominated in 78% of 57 raw milk samples from bulk milk tanks. Only when quaternary ammonium compounds were used as sanitizers did gram-negative rods predominate. Scroggins and Marshall (*4*) found that gram-negative rods constituted only 5% of the isolates rinsed with sterile water from milking equipment used in the present experiments. However, the necessity to hold milk at low temperatures for long periods makes it likely that psychrotrophic bacteria will become the dominant flora. We know that this group of bacteria is characterized by the ability to produce large amounts of hydrolytic enzymes, many of which are heat stable. Plate count methods are inadequate to rapidly and accurately enumerate these bacteria.

There is a wide variety of psychrotrophic bacteria that can get into milk, and the enzymes they produce are also highly variable. For example, six of seven strains of *Pseudomonas fluorescens* produced phospholipases C that differed in activation energy, heat sensitivity, optimal pH or response to cations (*2*).

Because of this variability, as well as the interacting effects of other microorganisms, somatic cells, inhibitory substances and temperature, it is probably impossible to devise a single test of milk quality that can have all the desirable attributes. No two samples of milk are exactly alike and each sample changes with age. Keeping quality depends both on numbers of bacteria and their biochemical activities.

Tolle et al. (*6*) published a report of extensive studies in which they examined numerous test of milk quality. These included pyruvate, dye reduction, alcohol coagulation and clot-on-boiling as well as total and selective bacterial plate counts. They concluded that the automated test for pyruvate gave promise of being highly

useful in determining the quality of milk. They also found that the enzymatic determination of pyruvate is of high specificity, that samples can be preserved for several days with trichloroacetic acid and that pyruvate is not destroyed by pasteurization.

Pyruvate is a central intermediary metabolite of most bacteria found in milk. It is produced in glycolysis through the Embden-Meyerhof Parnas, the Dickens-Horecker and the Entner-Doudoroff pathways, the former being primary for homofermentative lactic acid producers and the latter being primary for many psychrotrophs. Pyruvate is also produced from deaminated amino acids and from free fatty acids.

Tolle and Heeschen (*6*) constructed a model of pyruvate formation, excretion and utilization as may occur within microbial cells (Fig. 1).

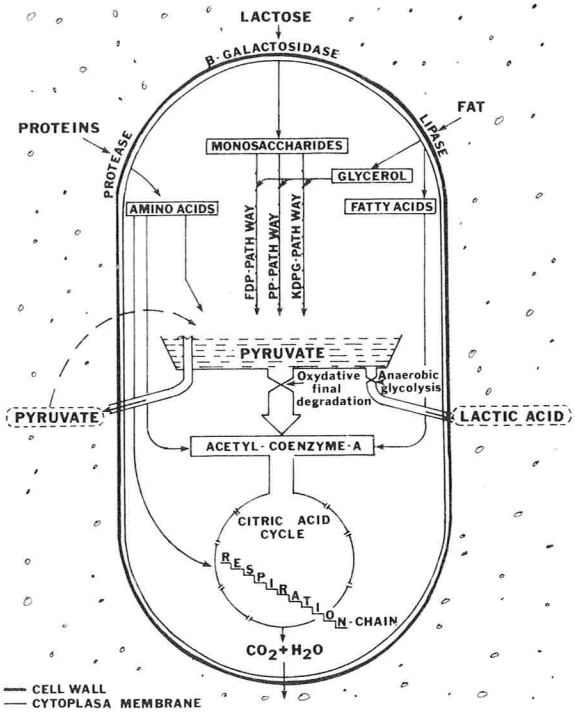

Figure 1. *Model of pyruvate formation, utilization and excretion by the microbial cell in milk (after: Tolle and Heeschen, Commission of the European Communities, No. 21. September, 1976).*

The present report relates to research done with the automated pyruvate method in which the objectives were to determine (*a*) whether on incubation of grade A raw milk for 24 h changes in pyruvate concentration were correlated with changes in bacteria counts, (*b*) the influence of somatic cells of raw milk on pyruvate content, (*c*) whether the pyruvate test could differentiate between 10-fold concentrations of several typical milkborne bacteria, (*d*), the pattern of pyruvate production by bacteria naturally in samples of pasteurized milk, and (*e*) patterns of pyruvate production by pure cultures of typical milk-borne bacteria in steamed skim milk.

The pyruvate method is based on the principle shown in Fig. 2. Pyruvate can be enzymatically reduced by lactate dehydrogenase and reduced nicotinamide adenine dinucleotide ($NADH_2$). The change in concentration of $NADH_2$ is measured colorimetrically at 340 nm, or it can be measured fluorometrically.

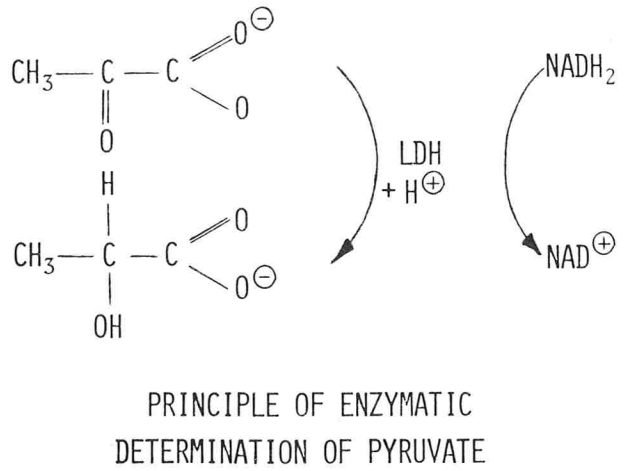

Figure 2. *Pyruvate is enzymatically converted to lactate with lactate dehydrogenase in the presence of $NADH_2$. Decrease in concentration of $NADH_2$ is measured colorimetrically at 340 nm.*

MATERIALS AND METHODS

Pyruvate analyses

Pyruvate concentrations were determined in a Technicon Auto Analyzer^R II (Fig. 3) composed of a Sampler IV, proportioning pump, two dialyzers, a single channel colorimeter, a strip chart recorder, and a voltage stabilizer. The colorimeter contained two 15-mm flow cells and 340-nm filters.

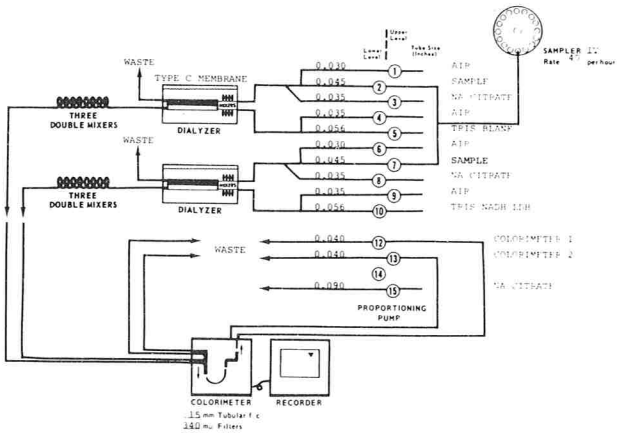

Figure 3. *Pyruvate Determination in AutoAnalyzer II System.*

Samples were pumped in a split stream at the rate of 0.8 ml/min with 0.42 ml/min of 7% sodium citrate, and the stream was segmented with air. Pyruvate dialyzed through type C membranes into a stream of 0.05% $NADH_2$ in tris buffer (pH 76.) or into a mixture of 0.05% $NADH_2$ and lactic dehydrogenase (1.6 units/ml) in tris buffer. The separate streams passed through individual cuvettes, and the difference in absorbance was recorded. Samples were tested at the rate of 60/h.

Raw milk

The sources of raw milk were the University of Missouri dairy herds. Samples were taken aseptically from individual quarters of Holstein and Guernsey cows or from sampling cocks of volumetric receiving jars

of two DeLaval pipeline milkers.

Samples from individual quarters (University dairy herds) were selected in pairs based on reactions to California Mastitis tests, one sample being taken from a non-reactive quarter and a second from a distinctly positive quarter of the same cow. These samples were later subjected to Wisconsin Mastitis tests (WMT) to provide objective estimates of somatic cell content. Cows were washed thoroughly with a warm spray containing 15-20 ppm iodophor and then dried with paper towels before samples were taken in sterile plastic bags.

Samples were selected randomly from receiving jars, and sampling cocks were deliberately not sanitized so samples would become contaminated. Each sample represented milk from a single cow and no more than four samples were taken from the same jar at any one milking.

All samples were immediately placed in crushed ice and reached the laboratory within 3 h. Initial pyruvate content was determined within 12 h.

At the time of pyruvate analyses, samples were plated in at least two consecutive dilutions and single plates of each dilution were incubated at 7 and 32 C to determine Psychrotrophic Plate Counts (PPC) and Standard Plate Counts (SPC), respectively (*1*). Aliquots were incubated at 5, 10, 15 and 20 C. After 24 h of incubation, pyruvate content was again determined and SPC and PPC were made on all samples incubated at 20 C and on samples incubated at other temperatures when pyruvate content increased by as much as 1 mg/l. Pyruvate difference (ΔP) was the pyruvate concentration after incubation minus the pyruvate concentration before incubation.

Data from Wisconsin Mastitis Tests (WMT) and plate counts were transformed to logarithms. Correlation coefficients were determined and t tests for differences between means performed using Statistical Analysis System programs (*5*).

Pasteurized milk

Market samples of fresh pasteurized milk were collected (eight in January and eight in March) and stored at 5 C. Initial pyruvate values were determined immediately upon receipt at the laboratory. Samples were plated in two consecutive dilutions and single plates of each dilution were incubated at 7 and 32 C to determine initial PPC and SPC Ten milliliters of each sample were pipetted into sterile test tubes and incubated for 24 h at 20 C for the pyruvate difference test. Pyruvate values of the milk held at 5 C were monitored for up to 17 days with SPC and PPC being determined at 5- to 8-day intervals.

Studies on pure cultures

Pure cultures of several typical milkborne bacteria were transferred to litmus milk and incubated at room temperature for 72 h. Following incubation, 1 ml of the culture was transferred to 99 ml of skim milk that had been autoclaved at 121 C for 5 min. Duplicates of four additional 1:10 dilutions in a series were made for each culture, and the SPC was determined. One set of tubes was held at 15 C and the other at 20 C. Pyruvate content of each sample was determined after 24 h.

Pure cultures used in the study of effects of extended storage were grown in litmus milk in the same manner as above. Skim milk was inoculated with 1% of these 24-h cultures and held at 20 C. Pyruvate values were determined for up to 7 days.

RESULTS AND DISCUSSION

Characteristics of the method

First experiments were designed to determine sensitivity and reliability of the method. The instrument was standardized to read over a range of 0 to 10 mg/l. The smallest division on the strip chart represented 0.1 mg/l. Analyses were reproducible within ± 0.1 mg/l when samples containing 5 and 10 mg/l of pyruvate in water were repeatedly tested. The coefficient of variation for 5 mg/l, the mid-range point, was 1.3%. The coefficient of variation was 1.6% for repeated analyses of a single milk sample which contained 3 mg of pyruvate per liter.

When 5 mg of pyruvate/l were added to five aliquots each of three samples of milk, an average of 95% was recovered, and the standard deviation was 10%.

The standard curve was linear as indicated by the correlation coefficient of 0.999 which was calculated from tests of six sets of standards made at five concentrations.

Studies on Grade A raw milk

The samples we used for these studies were chosen to represent three types of milk: (*a*) extra high quality — from clean normal quarters, (*b*) abnormal milk — from clean inflamed quarters, and (*c*) contaminated milk — from pipeline milker receiver jars.

Table 1 summarizes characteristics of the milk as measured by the SPC. None of the quarter samples and 8% of the jar samples had counts exceeding the Grade A limit of 100,000/ml. However, 56% of the jar samples had counts between 10,000 and 100,000/ml. Counts of quarter samples, especially those from normal quarters, were much lower. Averages of logarithms of the SPC were 270/ml for normal, 1,000/ml for abnormal and 16,000/ml for jar samples.

TABLE 1. *Percentages of standard plate counts by range and source of sample.*

Range of counts/ml	Source of samples		
	Jars	N. qtrs.	Abn. qtrs.
	%		
$>10^5$	8	0	0
10^4 -- 10^5	56	0	19
10^3 -- 10^4	30	31	36
10^2 -- 10^3	6	29	26
10^1 -- 10^2	0	33	17
$<10^1$	0	6	2
n^a =	71	48	47

[a]n -- The number of samples from each source.

PPC averaged 54/ml in samples from jars, but 35% of these samples contained between 100 and 1000 psychrotrophs per ml. No sample from any quarter had a PPC exceeding 100/ml, and only 16% had more than 10/ml. Initial PPC averaged 3 and 4/ml in the normal and abnormal samples, respectively.

We concluded, therefore, that contaminated milk from jars was at least as high in quality as market Grade A milk. Samples from quarters had very low bacteria counts, but those from abnormal quarters probably contained a disproportionately high number of mastitis bacteria compared with bulk milk.

Comparisons of milk from normal and abnormal quarters

Somatic cells, principally leukocytes, have a metabolic pool of pyruvate, as do bacteria, and numbers of somatic cells increase in milk in cases of inflammation of the mammary gland, the condition commonly known as mastitis. Therefore, it was important to determine and compare content of pyruvate in normal and abnormal milks. Mastitic milk usually contains many more bacteria than normal milk, but these bacteria grow

poorly at 20 C and below.

Table 2 shows averages of four characteristics of the two types of milk. WMT scores, initial content of pyruvate, and initial SPC were all significantly higher in milk from the inflamed quarters. The initial pyruvate

TABLE 2. *Characteristics of fresh milk from normal and abnormal quarters.*

Characteristic	Soucre of samples	
	N. qtrs.	Abn. qtrs.
	(Averages)	
WMT[a] Score (mm)	3.5[e]	16[f]
Initial PVA[b] Conc. (mg/l)	0.47[e]	1.34[f]
Initial log SPC[c]/ml	2.44[e]	3.02[f]
Initial log PPC[d]/ml	0.46[e]	0.54[e]

[a]WMT — Wisconsin Mastis Test
[b]PVA — Pyruvic Acid
[c]SPC — Standard Plate Count
[d]PPC — Psychrotrophic Plate Count
[e,f]Numbers on the same line with different superscripts are significantly different ($P < 0.05$).

concentration of milk from jars averaged 0.83 mg/l, which was intermediate between the average concentrations in normal and abnormal milk. This was higher than the base value of 0.5 mg/l which Tolle and Heeschen (*6*) recommended as standard for raw milks in Germany.

Correlation coefficients in Table 3 indicate that initial pyruvate content was significantly correlated with logarithms of WMT (upper line) scores of samples from

TABLE 3. *Correlation coefficients for long WMT vs. pyruvate in samples from jars and quarters.*

Tests	Correlation coefficients			
	Jar	All qtrs.	N. qtrs.	Abn. qtrs.
Log WMT[a] vs. IP[b]	.16	.62*	.30	.54*
Log WMT vs. Δ P[c]	.09	.46*	.17	.36*

* Significant correlation ($P < 0.05$).
[a]Log WMT — Logarithm of Wisconsin Mastitis Test score in mm.
[b]IP — Initial pyruvate concentration (within 12 hr of sampling).
[c]ΔP — Change in pyruvate concentration during 24 hr at 20 C.

quarters, but abnormal quarters were responsible for the correlation. Among samples from quarters, the logarithm of the WMT score was also correlated with the change in pyruvate concentration (ΔP) during incubation for 24 h at 20 C (lower line), but again abnormal samples were responsible for the correlation. Thus we reason that higher numbers of somatic cells or of bacteria, or both, in the abnormal milk were responsible for increases in pyruvate content. However, samples from receiver jars often contained milk from inflamed quarters, and there was no significant correlation of log WMT with pyruvate content of these samples.

Pyruvate vs. Standard Plate Count

Initial pyruvate (IP) content was weakly but significantly correlated with initial SPC of jar and abnormal samples (Table 4). However, ΔP was significantly correlated with initial SPC of only the abnormal samples.

TABLE 4. *Correlation coefficients for pyruvate vs. standard plate count and psychrotrophic plate count in samples from jars and quarters.*

Tests	Correlation coefficients			
	Jars	All qtrs.	N. qtrs.	Abn. qtrs
IP[a] vs I SPC[c]	.24*	.28*	.17	.34*
Δ P[b] vs I SPC	.03	.19	.12	.34*
Δ P vs Δ SPC[d]	.37*	.29*	.31	.44*
IP vs I PPC[e]	.03	.31*	.45*	.24
Δ P vs I PPC	.10	.37*	.40*	.27
Δ P vs ΔPPC[f]	.02	−.10	−.15	−.19

* Significant correlation ($P < 0.05$).
[a]IP — Initial pyruvate concentration.
[b]Δ P — Change in pyruvate concentration during 24 h at 20 C.
[c]I SPC — Initial Standard Plate Count.
[d]Δ SPC — Change in Standard Plate Count during 24 h at 20 C.
[e]I PPC — Initial Psychrotrophic Plate Count.
[f]Δ PPC — Change in Psychrotrophic Plate Count during 24 h at 20 C.

Pyruvate content increased significantly with increases in SPC except in samples from normal quarters.

Thus, data from SPC suggest that mastitis bacteria in abnormal milk are responsible for some of the increases in pyruvate content during incubation at 20 C.

Pyruvate vs. Psychrotrophic Count

Whereas with milk from normal quarters neither IP nor ΔP were correlated with the SPC, it was only among samples from normal quarters that we observed significant correlations (Table 4) of IP and ΔP with PPC. These observations suggest that psychrotrophs were important in the test only when there were relatively small numbers of mastitis bacteria and/or somatic cells present. Finally, the correlation coefficients for ΔP vs ΔPPC were quite small and sometimes negative. This suggested that psychrotrophic bacteria are variable in their abilities to produce pyruvate. However, data in Table 5 indicate that effects of psychrotrophs were minimal. They comprised, on the average, 1% or less of the viable bacteria in fresh samples. Although their numbers increased much faster during incubation than did total numbers, psychrotrophic bacteria averaged less than 10% of the population after incubation regardless of source of samples (SPC-PPC, Table 5). Therefore, in many samples effects of psychrotrophs were obscured by effects of mesophiles.

These observations suggest that had incubation at 20 C been continued for several more hours, psychrotrophic populations would have become dominant and their effects on pyruvate concentration might have been observed. Also, these samples were collected directly at milking; psychrotrophs had no chance to grow by the time tests were made.

Effects of temperature of incubation

Quantities of pyruvate in freshly drawn raw milk varied from negligible to 5.1 mg/l. This large variance in base value indicated that to determine the degree of

microbial contamination, samples would need to be tested initially then incubated at a temperature and time that would allow significant growth of undesirable bacteria before being tested again.

Since the most undesirable bacteria are psychrotrophic types, we chose to incubate samples at temperatures which were favorable for them and that would likely provide meaningful results the next day.

Data in Table 6 show changes in pyruvate concentrations during incubation for 24 h at 5, 10, 15 and 20 C. Mean quantities of pyruvate increased at all temperatures but standard deviations were large. Only about 0.1 to 0.2 mg/l more pyruvate was produced at 10 than at 5 C on the average. Depending on source of sample, incubation at 15 C produced about 0.5 mg/l more pyruvate than at 5 C, and incubation at 20 produced nearly twice as much pyruvate as incubation at 15 C.

As a group, samples from jars had a different population (<0.05) from those taken from quarters as shown by both total pyruvate produced and ΔP when samples were incubated at 15 and 20 C. The same tests were able to differentiate between samples from normal and abnormal quarters.

Differentiation of 10-fold dilutions of bacteria

Tolle and Heeschen (*6*) reported that quantities of pyruvate produced per bacterial cell are inversely proportional to the number of cells. With counts approximately 10^5/ml about 20,000 colony-forming units were represented by 0.1 mg of pyruvate/l, whereas with counts of about 10^6/ml some 100,000 colony forming units were represented by 0.1 mg of pyruvate/l. Their data are reproduced in Fig. 4.

We approached the problem somewhat differently by making 10-fold dilutions of pure cultures of typical milkborne bacteria and then testing to see which was the lowest concentration at which the dilutions could be differentiated with the pyruvate test.

SPCs revealed that initial concentrations of organisms approximated 10^5/ml in the lowest and 10^2/ml ($10^{2.23} \pm .41$) in the highest dilutions. Gram-positive organisms incubated at 15 C for 24 h generally caused little increase in pyruvate content with increasing initial concentrations (Fig. 5). At 20 C, several of the gram-positive cultures were more active in their pyruvate production (Fig. 6). Pyruvate content in cultures of

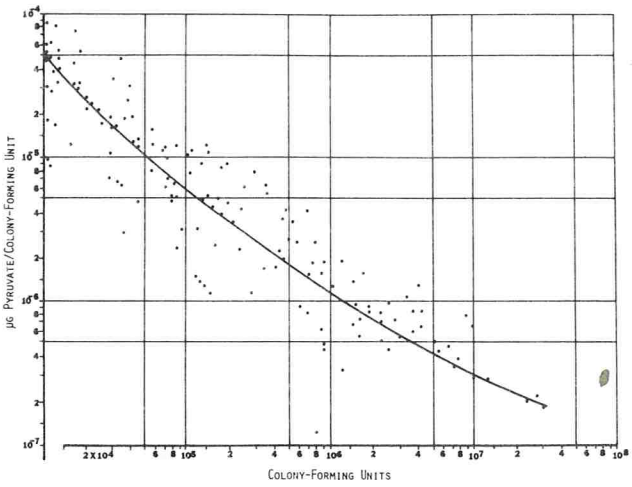

Figure 4. *Quantities of pyruvate per colony-forming unit with different numbers of colony-forming units (after: Tolle and Heeschen, Commission of the European Communities, No. 21, September, 1976).*

Streptococcus faecalis (S4) was the most influenced by initial number of organisms, with initial counts of 10^4/ml and 10^5/ml distinguishable by the pyruvate test. *Lactobacillus acidophilus* (L1) and *Micrococcus luteus* (CC5) also caused noticeable increases in pyruvate concentration although the magnitude of difference between 10^4/ml and 10^5/ml was not as great as that of *S. faecalis*. Neither *Streptococcus agalactiae* (S1), *Streptococcus thermophilus* (S2), *Staphylococcus aureus* (183B), *Micrococcus varians* (T6B), *Corynebacterium bovis* (CB), nor *Lactobacillus plantarum* (L4) produced significant increases in pyruvate content with increasing initial

TABLE 5. *Log means of standard and psychrotrophic plate counts of fresh and incubated samples*

Type of sample	Jars		N. qtrs.		Abn. qtrs.	
	SPC	PPC	SPC	PPC	SPC	PPC
Fresh	4.21	1.73	2.44	0.46	3.02	0.54
Incubated[a]	6.92	5.65	4.26	2.81	4.57	3.07
Change in count	2.71	3.92	1.82	2.35	1.55	2.53
SPC-PPC[b]	1.27		1.45		1.50	

[a]Incubated at 20 C for 24 h.
[b]SPC-PPC: The difference between Standard and Psychrotrophic Plate Counts of incubated samples for the three sources.

TABLE 6. *Effects of incubation temperature on changes in pyruvate concentration [mg/l].*

Temperature	Source of sample: Jar		N qtrs.		Abn. qtrs.	
	Pyruvate (mg/l)]					
	$\bar{X}$[1]	S. D.[2]	$\bar{X}$	S. D.	$\bar{X}$	S. D.
5	.56	.42	.18	.27	.15	.38
10	.69	.43	.28	.26	.31	.38
15	1.33	.93	.46	.29	.83	.79
20	2.26	2.12	.69	.35	1.35	1.26

[1] $\bar{X}$ - Mean.
[2] S.D. - Standard deviation.

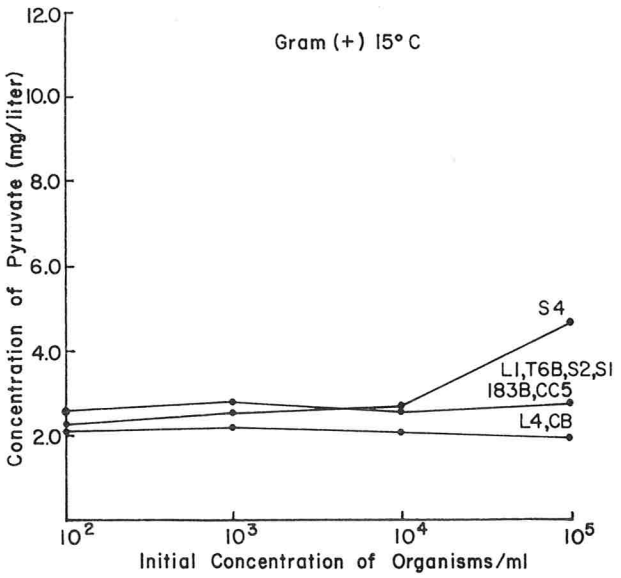

Figure 5. *Mean quantities of pyruvate produced by pure cultures of gram positive bacteria incubated at 15 C for 24 h after 10-fold dilutions were made to provide four concentrations per organism (n = 2).*

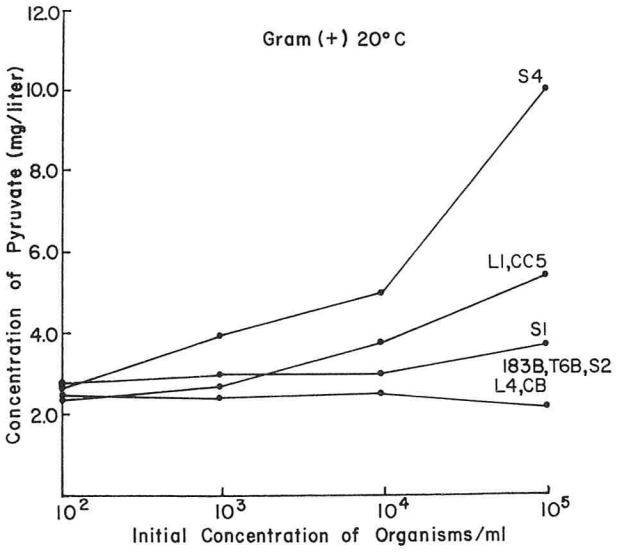

Figure 6. *Mean quantities of pyruvate produced by pure cultures of gram positive bacteria incubated at 20 C for 24 h after 10-fold dilutions were made to provide four concentrations per organism (n =2).*

concentrations of organisms at 20 C for 24 h.

The gram-negative enterobacteria, *Salmonella typhi* (St) and *Escherichia coli* (C5), showed no significant differences in pyruvate production among the four initial concentrations (10^2, 10^3, 10^4 and 10^5/ml) held at 15 C for 24 h (Fig. 7 and Table 7). At 20 C-24 h (Fig. 8 and Table 7), 10-fold concentrations of *S. typhi* still were not distinguished, however an initial concentration of 10^4/ml *E. coli* was distinguished from that of 10^5/ml.

The amounts of pyruvate produced by 10^3, 10^4 and 10^5/ml *Pseudomonas fluorescens* (P26) (initial counts) were all significantly different when samples were incubated at either 15 or 20 C for 24 h. An initial concentration of 10^4 *Pseudomonas fragi* (K1)/ml, was

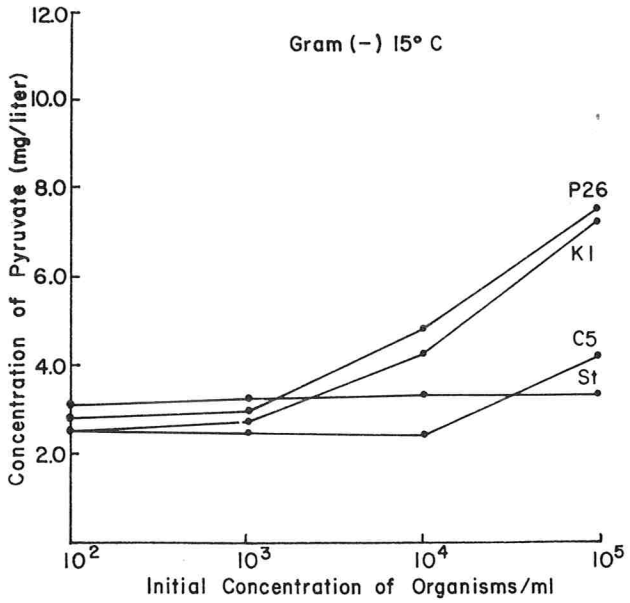

Figure 7. *Mean quantities of pyruvate produced by pure cultures of gram negative bacteria incubated at 15 C for 24 h after 10-fold dilutions were made to provide four concentrations per organism. (n =2)*

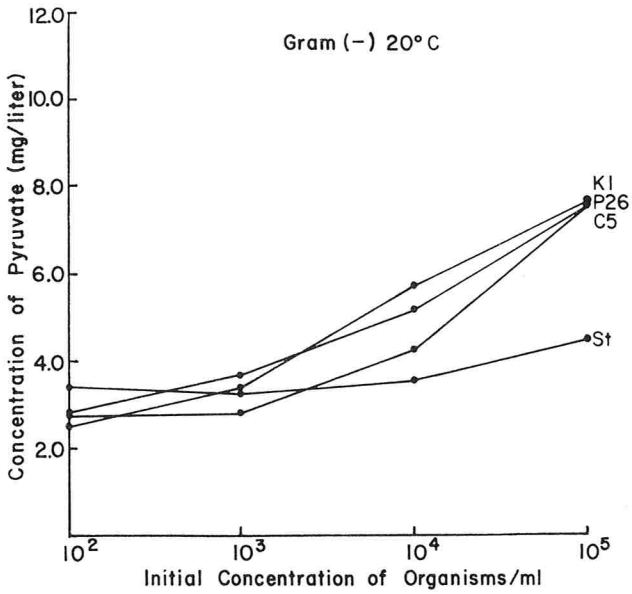

Figure 8. *Mean quantities of pyruvate produced by pure cultures of gram negative bacteria incubated at 20 C for 24 h after 10-fold dilutions were made to provide four concentrations per organism. (n =2)*

distinguished from that of 10^5/ml at 15 C for 24 h, and at 20 C-24-h the pyruvate produced by 10^2, 10^3, 10^4 and 10^5/ml *P. fragi* (initial counts) allowed each cell concentration to be distinguished. Since psychrotrophic bacteria are major spoilage organisms, the ability of the pyruvate test to differentiate small numbers of pseudomonads is of great importance.

Pasteurized milk and the Pyruvate Difference Test

There was no association between initial SPC and IP or between initial PPC and IP. Table 8 shows that the

TABLE 7. *Mean quantities of pyruvate produced by various initial concentrations of gram negative organisms incubated at 15 and 20 C for 24 h (n = 2).*

Temperature	Microorganism	Number of Bacteria/ml 10^2	10^3	10^4	10^5
15 C	*S. typhi*	3.10[a]	3.25[a]	3.25[a]	3.30[a]
	E. coli	2.45[a]	2.55[a]	2.40[a]	4.20[a]
	P. fluorescens	2.80[a]	3.00[a]	4.80[b]	7.50[c]
	P. fragi	2.45[a]	2.70[a]	4.20[a]	7.20[b]
20 C	*S. typhi*	3.40[a]	3.25[a]	3.60[a]	4.45[a]
	E. coli	2.75[a]	2.85[a]	4.30[a]	7.60[b]
	P. fluorescens	2.75[a]	3.70[a]	5.20[b]	7.55[c]
	P. fragi	2.45[a]	3.35[b]	5.75[c]	7.70[d]

[a,b,c,d]Numbers on the same line with different superscripts are significantly different ($P < 0.05$).

TABLE 8. *Relationship between bacterial numbers and quantity of pyruvate in pasteurized milk; two plating methods*

Range of initial Counts/ml	n	Mean IP[a] (mg/l)	Mean Δ P[b] (mg/l)
	Standard Plate Count		
$> 10^4$	3	2.33[c]	6.33[d]
10^3 — 10^4	15	2.55[c]	1.37[c]
10^2 — 10^3	25	2.54[c]	1.46[c]
	Psychrotrophic Plate Count		
$> 10^3$	5	2.36[c]	4.46[d]
10^2 — 10^3	4	2.23[c]	4.28[d]
10^1 — 10^2	6	2.23[c]	1.72[c]
$< 10^1$	29	2.68[c]	0.95[c]

[a]IP - Initial pyruvate.
[b]Δ P - Change in pyruvate concentration during 24 h at 20 C.
[c,d]Numbers in the same column with different superscripts are significantly different ($P < 0.05$).

average initial pyruvate content was similar for each range of counts. The ΔP (20 C-24-h) and initial SPC were slightly associated ($r = .30$; $P < 0.05$), however the ΔP (20 C-24-h) and the initial PPC were more strongly correlated ($r = .59$; $P < 0.0001$). A significant pyruvate difference was reached at a lower initial PPC as compared to SPC.

Extended storage of pasteurized milk

Figures 9-12 illustrate pyruvate production patterns in four individual milk samples held at 5 C for 17 days. Two types of response to pyruvate tests were observed among the 16 samples during extended storage. In one group pyruvate content increased slowly but persistently (Fig. 9 and 10). In the other group pyruvate was produced rapidly, but when the concentration reached about 10 mg/l, it began to decrease dramatically (Fig. 11 and 12). Initial pyruvate, SPC and PPC were not useful in predicting into which group the samples would fall. Yet a pyruvate difference test with incubation at 20 C for 24 h was a good indicator. Of the samples that had decreasing pyruvate content during storage, the mean ΔP (20 C-24-h) was 3.57 mg/l (S.D. = 2.07) whereas the mean ΔP was 0.96 mg/l (S.D. = 1.21) for samples that showed a gradual increase in pyruvate with time. After 17 days of storage, the SPC and PPC were higher in samples in which pyruvate concentrations had decreased. The average log SPC and PPC per ml were 8.60 (S.D. =.24) and 8.50 (S.D. =.33), respectively, for samples that showed a decrease in pyruvate concentrations, whereas for those that gradually increased in pyruvate, the mean values were 6.35 (S.D. =1.60) and 6.39 (S.D. =1.41), respectively.

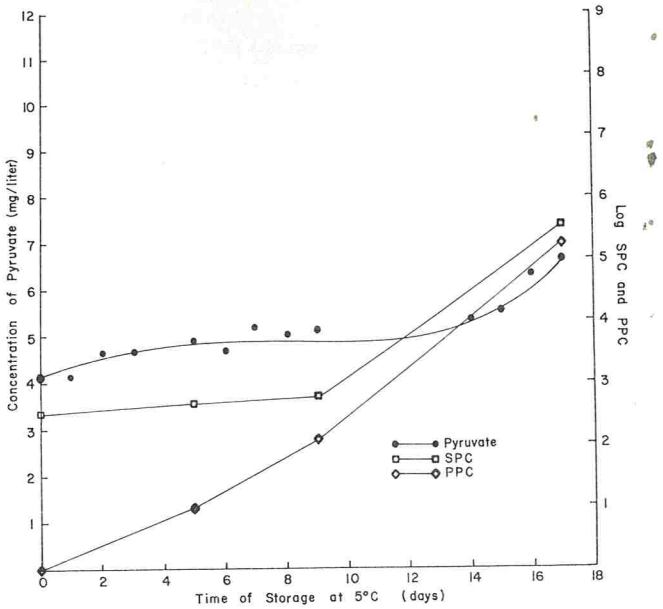

Figure 9. *Standard Plate Counts, Psychrotrophic Plate Counts, and pyruvate concentrations of Brand A pasteurized milk stored at 5 C for 17 days.*

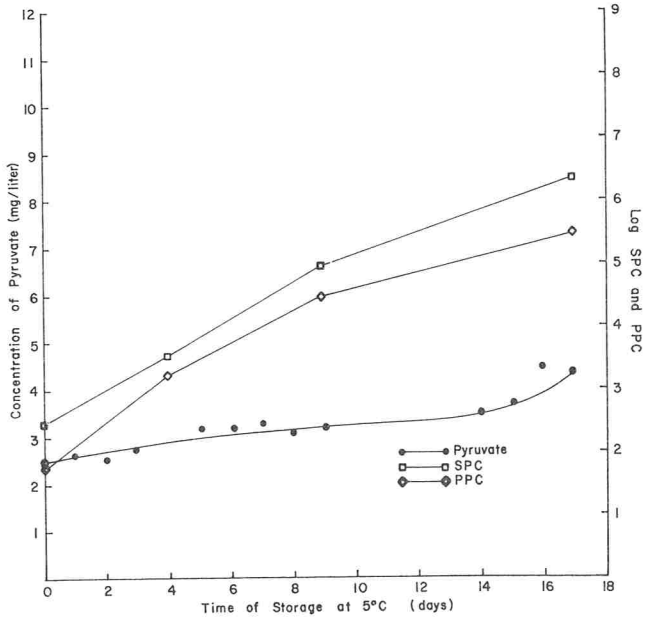

Figure 10. *Standard Plate Counts, Psychrotrophic Plate Counts and pyruvate concentrations of Brand B pasteurized milk stored at 5 C for 17 days.*

Dominant spoilage organisms were transferred to litmus milk from SPC plates of samples that had decreased in pyruvate. All of the organisms were gram-negative rods, oxidase-positive, catalase-positive, and produced no visible changes in litmus milk on incubation at 32 C for 48 h. They were presumed to be of the genus *Pseudomonas.*

Extended storage of pure cultures

Pure cultures of several gram-positive and gram-

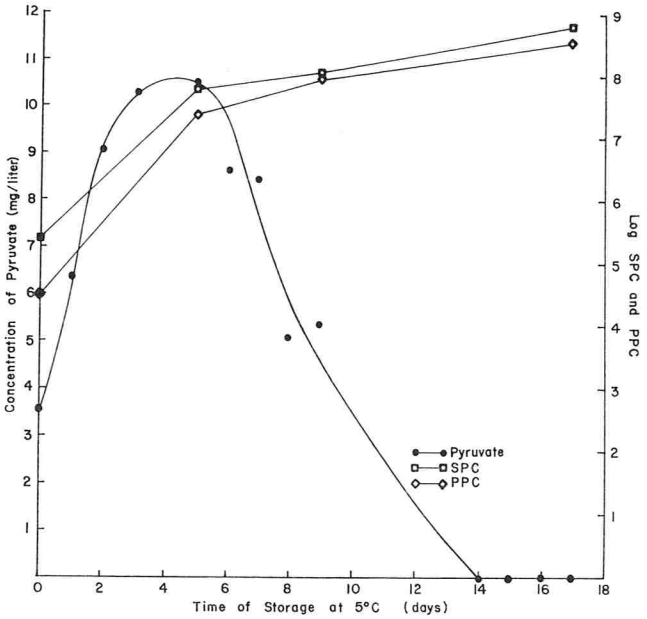

Figure 11. *Standard Plate Counts, Psychrotrophic Plate Counts, and pyruvate concentrations of Brand C pasteurized milk stored at 5 C for 17 days.*

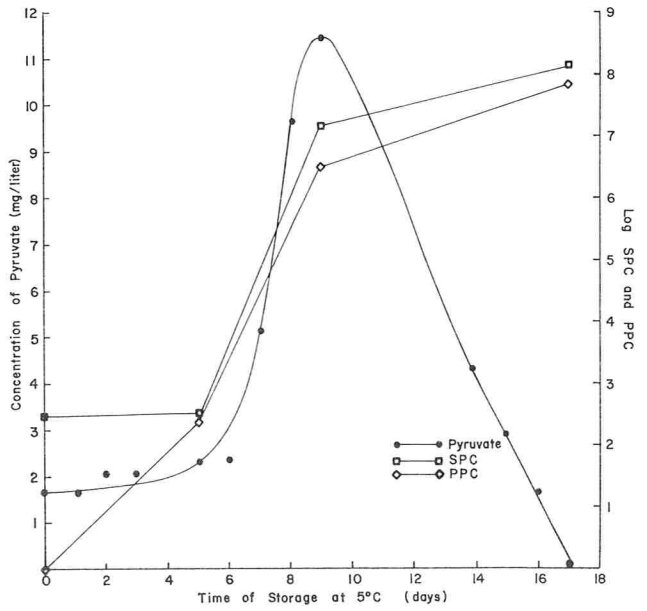

Figure 12. *Standard Plate Counts, Psychrotrophic Plate Counts and pyruvate concentrations of Brand D pasteurized milk stored at 5 C for 17 days.*

negative organisms were grown to study the pyruvate production of individual organisms at 20 C for up to 7 days. Pyruvate content generally increased in cultures of gram-positive organisms, with several production curves leveling off near 11.0 mg/l (Fig. 13).

There were two basic trends among the gram-negative organisms. The enterobacteria, *E. coli* (two strains) and *Enterobacter aerogenes,* sharply increased pyruvate content from Day 1 (Fig. 14). The concentration of pyruvate decreased, then slowly increased. In pure cultures of *P. fluorescens* and *P. fragi*, pyruvate content

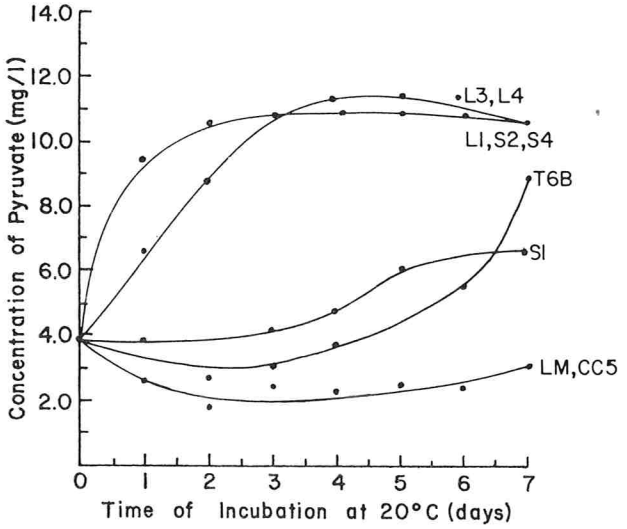

Figure 13. *Patterns of pyruvate production by gram-positive bacteria. L1, L3, and L4-lactobacilli; S1, S2 and S4-streptococci; LM-leuconostoc; CC5 and T6B-micrococci.*

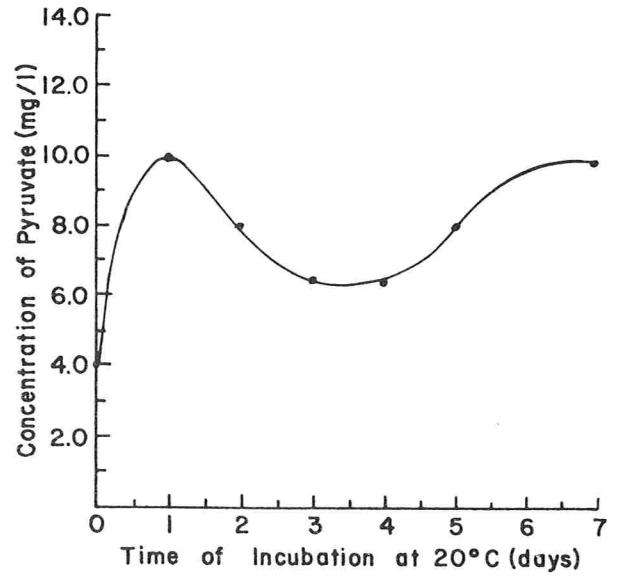

Figure 14. *Pattern of pyruvate production typical of both* Escherichia coli *and* Enterobacter aerogenes.

increased from Day 0 to Day 2, but then the concentration began to decline (Fig. 15). These observations support the previous observation that pseudomonads were the dominant organisms in samples of pasteurized milk in which pyruvate concentrations decreased markedly.

In evaluating the pyruvate test it is important to remember that no other test measures the same parameters. Therefore, limitations of the other tests must be considered in any comparison.

The quality test needed by the dairy industry would ideally detect small numbers of psychrotrophic bacteria and would give more weight to psychrotrophs that grow rapidly and produce large amounts of degradative enzymes. It might also give certain weight to mastitis bacteria and to somatic cells in raw milk. The pyruvate

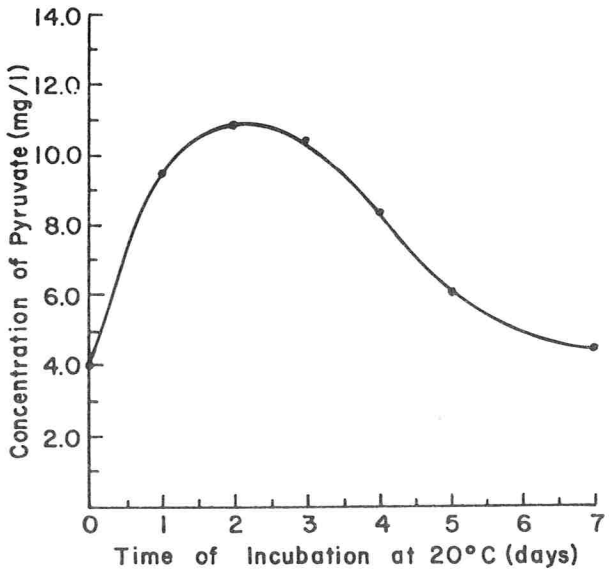

Figure 15. *Pattern of pyruvate production typical of both* Pseudomonas fluorescens *and* Pseudomonas fragi.

test appears to have these characteristics.

The present research indicates that gram-negative bacteria are detectable by the pyruvate difference test (20 C-24 h) in lower numbers than are gram-positives. Thus the test is biased toward detecting the most undesirable contaminants of milk. However, minimum numbers detectable approximate 100/ml of highly active bacteria. In fresh raw milk from receiving jars numbers of psychrotrophs ($\overline{\chi}$ =54/ml) were too few to be detected by the pyruvate difference test.

In raw milk in which there are numerous mastitis bacteria and somatic cells, one would expect the pyruvate difference test to be less sensitive to gram-negative rods. However, it does discriminate against abnormal milk.

The initial pyruvate concentration of fresh raw milk is influenced both by inflammation of the mammary gland and by external contamination. The contribution to the pyruvate pool by each factor differs from sample to sample, depending on proportions and activities of the various cellular constituents.

The mean content of pyruvate in fresh pasteurized milk, which averaged about 2.5 mg/l in our experiments, cannot be expected to reflect either the SPC or PPC. However, ΔP (20 C-24-h) is a relatively good indicator of PPC.

Some bacteria, mostly psychrotrophs, apparently metabolize pyruvate that has been released from cells. Whether this enters the Krebs cycle or is otherwise metabolized, and the reason for the change in metabolism has not been determined. However, decreases in pyruvate concentration are associated with the stationary phase of growth. Therefore, it might be expected that primary energy sources may have been exhausted when the decrease in concentration occurs. Evidently concentration of pyruvate is not the triggering mechanism, because once the maximal concentration is reached it does not stabilize but continues to decrease to an undectable quantity.

Were it not for this ability of some bacteria to use pyruvate, the test for pyruvate concentration in pasteurized milk would be a very good indicator of psychrotrophic count. It can serve as such when tests are done on consecutive days to reveal whether pyruvate concentration is increasing or decreasing.

An effective system of monitoring the keeping quality of pasteurized milk could be set up wherein numerous samples from each day's production could be stored at one or more temperatures. Daily tests of pyruvate content could be fed into a computer which would collate the data and present it in tabular or graphic form. From these data, management could make decisions regarding time and temperature constraints on distribution and on the sanitary status of fillers and milk lines.

Presently such decisions are usually based on the taste and odor or bacterial count of small numbers of samples stored at 7 C for 5-7 days. Interpretation of such results presents significant problems to management, especially when contamination is sporadic or localized. Obtaining a representative sample is the primary problem. For example, each container, filler valve, float tank, milk line and pasteurized storage tank is a variable. The lower the level of contamination and the closer it occurs to the container, the more variability in keeping quality among individual containers of product. Obviously, the larger the number of samples, the greater the probability that decisions of management will be correct.

Up to 120 samples per hour can be analyzed with the present pyruvate analyzer. Only a few milliliters of sample are needed for each test.

Since contaminants in equipment are likely to be dislodged as product first flows to containers, the first containers filled will likely carry the largest number of contaminants and such samples will likely have the shortest shelf life. However, there is potential for buildup of microorganisms and for external contamination. Therefore, periodic sampling during operation is advisable.

A logical program would involve sampling from each pasteurized storage tank, filler bowl and 10% of the filler valves at the beginning of each packaging period. Subsequently, single samples should be randomly taken from filler valves of each filler about every three hours.

Since sampling cartons may involve considerable product loss, samples from filler valves may be taken in plastic bags and formed containers may be tested periodically by the carton rinse test of Standard Methods (*1*). In addition, samples should be taken from cartons obtained for other test purposes.

As an example of the work load, assume that a plant fills containers 12 h/day on six fillers that have 80 filler valves and six pasteurized storage tanks. Twenty samples would be taken initially and about 20 would be taken later depending on scheduling. These samples should be tested on collection, stored at 7 C and then retested every

other day. If testing were continued for 8 days, the maximum number of samples tested per day would be 160.

An alternative to this is the performance of pyruvate difference tests and using the values obtained to predict shelf life. We are currently researching this application of the test.

Equipment for the test is expensive. Therefore, large numbers of samples are needed to justify purchase. Operational expenses for enzyme, $NADH_2$, miscellaneous chemicals, membranes, and tubing approximate 10 cents/sample under our conditions of use. They could be as low as 4 cents per sample if the instrument were used at capacity.

There are questions to answer yet regarding applicability of the automated pyruvate method to Grade A raw milk. We need to complete tests on a large number of bulk milk samples. We need to know what percentage of the pyruvate is contributed by somatic cells, mastitis bacteria and contaminants from the exterior of the cow and equipment. Since psychrotrophs usually are a small percentage of the microflora of fresh raw milk, we need to know the proper conditions for conducting the pyruvate test to determine when their presence and activities are excessive. However, the pyruvate test can fill the need for a quick and effective test of bulk tank raw milk received at plants. A value of 2 to 2.5 mg/l could serve as the cutoff. Extensive work by Tolle and Heeschen indicates that about 10^6 bacteria per milliliter are represented in West German raw bulk milk by this quantity of pyruvate. Sufficient numbers of samples need to be examined from the Grade A supply of this country to establish whether a different pyruvate value is applicable.

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Diffusion of Curing Brine in Tumbled and Non-Tumbled Porcine Tissue[1]

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ABSTRACT

Tumbling action and time of tumbling both increased the migration of individual cure components of sodium chloride, dextrose, sodium nitrite as well as the average brine (average migration of these components) in porcine tissue. In most instances the difference became apparent after 3 or 4 h (tumbled 10 min/h) of treatment and remained significantly ($P < .05$) different during the remainder of the 18-h treatment period.

Tumbling is the mechanical agitation of meat and the generation of impact energy on muscle tissue as the meat falls in a rotating drum (*11,13*). The primary objectives of this technique are to produce enough protein exudate to effectively promote cohesion during processing, enhance tenderness, insure juiciness, develop a uniform product with desirable slicing characteristics and increase yield (*3,5-9,13*). In addition, Viskase Limited (*12*) stated that a more even distribution of cure is obtained and Krause (*2*) found more diffusion of salt and nitrite after 18 h (10 min/h) of tumbling.

The purpose of this project was to compare the diffusion rate of sodium chloride, sodium nitrite and dextrose in tumbled and non-tumbled porcine tissue.

MATERIALS AND METHODS

Eight porcine semimembranosus muscles from eight hams were each divided equally into two pieces by cutting parallel to the bone. Each section of the muscle received a 6%, by weight, injection of brine. The injection was made parallel to the muscle fibers and delivered by a stitch needle drilled with extra holes in an attempt to deposit a core of cure in the center of the muscle. The brine was composed of the following ingredients by weight; 76.8% water, 16.5% sodium chloride, 6.6% dextrose and 0.092% sodium nitrite. After injection, one-half of all the semimembranosus muscle pieces were randomly assigned to the tumbling treatment and the other half to the non-tumbled treatment. The tissue was tumbled in an Ohio State University-constructed rotating stainless steel drum (56-cm diameter, 85-cm depth, three baffles) at 12 revolutions per minute for 10 min out of each hour for 18 h. The non-tumbled tissue was placed in a stainless steel container. Both tumbled and non-tumbled tissues were placed in a cooler at 2 ± 2 C for the 18-h treatment period. The tissue for each treatment was sampled at 0 h and each hour thereafter by taking a 1-cm slice perpendicular to the cure injection core. Sufficient slices were taken to have a fresh cut internal surface for each analysis. The experiment was repeated seven times.

The area of diffusion of salt (sodium chloride) was determined by the following technique. Eleven-cm Whatman number 42 filter paper was dipped in a 25% (wt/wt) solution of silver nitrate. The paper was then dried in an oven at 70 C. After slicing the muscle to be analyzed, the silver nitrate paper was pressed firmly against the tissue, removed from the tissue and the paper then was dried quickly (20 sec) in a Hobart 1250 R, 3000 watt microwave oven containing a dummy load of 227 g of tap (15 ± 2 C) water. After drying, the paper was exposed to a Thermolyne (LL-6515), 0.24 amperes, 60 Hz ultraviolet light for color development. A gray-black area on the paper was caused by the reaction of silver nitrate with sodium chloride to produce silver chloride and this compound darkened upon exposure to light. The colored area was traced on acetate paper and a compensating polar planimeter measurement of the area was used as an indicator of sodium chloride diffusion. This injection procedure and measurement technique resulted in a standard deviation of 0.72 cm^2 at zero time.

The area of diffusion of dextrose was determined by a similar technique except the filter paper was dipped in Fehling's solution [193 g of sodium potassium tartrate and 50 g sodium hydroxide in 500 ml of water (boiled and cooled water); 34.6 g of copper sulfate in 500 ml of water (boiled and cooled water); both solutions were aged for 24 h, mixed and the paper submerged], dried, pressed against meat tissue, removed from the tissue and the paper then was quickly dried in the microwave oven. Dextrose as a reducing sugar resulted in a yellow color (cuprous oxide) in the area that contained the sugar (standard deviation at zero time was 0.58 cm^2).

The area of sodium nitrite migration was determined by placing the sample in an aluminum pan and baking for 15 min in a 121-C oven. Sodium nitrite diffusion was determined by the area of pinkish-red cured pigment (nitrosohemochrome). The standard deviation at zero time was 0.13 cm^2.

The area values from sodium chloride, dextrose and sodium nitrite were averaged to yield an average brine area (standard deviation at zero time was 0.42 cm^2). Average linear distance of diffusion can be calculated from the injection site if the assumption is made that the migration pattern is circular in nature.

Analysis of variance (*1*) of the total experiment was used to determine the effect of tumbling, time (linear, quadratic or cubic) and their interaction. This analysis was also repeated for each hour and adjusted for time effects, to evaluate when tumbling became significant and the same least squares analysis technique was used to fit curves to the data.

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RESULTS AND DISCUSSION

The equations for the tumbled and non-tumbled treatments giving best fitting curves for salt (sodium chloride), dextrose, nitrite (sodium nitrite) and average brine (average of three curing ingredients) are shown in Table 1. These curves are graphed in Fig. 1 (salt), 2 (dextrose), 3 (nitrite), and 4 (average brine). Table 2 shows the significance test for tumbling at each time interval adjusted for time effects.

Salt migration for tumbled tissue was different ($P < .01$) from non-tumbled at the 3-h sampling period and remained significantly different until the 17-h sampling period (Fig. 1). The analysis of variance indicated that the salt area was larger ($P < .01$) for the tumbled than the non-tumbled samples for the average storage time (average time value). This was as suspected as 14 out of 19 sampling periods indicated significance. This agrees with Krause (*2*) who found higher salt levels for tumbled at three sample locations at graduated distances from the site of injection. Time affected the salt area ($P < .01$) and was cubic ($P < .05$).

Dextrose was not different ($P < .05$) at the average time value between tumbled and non-tumbled samples but the area became significantly larger for tumbled samples at the 13-h sampling period and remained significantly larger during the remainder of the experiment. Time was also significantly different and quadratic ($P < .01$).

The nitrite area was larger ($P < .01$) at the average time value for tumbled than for non-tumbled pork tissue. This agrees with Krause (*2*) who found that tumbling

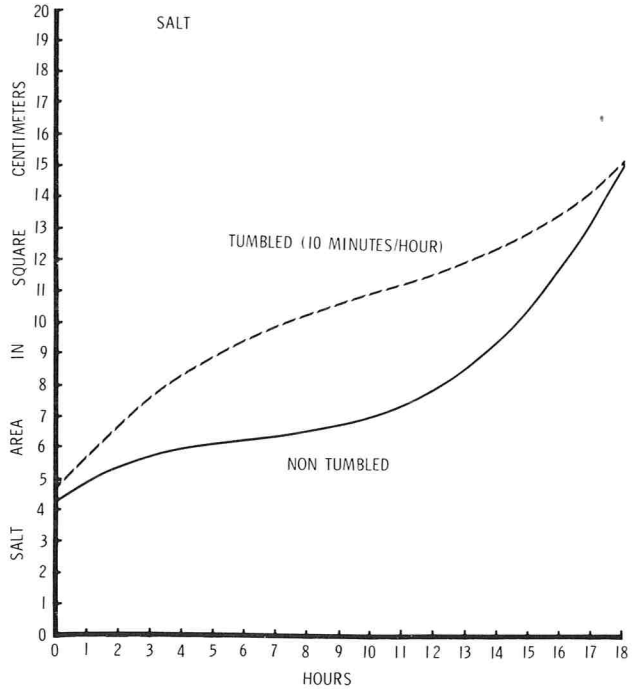

Figure 1. *Area of salt during curing process.*

increased migration of nitrite. When analyzed at each hourly sampling period, the nitrite area became larger ($P < .01$) after 4 h and remained larger ($P < .01$) throughout the processing period. The time effect was cubic ($P < .01$) in nature.

The average area for brine (average of three curing ingredients) was larger ($P < .01$) at the average time value

TABLE 1. *Polynomial curves for salt, dextrose, nitrite and brine*

Ingredient	Treatment	Equation[1]
Salt	Non-tumbled	Square Centimeters of Area = $4.3409618 + 0.6713156$ (hours) -0.0850975 (hours)^2 + 0.0045289 (hours)^3
	Tumbled	= $4.5801565 + 1.2739512$ (hours) $- 0.0947488$ (hours)^2 + 0.0031516 (hours)^3
Dextrose[2]	Non-tumbled	Square Centimeters of Area = $2.3455494 - 0.1489027$ (hours) + 0.0338759 (hours)^2
	Tumbled	= $2.2549548 - 0.2593708$ (hours) + 0.0592876 (hours)^2
Nitrite	Non-tumbled	Square Centimeters of Area = $3.6060182 + 2.0261898$ (hours) $- 0.1797330$ (hours)^2 + 0.0054249 (hours)^3
	Tumbled	= $2.9999049 + 3.2206919$ (hours) $- 0.2624715$ (hours)^2 + 0.0074954 (hours)^3
Average brine	Non-tumbled	Square Centimeters of Area = $3.3587710 + 0.7162476$ (hours) $- 0.0576830$ (hours)^2 + 0.0025966 (hours)^3
	Tumbled	= $2.9843230 + 1.5965655$ (hours) $- 0.1252810$ (hours)^2 + 0.0045384 (hours)^3

[1]All b values used are significant ($P < .05$)
[2]Cubic term was non-significant ($P > .05$)

TABLE 2. *Significance[1] of tumbling on ingredient area at each sampling period*

	Hour																		
Ingredient	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Salt	NS	NS	NS	**	**	**	**	**	**	**	**	**	**	**	**	**	*	NS	NS
Dextrose	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	**	**	**	**	**
Nitrite	NS	NS	NS	NS	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
Average brine	NS	NS	NS	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**

[1]NS — Non-Significant ($P > .05$).
*Significant at $P < .05$.
**Significant at $P < .01$.

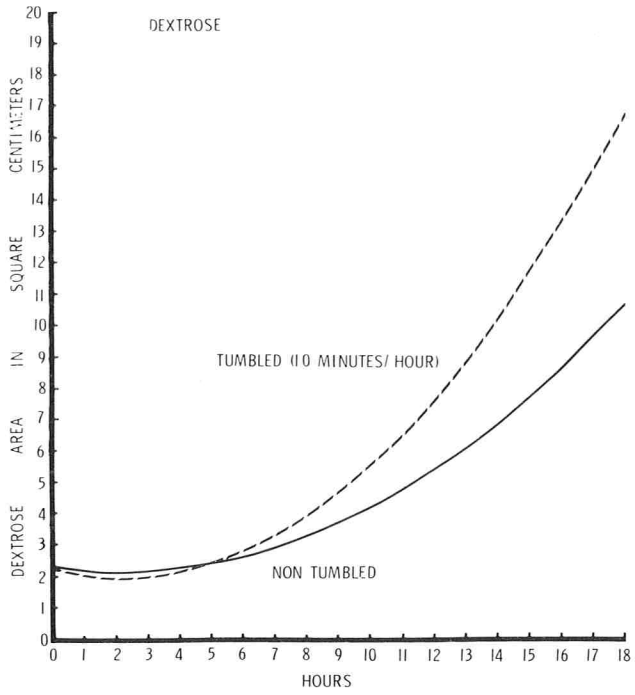

Figure 2. *Area of dextrose during curing process.*

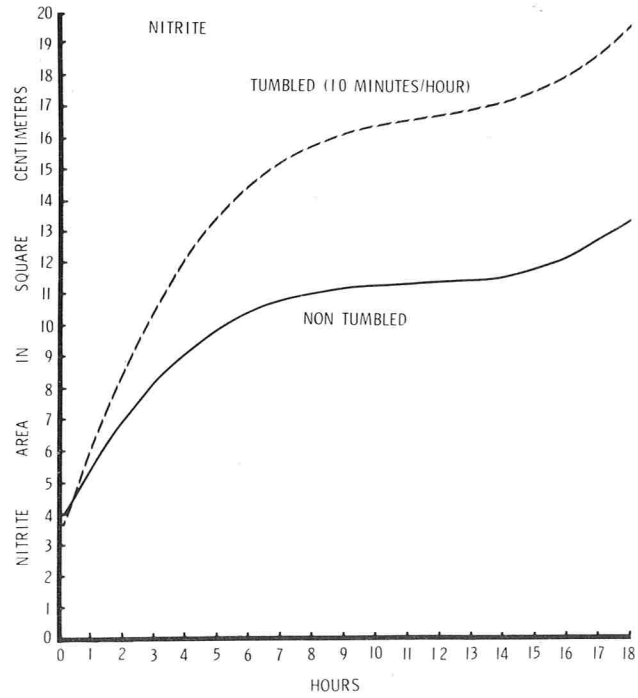

Figure 3. *Area of nitrite during curing process.*

for the tumbled tissue when compared to non-tumbled tissue. This is suggested by Rust and Olson (*5*) who found that hams that were stitched or artery pumped needed a holding period of seven days to achieve significant uniform cure distribution and by Mass (*4*) who claimed that tumbling can shorten curing time to 24 h. This also agrees with Viskase Limited (*12*) who reported that the function of tumbling is to uniformly distribute curing ingredients. The average brine area became higher

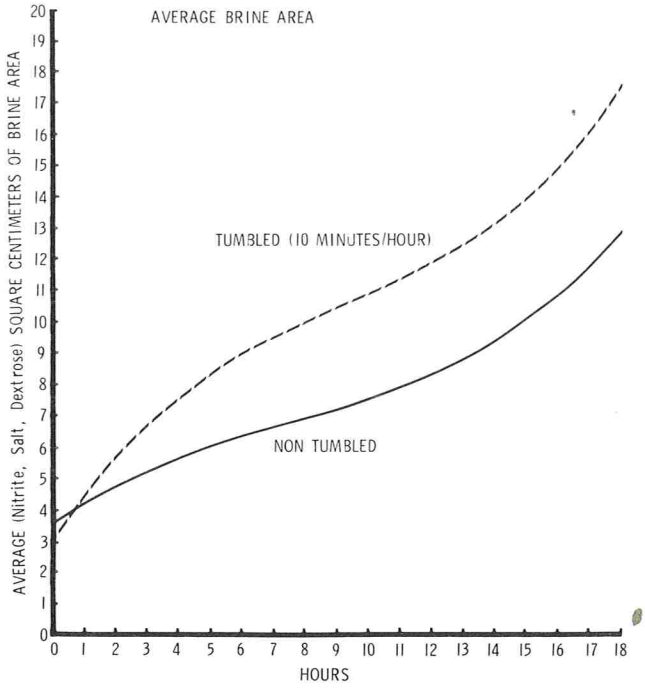

Figure 4. *Area of brine during curing process.*

($P < .01$) for tumbled tissue at the 3-h sampling period and remained higher for the remainder of the experiment. This agrees with Vartorella (*10*) who found that tumbling continuously for 30 min significantly increased cohesiveness scores of tumbled tissue. Time was significant for average brine area ($P < 0.01$) and was cubic ($P < .01$) in nature.

Further work is needed to evaluate the influence of different tumbling conditions and times on the brine diffusion rate.

ACKNOWLEDGMENT

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Book Review

Microbiology of Foods. Occurrence, Prevention and Monitoring of Hazards and Deterioration. D. A. A. Mossel. The University of Utrecht; Vakgroep Voedingsmiddelen van Dierlijke Oorsprong, Faculteit Diergeneeskunde, Biltstraat 172, Utrecht, The Netherlands, 1977, 165 p., $9.00.

This book is different. It is an admirable integration of the many and diverse interrelationships of microorganisms and the environment which develops into a complete understanding of Food Microbiology. The preface says that it is a revised version of an earlier manuscript. One that corrects a few minor errors and includes a few desirable changes. It also states that it is used by post graduate students, both "switch" and "refresher" attendants at the University of Utrecht.

The book is divided into five major subject areas or chapters. The organization is such that the text is efficiently back-referenced by the paragraph notation, i.e., 2.4.4 Viruses. In addition, the statements in the text are extensively documented. The 88 pages of text use a total of 2310 up-to-date references. Use of superscripts to indicate the documentation does not interfere with the reading of the text but does provide for efficient referral to the appropriate reference(s).

The first section is entitled, "General Principles of Microbiological Control," and is really an introduction to the book. Here Mossel points out that Food Microbiology is commonly considered to have the main areas of interest; protection against foodborne microbial diseases and prevention of food spoilage produced by microorganisms. He develops the possible interrelationships between these two categories of interest. He points out that all handling and processing activities affect the microbial content and emphasizes the inportance of quality assurance. Dr. Mossel prefers to include a discussion of all these interrelationships as the subject matter for Food Ecology. He then says "For the reasons presented above, the Microbial Ecology of Foods has been chosen as the central theme of this book."

Section Two is entitled, "Diseases of Microbial Origin Transmitted by Food." This comprehensive succinct chapter covers a variety of illnesses which commonly or occasionally have been related to ingestion of contaminated foods. The tables effectively collect pertinent data which efficiently supplement the text. Examples are Tables 4 and 6 which are entitled, "Amino Acid Decarboxylative Potentialities Encountered Among Bacteria" and "Review of the Morbid Effects of Some Mycotoxins." The chapter concludes with a brief discussion of "Helminthic Diseases" which is accompanied by a series of figures which describe the mode of spread by the particular agent.

Section Three is entitled "Microbial Deterioration of Foods." I particularly enjoyed this section as the author carefully integrated and described the various intrinsic and extrinsic factors which lead to food spoilage. He begins his discussion of the intrinsic factors affecting food spoilage with the complex problem of water content and water activity (a_w). This is adequately described and the discussion again supplemented with a series of tables which provide interesting additional information. Analytical discussions follow on acidity and buffering capacity, redox potential, an interesting discussion on nutrients, natural antibacterial constituents and structure. He discusses the influence of various types of processing and the expected spoilage patterns when sterilization is not achieved. His discussion of the various extrinsic factors (temperature, water vapor migration, gaseous environment) including the effect of an increased partial pressure of CO_2 is direct and to the point. He concludes this section with an interesting discussion entitled "Implicit Influences on Primary Spoilage Associations." Here he discusses and further integrates his comments of the earlier parts of this section and includes a discussion on particularly resistant types of organisms and their control.

Section Four is entitled, "The Control of the Microbial Quality of Foods." Here he degrades, and rightly so, the process of using a retrospective system for evaluating the microbiological examination of foodstuffs. He provides cogent arguments to support his thesis. In the place of the above mentioned system he advocates development and adherences to "Codes of Good Manufacturing and Distributive Practices" which is commonly abbreviated GMP. When such a system becomes operational, then monitoring becomes a valuable tool. He provides a meaningful discussion of the many ramifications on "Obtaining Raw Materials of Acceptable Microbiological Quality" even though he becomes philosophical in expecting to totally rid this earth of *Salmonella.*

It is understandable that in his discussion of "Food Processing by Heat" it is natural that much of the discussion relates to meat products. Here he discusses the importance and value of nitrite in obtaining safe shelf-stable products for cured meat products which had been midly cured. He refers the reader back to his statement in an earlier section where he discussed resistant types. "This is particularly the case with nitrites, certainly among the most useful chemical inhibitors used in foods since time immemorial."

This section continues with major subsections as follows (*a*) Food Processing by Compositional Modification. Here he emphasizes the interrelations between added substances (nitrate, NaCl, change in pH) and intrinsic and extrinsic properties of the food. An interesting presentation. (*b*) Temperature Control During Storage and Distributing. The author emphasizes the importance of good refrigeration control and points out that for many materials the shelf life is still short. Modification of the foodstuff may be attempted to further extend the shelf life.

He concludes this section by discussion "The Role of the Analytical Laboratory in Quality Assurance." Here Mossell points out that it is common to monitor the final product but he emphasizes that it is even more important to monitor the production lines. He goes into considerable detail.

The final major section of the book is entitled, "The Microbiological Monitoring of Foods." This section is well done and quite extensive. He emphasizes that the criteria used should be a minimum but appropriately they could differ widely for different foods. Professor Mossel analyzes the problem of good sampling and the statistical problems involved. Use of index organizing or groups of organisms is developed from a historical to a modern conceptual point of view. This section is perhaps the most detailed but it is a "must" reading to those involved in the use or the evaluation of index organisms as a sanitary tool.

The author concludes Section Five with a major sub-title, "Microbiological Reference Values for Foods." Most will quickly recognize that he has borrowed from the clinical sciences fields for this terminology. However, it is good. Hopefully this can be used in a

. . . *Continued on page 186*

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Role of Histidine and Tyrosine Decarboxylases and Mono- and Diamine Oxidases in Amine Build-Up in Cheese

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ABSTRACT

Presence of biologically active amines in foods has been recognized for about 20 years. Past research on amines such as tyramine and histamine has not investigated factors which account for the variable concentrations often found in food products. This research was designed to investigate the roles of tyrosine and histidine decarboxylase activities and mono- and diamine oxidases (MAO and DAO) in amine formation and catabolism in cheese. The data indicate that the presence of decarboxylases is probably not the limiting factor for amine build-up. Most cheeses contained decarboxylase activities for both tyrosine and histidine; however, tyramine and histamine were present in some cheeses which did not contain detectable levels of the corresponding decarboxylases at the time of sampling. Few cheeses possessed MAO or DAO activities. When the oxidases were present, the amine contents were usually lower. A Colby cheese containing tyramine and histamine with high MAO activity also possessed high tyrosine decarboxylase activity. It appears that a major limiting factor in formation of biologically active amines in cheese is the availability of free amino acids for decarboxylation. Dairy-related bacteria generally lacked the amine oxidases. However, five of six dairy-related cultures with highly active decarboxylases showed MAO or DAO activities. The culture showing the highest DAO activity, *Microbacterium lacticum*, possessed low tyrosine and histidine decarboxylase activities.

Monoamine oxidase (MAO) (EC 1.4.3.4) provides an important degradation pathway for biogenic amines (*10,16*). The enzyme was originally named "Tyramine oxidase", since the pressor amine, tyramine, was its first known substrate (*4*). Distribution of this enzyme in nature is widespread, being found in both bacteria and animals. *Sarcina lutea* is the most well-known bacterial source (*10,16*). Diamine oxidase (DAO) EC 1.4.3.6) also provides a degradation pathway for the biogenic amines. The discovery of therapeutic drugs that interfere with diamine oxidase activity has brought attention to this enzyme (*13*). Typical sources of DAO are pig liver, pig kidney, human placenta, blood plasma, and micro-organisms such as *Pseudomonas aeruginosa, Escherichia coli, Proteus vulgaris, Serratia marcescens, Sarcina flava,* and *Clostridium feseri* (*5,7*). Monoamine oxidase has been converted reversibly into a diamine oxidase (DAO) by treatment with cupric sulfate (*16*). The resulting DAO was active against putrescine and spermine, but not histamine (*16*). Kapeller-Adler (*8*) and other researchers (*2,15*) published adaptable radiometric assays for the oxidases.

Iproniazid, a monoamine oxidase inhibitor (MAOI), was introduced in 1951 as a chemotherapeutic agent for treatment of tuberculosis (TB). Six years later this drug and related compounds were hailed as therapeutic agents of unlimited possibilities for treatment of depression. These drugs were found to have favorable psychological effects on a variety of unrelated diseases, independent of the improvement of the disease (*1,3*). However, fear of disastrous complication, such as hepatic toxicity, and the sudden rise of blood pressure (cheese reaction), resulted in withdrawal of some of these compounds from the market and in formulation of control guidelines for the remainder. The observation that iproniazid inhibits MAO provoked a hypothesis on the mechanism of action to account for the euphoriant effects of the drug and provided an explanation of the cheese reaction (*4*). The drugs markedly enhance the pressor activity of the sympathomimetric amines. The tyramine threshold was shown to be potentiated 10 to 100 times, a result being that only 6 mg of tyramine is required to produce pressor effects (*3,6*). The drugs also interfere with DAO activity, which is an additive factor, since many of the diamines, such as histamine, are biologically active.

The presence of biogenic amines in foods has been confirmed by many researchers and was recently reviewed by Rice et al. (*14*). The factors limiting the concentrations of the amines in the products have not been explored. As part of a study to identify and quantitate biologically active amines in various foods, the tyrosine and histidine decarboxylase activities and the mono- and diamine oxidase activities in cheese and several dairy-related bactera were determined. The objectives of the study were: (*a*) to assess the relationship between the contents of the amines in cheese and the levels of decarboxylases and oxidases in cheese; and (*b*) to determine the role that dairy-related bacteria may play in metabolizing amines.

MATERIALS AND METHODS

Cheese amine contents

The 156 cheese samples used in this study were randomly obtained from commercial sources in the South and Midwest. The details of the amine extraction and quantitation techniques, along with the tyramine, histamine, and tryptamine contents of the various cheeses were reported earlier (*18*).

Bacterial cultures

Thirty-two dairy-related cultures were obtained from Miles Laboratories, Chrs. Hansen's Laboratory, Kraft Foods, and the University of Nebraska, North Carolina State University, University of Wisconsin, and University of Georgia. The maintenance conditions for the cultures were described in a previous publication (*17*).

Decarboxylase assays

The tyrosine and histidine decarboxylase activities were determined by an isotopic decarboxylase assay method adapted from the procedure of Levine and Watts (*9*). The procedure was described in a previous publication (*17*).

Oxidase assays

Porcine oxidases from hog kidney were used to provide reference standards of the mono- and diamine oxidases. These isolates were obtained by combining the method outlined by Otsuka and Kobayashi (*12*) for MAO with the method of Okuyama and Kobayashi (*11*) for DAO.

Monoamine oxidase was assayed isotopically using a modification of a procedure by Otsuka and Kobayashi (*12*). The reaction mixtures consisted of 2 ml of buffered sample (kidney isolate, cheese, or cultural media; 3:1, buffer: sample, 0.2 M phosphate buffer pH 4.5, 5.5, 6.5, 7.5, 8.5) and 100μl of [^{14}C-]tyramine (Amersham/Searle Corp., Arlington Heights, Ill.) (3×10^{-4} M tyramine•HCl, 1 μCi/ml) in a 16×100 mm tube. The reaction mixtures were incubated at 37 C for 60 min in a Dubnoff Metabolic Shaker. The reaction was stopped by addition of 0.4 ml of 2 M citric acid. Ten milliliters of a toluene scintillation fluor (4 g of 2,5-diphenyloxazoyl (PPO), 100 mg of p-bis (2-(5-phenyloxazoyl)) bezene (POPOP), and 1 liter of toluene (all Beckman, Fullerton, California) were added, the mixture was agitated twice with a Genie Vortex mixer for 30-sec intervals over a 5-min period, and then centrifuged at approximately $5{,}000 \times g$ for 15 min. The aqueous phase was frozen at –20 C for 20 min after which toluene layer was decanted into scintillation vials and the radioactivity determined with a Beckman Model LS-100C Liquid Scintillation Counter.

The assay for diamine oxidase followed the same procedures as for MAO except that [^{14}C-]putrescine (Abersham/Searle Corp.) was used as the substrate and the reaction was stopped by addition of excess $NaHCO_3$. The method was a modification of a procedure by Okuyama and Kobayashi (*11*). The diamine oxidase reaction on histamine is not as easily assayed as that of putrescine because of the difficulty in obtaining a non-ionized product (soluble in the non-polar solvent toluene) and an ionized substrate (insoluble in toluene). The MAO and DAO activities present in 58 of the cheese samples and in the 32 dairy-related cultures were determined.

RESULTS AND DISCUSSION

Table 1 gives the tyramine and histamine contents and the tyrosine and histidine decarboxylase activities of 156 cheese samples. One hundred and thirty-eight of the 156 samples were able to release $^{14}CO_2$ from tyrosine. Tyramine was found in measurable quantities in 148 of the samples examined. The highest tyrosine decarboxylase activities were found in the Medium Cheddar, Reblochon, and Colby varieties, which do not correspond to the cheeses with the highest tyramine contents, i.e., Edam, Swiss, Roquefort, Blue, Sap-Sago, Danbo, Tybo, Stilton, and German Golblock. It is apparent from the data that high levels of tyrosine decarboxylase did not lead to higher tyramine contents. One hundred and forty of the samples were able to release $^{14}CO_2$ from histidine. Histamine was detected in only 30 of the samples. The highest histidine decarboxylase activities were found in California Jack, Romano, Mimolette, Gietost, Danbo, and Stilton. There was no histamine detected in these samples. Again, it is evident that the histamine concentration is not dependent upon the level of decarboxylase in the finished cheese.

The consistent presence of tyrosine and histidine decarboxylase activities in cheese in the absence of corresponding quantities of the respective amines is an indication of the existence of a limiting factor(s), such as: an unavailability of tyrosine or histidine, presence of decarboxylase inhibitors, and/or presence of catabolism pathways for the amines. The mono- and diamine oxidases could provide such a pathway. Oxidase activities are especially sensitive to pH; thus a range of pH values were employed in the survey for mono- and diamine oxidases in the cheeses (pH 4.5 - 8.5). Only the assays from the pH 6.5 analyses are reported. Lower pH values resulted in either lower levels or the absence of the oxidases being detected. Table 2 and 3 present the amine contents and the decarboxylase and oxidase activities of 58 cheese samples. Of the 58 cheeses, 51 were Cheddars. Table 2 presents the tyramine contents and the tyrosine decarboxylase and the MAO activities of the cheeses. As indicated in Table 1, tyramine and tyrosine decarboxylase are unformly distributed. MAO was only detected in eight of the samples. The cheese with the highest level of tyrosine decarboxylase also had the highest level of MAO present. Cheeses of the same type containing MAO oxidase contained lower levels of tyramine. The inherent low pH of cheese probably limits the MAO activity.

Table 3 presents the histamine contents and the histidine decarboxylase and DAO activities of the various cheeses. As indicated in Table 1, the distribution of histamine is more limited than for tyramine, while histidine decarboxylase, like tyrosine decarboxylase, is widely distributed. However, DAO occurred more frequently than MAO, i.e., in 18 vs. eight cheese samples with MAO activity. Cheeses of the same type containing DAO, with one exception, contained lower levels of histamine. DAO was detected in eight of the nine sharp Cheddar samples. Since mono- and diamine oxidases do not appear in wide distribution in cheeses, they probably do not constitute a major limiting factor of cheese amine content.

Table 4 presents the decarboxylase and oxidase activities detected in the dairy-related cultures. Only the decarboxylase activities corresponding to the eight oxidase-positive cultures are presented. The decarboxylase activities in the 24 oxidase-negative cultures were published previously (*17*). Five of the 32 cultures surveyed showed MAO activity, while only four of the cultures showed DAO activity. Interestingly, five of these

TABLE 1. *Tyramine and histamine contents and the tyrosine and histidine decarboxylase activities of 156 cheese samples*

Cheese		Tyramine[a] (mg/g)	Tyrosine Decarboxylase[b]	Histamine[a] (mg/g)	Histidine Decarboxylase[b]
Cheddar					
Extra-sharp	(11)[c]	0.27 (10)[d]	1.19 (11)[d]	0.21 (5)[d]	0.71 (11)[d]
Sharp	(34)	0.21 (33)	2.49 (31)	0.11 (9)	2.05 (27)
Medium	(18)	0.24 (17)	16.12 (15)	0.14 (6)	2.31 (17)
Mild	(12)	0.09 (11)	1.64 (10)	0.19 (4)	1.16 (10)
Processed	(7)	0.11 (6)	1.90 (5)	ND	2.80 (6)
Smoked	(3)	0.12 (3)	0.29 (3)	ND	1.62 (3)
Colby	(8)	0.21 (8)	19.00 (7)	0.07 (3)	2.74 (8)
Edam	(2)	0.31 (2)	3.90 (2)	ND	3.85 (2)
Gouda	(6)	0.29 (6)	2.11 (5)	0.08 (1)	2.18 (6)
California Jack	(1)	0.13	2.00	ND	5.17
Swiss	(6)	0.41 (5)	0.74 (6)	ND	4.26 (6)
Roquefort & Blue	(7)	0.36 (7)	7.14 (6)	0.05 (3)	1.42 (6)
Camembert	(7)	0.12 (7)	5.71 (6)	0.07 (1)	0.88 (7)
Limburger	(1)	0.12	1.66	ND	0.15
Sap-Sago	(1)	0.52	0.16	2.6	0.86
Romano	(1)	0.14	1.08	ND	5.21
Parmesan	(1)	0.28	1.50	ND	4.64
Mozzarella	(1)	0.16	0.12	ND	0.35
Fontinella	(1)	0.10	0.05	ND	0.14
Cottage	(3)	ND[e]	0.99 (3)	ND	2.07 (3)
Mimolette	(1)	0.28	0.60	ND	6.32
Rehmkase	(1)	0.27	0.00	ND	0.25
Gourmandise	(3)	0.09 (3)	1.87 (2)	0.15 (2)	1.47 (3)
Gjetost	(1)	0.12	0.02	ND	5.01
German Blanco	(1)	0.10	0.68	0.28	0.03
Cheurotin	(3)	0.20 (2)	3.84 (3)	0.17 (1)	1.46 (2)
Danbo	(1)	0.62	2.24	ND	30.07
Tybo	(1)	0.66	1.66	0.98	0.13
Dofinio	(1)	0.25	0.65	ND	0.03
Graddoat	(1)	0.12	0.54	ND	4.47
Norwegian Jarlsburg	(1)	ND	2.60	ND	3.63
Port-Salut	(2)	0.15 (2)	2.06 (2)	ND	0.51 (1)
Reblochon	(1)	0.22	10.48	ND	4.78
Alpenjoi	(1)	0.10	0.00	ND	0.03
Stilton	(1)	0.46	0.63	ND	6.76
Muenster	(1)	0.14	0.11	ND	0.00
Boursault	(1)	0.11	0.73	ND	0.01
German Goldblock	(1)	0.33	0.00	ND	0.00
Brie	(2)	0.15 (2)	0.08 (2)	ND	0.19 (2)

[a]Average of positive samples.
[b]nMoles CO_2 released/min/g of cheese (average of positive samples).
[c]Number of samples.
[d]Number of positive samples.
[e]ND = not detectable below 10 μg tryamine or 50 μg histamine/g of cheese.

TABLE 2. *Tyramine contents and tyrosine and monoamine oxidase activities of 58 cheese samples*

Cheese		Tyramine (mg/g) Range	Tyramine (mg/g) Average	Tyrosine decarboxylase[a]	Monoamine oxidase[b]
Cheddar					
Extra-sharp	5-ON[c]	0.17-0.45	0.31	1.13	0
Extra-sharp	1-OP	0,27	0.27	0.36	2.2
Sharp	13-ON	ND[d]-0.76	0.25 (12)[e]	3.79 (12)[e]	0
Sharp	4-OP	0.04-0.24	0.12	1.43 (3)	0.7
Medium	14-ON	ND-0.66	0.25 (13)	19.05 (12)	0
Mild	7-ON	ND-0.5	0.13 (6)	2.34	0
Mild	1-OP	0.2	0.2	1.65	0.5
Processed	5-ON	ND-0.22	0.16 (4)	2.64 (4)	0
Smoked	1-OP	0.08	0.08	0.06	0.67
Colby	3-ON	0.10-0.56	0.27	24.8 (2)	0
Colby	1-OP	0.20	0.20	52.54	84.3
Limburger	1-ON	0.12	0.12	1.66	0
Danbo	1-ON	0.62	0.62	2.24	0
Reblochon	1-ON	0.22	0.22	10.48	0

[a]nMoles CO_2 released/min/g of cheese (average of positive samples).
[b]pMoles tyramine oxidized/min/g of cheese (average of positive samples).
[c]Number of samples, ON = oxidase negative, OP = oxidase positive.
[d]ND = not detectable below 10 μg/g of cheese.
[e]Number of positive samples.

TABLE 3. *Histamine contents and histidine and diamine oxidase activities of 58 cheese samples*

Cheese		Histamine (mg/g) Range	Histamine (mg/g) Average	Histidine decarboxylase[a]	Diamine oxidase[b]
Cheddar					
Extra-sharp	6-ON[c]	ND-0.78[d]	0.22 (2)[e]	1.18	0
Sharp	9-ON	ND-1.26	0.29 (5)	3.17	0
Sharp	8-OP	ND-0.32	0.04 (1)	2.69	9.2
Medium	11-ON	ND-0.9	0.14 (2)	2.58	0
Medium	3-OP	0.22-0.6	0.35	0.87	22.2
Mild	6-ON	ND-1.26	0.27 (2)	1.45 (5)[e]	0
Mild	2-OP	ND-0.32	0.16 (1)	2.45	24.0
Processed	3-ON	ND	ND (0)	4.28	0
Processed	2-OP	ND	ND (0)	3.14	7.6
Smoked	1-ON	ND	ND (0)	4.75	0
Colby	3-ON	ND-0.38	0.13 (1)	2.71	0
Colby	1-OP	ND	ND (0)	3.94	6.1
Limburger	1-OP	ND	ND (0)	0.15	34.1
Danbo	1-ON	ND	ND (0)	30.07	0
Reblochon	1.OP	ND	ND (0)	4.78	0.9

[a]nMoles CO_2 released/min/g of cheese (average of positive samples).
[b]pMoles putrescine oxidized/min/g of cheese (average of positive samples).
[c]Number of samples, ON = oxidase negative, OP = oxidase positive.
[d]ND = not detectable below 50 μg histamine/g of cheese.
[e]Number of positive samples.

TABLE 4. *The tyrosine and histidine decarboxylase activities and the mono- (MAO) and diamine (DAO) oxidase activities of various dairy-type bacteria*[a]

Culture	Tyrosine decarboxylase[b]	Monoamine oxidase[c]	Histidine decarboxylase	Diamine oxidase[c]
Escherichia coli	0.3	1.2	0.25	0.0
Leuconostoc cremoris	19.4	0.0	0.0	4.1
Microbacterium lacticum	0.5	0.0	0.05	13.3
Streptococcus cremoris	1.2	0.0	0.15	2.0
Streptococcus diacetylactis	0.7	0.3	0.26	0.0
Streptococcus lactis (1)[d]	1.0	0.9	0.0	0.0
Streptococcus lactis (2)	12.6	27.3	0.0	1.4
Streptococcus liquefaciens	31.0	1.3	0.05	0.0

[a]Thirty-two cultures were surveyed, 27 showed no MAO activity and 28 showed no DAO activity. The decarboxylase activities of these cultures was published previously (*17*).
[b]nMoles CO_2 released/min/ml of skim milk culture.
[c]pMoles amine oxidized/min/ml of resting cells from TYEM.
[d]Culture number.

oxidase positive bacteria represent species that are employed in cheese starter cultures. Two of the other oxidase positive bacteria, *Escherichia coli* and *Streptococcus liquefaciens*, are microorganisms that are apt to enter a fermentation product by faulty hygiene. In a previously published survey of 38 dairy-related bacteria, six were shown to possess highly active decarboxylase activities. Five of these cultures produce mono- or diamine oxidase, viz., *E. coli, Leuconostoc cremoris,* two strains of *Streptococcus lactis,* and *S. liquefaciens.* The culture showing the highest DAO activity, *Microbacterium lacticum,* had only a slight ability to release $^{14}CO_2$ from histidine and tyrosine. It is surprising that the cheeses, with their diversity of bacterial species, did not contain higher oxidase activity.

This study indicates that most cheeses would possess sufficient tyrosine or histidine decarboxylase activity to rapidly convert the corresponding amino acids to tyramine or histamine. In view of the absence of detectable levels of mono- or diamine oxidase in most cheese samples, it is unlikely that oxidase conversion of the amines to non-physiologically active compounds significantly affects tyramine or histamine levels in cheese. It appears that a major limiting factor in formation of biologically active amines in cheese and other fermented foods is the availability of free amino acids for decarboxylation. This would be dependent upon the amount of proteolysis occurring during the fermentation. Since some cheeses did possess oxidase activity and tended to contain lower amine levels, the possibility exists that amine contents could be controlled through use of dairy starters having oxidase capability. Whether or not such cultures could be developed needs to be determined.

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manner that is less obnoxious than the variety of expressions currently used with varying degrees of success. He presents the principles involved, discusses the ways and means of developing, and the significance for reference values for a variety of food products. The several tables that are associated with this discussion are superbly used to substantiate the several points.

This book belongs in the hands of all Food Microbiologists and regulatory agents who are concerned with the microbiology of foods. It is a book that needs to be carefully read and thoroughly digested. The excellent documentation is a real assistance so that one can readily find greater detail when and where that is necessary. It also belongs on the shelves of all libraries that have a clientele of people concerned with Food Science or Food Microbiology.

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Fouling of Heat Transfer Surfaces by Solutions of Egg Albumin

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ABSTRACT

A major problem which can be encountered when biological fluids are heated is the deposition of components from the fluid on the heating surface. This results in loss of efficiency in the heat transfer process and usually contributes to requirements for more severe cleaning regimes to return the surface to the original clean condition. Since proteins have been implicated as a major source of the problem in heat exchanger fouling, we studied the effect of process and fluid variables on deposition from protein solutions. An electrically heated stainless steel surface was exposed to egg albumin solutions flowing at a variety of temperatures, fluid flow rates, and concentrations. The system for studying the rate of burn-on is unique and has been described elsewhere. The surface temperatures of the stainless steel surface could be monitored and controlled to any desired temperature. Results of the study indicated: (*a*) fouling rate increased with an increase in protein concentration, (*b*) increasing the fluid flow rate did not have a significant effect on rate of fouling, (*c*) increasing the temperature difference increased fouling rate, and (*d*) surface finish did not influence fouling.

When biological fluids are heated, deposition of thermally unstable materials on heat transfer surfaces is of great practical importance. This phenomenon is called fouling. Severe cleaning problems may result and manual cleaning of some deposit components may be necessary for returning the surface to the original clean condition. An equally important effect is the possible limitation of the time for which a heat transfer operation can be operated without intermittent cleaning. Any limitation is economically undesirable since it reduces the availability of equipment and increases the processing cost. The operating time may be restricted either through deposits obstructing flow passage in the heat exchanger, causing excessive pressure drop, or through a reduction of the heat transfer coefficients for heating surfaces, making it increasingly difficult to maintain the required processing temperature.

Although deposit formation is of great importance for operation of heat treatment plants, processes involved in fouling (burn-on) are still not well understood. Many operational factors affecting the rate of fouling of a heated surface have been identified either by observation of commercial equipment or experimental determination. The most widely recognized factors are (*a*) flow rate of the heated material (*6,8,9,14*), (*b*) temperatures of the surface on which foul forms (*5,6,8,14*), (*c*) bulk fluid temperature (*8,14,18*), and (*d*) type and nature of the surface on which the foul forms (*8,14,18*).

Studies on the fouling of biological fluids have been limited. Investigations have most frequently been applied to fouling of heat exchangers by milk (*1,3,4,7,12*) and tomato products (*14*). In these studies, attempts were made to develop equations to predict fouling rates. However, due to the complex nature of fouling processes and equipment limitations, fouling rate could not be quantified and a complete investigation on the effect of operational variables on the rate of fouling could not be conducted. In this study, dilute egg albumin solutions were heated in an electrically heated double tube heat exchanger and the effect of process and fluid variables on deposition from protein solution were investigated.

MATERIALS AND METHODS

Fouling experiments were done in a laboratory-scale, electrically heated, double tube heat exchanger under constant heat flux conditions. Description and characterization of the experimental system are in an earlier paper (*11*). The fouling process is initiated by continuously circulating a test solution through the annular passage of the heat exchanger. The fluid which comes in contact with the heated stainless surface is subjected to conditions conducive to fouling and a layer of scale is deposited.

A concentrated egg albumin solution was freshly prepared before each fouling experiment by dissolving a known amount of soluble egg albumin impalable powder (Fisher Scientific Company, Fair Lawn, NJ) in 500 ml of distilled water. The protein solution was then centrifuged in a clinical centrifuge (International Equipment Company, Needham Heights, MA) at 2600 rpm (ca. $800 \times g$) for 5 min to remove any undissolved material. To begin the fouling experiment, the heat exchange apparatus was first allowed to equilibrate and stabilize at certain operating conditions with 15 liters of circulating distilled water. The concentrated egg albumin solution was then introduced to the feed tank and the experiment initiated. The current and electrical potential across the heat exchanger were then measured at convenient time intervals. Measurements allowed determination of the resistance and therefore the temperature of the stainless steel test section (*11*). The fouling process was followed by calculating the fractional reduction of the overall heat transfer coefficient of the system. When the overall heat transfer coefficient was reduced to 70% or less of its original value, the experiment was terminated. The power input could be maintained at a

constant value throughout each fouling experiment with less than 5% variation. Fluid flow rate was determined by collecting and weighing the fluid over a fixed time interval. The concentration of protein in the test solution was determined by drying 10 ml of protein solution in a vacuum oven (at 70 C, 730 mm Hg vacuum) until a constant weight was reached.

In this study, conditions were set to evaluate effects of operational variables on fouling. A set of half factorial designed experiments was arranged to identify the significance of heat exchange surface temperature, fluid flow rate and fluid bulk temperature on the rate of foul of egg albumin solutions. A more detailed study on each operational variable was also made.

Using dilute egg albumin solutions of 0.18 ± 0.01% (wt/vol), the effect of fluid velocity on the fouling rate was investigated by varying the average fluid velocity from 25.1 cm/sec to 40.9 cm/sec (nominal Reynolds numbers of 9700 and 15900, respectively). The fouling experiments were done at a heat exchange surface temperature of 73.5 ± 1 C and a fluid bulk temperature of 41.7 ± 1 C.

Nine fouling experiments at various heat exchange surface temperatures were done with 0.18 ± 0.01% (wt/vol) egg albumin solutions. Fluid velocities and fluid bulk temperatures were maintained at 24.8 ± 05 cm/sec and 41.5 ± 1 C, respectively. Heat exchange surface temperatures ranged from 66.2 to 74.6 C.

Fouling experiments were also done at a heat exchange surface temperature of 70.0 ± 2 C, an average fluid velocity of 24.5 ± 0.5 cm/sec and a constant fluid bulk temperature between 29.0 and 41.5 C.

Previous studies on fouling processes have focused primarily on effect of operational variables on rate of fouling since alteration of the fluid being processed or modification of equipment surfaces were generally difficult. In this study, the concentration of protein in the test solution was varied and fouling rate was detemined to gain a better understanding of the fouling process. Also the heat exchange surface was modified and the effect of the nature of heat exchange surface on rate of protein deposition was studied. To study the effect of protein concentration on the fouling rate, fouling experiments were done with a heat exchange surface temperature at 70.0 ± 2 C, fluid bulk temperature at 41.0 ± 1 C and an average fluid velocity of 25.1 ± 0.5 cm/sec. The experimental variable was protein concentration of the egg albumin solutions which ranged from 0.094 to 0.240% (wt/vol).

The heat exchange surface was modified by three different treatments. The surfaces of two stainless steel probes were given a conventional number 4 or number 7 finish (Engineering Department, Crepaco Inc., Lake Mills, WI). A commercially available buff treatment (mirror finish) was given to the surface of a third probe (Mechanician Shop, University of Wisconsin, Madison, WI). The three differently finished test sections were used in fouling experiments using 0.18 ± 0.01% (wt/vol) egg albumin solution. Experimental conditions were set at heat exchange temperature of 70.0 ± 2 C, fluid bulk temperature of 41.5 ± 1 C and a fluid velocity of 25.4 ± 0.5 cm/sec. A fourth stainless steel test section was coated with a commercially available, food grade, phenolic resin-bonded polytetrafluorethylene coating (teflon), "Emralon" 311^R (Acheson Colloids Company, Chicago, IL). The "Emralon" 311^R was applied on the stainless steel test section by the Acheson Colloids Company Laboratory (Port Huron, MI) and the final coating thickness was measured to be 0.015 mm (0.6 mil). Six fouling experiments were completed with the fluoropolymer-coated test section using 0.18 ± 0.01% (wt/vol) egg albumin solution. The fluid bulk temperature and fluid velocity were held constant in these experiments at 41.5 ± 1 C and 24.8 ± 0.5 cm/sec, respectively. For each experiment the stainless steel test section was maintained at a constant temperature between 72 and 79 C. A fifth probe was coated with Dow Corning^R 24, a water dilutable food grade, nonionic emulsion containing 35% dimethyl polysiloxane (Dow Corning Corporation, Midland, MI). From company literature, a film thickness of 0.3 micron of the release agent is believed to be sufficient to provide lubrication to prevent or at least retard protein deposition. The 35% emulsion solution was diluted to 8% with distilled water and a stainless steel test section was dipped in the diluted emulsion. Free liquid was allowed to drain before the test section was dried in a vacuum oven at 70 C, the Dow Corning^{R}24 treated stainless steel test section was then subjected to fouling conditions.

RESULTS AND DISCUSSION

Ling (*10*) analyzed the heat transfer characteristics in a system prone to fouling and suggested that if fouling is allowed to proceed under a constant heat flux condition, the fouling rate can be characterized by the rate of change of the reciprocal of heat transfer coefficient. Thus

$$\text{Rate of fouling} = \frac{d}{dt}\left(\frac{1}{h}\right)$$

where h = instantaneous heat transfer coefficient

The constant fouling rate period can be seen by examining a plot of h_0/h versus time, as in Fig. 1. Generally, the relationship can be described as consisting of three periods: (*a*) an initial induction period (AB), (*b*) a constant rate period (BC), and (*c*) a falling rate period (CD). It is suggested that the initial induction period (AB) is the result of the heterogenous nucleation process of protein on the heated metallic surface and the falling rate period (CD) is caused by deviation of fouling conditions from the original constant heat flux situation period (BC) was used to quantify the fouling rate by calculating the slope of the 1/h versus time curve after the induction period (*10*). Fouling rates for all the fouling experiments are in Table 1.

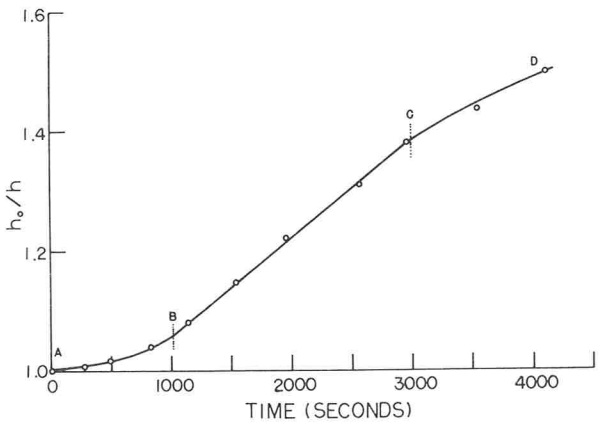

Figure 1. *Heat transfer resistance as a function of time during fouling.*

Analysis of the results obtained from the experiments done in the half factorial design provided information on the effect of surface temperature, fluid flow rate of and fluid bulk temperature on fouling rate. Applying Yates algarithm (*21*) to the fouling rate data for the first four experiments in Table 1, the main effect of these three variables on fouling rate was quantified. Surface temperature greatly affected fouling rate. When the surface temperature was increased from ca 70.5 to 74 C, the fouling rate increased by 100% (± 20%, 95% confidence interval). On the other hand, doubling the fluid flow rate did not have a statistically significant effect on rate of fouling. Similarly, increasing bulk fluid temperature

TABLE 1. *Fouling rate as a function of experimental conditions*

Test condition	Experimental conditions: T_s[a] ± 2 C	T_b[b] ± 1 C	Average fluid velocity ± 0.5 cm/sec	Protein concentration ± 0.01% (wt/vol)	Fouling rate[c]
Half factorial design	70.0	38.0	24.8	0.18	3.76×10^{-2}
	70.8	42.2	14.4	0.18	3.91×10^{-2}
	73.8	41.5	24.7	0.18	6.85×10^{-2}
	74.5	38.2	14.6	0.18	8.11×10^{-2}
Effect of fluid flow rate	73.2	41.5	25.1	0.18	4.27×10^{-2}
	74.0	41.7	35.4	0.18	3.61×10^{-2}
	73.2	41.7	40.9	0.18	3.46×10^{-2}
Effect of surface temperature	70.0	42.0	24.9	0.18	3.74×10^{-2}
	69.5	41.5	24.7	0.18	3.80×10^{-2}
	71.2	41.5	24.4	0.18	3.39×10^{-2}
	73.7	41.5	24.7	0.18	6.87×10^{-2}
	74.6	41.0	24.4	0.18	8.33×10^{-2}
	66.2	41.5	25.5	0.18	1.62×10^{-2}
	69.2	41.5	25.9	0.18	3.36×10^{-2}
	69.6	42.3	24.9	0.18	3.55×10^{-2}
	73.2	41.2	25.1	0.18	4.41×10^{-2}
Effect of fluid bulk temperature	69.0	29.0	25.1	0.18	9.58×10^{-2}
	70.9	30.8	25.1	0.18	7.52×10^{-2}
	70.0	38.0	24.8	0.18	3.76×10^{-2}
	70.0	41.5	24.9	0.18	3.74×10^{-2}
Effect of protein concentration	70.4	42.0	25.2	0.094	0.83×10^{-2}
	70.1	42.2	24.9	0.10	0.99×10^{-2}
	70.0	42.0	24.9	0.18	3.74×10^{-2}
	71.1	42.0	24.3	0.24	10.17×10^{-2}
Effect of Surface Modifications					
(*a*) Mechanical finishes					
Number 4 finish	69.8	42.0	24.9	0.18	3.73×10^{-2}
Number 7 finish	69.2	41.5	25.8	0.18	3.49×10^{-2}
"Mirror" finish	69.5	42.0	24.8	0.18	3.61×10^{-2}
(*b*) Teflon coating	67-72	42.0	24.8	0.18	no fouling observed
(*c*) Dow Corning R24 coating	73.7	42.0	25.5	0.18	5.64×10^{-2}
	70.5	42.0	18.4	0.18	5.55×10^{-2}
	70.6	41.5	24.8	0.18	no fouling observed

[a] T_s represents the heat exchange surface temperature.
[b] T_b represents the fluid bulk temperature.
[c] 95% confidence interval = $\pm 0.7 \times 10^{-2}$. Unit = $cm^2 - °K/cal$.

from 38 to 41 C did not have a significant effect. The effect of both of these parameters, however, will be explored in greater detail in later experiments.

To verify the observation that fluid flow rate did not affect rate of fouling a further investigation was completed in which the average fluid velocity varied from 25.1 ± 0.5 cm/sec (Re = 9700) to 40.9 ± 0.5 cm/sec (Re= 15900). These results (the second series of data in Table 1) confirmed that fluid flow rate did not significantly affect rate of fouling. The actual data are in Fig. 2. as fractional reduction in the overall heat transfer coefficient for each experiments versus fouling time and it can be seen that there is no difference between the three trials. Therefore, it can be concluded that the rate of fouling of dilute egg albumin solutions will not be affected by fluid flow rates, at least for fluid film Reynolds numbers ranging from 6000 to 16000.

The fact that velocity in the range studied here does not affect fouling rate suggests that fouling for egg albumin solution is not diffusion controlled. Using the analogy between heat and mass transfer (*3*), if the fouling rate were diffusion controlled, the rate of fouling should be proportional to the 0.8th power of the fluid Reynolds number. If protein deposition were diffusion controlled,

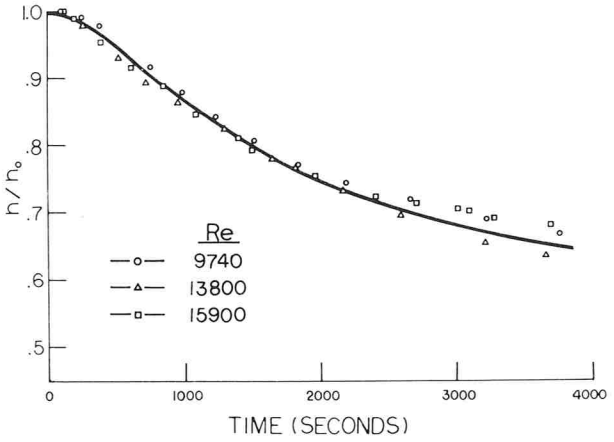

Figure 2. *Fraction of heat transfer remaining as a function of time for various flow rates.*

an increase in fluid film Reynolds number from 9,700 to 15,900 should result in the fouling rate increasing 1.48 times. This magnitude of increase in fouling rate is certainly large enough to be detected by the experimental system. Therefore, it must be concluded that fouling rate is not governed by the mass diffusion rate of protein to the heating surface.

Although deposition of protein on the heated surface was found to be independent of fluid flow rate, the

importance of fluid velocity on scale removal must also be considered. Kern and Seaton (*9*), in describing fouling, first introduced a removal term which was directly proportional to the product of the fluid shear stress and scale thickness. Using the same concept, Taborek et al. (*18*) described the removal term as a function of wall shear stress, scale thickness and scale strength. In these analyses the scale removal rate is proportional to scale thickness and therefore the amount of scale removal increases as the scale thickness increases leading to an asymptotic fouling resistance value. Suitor et al. (*17*) analyzed available data from fouling studies on $CaCo_3$ and concluded that asymptotic fouling will occur only when a threshold shear stress is exceeded. This threshold shear stress should be dependent on the mechanical strength of the scale. In our case, the mechanical strength of the protein deposit is unknown, but it is still interesting to evaluate wall shear stresses for this heat exchange system. Using the Prandtl mixing-length model (*13*), the wall shear stress at fluid Reynolds numbers of 9,700 (25.1 cm/sec), 13,800 (35.4 cm/sec) and 15,900 (40.9 cm/sec) were estimated to be 0.425 N/m^2, 0.780 N/m^2 and 0.984 N/m^2, respectively. If the threshold shear stress of the protein deposit is less than 0.984 N/m^2, at least one of the fouling experiments should show the effect of scale removal. However, the almost identical rate of change of thermal resistance under these wall shear stresses indicated that 0.984 N/m^2 is still too low to cause any significant scale removal.

Further studies were completed to show the influence of heat exchange surface temperature on the fouling rate of egg albumin solutions. Figure 3 presents three representative h/h_0 against time curves, representing surface temperatures of 66.2, 70.0 and 74.6 C. These data and the results of an additional six fouling

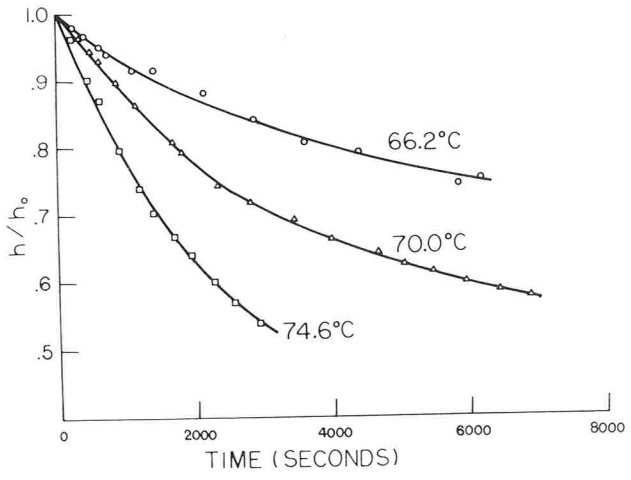

Figure 3. *Fraction of heat transfer remaining as a function of time for various surface temperatures.*

experiments with a heat exchange surface temperature ranging from 66.2 to 74.6°C (Table 1) show a definite direct effect of the heat exchange surface temperature on the rate of fouling. To assess if the fouling process exhibited a conventional dependence on temperature described by the Arrhenius equation, the logarithm (base e) of the fouling rate was plotted against the reciprocal of absolute heat exchange surface temperature, a conventional Arrhenius plot. The result, Fig. 4, would suggest that the fouling rate obeys the Arrhenius model for temperature dependence. Linear regression analysis of

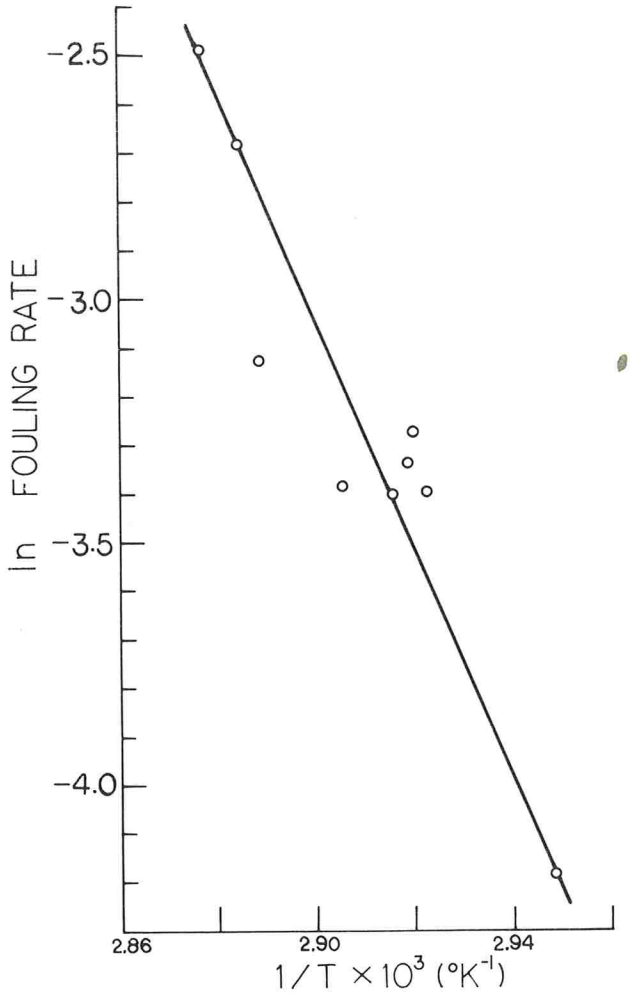

Figure 4. *Arrhenius plot relating fouling rate to heat exchange surface temperature.*

these data resulted in a calculated Arrhenius activation energy of 40 ± 6 Kcal/mole. The Arrhenius relationship between fouling rate and temperature indicates that the protein deposition has a kinetic character and is sensitive to the variation of the heat exchange surface temperature. The rate of foul of egg albumin solution does not appear to be controlled by the rate of denaturation of egg albumin since the activation energy for thermal denaturation of egg albumin at 65 C was reported to be 132.0 Kcal/mole (*16*). This is nearly four times greater than the estimated 40 Kcal/mole for the fouling process. Thus it is probable that deposition and attachment of the protein molecules on the wetted surface is the rate determining reaction for the fouling process.

In the half factorial design, fluid bulk temperatures

between 38.0 and 41.5 C did not significantly influence the fouling rate. However, when the fluid bulk temperature was lowered from 40 to 30 C, a doubling of fouling rate was observed as shown in the fourth set of experimental data presented in Table 1.

The fouling curves are presented in Fig. 5. It is suggested that the larger fouling rate at a lower fluid temperature is caused by the higher concentration of native protein in the test solution. While denaturation of protein occurs

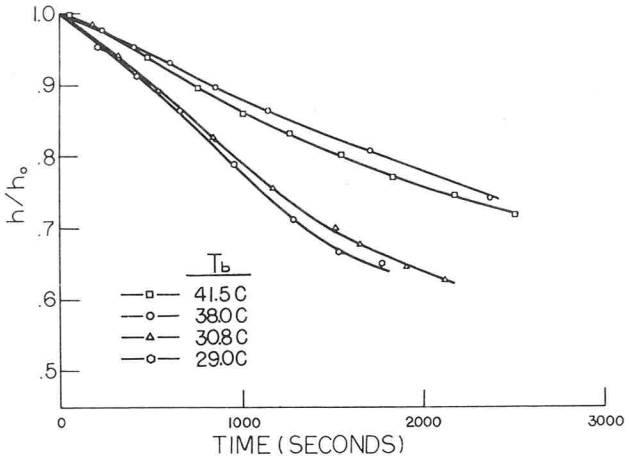

Figure 5. *Fraction of heat transfer remaining as a function of time for various fluid bulk temperatures.*

at all temperatures, the rate of protein denaturation varies drastically with temperature. Egg albumin, with an activation energy for thermal denaturation of 132.0 Kcal/mole, will have a denaturation rate 1100 times faster at 40 than 30 C. This large difference in protein denaturation rate could result in a difference in native protein or unaggregated protein at the heat transfer surface. For fluid bulk temperatures at 42 and 38 C, the denaturation rate is 15 times slower at 38 than at 42 C and it appears that difference was too small for detection in the initial series of experiments.

The above explanation leads to the implication that the native undenatured protein must be present at the heated surface for formation of protein scale. It is reasonable to suspect that when protein molecules are denatured and subsequently precipitate, it is more difficult for these solid particles to be physically adsorbed onto the heated surface. Aggregation of the denatured protein molecules in the fluid bulk would further reduce fouling. To test this hypothesis, egg albumin solution was preheated before a fouling experiment to denature the protein. When the concentrated egg albumin preparation was preheated in a water bath at 60 C for 20 min before it was used for a fouling experiment, fouling was greatly reduced and less than 7% reduction in the overall heat transfer coefficient was observed after 4000 sec of fouling at a heat exchange temperature of 74 C. When the concentrated protein preparation was heated at 60 C for 30 min before the fouling experiment, no measurable fouling was obtained within 4000 sec of fouling at a heat exchange surface temperature of 73 C. Preheating the protein solution at 60 C aggregated most of the native protein and when the solution was subjected to fouling conditions after the heat treatment, available native protein was too low to cause a measurable fouling resistance even though the total protein concentration remained at 0.18% (wt/vol). This reduction in fouling rate through forewarming treatments to denature proteins is an unintentional benefit derived from pretreatment given to milk before vacuum concentration (*19*).

A series of experiments was done to determine the effect of protein concentration on the fouling rate. This was accomplished by changing the amount of egg albumin in the test solution. Results given in the fifth series of data in Table 1 indicate that the rate of foul increases with increasing protein concentration. To estimate the order of reaction with respect to protein concentration, the logarithmic (base e) values of fouling rates were plotted against the logarithmic (base e) values of protein concentrations (Fig. 6). From the linear nature of this plot, the order of the reaction was estimated to be 2.6 ± 0.4. Since thermal denaturation of protein is generally believed to follow a first order process (*20*), the higher reaction order obtained for the fouling process also leads to the conclusion that protein denaturation per se is not the rate controlling reaction for the fouling process.

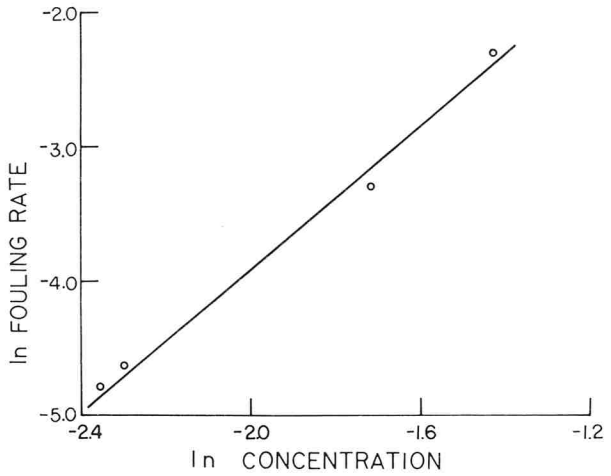

Figure 6. *Determination of reaction order for the fouling process of egg albumin solutions.*

The rate of foul $[\frac{d}{dt}(1/h)]$ defined in this study is measured in each fouling experiment during the period of constant protein deposition, section BC, Fig. 1. Since this constant deposition rate occurs after the nucleation process and consequently after the heat exchange surface has been covered with a layer of protein scale, modification of the heat exchange surface would not be expected to change the mechanism of protein deposition on the wetted proteinaceous surface. Modification of the heat exchange surface would presumably only influence the fouling process during the initial heterogeneous nucleation period or possibly by changing the rate of scale removal.

Several experiments were done with various stainless steel surface finishes or treatments and the results are presented in the sixth section in Table 1. From these results, there appeared to be no difference in the rate of fouling when the surface finish of the stainless steel was changed although the differences in roughness of the three are quite large and can be observed with the naked eye. When viewed under a scanning electron microscope (*10*) at 1000 and 3000 magnification, rugosities were estimated to be about 3 μ for the number 4 finished surface, 1.5 μ for the number 7 finished surface and 1 μ for the "mirror" finished surface. The actual data from the fouling experiment are in Fig. 7. Not only were the

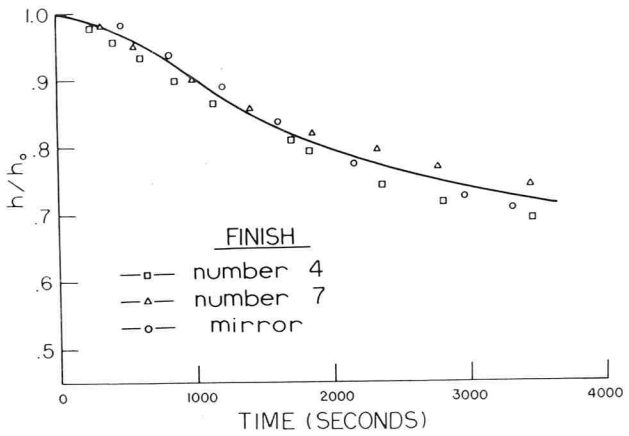

Figure 7. *Fraction of heat transfer remaining as a function of time for stainless steel surfaces of various finishes.*

fouling rates not significantly different, it is also observed from Fig. 7 that the induction periods were very similar. The relative roughness of the surface apparently did not differ enough to cause a detectable change in the nucleation process during the initial stage of the fouling experiment. Furthermore, there apparently was no significant change in the rate of removal of the protein deposit because the three systems behaved similarly throughout the fouling experiment.

When the stainless steel surface was coated with teflon, no fouling was observed (Table 1). Fluorine-containing polymeric substances have low inter-molecular forces at the air/solid interface and therefore form surfaces with extremely low free energy. These surfaces are generally difficult to wet and tend to exhibit a nonadhesive character and low coefficients of friction. Fouling of 0.18% (wt/vol) egg albumin was never observed within 2 h of heating at heat exchange surface temperatures of 67 to 72 C when the "Emralon" 311^R coated test section was used. This indicates that the fluoropolymer coating is effective in preventing nucleation of protein scale on its surface under fouling conditions used in the experiment.

Although this might suggest that the solution to fouling is to coat all heat transfer surfaces with teflon, there is a major disadvantage in using fluoropolymeric coatings on heat exchange surfaces. The polymeric coating can contribute a significant thermal resistance to the overall heat transfer coefficient of the heat exchanger. Polytetrafluoroethylene has a thermal conductivity of 5.8×10^{-4} cal/sec-cm-°K (0.14 Btu/ft-hr-°F) (*15*) and a 0.015-mm coating will introduce an equivalent surface heat transfer coefficient of 0.38 cal/cm^2-sec-°K (2800 Btu/ft^2-hr-°F) to the system. For a system that has a clean heat transfer coefficient of 0.041 cal/cm^2-sec-°K (300 Btu/ft^2-hr-°F), about a 10% increase in heat transfer resistance will be introduced by a polytetrafluoroethylene coating.

The effectiveness of coating the heat transfer surface with an emulsifier (dimethyl polysiloxane, Dow Corning^R 24) is difficult to assess (Table 1). The data on each of the three fouling experiments using the emulsifier treated heat exchange surface are in Fig. 8. On one occasion, at a

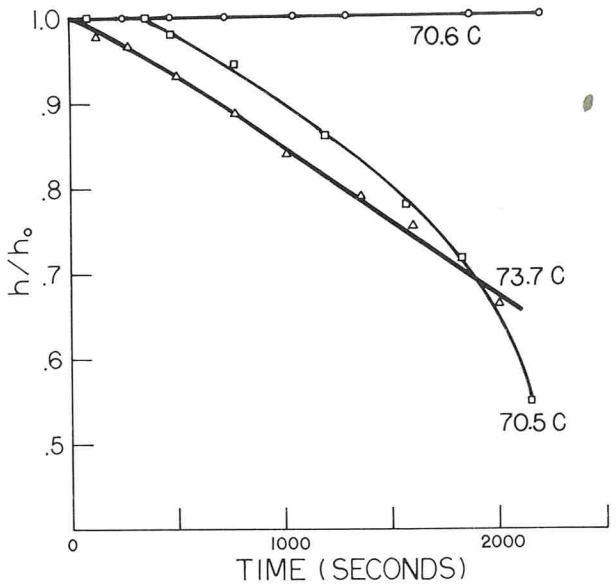

Figure 8. *Fouling curves for silicone-coated [Dow Corning ^{R}24] heat exchange surface.*

heat exchange surface temperature of 70.6 C no fouling was observed within 2000 sec of heating. This experiment suggested that the release-agent is capable of preventing scale deposition. However, in a similar experiment at a heat exchange surface temperature of 73.7 C, a typical fouling rate (5.64×10^{-2} cm^2-°K/cal) was measured. Therefore in that experiment, treating the heat exchange surface had no effect on the fouling rate. The third experiment, done with a heat exchange surface temperature of 70.5 C, unexpectedly resulted in a fouling rate greater than those obtained from an untreated stainless steel surface. Observations of the surface during the fouling experiment revealed that scale formation on the heated surface was irregular and uneven. Hot spots on the heat exchange surface due to uneven coating of protein deposits probably existed. This irregular behavior could have been the result of the uneven coating of the releasing agent on the surface. Rinsing the test section with the Dow Corning^R 24 emulsion does not guarantee a complete and uniform film formation on the

metal surface. An area which is not coated would be susceptible to scale deposition and the nonuniform coating of the scale would lead to an unpredictable fouling behavior. An unintentional complication in these experiments with the release-agent coating is that before each fouling experiment, water was allowed to circulate through the heat exchange system for thermal and electrical stabilization of the system. The circulating water may wash away part or all of the coating before the experiment is actually started. However, if the water would remove the release-agent, it would be anticipated that circulating the test solution would also. Consequently this would not be a long-lasting measure to prevent fouling. Results of the experiments suggest that coating with a release-agent such as Dow Corning $^{R}24$ is capable of preventing or retarding protein deposition on heated surfaces. However, the surfaces must be properly coated and the difficulty of coating the heat exchange surface encountered in this study suggests the need for further research on developing acceptable coating procedures.

SUMMARY AND CONCLUSIONS

The fouling process associated with heating egg albumin solution was investigated using an electrically heated double tube heat exchanger. The effects of operational variables (heat exchange surface temperature fluid velocity and fluid bulk temperature) on the rate of foul were determined. To compare fouling rates, the rate of foul for each experiment was quantified by measuring the rate of change of the reciprocal of the overall heat transfer coefficient $[\frac{d}{dt}(1/h)]$ after the induction period of the fouling process. The observation that the fouling rate was independent of fluid velocity suggested that fouling from egg albumin solutions was not controlled by mass diffusion of protein to the heated surface. Furthermore, the largest fluid velocity used in these experiments (Re = 15900) did not result in scale removal due to shear stresses. In conventional heat exchange equipment, fluid velocities are usually insufficient to produce shear stresses at equipment surfaces which would result in scale removal.

While mass diffusion was not the mechanism controlling the rate of foul, the fouling process did exhibit some kinetic character. A classical Arrhenius relationship was observed between the heat exchange surface temperature and the fouling rate. An activation energy of 40 ± 6 Kcal/mole was determined for the fouling process. This low activation energy found for the fouling process (compared to the 132 Kcal/mole reported for thermal denaturation of egg albumin) suggested that the fouling rate was controlled by the deposition reaction of protein on the wetted surface rather than the denaturation reaction of the protein. Furthermore, experiments using egg albumin solutions at various concentrations indicated that the order of reaction for the process was about 2.6. This also suggested that the fouling process was not controlled by denaturation of protein since thermal denaturation of protein is generally thought to follow first order kinetics.

The chemical kinetic nature of the fouling process provided a possible explanation for the observed increase in fouling rate when the fluid bulk temperature was reduced substantially. When the fluid bulk temperature was reduced from 40 to 30 C, the rate of fouling doubled. It was suggested that the rate of protein denaturation at 30 C was so much lower than at 40 C (1100 times) that a difference in native, unaggregated protein concentration in the egg albumin solutions resulted. The native protein in solution is believed to be responsible for scale formation on heated surfaces and the speculation was substantiated by preheating the protein solution before subjecting it to fouling conditions. Preheating at 60 C for 30 min denatured most of the protein in solution and no scale formation was detected in 4000 sec under typical fouling conditions.

The effect of the nature of heat exchange surfaces was also studied. The mechanical finishes (number 4, number 7 and "mirror") applied to the stainless steel heat exchange surface did not have any effect on the fouling process. Coating the heated surface with a fluoropolymer (teflon) "Ermalon" 311^{R} was effective in preventing protein deposition under the fouling conditions used in this study. Experiments were also made using stainless steel section coated with a silicone emulsion, Dow Corning 24^{R}. Results generated by these fouling experiments were unpredictable. There is some evidence that the release-agent is effective in preventing or retarding protein deposition but uniform coating of the heat exchange surface was difficult to accomplish. Until a satisfactory scheme for coating the surface is developed, the advantage of using the release-agent to prevent protein deposition on heat exchange surfaces remains questionable.

In several places in this paper reference is made to "native protein" and to the necessity of having native protein for fouling to occur. We did not actually measure the concentration of native protein and it is possible that some denatured protein could have been present. Hence, reference to "native protein" should imply native and unaggregated protein.

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Patulin Production by Species of *Aspergillus* and *Penicillium* at 1.7, 7.2, and 12.8 C

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ABSTRACT

Two strains each of *Aspergillus clavatus* (NRRL 1980 and ATCC 9599), *Penicillium claviforme* (NRRL 1001 and 1002), *Penicillium expansum* (FM 1071 and NRRL 973), and *Penicillium patulum* (ATCC 24550 and FM 1172); and one strain of *Penicillium griseofulvum* (NRRL 2300) were inoculated into potato-dextrose broth and incubated at 1.7, 7.2, and 12.8 C for 110, 84, and 55 days, respectively. All cultures grew at all temperatures. Patulin production by *P. griseofulvum* and *P. claviforme,* NRRL 1001, was limited or inhibited at 1.7 C, whereas at 7.2 C only *P. griseofulvum,* NRRL 2300, failed to produce toxin. Patulin was produced at 12.8 C by all nine cultures.

In recent studies by Torrey and Marth (*14, 15*), *Aspergillus* and *Penicillium* species dominated molds isolated from home stored foods, both refrigerated and non-refrigerated. The mycotoxins produced by some of their isolates were aflatoxins, kojic acid, Ochratoxin A, penicillic acid, and patulin (*14*). When these isolates were tested for their ability to grow at refrigeration temperatures (*15*), no growth was detected at 8 C in *Aspergillus* species, but *Penicillium* isolates grew at 5 C if pregerminated at higher temperatures. The mean temperature limit of home refrigerators was noted as 3.9 to 11.9 C (*15*).

Several foods are known to have an indigenous flora of toxigenic species of *Aspergillus* and *Penicillium* (*1, 2, 3, 6, 8, 16, 17*), and the ability of several toxigenic strains to grow and produce toxin in refrigerated and controlled atmospheres is well documented (*1, 2, 3, 5, 7, 8, 10, 13*). *Penicillium expansum* is frequently encountered as an invader of fruits stored at refrigeration temperatures (*1, 7, 8, 10*). This species is also one of the better known producers of patulin—a toxic and carcinogenic metabolite of several species of *Aspergillus* and *Penicillium* (*4*). Patulin is stable in aqueous solution in the pH range of most fruit products and resists thermal destruction in the pH range of 3.5 to 5.5 (*9*). *P. expansum* can grow and produce patulin in the temperature range of 0 to 30 C (*12*), but for most patulin-producing species, temperatures limiting or restricting toxin production are not known.

This research explored the ability of nine strains of patulin-producing aspergilli and penicillia in five species to produce toxin at commonly used refrigeration temperatures. Because of the large number of natural in vivo substrates that would be suitable for this study, the scope was limited by the choice of a substrate known to maximize in vitro patulin production (*11*).

MATERIALS AND METHODS

Cultures used in this study were from the American Type Culture Collection (ATCC), Rockville, Md., the Northern Regional Research Center (NRRL), U.S. Department of Agriculture, Peoria, Ill., and the Food Microbiology Branch (FM), Division of Microbiology, Food and Drug Administration, Washington, D.C. They were: *Aspergillus clavatus* NRRL 1980 and ATCC 9599; *Penicillium claviforme* NRRL 1001 and 1002; *P. expansum* FM 1071 and NRRL 973; *Penicillium griseofulvum* NRRL 2300; and *Penicillium patulum* ATCC 24550 and FM 1172. Spores were produced from these cultures on potato-dextrose agar in 5-liter bottles and harvested in potato-dextrose broth with sterile glass beads.

Potato-dextrose broth (*12*) was dispensed (50 ml/flask) into 300-ml Erlenmeyer flasks and autoclaved for 30 min at 121 C. The spore suspension was added to each flask to produce a concentration $> 10^6$/ml. Each flask was shaken vigorously and incubated at 1.7, 7.2, or 12.8 C for 110, 84, and 55 days, respectively. Sampling intervals were 10 days at 1.7 C, 7 days at 7.2 C, and 5 days at 12.8 C.

Temperatures used in this study were provided by Model 805 incubators manufactured by Precision Scientific. Although rated 5 to 55 C, two of our incubators achieved and held 1.7 C without difficulty. Temperatures were adjusted and monitored with both mercury thermometers (permanently in place) and a YSI Model 425C Telethermometer. To minimize exposure to temperatures above those provided by the experimental atmospheres, each flask was preincubated before inoculation at the test temperature to be used.

At appropriate sampling intervals, duplicate broth cultures for each fungal species at each temperature were filtered through tared Whatman #2 filter paper. The paper and mycelial mat were dried for 20 to 24 h at 80 C and weighed to determine the mass of the fungal mat. This procedure was used as a semiquantitative measure of growth, and results expressed as milligrams per flask.

Patulin was determined, using 5-ml samples of filtered culture broth extracted three times with equal volumes of ethyl acetate. The extracts were dehydrated with anhydrous sodium sulfate and evaporated in vacuo at 45 C. The residue was dissolved in chloroform and spotted on thin layer silica gel (0.25 mm) along with patulin standards dissolved in chloroform. Plates were developed in benzene: methanol: acetic acid (90:5:5), and spots were made visible by forming the phenylhydrazine derivative (*10*). Quantitation was by visual comparison with standard

spots. The lower detection limit of the procedure was 0.1 μg/ml. Positive results are reported as micrograms per milliliter, and negative results as <0.1 μg/ml.

RESULTS AND DISCUSSION

All cultures germinated and grew at all temperatures. Maximum growth occurred at the highest incubation temperature (12.8 C) in only three cultures: *P. griseofulvum* NRRL 2300, *A. clavatus* NRRL 1980, and *P. expansum* NRRL 973. In all others, growth at 7.2 C was better than or equal to that of the other two temperatures. Growth was suppressed at 1.7 C in *P. griseofulvum* NRRL 2300, and *P. patulum* ATCC 24550. The data are summarized in Fig. 1.

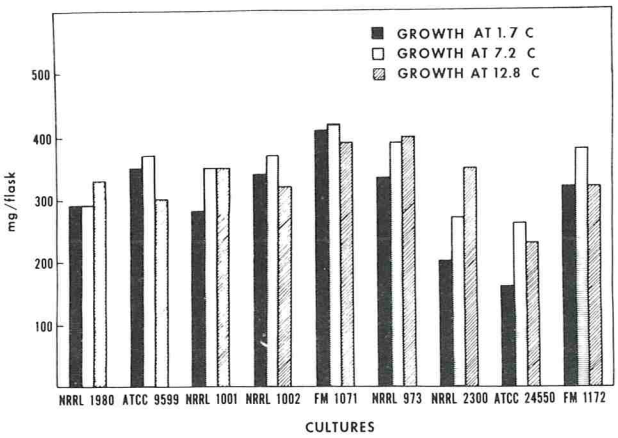

Figure 1. *Maximum culture growth in milligrams per flask.*

All cultures except *P. claviforme* NRRL 101 and *P. griseofulvum* NRRL 2300 produced >50 μg/ml of patulin at all temperatures. Both of these exceptions produced appreciable levels of toxin at 12.8 C but not at 7.2 nor 1.7 C. In contrast, *A. clavatus* ATCC 9599, *P. expansum* FM 1071, and *P. patulum* FM 1172 accumulated the highest toxin concentration at the lowest growth temperature (1.7 C). *A. clavatus* NRRL 1980, *P. claviforme* NRRL 1002, *P. expansum* NRRL 973, and *P. patulum* ATCC 24550, accumulated the highest concentration of toxin at 7.2 C. These data are presented in Fig. 2.

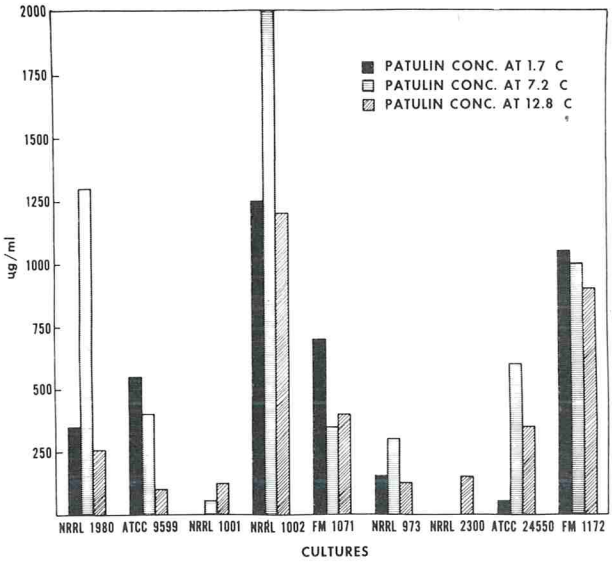

Figure 2. *Maximum patulin concentration in micrograms per milliliters culture broth.*

Table 1 gives times at which initial patulin detection, maximum toxin concentration, and maximum culture growth occurred. Maximum patulin concentration usually coincided with or followed closely maximum growth. As expected, there was considerable variation in growth and toxin production rates, both between species and between strains within a species. The most important data presented are those showing time of initial toxin detection. Patulin was detected first from 10 to 80 days at 1.7 C, 7 to 28 days at 7.2 C, and 5 to 35 days at 12.8 C. While time for maximum toxin accumulation did not coincide with initial detection, these data show that at all temperatures used, the potential for production of patulin during short (5 to 10 days) holding periods exists.

These results show the ability of all the *A. clavatus, P. expansum,* and *P. patulum* cultures and one of two *P. claviforme* cultures to produce large amounts of patulin on a suitable substrate held at the most frequently used refrigeration temperatures. The potential exists for substantial patulin production by both primary invaders such as *P. expansum* and *P. claviforme* and opportunis-

TABLE 1. *Time required for patulin detection, maximum patulin concentration, and maximum culture growth*

Culture identification	Day patulin initially detected			Day patulin maximum detected			Day maximum culture mass detected		
	1.7 C	7.2 C	12.8 C	1.7 C	7.2 C	12.8 C	1.7 C	7.2 C	12.8 C
A. clavatus									
NRRL 1980	30	14	15	100	63	35	100	63	25
ATCC 9599	80	21	10	100	77	55	100	63	40
P. claviforme									
NRRL 1001	40	28	5	100	28	20	80	49	20
NRRL 1002	10	7	5	50	42	30	50	35	30
P. expansum									
FM 1071	30	14	5	100	35	40	70	42	20
NRRL 973	50	14	5	100	70	25	110	49	35
P. griseofulvum									
NRRL 2300	ND[a]	ND	35	ND	ND	55	80	70	50
P. patulum									
ATCC 24550	20	7	5	80	42	20	70	42	20
FM 1172	30	14	5	80	28	30	80	35	20

[a]None detected.

tic decay organisms such as *A. clavatus* and *P. patulum,* when foods are stored for long periods at low temperatures. The mean temperatures of home refrigerators (*15*) would provide the opportunity for growth and substantial patulin production by seven of the nine mycotoxigenic strains tested. Whereas these in vitro studies cannot necessarily be extrapolated to in vivo conditions (*12*), they do indicate the ability of patulin-producing fungi to grow and express toxigenicity at ≤12.8 C is not unusual.

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Errata

Isolation and Identification of Lipolytic Microorganisms Found on Rough Rice from Two Growing Areas

ANTHONY J. DeLUCCA II, STEPHEN J. PLATING, and ROBERT L. ORY

This paper appeared on pages 28-30, Vol. 41 (January 1978) of the *Journal of Food Protection.* Tripticase Nutrient Agar should replace Triplicate Nutrient Agar in the Microbiological examination: Bacteria sub-section of the Materials and Methods section of this paper.

Journal of Food Protection Vol. 41, No. 3, Pages 198-200 (March, 1978)

Survey of Soft and Semisoft Cheese for Presence of Fecal Coliforms and Serotypes of Enteropathogenic *Escherichia coli*

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ABSTRACT

Soft and semisoft cheese varieties including Camembert, Brie, brick, Muenster, and Colby were analyzed for fecal coliforms and serotypes of enteropathogenic *Escherichia coli* (EEC). Analysis for EEC was done using both direct streak and two enrichment procedures so that atypical strains, if present, would be recovered. Of 106 samples collected during the summer of 1977, 57.5% contained less than 100 fecal coliforms/g and 17.0% contained over 10,000 fecal coliforms/g. Serotypes of EEC were not detected in any of these samples.

Much of the past research concerning coliforms in cheese dealt with prevention of gassy defects and with significance of coliform contamination as an index of unsanitary manufacturing practices (*2,16*). However, since the occurrence of an outbreak of foodborne disease caused by enteropathogenic *Escherichia coli* (EEC) and associated with consumption of soft-ripened cheese (*10*), presence of coliforms in market cheese has taken on added significance. EEC have the ability to grow during the manufacture of soft and semisoft cheese and to survive in the ripened product (*5,6*). EEC, found as a natural contaminant in soft-ripened cheese, can grow in the ripened cheese at refrigeration temperatures (*3*).

An improved screening procedure for serotypes associated with EEC was developed by Mehlman et al. (*11*). Previous methods were inadequate because they failed to detect slow-lactose fermenting strains of EEC and strains which failed to grow at the elevated temperatures used (*11,13*). We used the improved methodology and thus we hope to present a more accurate estimate of the extent of EEC contamination in market cheese. Additionally, information is reported on fecal coliforms in cheese.

MATERIALS AND METHODS

Sample collection

Samples were collected from retail grocery and cheese stores in Madison, Wisconsin and vicinity during the summer of 1977. Both sliced and unsliced cheeses were selected, and representative samples of a variety of brands were obtained. Samples were refrigerated in the laboratory for a maximum of 6 days before analyses were done. Types of soft-ripened cheese that were sampled included Camembert and Brie. The semisoft cheeses that were sampled included brick, Muenster, Colby, farmer's cheese, and Monterey Jack.

Analysis for fecal coliforms

Numbers of fecal coliforms were estimated by a 3-tube Most Probable Number (MPN) procedure as described by Fishbein et al. (*4*) in the *Compendium of Methods for the Microbiological Examination of Foods.*

Analysis for serotypes of enteropathogenic E. coli

Analysis for serotypes of EEC was done by the method described by Fishbein et al. (*4*) which is similar to that proposed by Mehlman et al. (*11*). This method is qualitative and involves two separate enrichments, as well as a direct streaking of the sample.

Two 25-g portions of the sample were blended with 225 ml of MacConkey broth (Difco) and 225 ml of nutrient broth (Difco) each for 30 sec using a Waring blendor. The 1:10 dilution in nutrient broth was then streaked onto Eosin Methylene Blue (EMB, Difco) and MacConkey (Difco) agars, and these plates were incubated at 35 C for 24 h. After incubation, 10 typical *E. coli* colonies were picked from the EMB agar and 10 colonies unable to use lactose were picked from the MacConkey agar. These were placed on Blood Agar Base (Difco) and then screened serologically. A slide agglutination test with polyvalent A, B, and C antisera (Difco) was used.

Enrichments involved incubation of the MacConkey broth mixture for 20 h at 35 C, and then transferring one loopful of this mixture to 30 ml of lauryl sulfate tryptose broth (LST, Difco). This was incubated at 44 C for 20 h. The nutrient broth mixture was incubated for 6 h at 35 C, then one loopful was transferred to 30 ml of enteric enrichment broth (EE broth, Difco). This was then incubated at 41.5 C for 18 h.

The LST and EE enrichments were neutralized with sterile 10% $NaHCO_3$ before serological screening. Slide agglutination tests were done on the enrichments using polyvalent A, B. and C antisera. LST enrichments with positive agglutination tests were streaked onto EMB agar and EE broth enrichments with positive agglutination tests were streaked onto EMB and MacConkey agars for isolation of individual colonies. Isolates thought to be *E. coli* and obtained from enrichment cultures and from direct streak plates were subjected to biochemical tests necessary for identification of *E. coli* as described by Mehlman et al. (*11*) and Fishbein et al. (*4*).

RESULTS AND DISCUSSION

Occurrence of fecal coliforms

One hundred and six different retail samples of cheese were analyzed for EEC and fecal coliforms. Twenty four

different brands of cheese were obtained. The distribution of fecal coliforms in these samples is given in Table 1. Samples of soft cheese included imported brands, these being from France and Denmark. Five of the imported samples of soft-ripened cheese were processed in cans, and these all had less than 10 fecal coliforms/g. The five samples of soft-ripened cheese which contained over 10^4 fecal coliforms/g were all from the same domestic manufacturer from whom we obtained a total of 7 samples. Two of these samples contained about 10^6 fecal coliforms/g and appeared gassy. However, they could have been considered edible by some consumers. Fortunately, serotypes of EEC were not isolated from these samples. Six different brands of soft-ripened cheese were analyzed; only one brand yielded samples exceeding 1000 fecal coliforms/g.

TABLE 1. *Distribution of fecal coliforms in soft-ripened and semisoft market cheese*

Sample description	No. of samples	MPN per gram				
		10	10-100	101-1000	1001-10,000	$> 10^4$
		—(% of samples in each group)—				
Soft-ripened[1]	25	60	12	8	0	20
Semisoft						
Brick	28	32.1	21.4	14.3	14.3	17.9
Colby	26	42.3	19.2	15.4	3.8	19.2
Muenster	18	38.9	5.6	16.7	22.2	16.7
Misc.[2]	9	22.2	22.2	22.2	33.3	0
Total	81	35.8	17.3	16.0	14.8	16.0
Sliced[3]	17	35.3	23.5	11.8	11.8	17.6
Whole[4]	64	35.9	15.6	17.2	15.6	15.6
Total soft and semisoft	106	41.5	16.0	14.2	11.3	17.0

[1]Includes Camembert and Brie varieties
[2]Includes farmer's and Monterey Jack varieties
[3]Sliced brick, Colby, and Muenster varieties
[4]Unsliced semisoft cheese

Distribution of fecal coliforms in semisoft cheese is given in Table 1. Sixteen percent of the samples contained over 10^4/g and 52% contained less than 100/g. Distribution of fecal coliforms among the different varieties was similar. Also, the distribution was similar for sliced and whole cheese. Nine samples of semisoft cheese contained over 24,000 fecal coliforms/g with one as high as 2×10^6/g. These samples included aged brick (Bierkaese), mild brick, Colby, and Muenster varieties. Several samples with over 10^4 fecal coliforms per gram were gassy, but most were not.

The overall level of fecal coliform contamination we observed was much higher than that found by Collins-Thompson et al. (*1*) and Jones et al. (*9*). Collins-Thompson et al. (*1*) reported only 0.8% of semisoft and 2.1% of soft cheese samples contained over 1600 fecal coliforms/g. A possible explanation for this difference is that the occurrence of fecal coliforms in pasteurized dairy products is seasonal, with greatest numbers appearing during the summer months (*9*). Our study was done exclusively during the warmest (heat wave) part of the summer. Thus our survey only gives an estimate of contamination occurring in cheese at a particular location and at a specific time of year. However, even with our small sample size, the high levels of contamination we found, whether normal or not, are still cause for concern, because they indicate that an undesirable situation existed which could be repeated. The actual number of *E. coli* present may be lower than the estimated fecal coliform population. Hall et al. (*8*) found that only 40.4% of EC-positive tubes from MPN tests for fecal coliforms in market foods contained *E. coli.*

Occurrence of EEC serotypes

No serotypes of enteropathogenic *E. coli* were isolated from the cheese samples. This is fortunate considering the large amount of coliform contamination present in the samples. This result is also encouraging because the EEC enrichment procedures used were designed to recover slow-lactose fermenting and temperature-sensitive as well as typical EEC. However, the sensitivity of the methods used for these tests has not been determined. Other studies have also found a very low incidence of EEC serotypes in pasteurized dairy products (*9,14,15*), though their methods might not have recovered atypical strains (*13*). Jones et al. (*9*), in a survey of coliforms in Canadian pasteurized dairy products, found three EEC serotype isolates which was 2% of the *E. coli* isolates examined. The low pathogenic potential of *E. coli* isolated from foods is discussed by Mehlman et al. (*12*). Of *E. coli* isolates from cheese involved in recent EEC foodborne gastroenteritis outbreaks, only 14% were invasive and 2% toxigenic.

Goldschmidt and DuPont (*7*) as well as Mehlman et al. (*12*) have presented evidence that pathogenicity and serotype are not as closely correlated as once thought. The fact that serotypes of EEC were not isolated from our samples does not completely prove their safety for at least two reasons: (*a*) EEC not belonging to the serotypes for which we tested could be present, and (*b*) EEC strains could have been present as a small fraction of the *E. coli* population in a particular sample, and during enrichment could have been overgrown by the other strains. Cheese containing 10^5 EEC/g has been implicated in causing foodborne illness (*10*), so some of our samples would contain close to hazardous levels of coliforms if EEC strains had predominated.

Large numbers of coliforms in cheese can result from excessive post-pasteurization contamination of milk, or excessive growth during manufacture prompted by low starter culture activity (*5*). The possibility of coliform growth occurring because of inadequate refrigeration during retail handling of cheese should be investigated. Though EEC contamination in pasteurized dairy products may be rare, the common occurrence of large numbers of coliforms in soft and semisoft cheese represents a potential hazard. This indicates a need for more concern on the part of the dairy industry to prevent contaimination of cheese with coliforms and thus to prevent additional outbreaks of foodborne illness caused by EEC.

ACKNOWLEDGMENT

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Amendment to the 3-A Sanitary Standards for Centrifugal and Positive Rotary Pumps for Milk and Milk Products, Number 02-06

Number 02-07

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

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

The 3-A standards for centrifugal and positive rotary pumps, Number 02-06, are amended as set forth below.

Re-write section C.1.7 of *MATERIALS* to read as follows:

C.1.7

Pump impellers or rotors, and cases or stators, which operate in conjunction with a metallic counterpart, and the sealing faces of rotary seals may be covered with a ceramic material.

C.1.7.1

Where materials having certain inherent functional properties are required for specific applications, such as rotary seals, carbon may be used.

C.1.7.2

Carbon and ceramic materials shall be inert, non-porous, non-toxic, non-absorbent, insoluble, resistant to scratching, scoring, and distortion when exposed to the conditions encountered in the environment of intended use and in cleaning and bactericidal treatment.

Note: This Amendment will be included in the reprint, Number 02-08.

Journal of Food Protection Vol. 41, No. 3, Pages 201-219 (March, 1978)

New Methods for Microbiological Analysis of Food

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ABSTRACT

This review article discusses the application and implications of automated or semi-automated instrumentation as well as miniaturized methods which can be used to detect and characterize microorganisms of importance in the food industry. The instrumentation section includes techniques involving turbidometry, radiometry, fluorometry, immunology, and chromatography. Miniaturized methods include various diagnostic kits and procedures. Instruments and techniques such as immobilized enzyme analysis and nuclear magnetic resonance, which have potential applications to this area of microbiology, are also mentioned.

Interest in new methods for identification of microorganisms and bacterial pathogens in particular has increased greatly in recent years due to the awareness of, the need for, and the potential of rapid methods and automation in microbiology[3]. In 1971, a report by the U.S. National Institute of General Medical Sciences presented the status of mechanization and automation in the clinical laboratory. Automation in microbiology was one of the topics reviewed (*107*). Goldschmidt also published one of the early reviews on instrumentation (*66*). Richardson (*156*) summarized the developments of automation in the dairy industry laboratories.

These topics have been discussed in detail in two international symposia: The International Symposium on Rapid Methods and Automation in Microbiology first held in Stockholm, Sweden in 1973 and then again in Cambridge University, England in 1976. Proceedings of the former were published in two volumes: *Automation in Microbiology and Immunology* (*85*) and *New Approaches to the Identification of Microorganisms* (*86*). Another symposium was held in Kiel, Germany in 1974 with the title, Automation of Microbiological Food Analysis. Another review article on automated methods and data handling is found in the 1976 *Annual Reviews of Microbiology* (*93*). The book, *Modern Methods in Medical Microbiology: Systems and Trends* (*148*), also contains a good discussion. The journal *Analytical Chemistry* and other journals contain scattered review articles. For example, the 5th International Conference on Immunofluorescence and Related Staining Techniques is reported in detail in the *Annals of the New York Academy of Science* (*8*).

We have attempted to summarize the highlights of some automated or semi-automated microbiological tests and rapid methods relevant to the food industry. Hopefully, some of these methods could be adopted and routinely used by the food industry.

NEW APPROACHES AND AUTOMATION IN MICROBIOLOGY

Generally, the approach to developing new methods for detection or characterization of microorganisms involves either a search for new ideas and related instrument development or improvement of existing microbiological procedures. The impetus for development of these new techniques comes mainly from the clinical needs for rapid detection, identification and antibiotic sensitivity testing of various microorganisms. However, the space program and various governmental and industrial laboratories have also contributed greatly in this area.

Automation in dilution and dispension

In most laboratories, much time is spent in routine manipulations involving media preparation, plate pouring, specimen dilution and inoculation, and various staining procedures. There are several instruments now available which help with these mundane tasks.

Sharpe et al. (*170,171*) report on three instruments manufactured by A. J. Seward & Co., London. These are the Colworth 2000, the Colworth "Stomacher" and the

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[2] *Pennsylvania State University.*
[3] *The mention of various instruments and manufacturers in this review does not imply any endorsement by the authors or this journal. They are for reference only. Similarly, pictures of the various instruments are used merely to illustrate the various types of instrumentation available. This does not imply that those selected are superior to the others.*

Dropette. The "Stomacher" breaks and mixes raw samples such as meats, cheese, beans, peas, etc. The sample is placed in a sterile ethylene bag with a small amount of diluent. Paddles then massage the bag externally, creating violent shear forces which pulverize the specimens.

The Dropette is a diluter-dispenser which distributes samples in 0.1-ml agar droplets in empty petri plates. A viewer magnifies the drops.

The Colworth 2000 is a programmable instrument which takes a pre-determined volume of sample and makes aseptic dilutions (up to eight times) in as many as four media. It mixes these in molten agar, pours petri plates, prints the sample number on the plates, and stacks them for incubation. This takes 15 sec per plate. Fisher Scientific Company (Pittsburgh, PA) markets a Technomat filler which fills and stacks 700-1200 petri plates per hour.

Sharpe and Jackson are of the opinion that automation of existing routine procedures and manual methods sufficiently close to conventional microbiology are essential rather than automated biochemical or physical tests. They have directed their research toward these ends (*170-173*). However, as will become apparent, most of the automated instrumentation of today involves these latter parameters.

Tomtec Company (Orange, Conn.) produces an Autostreaker (Fig. 2) which streaks 1 to 10 plates of various media, selects the desired plates, streaks for isolation or count, labels with specimen number and sorts the finished plates for the correct incubator. It is equal to or better than a manual method (*199*). Others have also reported automated inoculating and streaking techniques for agar plates (*102,203,218*). Fisher Scientific Co. (Pittsburgh, PA) markets a Roto-plate turntable for manual streaking of petri plates.

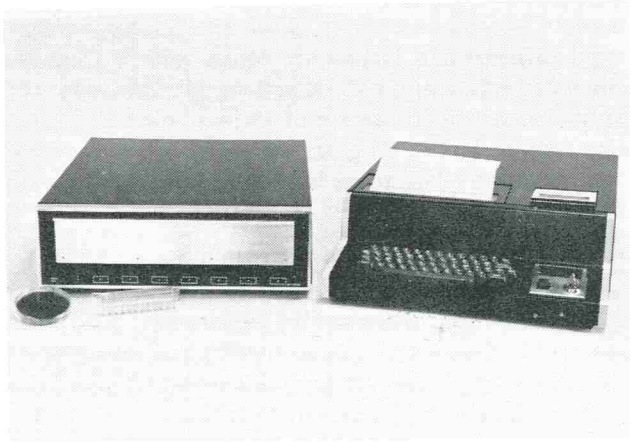

Figure 1. *R.S.B.A. [Rapid Sequential Bacteriological Analyzer]. An instrument for automated microbial kinetic studies. With permission from Akro-Medic Engineering Co., Denville, N.J.*

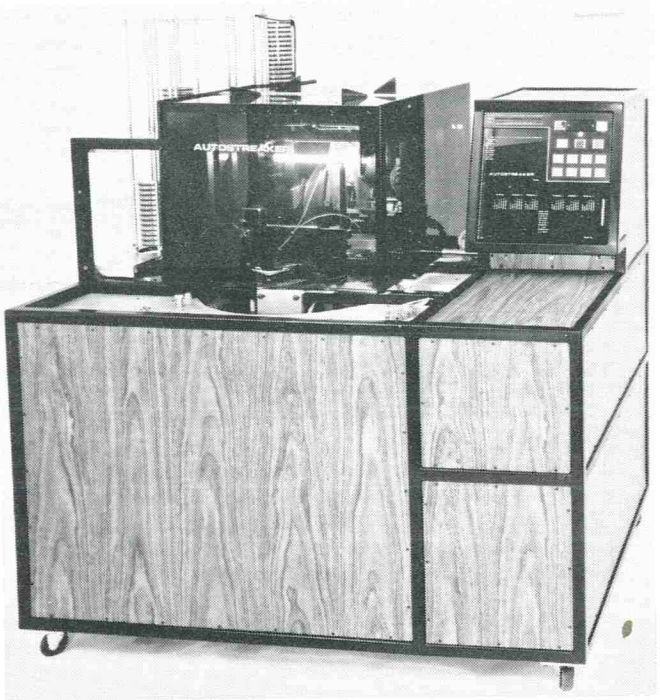

Figure 2. *Autostreaker. An automated instrument to streak bacteria into agar surfaces. With permission from Tomtec Co., Orange, CT.*

Malligo (*115*) developed a high speed scanner which counted colonies on agar plates. Several companies have marketed automated and semiautomated colony counters. Among these are the Petriscan, (American Instrument Company, Silver Spring, MD), Fisher Scientific Co. model 480 Bacterial Colony Counter, New Brunswick Scientific (Fig. 3, New Brunswick, N.J.), and the 3M automated colony counter (Curtin Matheson Company, Houston, TX). Fisher Scientific Co. markets the Fisher-Lilly Antibiotic Zone Reader II, to speed up reading of antibiotic zones (Fig. 4).

Fung (*56*) and Ryan (*165*) reported on an automated slide staining machine, now called the Dynastainer (Fig. 5 and 6) marketed by Cooke Laboratory Products (Alexandria, VA). This instrument can efficiently perform gram, acid-fast, and other stains seven to eight times faster than manual operations. Wilkins (*214*) designed an automated staining device for single slides.

Rarick et al. (*151*) used a multiple inocula replicator to determine carbon substrate utilization patterns of *Alcaligenes* species. Many companies have marketed automated pipettors and dilutors for rapid delivery and/or dilution of specimens, antibiotics, antigens, and various other fluids. Some of these included the Microtiter system (Dynetech Lab Incorporated, Alexandria, VA), Titertek (Fig. 7; Flow Lab., Rockville, MD), Medimixes (Linbro Chemical Co., Inc., New Haven, CT), Ultrolab dilutor (LKB, Washington, D.C.), Bioreactor (Biomedica, Geneva, Switzerland), Micromedic (Micromedic, Philadelphia, PA), Eppendorf Dilutor-Dispensor (Fig. 8, Brinkman Instruments Inc., Westbury, NY). This

Figure 3. *Automated colony counter. With permission from New Brunswick Scientific Co., Inc., New Brunswick, NJ.*

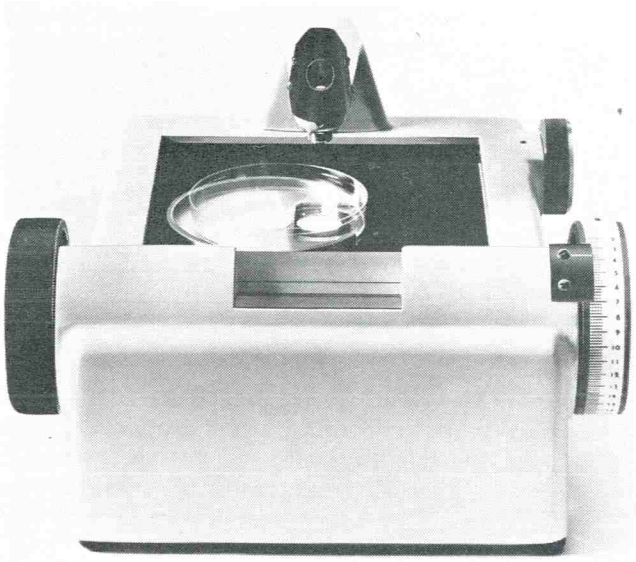

Figure 4. *Fisher-Lilly autobiotic zone reader 111. With permission from Fisher Scientific Co., Pittsburgh, PA.*

Figure 5. ***The Dynastainer. An automated gram-staining machine.*** *With permission from Cooke Lab. Products, Alexandria, VA.*

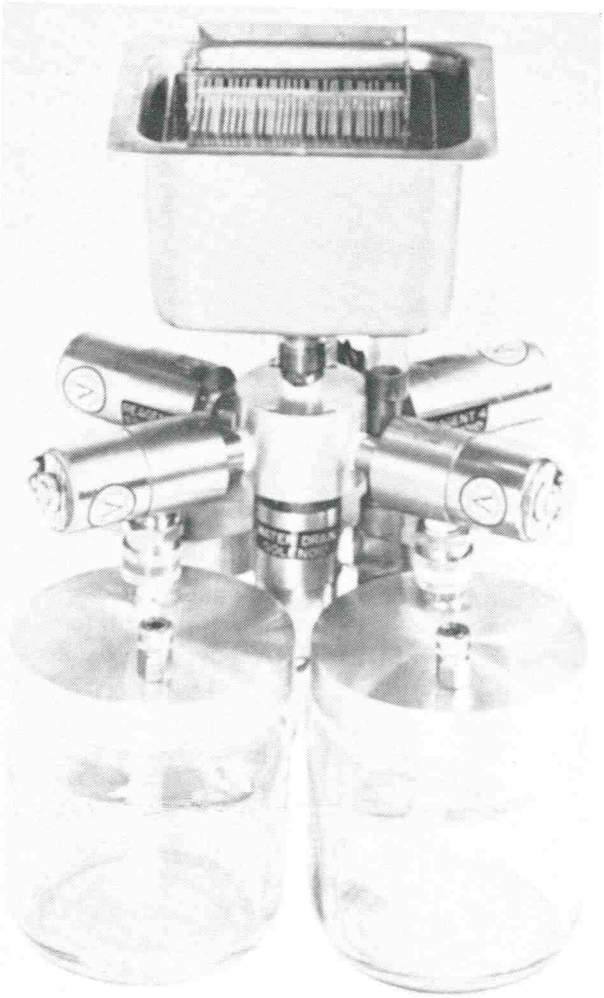

Figure 6. *Automated gram staining machine showing the internal compounds of the machine. With permission from Cooke Lab. Products, Alexandria, VA.*

last company also markets a sample concentrator and another dilutor ("Brand"). An example of laboratory operations of these microsystems is presented in Fig. 9. Microbiological Associates (Walkersville, MD) markets a multiple automated sample harvestor and washer (MASH II). Trotman (*202*) reported on one of the first automatic serial dilutors. Barbaree et al. (*13*) recently did a comparative study on the accuracy of several of these systems. O'Brien et al. (*135*) evaluated several automatic diluting and pipetting instruments for serological studies on influenza. Science Spectrum (Santa Barbara, CA) is now marketing a programmable action incubator/sampler (PAIS) which is controlled by a standard laboratory computer. This instrument provides independent programmable dispensing, incubating and sampling control of up to 600 small (2 ml) tubes. It may also be used in conjunction with continuous flow chemical analyzers.

There are a number of new approaches to studying pathogens which involve measuring growth of organisms in the presence of various antibiotics. Other methods involve physical parameters such as light scattering or impedance changes in the absence of growth per se. In some, both may be combined.

Antibiotic sensitivity determinations in liquid media can be miniaturized. Ways have been devised to dilute or dispense various concentrations of antibiotics into miniaturized wells. A standardized number of organisms is dispensed into these wells by a convenient multipoint inoculator. After an incubation period, the minimal inhibitory concentration (MIC) of various antibiotics can be ascertained by observing the absence of growth (the end point) in each series of wells.

A machine called the Dynatiter (Fig. 10, Cooke Laboratory Products, Division of Dynatec, Inc., Alexandria, VA) can automatically dilute antibiotics and add nutrient and test solutions for rapid MIC studies. Other immunological studies can also be done. These plastic plates are called Microtiter plates and have 8 × 12 wells. Several other manufacturers make similar plates, many with various sizes or shapes of wells. The Dynatiter also can do a variety of immunological and serological tasks. An instrument called the Autotiter (Ames Co., Elkhart, IN) also performs similarly. Recently Cooke Laboratory Products designed an instrument, the MIC-2000 (Fig. 11) that dispenses various concentrations of antibiotics into the Microtiter plates for MIC determinations. Along with an automatic multipoint inoculator system and a convenient reading aid, this system offers a convenient package for determination of MICs. Various other dilution machines and procedures exist for rapid dilution and dispensing of antibiotics or other substances to the dilution series.

Automation in turbidity measurements

Another method of antibiotic sensitivity testing involves comparison of changes in light scattering which

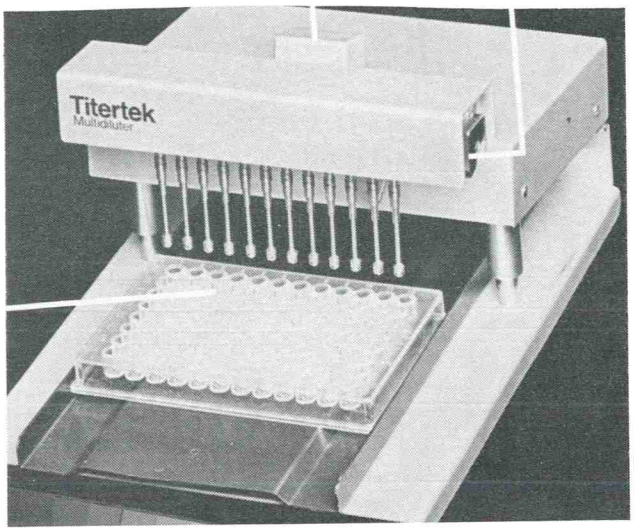

Figure 7. *Titertek. A multidilution machine. With permission from Flow Lab., Rockville, MD.*

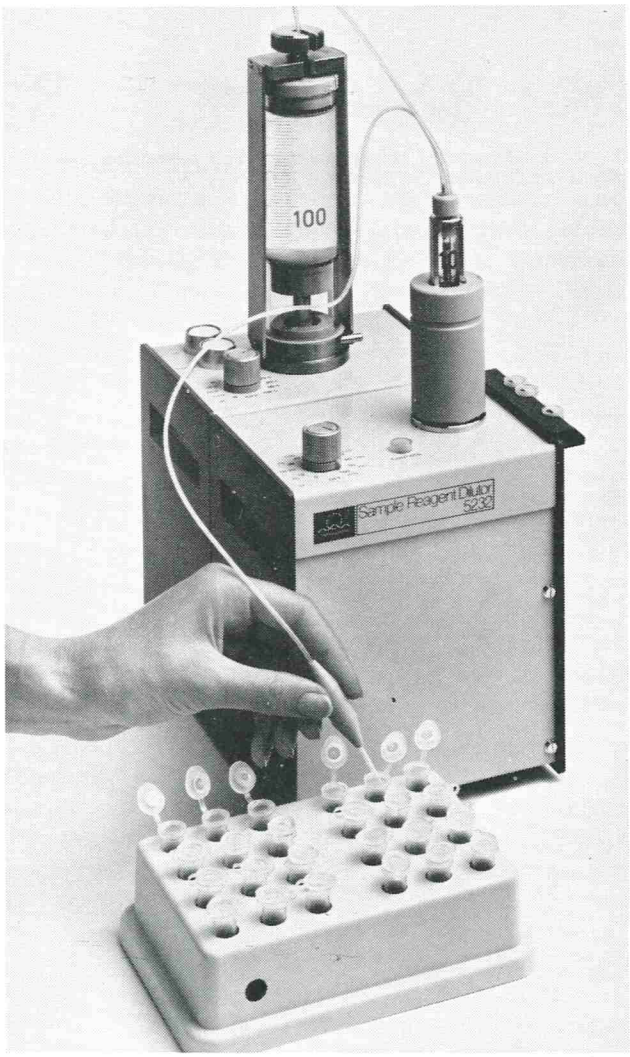

Figure 8. *Eppendorf Dilutor-Dispensor. With permission from Brinkman Instrument, Inc., Westbury, NY.*

Figure 9. *Microtiter system in use. With permission from Center for Disease Control, Atlanta, GA.*

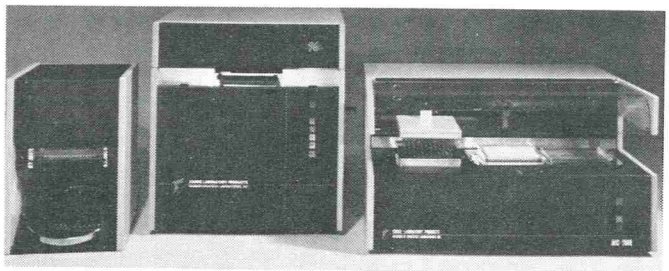

Figure 11. *MIC-2000. A system for rapid determination of MIC. With permission from Dynatech Inc., Alexandria, VA.*

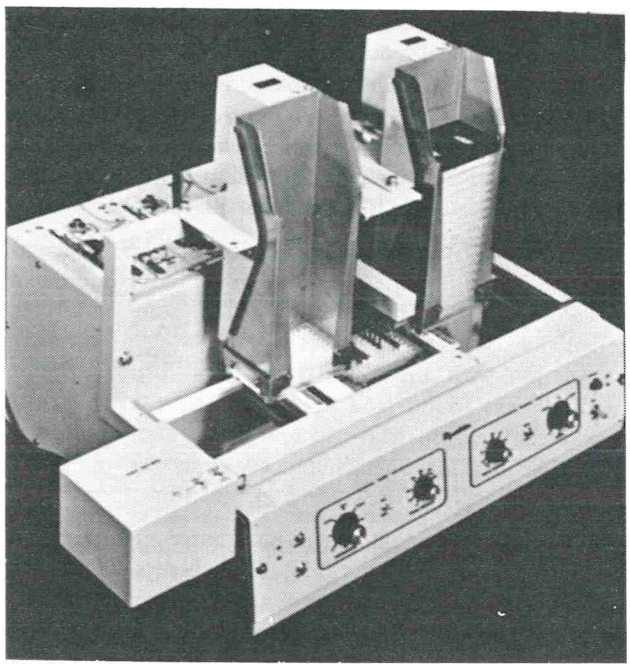

Figure 10. *Dynatiter. An automated machine for pipetting, dilution, and delivering specimens, antibiotics, antigen, antibodies, and other fluids. With permission from Dynatech Inc., Alexandria, VA.*

Figure 12. *Autobac 1. An automated machine for performing antibiotic sensitivity tests. With permission from Pfizer Diagnostic Inc., New York, NY.*

occur as organisms respond to a range of antibiotics in nutrient solutions. An elaborate system, the Autobac 1 (Fig. 12, Pfizer Diagnostics, New York) was designed for this purpose (*122,144*). Bacterial isolates are first dispensed into liquid and adjusted to the correct turbidity before they are added to a specially designed 13-cell chamber (a cuvette model). Twelve of the cells each contain a different antibiotic disc while the other cell serves as the growth control. The cuvette model is incubated in the machine. After 3 to 4 h, light scattering ability of each cell is monitored and matched against the control cell. A computer printout presents the antibiogram of the organism. When a prolonged incubation time is required, the module is scanned at a later time. A collaborative study was made by several laboratories in 1975 (*197*). Other evaluations with this instrument showed a 90% agreement for gram-negative organisms and a 93% agreement with *Staphylococcus aureus* when compared to the standard Bauer-Kirby method (*194*). However, some major discrepancies were observed for some organisms on certain antibiotics, such as methicillin and clindamycin, by these authors as well as others (*34,71*). Due to the efficiency of the system, Sielaff (*177*) used the antimicrobial sensitivity profiles (antibiograms) generated by the Autobac 1 system in conjunction with a suitable computer program to rapidly identify bacterial isolates. He reported a 95-97% accuracy when 481 clinical isolates were studied, compared to conventional laboratory methods. He also used the Autobac 1 to identify bacteria, on the same day as isolated, by using susceptibility to inhibitory substances such as dyes, various inorganic and organic compounds and anti-neoplastic agents. He claimed a 95% accuracy with 33 gram-negative organisms in a 3 to 5 h period (*176*). Use of antibiograms for rapid presumptive identification is gaining acceptance. However, the fact that resistance transfer factors can be passed easily among various genera of bacteria should indicate

extreme caution. The Autobac 1 has been modified to test ampicillin resistance in *Hemophilus influenzae* isolates by using Eugonic broth and XV strips. Results were obtained in 4 h and compared favorably to those of the Bauer-Kirby method (*192*). Goodyear combined radio-metric procedures (Bactec) with the Autobac 1 system for rapid identification and susceptibility testing of *Enterobacteriaceae* isolated from blood (*69*).

The MS-2 (Abbott Diagnostic Products, N. Chicago, IL) is another automated instrument using turbidometric measurements in determining MIC and antibiotic sensitivity patterns of microorganisms. An excellent correlation with the Bauer-Kirby and broth dilution methods was found (*187,191*). This instrument is also marketed for industrial use under the name of RSBA (rapid sequential bacteriological analyzer) by Akro-Medic Engineering, Inc. Wharton, NJ. (Fig. 1).

Berg (*16*) reported on a semi-automated turbidometric method for several microbiological assays (antibiotic susceptibility testing and response to vitamins).Turbidometric techniques have been used to determine antimicrobial susceptibility of yeasts (*62*). Schoon et al. (*167*) reported an automated light-scattering instrument to detect growth of organisms in agar-filled capillary tubes. This was similar to the instrument developed by Bowman (*20*). In 1969, Kuzel at the Eli Lilly Company (Indianapolis, IN) designed an instrument, the Autoturb (*112*). This automated system for analytical microbiology employs a continuous flow spectrophotometer to evaluate changes in the turbidity of microorganisms exposed to antimicrobials or to growth factors. It is still in use today (*211*). Growth, or the lack of it, can also be measured turbidometrically by fiber-optic colorimetry (*159,207*). Early pioneers in development of this type of instrumentation included Alper and Sterne (*5*).

An ambitious system, the Automicrobic System (Fig. 13), which detects, identifies, and enumerates bacteria and yeasts in urine specimens without an initial isolation procedure was developed by the McDonnell Douglas Corporation (East St. Louis, MO) and marketed in 1976 by the Fisher Scientific Company. This system consists of a small flat cuvette (Fig. 14) with 20 tiny culture wells (called "Identi-Paks"). Sixteen of these contain freeze-dried highly selective media. Urine is introduced into the cuvette pneumatically. The cuvettes are then placed in a reader-incubator. Final results are printed in 12 to 15 h after changes in optical absorption of each well (*100*). Some pseumonads did not grow. Growth of other organisms that the instrument was not designed to detect could be observed if their initial concentration was 70,000/ml or greater (*181*). Microbiological evaluation of the system showed an overall high accuracy (90% or above) compared to conventional methods for most test organisms (*3,186*). This system seems to hold good promise as a high capacity (240 clinical specimens) automated microbiological identification system. It is the first one to bypass routine isolation of organisms

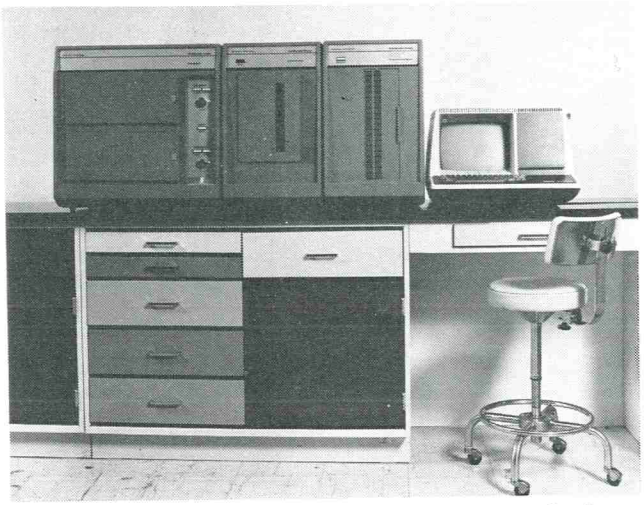

Figure 13. *Automicrobic System. An automated system for detection, identification, and enumeration of bacteria and yeast. With permission from Fisher Scientific Co., Pittsburgh, PA.*

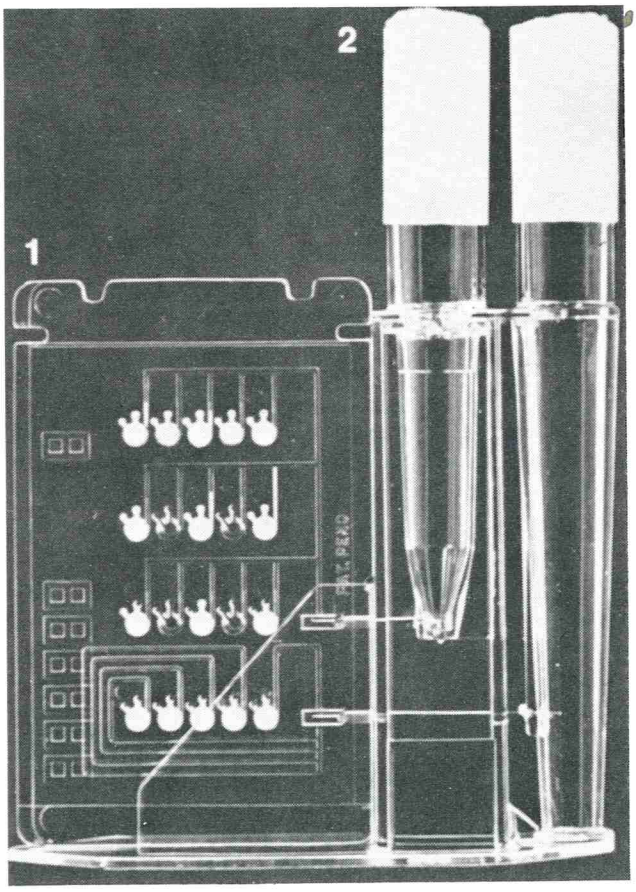

Figure 14. *Automicrobic System. The cuvette of the automicrobic system which is able to determine types and number of bacteria in clinical specimens. With permission from Fisher Scientific Co., Pittsburgh, PA.*

the first one to by pass routine isolation of organisms before identification. A recent collaborative evaluation by six laboratories reported that 98% of *Escherichia coli* and 100% of Group D streptococci were identified by this device (*181*). "SensiPaks", an antibiotic sensitivity testing

capability of this instrument, was reported to have a 90-97% agreement with the Bauer-Kirby method (*52*).

Another ingenious and unique method of bacterial identification employs differential light scattering. In this system, a laser beam is focused on a bacterial suspension and the scattered light (due to various components of the bacterial cell) is collected by a rotating detector. The intensity of light collected at various angles provides a specific pattern. Since different bacteria scatter light differently, due to their unique surface configurations, the patterns can be used for identification when compared to standards. Moreover, when the organisms are challenged with certain antibiotics or other antimicrobial agents, the cell structure undergoes detectable changes. These changes can often be observed within minutes. These instruments were designed by Wyatt (*220,221*) and are marketed by Science Spectrum (Santa Barbara, CA) as Differential I (Fig. 15), II and III. Recently, the Differential III photometer has been used in bioassays to determine the presence of veterinary drugs

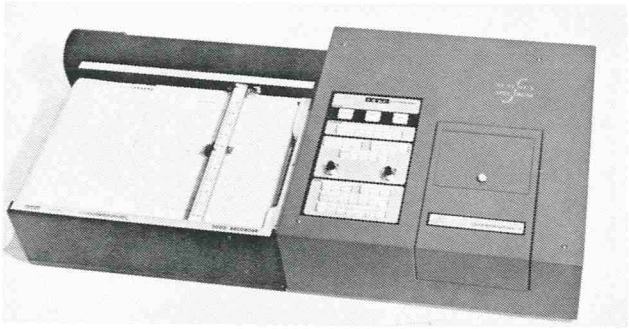

Figure 15. *Differential 1. An automated machine using light-scattering photometer for studying bacterial cells. With permission from Science Spectrum, Inc., Santa Barbara, CA.*

in tissues and secretions of food-producing animals. Parallel tests were done on 172 bovine specimens, in conjunction with the USDA. These included milk, serum, urine and bile. This instrument identified 57 positive tissues compared to 26 with standard methods (*222,223,224,*) and seems to be an excellent technique.

Laser beam spectrophotometry (Fig. 16) has also been used to detect microbial contamination in pharmaceuticals such as bovine serum albumin (*210*). Laser-nephelometry was stated to be a better technique for quantitation of immunoglobuin than radial immunodiffusion assays as the molecular size of the immunoglobin has no effect on the assay (*125*).

Other biophysical and biochemical approaches

Radiometry and microcalorimetry are two other approaches to rapidly determine growth of bacteria in clinical and food samples. The radiometric method is based on the premise that in a suitable medium containing radioactive glucose (or other compounds), microorganisms will liberate radioactive CO_2 as they metabolize the compound (*39*). The liberated gas is passed through an ionization chamber and can be determined. Usually [^{14}C] compounds are employed. Bactec (Fig. 17), designed and marketed by Johnston Laboratories, Inc. (Cockeysville, MD), is a completely automated system for radiometric determination of microbial growth. The degree of radioactive CO_2 production can be correlated with growth and is translated into a "growth index" on the instrument. A large body of literature exists for radiometric detection of bacterial growth and enzyme activity from clinical and environmental specimens (*24,36,54,97,104,145,149,153, 157,162,168*). The Bactec 310 was used with labeled sodium formate to determine the microbial acceptability of cooked vegetables (*163*). This instrument has also been used to determine the microbiological burden of raw hamburger samples (*147*). Randall (*150*) discusses the use of the Bactec for sterility testing and for detection of foodborne bacteria, including *Salmonella, Staphylococcus, Clostridium sporogenes* and *Clostridium botulinum*. The Bactec technique is basically designed for fermentative bacteria. To make use of this procedure for "non-fermentors," a special radiometric medium was developed which allowed rapid detection of *Pseudomonas* and *Alcaligenes* (*146*).

Patulin, a toxin produced by *Penicillium patulum* in foods and feeds can be labeled with [^{14}C]acetate so that its metabolism and mode of action can be studied (*133*). The ability of microorganisms to utilize substrates in the presence of herbicides or to degrade herbicides can also be studied using [^{14}C] compounds (*70*).

Two radiometric procedures were incorporated into the biology instrument placed aboard the Viking Lander which landed on Mars (Fig. 18 and 19). This instrument has a volume of 1 f^3 and weighs 34.8 lb. It is the first experimental device placed on a planet in the increasingly active search for exterrestrial life. One nuclear detection system analyzed Martian soil for incorpation of [-^{14}C] into cellular components. Another, similar to the Bactec, detected release of $^{14}CO_2$ after addition of several labelled organic compounds. A third device, a gas chromatograph, sampled the gaseous composition of the environment above the soil after an enriched medium was added (*2,128*). Figure 20 illustrates the three processes in the biological package in Viking Lander.

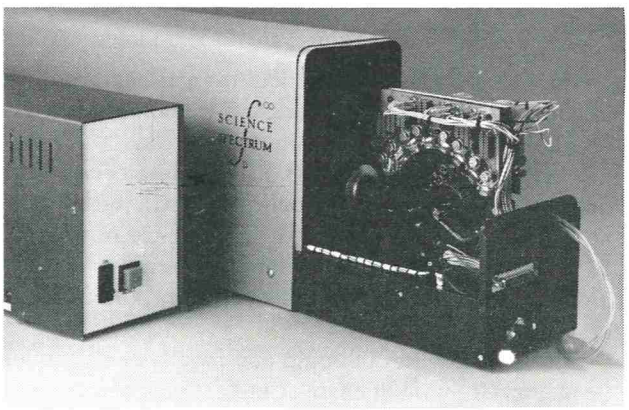

Figure 16. *Lazer beam Spectrophotometer. An automated machine to detect microbial contamination and pharmaceuticals. With permission from Science Spectrum, Inc., Santa Barbara, CA.*

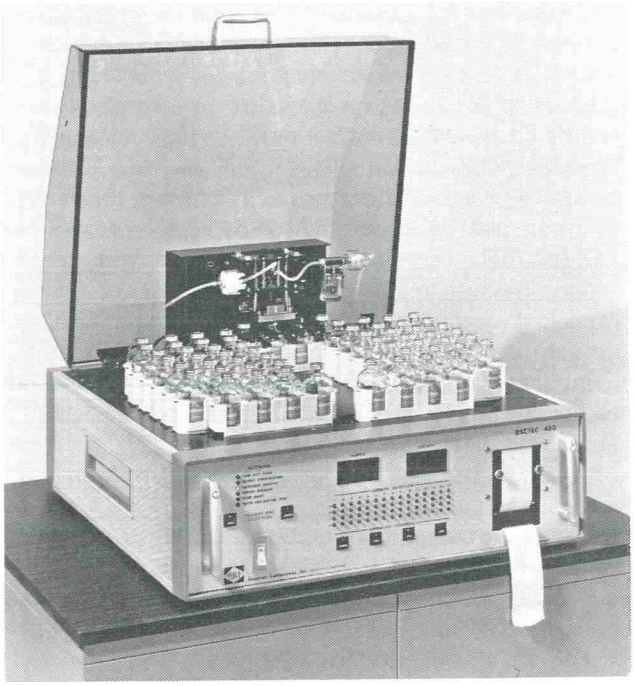

Figure 17. *Bactec 460. A machine utilizing radiometry to study microbial growth. With permission from Johnston Lab., Inc., Cockeysville, MD.*

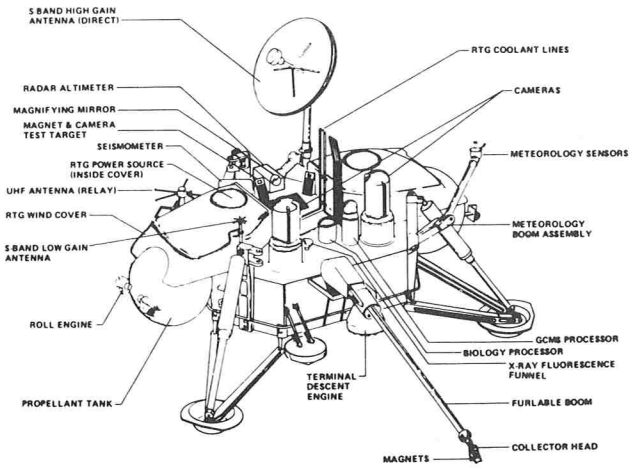

Figure 18. *Viking Lander. With permission, TRW System, Inc., Redondo Beach, CA.*

More recently, radioactive iodine [^{125}I] has been used in conjunction with immunoassays (radioimmunoassays or "RIA") to assay staphylococcal toxins (*123*), and detect them in foods (*139,143*). RIA has also been used to detect the M antigens of histoplasmin (*152*), the mannans of *Candida albicans* (*208*) and the presence of aflatoxin B_1 (*33*). As regulations governing the presence of toxins and food additives become more prevalent, these types of assays will be used often.

Bacterial activities in liquid and food systems can also be measured by small changes in heat production due to bacterial growth (*166*). When more refined instruments are used (Fig. 21, LKB Microcalorimeter, LKB, Washington, D.C.), microcalorimetry can even be used to

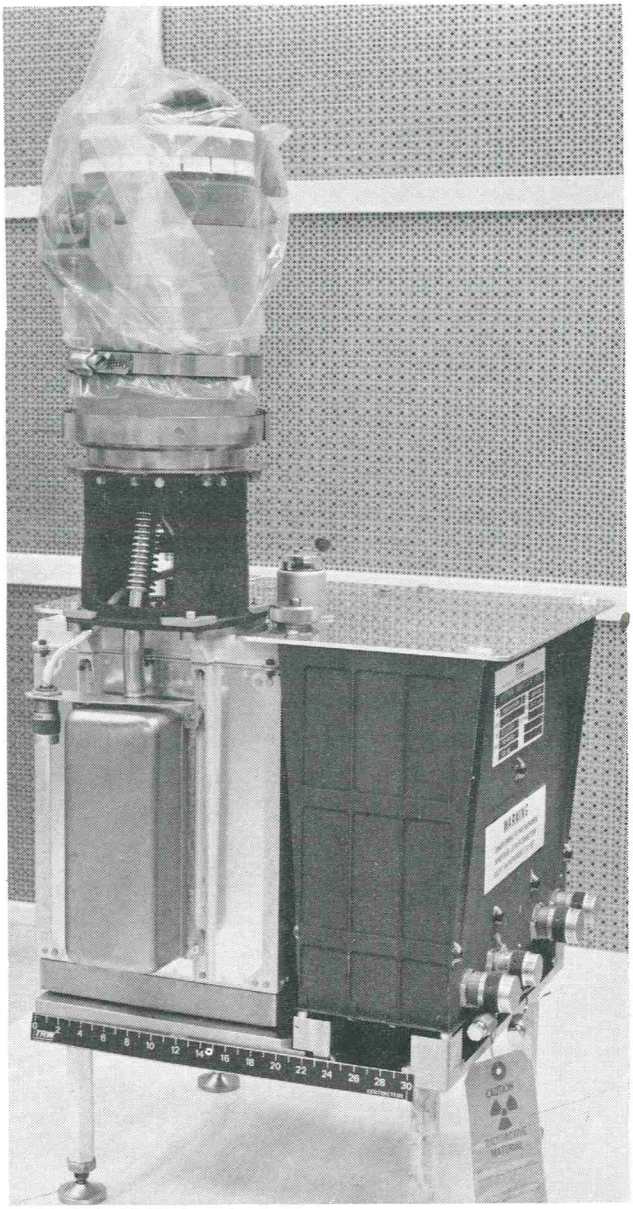

Figure 19. *Viking Lander. Biological package. With permission from TRW System, Inc., Redondo Beach, CA.*

identify bacteria (*164,190*). Bowling et al. (*19*) reported that there are characteristic profiles for different species of the *Enterobacteriaceae* based on patterns of heat production during growth. These could be used for identification. Belaich et al. (*14,15*) used microcalorimetry to study anaerobic growth of *E. coli* and its affinity for various substrates. An attempt to use both microcalorimetry and radiometry for rapid detection of foodborne organisms was made by Lampi et al. (*113*). In addition to studying pure cultures, they reported that there was a high degree of correlation between viable cell count and radiometric measurements as meat loaf underwent spoilage. They did not report a practical usefulness of microcalorimetry in food systems.

Alteration in electrical circuitry can also be used to detect and enumerate microorganisms. The Coulter

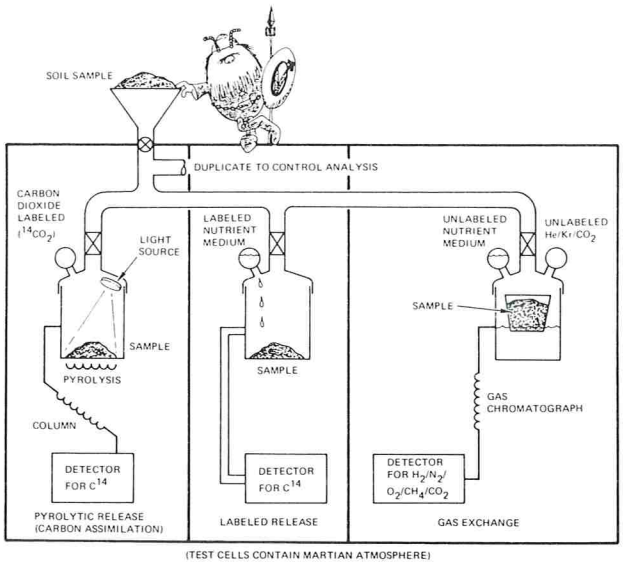

Figure 20. *Viking Lander, Biological package, illustrating the 3 procedures to detect life on Mars. With permission from TRW System, Inc., Redondo Beach, CA.*

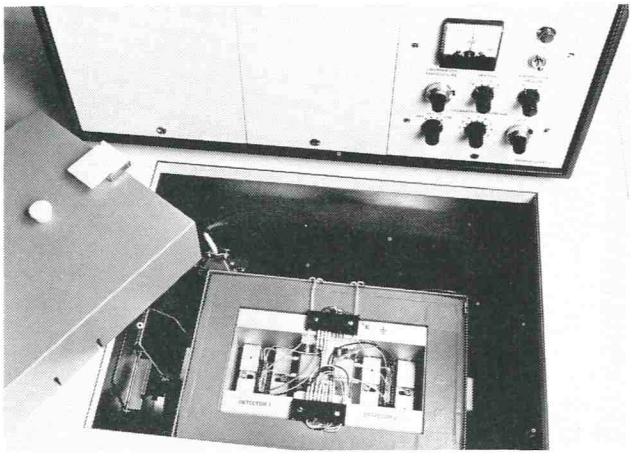

Figure 21. *LKB Microcalorimeter. This instrument measures heat generated during bacterial growth. With permission from LKB Instrument, Inc., Rockville, MD.*

Counter (Coulter Electronics Inc., Hileah, FA) has been used to determine the concentration of microorganisms in various fluids (*7,108.204*). It has been used to study growth of microbacteria (*120*) and to study the effect of both inhibitory and growth-promoting agents on cells (*110*). Goldschmidt et al. (*67*) used a Beckman R411 Dynagraph (Beckman Instruments, Inc., Fullerton, CA) to measure electrical resistance changes in pressure gauges when studying the effect of bacterial enterotoxins on gut motility in vivo. Goldschmidt and Wheeler (*68,213*) used simple instrumentation to determine electrical changes due to the presence of microorganisms in urine in the absence of growth. They suggested its use in water analysis and antibiotic sensitivity testing. Growth of microorganisms in a medium is reflected by changes in the conductivity, resistance and capacitance of that medium (*68*). The sum of these changes, called impedance, can be measured and used as an indication of stimulation or inhibition by various agents. This principle has been exploited for antibiotic sensitivity testing and to measure growth (*27,205*). There are presently two instruments available which measure impedance changes. The Bactobridge (distributed in North America by H.E.M. Research, Inc., Rockville, MD) and the Bactometer 32, an automated instrument marketed by Bactomatic, Inc. (Palo Alto, CA). Kahn et al. (*105*) used the Bactometer to detect bacteria in blood and spinal fluids of children. They claimed that the average detection time was 8.5 h compared to 24 h for conventional methods. Other applications include screening for high level of contamination of milk (*44*), monitoring of bacteria (*82*), and detection of fecal coliforms (*126*). The presence of 100,000 organisms or more in various fluids such as beer, can be detected within an hour. This type of instrumentation shows great promise for the food industry.

Bioluminescence and chemiluminescence are two procedures used to detect bacteria. The luciferin-luciferase system detects the presence of adenosine triphosphate (ATP) in bacteria. The amount of light generated is proportional to the amount of ATP present (*30*). The method has been used to detect bacterial growth in blood cultures and urine (*75,169*). It has also been used to determine microbial activity in coal strip-mining soils (*88*) and to measure the biomass in an eutropic lake (*188*). The method by which hydrostatic pressure limits colonization of deep water and sediments by non-barotolerant organisms has been investigated by this method. ATP was found to be the limiting factor—the increase in pressure stimulated ATPase (an enzyme which breaks down ATP), resulting in inefficient energy metabolism (*118*). Several companies manufacture instruments used to detect these luminescent reactions. They include the DuPont 760 Luminescent Biometer, (DuPont, Wilmington, DE), Lab-line ATP Photometer (Lab-Line Instruments Inc., Melrose Park, IL) and the Chem-Glo Photometer (American Instrument Co., Silver Spring, MD). A scintillation spectrometer can also be used in this procedure (*189*).

One important aspect of this assay is the need to destroy ATP of non-bacterial origin in the biological samples before measuring luminescence. It has also been noted that the ATP content of bacterial cells is not constant. Since the test is based on the premise that there is a constant amount of ATP per cell, results can be misleading. The age of cells, exposure to deleterious compounds, etc., all affect the ATP content. For this reason, several workers have started using procedures involving chemiluminescence. These are based on the presence of iron-porphyrin compounds in the bacterial cell. These porphyrins appear to remain at a constant level in the cell regardless of environmental conditions or age of the cells. When luminol (or myeloperoxidase) and hydrogen peroxide are added to bacterial cells, light is generated. As with bioluminescent reactions, the amount of light generated is proportional to the amount of

iron-porphyrin compounds present and can be equated with bacterial concentration (*4,132,193*). This technique has been automated (*138*). Using both bioluminescene and chemiluminescence, the Boeing Co., (Houston, TX) and the Goddard Space Flight Center have recently developed automated flow instrumentation for use by NASA at the Johnson Space Center for their water monitoring systems (*40,137,195*). This system has potential in other areas. The Goddard Space Flight Center has been interested in bioluminescence instrumentation to monitor aerospace water for potability since 1967 and has designed an automated system for this procedure (*180*).

Fluorescent staining techniques are several magnitudes more sensitive than are visual or chromogenic methods. Therefore, these methods are becoming more prevalent in rapid detection procedures. They have been used in counting bacteria (*89,192*). Laser-flow microfluorimetry has been used to measure composition distribution in *Bacillus subtilis*. The relative amounts of proteins and nucleic acid in individual cells were determined for a population of over 100,000 cells (*11*). Most often, for even greater sensitivity and selectivity, fluorscence is combined with immunological techniques. In 1969, Goepfert and Insalata (*65*) were reviewing the use of fluorescent-antibody techniques in the rapid detection of salmonellae in foods and feeds. Clostridia were being identified by this procedure in 1970 (*49*). Fluorescent antibody tests have been used to detect salmonella in poultry floor or nest litter (*185*) and enteropathogenic *E. coli* in waste water (*1*). Cherry et al. (*32*) reported on use of the fluorescent antibody test to detect salmonellae in foodstuffs, feces and water. By 1975, workers were evaluating commercial poly-valent fluorescent antibody preparations used on screening foodstuffs for salmonellae (*53*). This technique has also been used to detect bacterial contaminants which cause beer spoilage (*41,42*). A direct fluorescent antibody test was used to detect *Clostridium colinum* in sections of liver and intestine from chickens with ulcerative enteritis (*17*). Good progress has been made in automating these fluorescent antibody tests (*116,196*) for *E. coli, Salmonella, Shigella*, and other organisms found in food products. Viruses that infect poultry (*142*) and other animals (*174*) are also detected by these methods. Fluorescent antibody techniques, of course, are widely used in the clinical microbiology laboratory to detect many pathogens and will not be discussed here.

Immunological techniques have also been combined with various electrophoretic techniques such as counter-immunoelectrophoresis (*96*), immuno-electro-focusing (*91*), etc. Most of the books and review articles mentioned in the introduction contain chapters on immunological and electrophoretic techniques, both manual and automated. Many companies have marketed automated instruments for these procedures (e.g. Micro-Medic, Rhom and Haas, Philadelphia, PA) and for automated scanning (Gelman Instrument Co., Ann Arbor, Michigan). Laser beam densitometers are also available for scanning tubes, gels, etc.

Use of various types of chromatography in the identification of bacteria, especially anaerobic organisms, is well known (*43,90,127*). As with immunological and electrophoretical techniques, many reviews are available (*85,86,148*). Clinical Analysis Products Company ("Capco" Fig. 22, Sunnyvale, CA) markets an anaerobic bacteriology system called the Dohrman Anabac. This instrument is in use in many clinical laboratories.

Figure 22. *Anaerobe Identification System, Model 700. An instrument to detect the presence of microorganisms and their activities in various biological materials by gas or gas-liquid chromatography. With permission from Clinical Analysis Product Corp., Sunnyvale, CA.*

Presence of microorganisms and their activities in various biological materials has been detected by gas or gas-liquid chromatography. These range from rapid confirmation of *Clostridium perfringens* (*6*), and detecting presence of lactic acid in the spinal fluid of bacterial meningitis patients (*21,22*), to identification of auxin production by phytopathic fungi (*73*). Gas chromatography together with [^{14}C]-labeled substrates also play an important role in studying the interactions between soil microorganisms and plant growth. These range from stimulation (gibberellin production) to inhibition (phytotoxins) (*114*).

Pyrolysis in combination with gas or gas-liquid chromatography provides another means for rapid identification of bacteria or bacterial products and the method has been successfully automated (*109,124,140*). Of importance to food microbiologists, Emswiler and Kotula (*50*) were able to correctly classify *Salmonella* serotypes by analyzing pyrochromatograms of flagella and DNA. Using this combination of pyrolysis and gas chromatography, an instrument named the Bacterial ID System 700 series is marketed by Chemical Data System Inc., Oxford, PA. Most major instrument companies market various types of chromatographs. Chromatape, the first fully automated system for thin-layer chromatography, is marketed by J.T. Baker (Phillipsburg, N.J.). This has potential for food industry laboratories.

There are several scientific journals devoted to various aspects of chromatography. These include *Journal of Chromatographic Science, Journal of Gas Chromatography,* and *Journal of Chromatography. Analytical Chemistry* and *Analytical Biochemistry* also carry many articles on chromatographic procedures as well as such other areas as electrophoresis and enzyme assays.

There are many other techniques that are only now beginning to attract attention in the areas of instrumentation and automation or rapid detection methodology in general. Some of these methods have already been applied to the detection of food spoilage organisms while others, as yet, are still in their infancy in basic science, food science, or medical microbiology laboratories. A few examples are mentioned here. An indirect enzyme-labelled antibody technique (ELAT) was used to detect salmonellae in samples of pancake mix and non-fat dried milk (*111*). Immobilized enzyme systems (with enzymes attached to beads or gels) have been used to study enzymatic activity on various substrates (*38,46*). Often immunoperoxidase is employed in enzyme-linked immunoabsorbent assays (ELISA). These are becoming more prevalent (*29,51*) and have been used to detect *Trichinella spiralis* infections in naturally infected slaughter pigs (*206*) and to detect the presence of enteroviruses (*87*). It has been shown that polio virus can persist in soil and on vegetables grown in soil previously flooded with virus-seeded sewage sludge or effluent. The virus was recovered form crops such as lettuce and radishes 23 days after the plots were flooded (*198*). No doubt rapid assays for polio and enteroviruses employing ELISA or radioimmunoassays will become more important in the future. The technique of "co-agglutination" allows identification of *Salmonella* and *Shigella* directly on the primary isolation plate using drops of Protein-A-containing staphylococci sensitized with specific antibody (*47*). Ion sensitive electrodes will be used to sense volatile gases and other products in the detection and characterization of microorganisms (*215*). Nuclear magnetic resonance, proton magnetic resonance and mass spectroscopy in conjunction with chromatography will also be used in the future. It will be interesting to watch development of these methods.

DIAGNOSTIC KITS

Another area in the research and development of new methods for detecting and characterizing microorganisms centers on convenient and rapid diagnostic kits.

Agar-based kits

The one closest to the conventional tests is the r/b system (Diagnostic Research, Inc., Rosalyn, NY). This consists of the basic two-tube r/b system which uses eight agar-based reactions coupled with the two-tube "Expanders System" which utilizes another six solid media tests. A special sterile needle is used to stab-streak the suspect bacterial colony into the tubes. Reading of reactions is made after incubating tubes 18 to 24 h at 35 to 37 C. Results can be compared with the "Enteric Analyzer" (provided by the manufacturer) for ease of interpretation. Reliability of this system compared with the conventional methods for identifying enteric organisms was cited at 90-95% (*94,182,183*). Studies by O'Donnell et al. (*136*) and Brown (*23*) indicated a 99-100% degree of accuracy when certain species were examined. Martin et al. (*117*) however, could only indicate a 45% accuracy (here, the authors only used accurate identification of the organisms under study).

The Enterotube system consists of 11 agar based tests housed in a compartmented plastic tube (Roche Diagnostics, Nutley, N.J.). Inoculation is done by touching an isolated bacterial colony with the tip of a special needle of the kit and pulling the needle through the kit, depositing the bacteria in the various chambers in the process. After an 18 to 24 h incubation, appropriate reagents are added to some of the chambers and reactions are recorded. To facilitate identification of unknowns, a convenient booklet (ENCISE) is available. This contains special codes and keys to identify the enteric microorganisms. A computer consultation service is available for helping the technician to identify the cultures. This system is easy to use and seems to have wide acceptance. The disadvantage of the system involves the fact that only 11 tests are available and the kit has a short shelf life. The original system was designed for *Enterobacteriaceae*. Recently (1976), a new system has been marketed for non-fermenting organisms. Compared with the conventional tests, the Enterotube, in combination with ENCISE identification system was 91-98% accurate. It provided rapid and efficient identification of *Enterobacteriaceae* (*48,95,141,201,219*). Both the r/b system and the Enterotube have the same disadvantages of a short shelf life (the agar tends to dehydrate easily) and lack of flexibility in the types of tests. Medical Diagnostics, Corning Company (Corning, N.Y.) markets a yeast system Uni-Yeast Tek (Fig. 23).

Dehydrated media kits

Dehydrated media or paper discs impregnated with media prolong shelf life of diagnostic kits. The Auxotab System (Wilson Diagnostic, Inc., Glenwood, IL) consists of a series of carded bacterial identification systems each

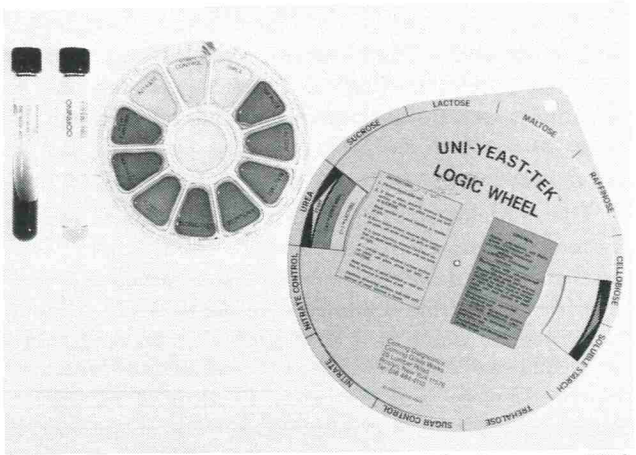

Figure 23. *Uni-Yeast-Tek. A system to identify yeast. With permission from Corning Medical Diagnostics, Corning, NY.*

containing 10 reagent-filled capillary chambers. After addition of cell suspensions, results can be obtained and common pathogens identified within 7 h. Since these tests depend only on rapid biochemical reactions, aseptic technique is not necessary and bacterial multiplication is not essential. This kit is stable for 18 months. The Auxotab System has been used to study various species of *Enterobacteriaceae* (*12,35*) and a wide range of aerobic and anaerobic organisms (*25,101*). More recent evaluations indicate that the Auxotab System had an accuracy of 87% (*154*) initially. After the system was improved, an accuracy of 96% was noted by the same workers (*155*).

A series of dehydrated systems called API are marketed by Analytab Products (Division of Ayerst Laboratories, Inc., Plainview, NY). The API 20 E (20 tests) and the API 50E (50 tests) are used for identification of *Enterobacteriaceae*. The API 20A is an anaerobic system, the API 20C identifies clinical yeasts, and the API 3S or 10S detects the possible presence of enteric pathogens in stool cultures (in 5 h). The dehydrated media are housed in small plastic chambers or "microtubes" on a convenient plastic card. Suspensions of the organisms are introduced into the individual chambers. After a 18 to 24-h incubation period, appropriate reagents are added and reactions are recorded. The data are matched with the API Coder and API Profile Register for identification. To facilitate accurate identification, the company also has a computer service to help with interpretation of the data, either by telephone or direct use of the computer bank. Overall accuracy of the API 20E system was cited at 93-96% (*178,184,209*). The API 20E and the r/b compared favorably with each other and with the conventional laboratory methods (*10*). The anaerobic API 20A system has been evaluated by several workers. One group cited an accuracy of 90% (*84*) and the other group indicated a correlation of between 85 and 91% (*129*). The API 50E was useful in studying *Salmonella* biotypes at an intrasubspecific level (*200*). The API 20 C system has also been evaluated for yeasts (*26*). It is easy to use, has a long shelf life (2 years), but the system is not sterile. Therefore, some difficulties may develop when tests are incubated for 48 h or longer. Several other workers feel that additional morphological tests such as germ tube formation are also needed as a supplement (*37,212*).

The next two systems described in this article have both flexibility and storage stability (1-2 years). The PathoTec System (General Diagnostic, Morris Plain, NJ) consists of 12 different paper strips that are impregnated with different substrates or compounds. Each may be purchased separately. An isolate is suspended and pipetted into a series of test tubes, each containing a different strip. Aseptic techniques are not necessary since only enzymatic reactions are utilized and color changes in the strips can usually be detected within 4 h. For example, reagent strips with hemin, NAD or both were used to characterize *Hemophilus* species within 4 h (*45*). The accuracy of this system is approximately 95% (*18,76,161*). The advantage of the PathoTec System is speed and flexibility as the laboratory uses individual strips only when needed. However, the time involved in preparation of tubes, etc. makes the system less desirable in terms of operational convenience. It seems easy enough to design a kit for this system, but in so doing flexibility would have to be sacrificed.

The newest entry into the market is the Minitek System (BioQuest, Cockeysville, MD). It consists of a 12-well plastic plate, a dispenser which allows ease and flexibility in dispensing of the substrate-impregnated discs into the plates, and a convenient plunger to introduce 0.1 ml of bacterial suspension into the well. After a suitable incubation, reactions are recorded. These results can then be compared with a color "Coder" to identify unknowns. At least 35 different substrates are available for use in the system, providing the user with a good choice of tests. The accuracy of this system is about 95-97% (*79,106*). It is reliable with organisms that are not members of the *Enterobacteriaceae* (*179*). The Minitek system can also be used with anaerobes (*64,80,81*). One hundred and eight clinical isolates were also correctly identified by McCarthy et al. (*121*) compared with the more tedious conventional anaerobic cultivation techniques. Yeasts can also be detected by the Minitek auxanogram method with an accuracy of 95% (*28*).

Comparative studies of kits

The ability of these kits to identify unknown microorganisms has been studied extensively by both manufacturers and various impartial microbiologists. Depending upon the investigators, many kits were found to have a 90% or higher degree of accuracy (especially after suggested improvements were made). One commercial product, not mentioned in this review, was withdrawn from the market due to consistently poor testing and evaluation. Although studies mentioned earlier in this review contain some comparative data, several others are of particular interest. Nord et al. (*134*) conducted a comprehensive study of five kits-API, Enterotube, Auxotab, Pathotec and r/b. They compared them with conventional procedures using 329 strains of *Enterobacteriaceae*. The kits were ranked according to

accuracy as follows: Enterotube (97%), API (91%), PathoTec (77%), and Auxotab (42%). In another study, Moussa (*130*) similarly compared API, PathoTec and Enterotube ranking them as: Enterotube (99.2%) API, (92.2%) and PathoTec (80.5%). PathoTec and Auxotab have since modified their kits and would probably rank higher.

A comparison between API and Minitek reported a 97.6% accuracy for Minitek and a 95.3% accuracy for API (*9*). When these two methods were compared to conventional methods for the identification of 175 anaerobes, the percentage correlation of both positive-negative reactivities with the API Anaerobic System ranged from 70-99% and that with the Minitek from 97.1-100% (*80*). When r/b and Minitek systems were compared with 294 isolates, an accuracy of 85% was reported for both methods (*175*). Several other recent comparative studies seem to indicate that Minitek has a slight edge in accuracy and flexibility (*28,76,160*).

An interesting "double blind" identification study was made by three hospital laboratories, each contributing 130 isolates. Each tested 100 randomly chosen "blinds" from the pool of cultures, using the conventional methods, r/b system and the API 20 system. The results indicated that all three methods gave identical genus and species identification with more than 90% of the isolates (*10*).

Cost Analysis

It is of interest to examine the relative cost of these systems compared with conventional methods. The only data available are for the family *Enterobacteriaceae.* For the conventional seven-tube (10 test) and the 17-tube (20 test) kits, the cost to identify one isolate is $3.60 and $7.98, respectively. For a similar identification using API 10S and API 20E, the costs are $2.33 and $3.02, respectively (*158*). Another estimation for each identification would be: API 20 ($2.17), API 50 ($4.88), Enterotube ($1.85), Minitek 12 test ($1.83), Minitek 34 test ($4.74), and r/b tubes system ($2.03) (N.A. Cox, personnal communication). These latter values are based on the purchase of kits in 100 unit lots. It appears that these commercial diagnostic kits are less expensive and more convenient than the conventional procedures while still offering accurate identification.

MINIATURIZED MICROBIOLOGICAL TECHNIQUES

Many of the recently developed diagnostic kits can be called "miniaturized" microbiological techniques. Summaries of miniaturized microbiological methods have been made by Hartman (*83*) and Fung and Hartman (*57,58*). Weaver (*103*) was among the first to use the miniaturized methods for rapid identification of bacteria including the clostridia.

The system developed by Fung (*55*) for identification of bacterial isolates involves miniaturization of tests as well as inoculation of many organisms into a variety of test substrates. In general, this system involves preparation of a master plate containing many pure cultures, then aseptic multiple inoculations of these into liquid or solid media contained in microtiter plates. After incubation, biochemical changes are observed and used to identify the unknown microorganisms with the aid of various diagnostic schemes.

Microtiter plates containing 96 wells (each holding 0.35 ml) or other vessels with multiple wells can be used to cultivate the organisms. The plates can be sterilized by soaking in 500 ppm hypochlorite solution for 1 h (followed by rinses in sterile distilled water), ultraviolet treatment or Cobalt-60 irradiation (*98*). Liquid, semisolid and solid media can be added to the plates by a hand pipette or by automatic pipetting machine. A large petri dish (15 × 150 mm) can also be used for solid media.

A multiple inoculator facilitates mass transfer of test cultures into the wells or onto solid media in the petri plates. This can be purchased or constructed by fixing 96 stainless steel pins (27 mm long) into woodblocks or other materials in the same pattern as the microtiter plate. Pin heads are suitable for inoculation into liquid media and solid agar while pinpoints are suitable for stabbing into agar or semi-solid agar. The inoculator is sterilized by alcohol-flaming and used to transfer organisms from the master plate to test media. Since microtiter plates are now used in many laboratories for a variety of techniques, ranging from immunological to tissue culture procedures, automated instruments are now available for this method (see the earlier section on dilution and dispersion). Micro-media Systems, Potomac, MD markets a similar system using micro-titer type plates (Fig. 24).

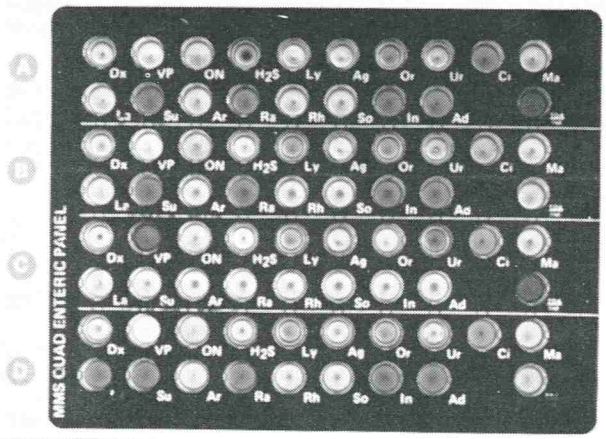

Figure 24. *Micro-ID Panel. A miniaturized biochemical testing system. With permission from Micro Media System, Inc., Potomac, MD.*

Fung has used this technique for carbohydrate tests (*59*), IMViC tests (*60*), litmus milk tests (*61*), and others in studying a variety of gram-positive and gram-negative bacteria of public health significance. This system has also been used for rapid and convenient mass identification of streptococci (*78*), *Enterobacteriaceae* (*131*), fish pathogens (*31*), streptococci, lactobacilli, and gram-negative rods from animal sources (*98,99*) and anaerobic microbiology (*216,217*). The MIC levels of drugs active against fungal pathogens have also been

determined (*63*) as well as antigen typing (*74*) and antimicrobial susceptibility testing (*72,119*). The tests described by these investigators cover the whole gamut of diagnostic microbiology, indicating that most tests can be successfully miniaturized.

Advantages of this type of system include low operating cost, flexibility of tests, mass production of data, speed of reactions, and a saving of time and space. Disadvantages include the need to have large numbers of organisms to test at any one time and technicians who are well trained, experienced and have dexterity. With the advent of automated and semi-automated instrumentation in this area, the need for dexterity is eliminated. The design for "kits" geared for specific laboratories will reduce the number of complete plates to be prepared, i.e. a design for eight of 12 tests in one Microtiter plate could be used for studying eight unknown organisms at one time.

Data analysis

The capability of computers to collect data, analyze them, extrapolate from them, convert them into desired units, and store them for almost instant recall has revolutionized almost every area of human endeavor. In the last decade, refinements in the electronics industry resulting in inexpensive calculators and computers have given the needed impetus for an explosive increase in automated instrumentation for the laboratory.

The biochemical and biophysical "fingerprints" of microorganisms can be stored in data banks and used for presumptive identification (*77,177*). Many clinical microbiology laboratories computerize their data for reports and epidemiological purposes. They can pinpoint trouble spots in a hospital and evaluate potential or real epidemics. Thus the potential for collation and dispersion of microbiological data is almost infinite. Books and reviews mentioned in the introduction of this article contain sections dealing with this subject (*85,86,148*).

CONCLUSION

A variety of methods is discussed in this review. Mentioned are methods used to identify and detect microorganisms and their metabolic products. Some of these are immediately applicable to food industry laboratories and others have future potential for adoption. In the final analysis, it is the responsibility of the laboratory director to decide which procedures or methods to use in place of, or in conjunction with, conventional techniques. A word of caution, however, before venturing to use new procedures; there should be a careful evaluation and thorough investigation of the advantages and disadvantages as well as the potential limitations of the system in light of the requirements of the individual laboratory. Many of the automated instruments are expensive and complex. In case of mechanical failure, alternative methods should be readily available.

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A Perspective on Milk Intolerance

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ABSTRACT

Lactose-intolerance is defined as the occurrence of gastrointestinal symptoms after ingestion of a test dose of lactose, usually 50 g in aqueous solution. The amount of lactose in an 8-oz. glass of milk (i.e., that amount normally considered a serving when making dietary recommendations) is only 12 g and is present in a mixture of proteins, fats, salts, vitamins and water. Unfortunately, many people, both in the scientific and health communities, believe lactose-intolerance implies intolerance to milk because of its lactose content. The result has been unwarranted recommendations to reduce or restrict milk consumption for people who may be lactose-intolerant but able to tolerate a normal serving of milk. This may mean an unnecessary loss of milk's important nutrients. Milk has long been recognized as a valuable food in feeding programs directed at overcoming protein-calorie malnutrition and maintaining a nutritionally balanced diet for all people. Milk is a major supplier of three nutrients — calcium, riboflavin and vitamin A — which are commonly found lacking in the American diet. For those people who are truly milk-intolerant, suitable alternatives include cultured dairy products (such as some yogurts), and cheeses, the recommendation to consume smaller quantities of milk more frequently throughout the day, and lactose-hydrolyzed milk. Possible beneficial effects of milk containing bacterial cultures such as *Lactobacillus acidophilus* also are being studied.

Lactose-intolerance (LI) has been the basis for many dietary recommendations calling for reduction or total restriction of milk. The argument put forward is that individuals judged to be lactose-intolerant, based on a lactose-tolerance test (LTT), should not consume milk because it contains lactose. This is unfortunate. The LTT is based on consumption of an unusually large dose of lactose (50 g — the amount in a quart of milk) and does not relate symptoms to an 8-oz. glass of milk (i.e., 12 g of lactose — the amount in milk normally considered a serving when making dietary recommendations). This leads, in many instances, to an unwarranted restriction of milk and the valuable nutrients it provides. Those who do call for restrictive measures often fail to put forward suggestions as to how much milk can be tolerated and simply suggest non-dairy alternatives.

Milk has long been recognized as virtually a nutritionally complete food. Its total nutritional value is greater than the sum of its parts because of complex nutrients interactions. Milk has been accepted universally as a nearly complete food for artificial feeding of infants starting at age 4 to 6 months (*23*). Milk, as well as other dairy foods, continues to make a significant nutritional contribution to growing children and adults as part of a mixed diet (*13,40*). Milk also has been recognized as providing valuable nutrients in those areas where protein deficiency and protein-calorie malnutrition are widespread (*10,32,41*). In a time when many people are consuming less than recommended amounts of various nutrients both in the United States and around the world, it is unfortunate that recommendations which reduce the contributions of milk and milk products to the diet are being made based on unsubstantiated claims. But the problem is complex!

No doubt some of the confusion stems from the fact that milk is the only natural dietary source of lactose, and that lactose often is referred to as milk sugar. This leads many to the erroneous conclusion that lactose-intolerance equals milk-intolerance (MI). Further confusion is caused by the indiscriminate use of terms used to describe various aspects of lactose maldigestion. For purposes of this paper the following definitions will be used (*41*):

1. *Low lactase activity — lactase deficiency* (*LD*): Less than two units of lactase activity per gram wet mucosa. This can be demonstrated in either of two ways: (*a*) directly by examination of biopsy material, or (*b*) indirectly by lactose-tolerance test (LTT) — "flat" blood sugar curve, i.e., rise of 25 mg% or less following ingestion of standard lactose dose: 50 g/m² or 2 g/kg in children.

2. *Lactose malabsorption* (*LM*): Reduced absorption of lactose, a consequence of LD determined by LTT described above.

3. *Lactose intolerance* (*LI*): Clinical signs (abdominal pain, diarrhea, bloating, flatulence, etc.) following ingestion of lactose, mixed in water in standard dose or less, in a person with proven LM.

4. *Milk intolerance due to lactose content (MI)*: Clinical signs similar to those in LI consistently following a few hours after ingestion of milk or mik-containing products in usual amounts in a person with proven LM.

Of practical importance, is whether MI equates with LI. Many factors may affect an individual's ability to properly digest lactose; of these, both age and ethnic background have been studied in most detail. Few studies have tested the tolerance of individuals to a dose of lactose similar to that found in an 8-oz. glass of milk, and even fewer studies have investigated the tolerance to milk per se.

Simooms et al, (46) raise three important considerations about practical research concerned with MI: (*a*) whether a significant number of children who are lactose malabsorbers also are milk-intolerant, (*b*) the amount of milk or lactose required to bring about symptoms in children who are lactose malabsorbers, and (*c*) the significance of the symptoms in terms of severity and likely detrimental effects on the child. It is these last two considerations which make determination of MI so difficult. Development of symptoms is a highly variable parameter. It is related to the dose of lactose encountered and the form in which it is taken (i.e. milk or water solution). As well, evaluation of severity of symptoms must be accomplished on an individual and personal basis. Practically speaking, should an individual be considered intolerant to milk if he himself does not consider the symptoms severe enough to warrant the elimination of milk from his diet? This brings us back to the first of Simoon's considerations — that of the number of individuals who are lactose malabsorbers and milk-intolerant. Regardless of the number or percentage of a population who may truly be milk-intolerant, recommendation restricting milk's use in the diet is relevant only to them. It is certainly unwise to restrict use of milk in the diet for entire populations when a substantial number of these people can benefit from milk's nutritional contribution. The answer, then, is to properly identify those individuals who truly cannot tolerate useful quantities of milk, and then to make dietary recommendations specifically for them.

Another relevant consideration is the effect of MI on utilization of other nutrients in milk. Bedine and Bayless (*4*) speculate that if all the lactose is not being properly digested then perhaps other nutrients are similarly under-utilized. There is a paucity of information in the literature which supports this contention. In fact, several studies (*7,8-11*) have shown that the protein and fat of milk are utilized as well by lactose malabsorbers as by absorbers. The event of diarrhea may alter this pattern; however, occurrence of diarrhea as a result of MI is extremely rare. It is the purpose of this paper to put milk intolerance into perspective so that those affected by MI can be properly counseled and those who can enjoy the nutritional benefits of milk are not needlessly denied this privilege.

A BRIEF BACKGROUND OF THE PROBLEM

Lactose is a disaccharide made up of the sugars galactose and glucose. The major dietary source of lactose is milk — thus the term milk sugar. Lactose serves as an important source of calories especially for the infant. Lactose also has been shown to have a beneficial effect on calcium absorption (*1*) and it has been speculated that it also may enhance absorption of other cations (*25,30*). Since lactose is slowly absorbed in the intestine, it has been reported to promote proliferation of intestinal bacteria capable of synthesizing vitamins like biotin, riboflavin, folic acid and pyridoxine (*31*).

To be properly digested, lactose must be hydrolyzed into its component sugars — galactose and glucose. This is accomplished by the action of the enzyme lactase which has been associated with the brush border of the small intestine. Detailed information regarding digestion of lactose has been given elsewhere (*18*). The physiological consequences of low intestinal lactase activity resulting in lactose malabsorption also have been described elsewhere (*18*). Suffice it to say that the clinical results of these events include occurrence of watery, fermentive acid diarrhea, flatulence, cramps, and abdominal bloating.

Because milk is distributed so widely in both international and domestic feeding programs, and many of the individuals who participate in these programs belong to population groups which have a high incidence of LI, some have questioned the value of milk in these programs (*17,29*). Nevertheless the Protein Advisory Group Ad Hoc Working Group on Milk-Intolerance concluded in 1972 (*41*) "Milk is considered a virtually complete food and in the developing areas of the world, where protein deficiency is widespread and protein-calorie malnutrition a serious childhood problem, the use of milk for child feeding programs is strongly advocated.... It would be highly inappropriate on the basis of present evidence to discourage programs to improve milk supplies and increase milk consumption because of the fear of milk-intolerance." These sentiments were reiterated by the Food and Nutrition Board — National Research Council (*32*). In 1974, the Committee on Nutrition, American Academy of Pediatrics agreed fully with these statements (*10*).

One may wonder, then, why all the confusion? The confusion exists because MI does not equate LI and most recommendations are based on data where only with LI has been measured. Let us now evaluate some of the recent literature in this field.

WHAT DOES THE LITERATURE CONTAIN?

Studies which measure the prevalence of lactose malabsorption in various population groups are numerous and have been adequately reviewed elsewhere (*19,27,44*). There is little doubt, after perusing such literature, that the prevalence of LM is high (i.e.

60-100%) in most non-white peoples around the world. Simoons et al. (*46*) suggest the incidence of LM is low (i.e. 0-35%) among relatively few of the world's peoples. Theories which purport to explain why some people develop LM and some do not are currently being debated and are beyond the scope of this review. However, the reader is directed to other references (*18,26,43-45*). Nevertheless, the practical significance of these findings is questionable.

How practical are measurements of LI and LM?

Flatz et al. (*12*) investigated the prevalence of LI among 114 healthy individuals living in northern Thailand. LI was widespread among this group — almost 100%. The authors concluded, however, "that the maximal safe dose of cow's milk for all age groups of lactase deficient individuals is 300 ml, corresponding to about 14 g of lactose." They also concluded that lactose intolerance is not related to malnutrition. Bell et al. (*5*) investigated the prevalence of LM among 17 Eskimo adults and 27 Eskimo children. Sixty-five percent of the adults and 55% of the children were classified as having LM. Despite the high incidence of LM, 95% of the adults and 96% of the children could consume at least one cup of milk without any adverse effects. These authors point out the practical limitations of the 50-g lactose load test as an indicator of tolerance for milk or milk-products. They say, "When the lactose tolerance test is used to test for tolerance to dairy foods, the use of this unrealistically large dose of lactose leads to a spuriously high estimate of intolerance for such foods." Extrapolation of MI from LI or LM based on LTT is a dangerous step and if not done cautiously will result in unwarranted restriction of milk in the diet.

Mitchell et al. (*29*) studied 33 randomly selected healthy black adolescents ranging in age from 11 to 18 years. Thirteen of the subjects had flat sugar curves and developed symptoms to the 50-g LTT and thus were classified as LI. Seven, or 54%, of the LI subjects also developed symptoms after consuming 8 oz. of milk although none had diarrhea. In addition, symptoms were much less severe when lactose was consumed in milk as opposed to an aqueous solution as in the LTT. The authors concluded that as many as 50% of LI teenagers might be expected to be symptomatic after drinking 8 oz. of milk without other food and that MI should be considered in the nutritional planning for teenagers especially those in populations with a high prevalence of LI. Nevertheless, only one of the MI individuals in this study said he would stop drinking milk because of the symptoms. Practically speaking then, if one considers the importance of severity of symptoms as Simoons et al. (*46*) suggest, only one of the LI individuals could objectively be considered MI. That the form (i.e. aqueous solution or in milk) of lactose and the amount of the challenge greatly affect the number and severity of symptoms also was demonstrated by Leichter (*24*). In his investigation, 21 healthy Canadian adults of different ethnic backgrounds were given a LTT. Eleven of the 21 were found to be LI — nine of the individuals experienced intense symptoms, one exhibited moderate symptoms and one only mild symptoms. When these 11 LI subjects consumed 1050 ml of whole milk (equivalent in lactose to that in a LTT), four showed moderate symptoms, six showed slight symptoms and one was asymptomatic. Eight of the LI subjects also were challenged with 500 ml of whole milk and four of the eight were asymptomatic. Five hundred milliliters of whole milk still is about twice the amount normally considered a serving when making dietary recommendations.

What is the effect of dose and form of lactose?

Paige et al. (*37*) investigated the effect of various doses of lactose on eight Peruvian Mestizo siblings aged four to 11 years. The subjects were challenged with three doses of lactose - 2.0 g, 1.0 g and 0.5 g/kg of body weight - in aqueous solution. All eight reported symptoms to the 2.0 g/kg dose and five reported symptoms to both of the lower doses. In all cases, symptoms to the lower doses were less severe than with the highest dose. One only can speculate based on the data of Mitchell et al. (*19*) and Leichter (*24*) that the symptoms would have been even less severe had the children been given milk per se. Paige et al. (*36*) also have investigated the possible beneficial effects of lactose hydrolyzed milk for LI individuals. The study group included 32 black youngsters aged 13-19 years. Twenty of the 32 subjects were classified as being LI. Only three of these youngsters noted symptoms to an 8-oz. glass of milk! Three subjects (two of the three from above) also noted symptoms to 90% lactose-hydrolyzed milk while all youngsters were asymptomatic with the 50% lactose-hydrolyzed milk! These findings bring up another interesting controversy, the interpretation of findings when a proper control is not conducted. In the present situation, two of the three subjects symptomatic to milk also were symptomatic to 90% lactose hydrolyzed milk but not to 50% lactose-hydrolyzed milk. This casts doubts as to the role of lactose in eliciting these symptoms, and the classification of these individuals as milk intolerant.

Kwon et al. (*21*) examined the relationship between lactose intolerance as determined by the LTT and MI by clinical symptomatology to graded amounts of milk with and without lactose. Two interesting findings resulted from this study. First, 45% of the LI subjects were asymptomatic for milk while 46% of the lactose tolerant subjects displayed symptoms. Furthermore, 75% of the symptomatic subjects responded to lactose-free milk or to one glass of lactose-containing milk but not to two. The authors agree with Bell et al. (*5*) that the LTT does not predict symptomatic response to milk consumption and clearly the report of symptoms by test subjects given milk does not necessarily imply intolerance to the lactose of milk. These findings emphasize that caution must be taken when extrapolating from a LTT to milk intolerance.

Garza and Scrimshaw (*15*) investigated the prevalence of LI as revealed by the standard LTT and compared

these results with occurrence of intolerance to graded amounts of milk in 69 black and 30 white children. Of the black children studied, lactose-intolerance ranged from 11% of those four-five years old to 72% of those eight to nine years old, yet no child was intolerant to 240 ml of milk. Furthermore, no differences were found between the milk intakes of black lactose-tolerant and black lactose-intolerant children. Thus their data do not support the view of others (*34,35*) that milk consumption is directly influenced by whether or not a child is lactose-intolerant. Sahi (*44*) also found no differences in milk consumption between lactose malabsorbers and absorbers.

Stephenson et al. (*48*) designed a study to gain insight regarding milk consumption during school lunches, preferences for certain dairy products, milk consumed outside the school environment, and factors affecting the above. Two hundred and twenty-two students, grades 1-6, were studied. Approximately half the students were black, the other half white. There was an equal number of boys and girls from both races. Lactose-tolerance tests were not conducted; however, the prevalence of lactose-intolerance among black people has been well established and has been alleged to affect the milk drinking habits of these children (*34,35*). Stephenson et al. (*48*) found no differences in milk consumption due to race and the entire population studied consumed on the average 75% of the 1/2 pint of milk offered during the school lunch. In addition, they reported drinking an average of three glasses of milk a day and the vast majority reported liking milk and a number of other dairy products.

Reddy and Pershad (*42*) also investigated the effect of dose and form of lactose on development of symptoms in both adults and children residing in India. In their study no correlation was found between symptoms of intolerance, rise in blood sugar following a lactose load and intestinal lactase levels. Twenty of the 54 children were intolerant to a lactose load of 2 g/kg, and when eight of these children were retested with a dose of 1 g/kg, all were asymptomatic. Only four of the 20 LI children had symptoms when milk containing an equivalent amount of lactose (i.e., 2 g/kg) was given and even in these children symptoms disappeared when the milk was given in divided doses. All children could consume 200 ml of milk containing 15 g of lactose at a time without any adverse effects. The authors concluded that lactose intolerance does not necessarily imply milk-intolerance. Also, the incidence of lactose intolerance and low levels of lactase activity are not reliable guides to assess milk-tolerance and use of skim milk to improve the dietaries of poorer sections of the population should not be based on these considerations.

Newcomer et al. (*33*) studied the tolerance to lactose of 59 lactase-deficient American Indians. Symptoms were assessed by a "blinded" observer (i.e., observer did not know how much lactose the subject was getting) and under conditions of this study a modest amount of lactose, equivalent to that present in 1 to 1½ glasses of milk was well tolerated by the lactase-deficient American Indian. Vlachos et al. (*50*) reported that although the frequency of lactose-intolerance is high in Greek children, they have no symptoms after drinking milk.

Stephenson and Latham (*47*) performed LTT on 35 healthy adults. Nineteen were female and 16 were male, and 10 were non-Caucasian. Fifteen of the subjects were lactose-intolerant. All of the intolerant subjects consumed at least 15 g of lactose in water or milk with no or only mild symtoms and the majority could consume much more. Eighty-six percent of the LI subjects reported they would not stop drinking milk regularly with the discomfort they felt and two reported they would stop regular milk drinking with such symptoms, both received 30 g of lactose. The authors concluded that their attempt to classify persons as lactose-tolerant or intolerant, lactase deficient or normal may be convenient clinical definitions but seen in terms of milk-tolerance, to imply a false dichotomy, and are therefore inadequate operational definitions. They, as Garza and Scrimshaw (*15*) and Sahi (*44*), found no significant differences in milk consumption or milk preference between the tolerant and intolerant groups.

Jones and Latham (*20*) have reviewed the data concerning the implication of LI in children. The authors conclude that the evidence is quite clear that low lactase activity levels and flat tolerance curves are common in children of African, Asian and Latin American origin. They go on to say, however, that most of the lactose intolerant children can consume useful quantities of milk without serious symptoms developing.

How important is proper testing protocol?

More recently Haverberg et al. (*16*) and Kwon et al. (*22*) have investigated the tolerance of adolescents of differing ethnic backgrounds to lactose-containing and lactose free milk. What's novel about these studies is that they both use a double blind procedure. In such a procedure the subjects are "blinded" as to which dietary treatment (i.e., lactose-containing or lactose-free milk) they are receiving and the experimenter is "blinded" as to which subjects are lactose intolerant or lactose tolerant. Haverberg et al. (*16*) studied 110 healthy adolescent volunteers ranging in age from 14 to 19 years; 58 were black, 44 were white and eight were of Latin American descent. The authors concluded from their study that at the one-glass level of intake, the risk of experiencing gastrointestinal symptoms from drinking milk appears to be relatively small for lactose malabsorbers and not significantly different statistically from the response to lactose-free milk under the conditions of the study. They stress the point that more studies utilizing the placebo method (i.e. lactose-free milk) must be conducted to understand the nature and extent of the causal relationship that exists between lactose malabsorption and milk intolerance. Finally they say that the true prevalence of MI secondary to LM cannot be determined

in any other way except "double blind" studies. Kwon et al. (*22*) studied 87 healthy adolescents ranging in age from 14-19 years, 26 blacks, 56 whites and five orientals. The prevalence of MI secondary to LM among lactose malabsorbers was found to be zero percent at the one glass level of intake and 16% at the two glass level of intake. The authors go on to say that even at the two-glass level of intake, when considering the clinical significance of MI secondary to LM, the nature and severity of symptoms reported by intolerant individuals rarely warrant serious consideration.

What is the effect of LI or LM on other nutrients in milk?

Bedine and Bayless (*4*) have suggested that individuals who are lactose intolerant may, not receive the full benefits from milk's nutrients. They speculate that if all of the lactose is not being properly digested, then perhaps other nutrients are similarly under-utilized. Research done at several laboratories, however, does not bear this out.

Calloway and Chenoweth (*8*) have shown that the protein and fat of milk are utilized as well by lactose malabsorbers as by absorbers. These authors also have evidence that calcium, magnesium, and phosphorus also are equally well utilized by both lactose absorbers and malabsorbers (*9*). Debongnie et al. (*11*) have confirmed the data on protein and fat as has Bowie (*7*). Bowie conducted his study on 20 children with kwashiorkor. Thirteen of the 20 had LM and severe diarrhea on milk-based diets. Fat absorption and nitrogen retention were unaffected and the author concluded that milk and milk products should continue to be used in programs to eliminate malnutrition. Bond and Levitt (*6*) have shown that perhaps 85% of the breakdown products of lactose in the colon are absorbed across the colonic mucosa and Calloway and Chenoweth (*8*) reported only a 4% energy loss due to lactose malabsorption. While one may speculate that the nutrients in milk may not be utilized fully due to LM or LI the data to support this hypothesis are not available.

WHAT ARE THE ALTERNATIVES?

Regardless of the number of people who are truly intolerant to milk, these people must be identified and given proper dietary counseling. It is estimated (*28*) that in 1976 dairy foods excluding butter contributed 75.0% of the calcium, 38.6% of the riboflavin, 34.8% of the phosphorus, 21.9% of the protein, 21.6% of the magnesium, 20.1% of the vitamin B_{12}, 13.0% of the vitamin A value, 10.3% of the vitamin B_6 and 8.6% of the thiamin available for the U.S. civilian consumption. It has also been shown (*39*) that calcium, vitamin A, and riboflavin are nutrients which may often be lacking in the American diet. It becomes obvious, then, that milk plays an important role in supplying nutrients which often may be consumed in less than recommended amounts. The major finding of the Ten-State Nutrition Survey was that "a significant proportion of the population surveyed was malnourished or was at high risk of developing nutritional problems." Phillips and Briggs (*39*) have postulated declining milk consumption as a possible factor contributing to poor nutritional status.

Suitable alternatives for the milk-intolerant individual, then, must supply the same nutrients as does milk. Lactose-hydrolyzed milk has been shown to be a suitable alternative in most cases (*20,37,38,49*). In addition, other dairy products such as cheese and fermented dairy products also are well tolerated by truly milk-intolerant individuals (*2,14*). Perhaps the first recommendation for these individuals should be to determine for themselves just how much milk they can tolerate. Bayless and Huang (*3*) found that these children can probably tolerate smaller quantities of milk with or without food, although ingestion of one cup of milk may cause discomfort. Finally there is anecdotal evidence that such culture-containing products as milk containing *Lactobacillus acidophilus* may be well tolerated by milk-intolerant individuals. Currently the National Dairy Council is sponsoring research at the Mayo Clinic to investigate the possible beneficial effects of such a product for milk-intolerant. individuals. This project is a well-controlled double blind study and should provide definite conclusions as to the efficacy of milk containing *Lactobacillus acidophilus* in this regard.

CONCLUSION

Presently there is no evidence to alter the conclusions of the Protein Advisory Group of the United Nations (*41*), the National Research Council (*32*), and the Committee on Nutrition of the American Academy of Pediatrics (*10*) that milk is a nutritionally important food and that fear of milk-intolerance should not alter its use in feeding programs. On the contrary, there is a wealth of data which encourages milk's use in overcoming protein-calorie malnutrition and in maintaining a nutritionally balanced diet for all people. It is clear that lactose-intolerance does not imply milk-intolerance, and that milk-intolerance cannot be predicted by the same clinical tests used to diagnose lactose malabsorption or lactose-intolerance. For those individuals who are truly intolerant to milk, there is a wide variety of dairy products which are tolerated and which provide the same nutrient profile as the milk from which they are manufactured.

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The Hypocholesteremic Effect of Milk — A Review

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ABSTRACT

Each Maasai tribesman in Africa ordinarily consumes 4-5 liters of fermented whole milk per day in addition to substantial quantities of meat. In spite of this diet high in saturated fat and cholesterol, the Maasai have low serum cholesterol levels and a very low incidence of clinical coronary heart disease. In studying this paradox, Mann and co-workers serendipitiously discovered that there is apparently a milk factor (MF) in the fermented milk responsible for the low serum cholesterol levels of the Maasai. A hypocholesteremic effect of fermented whole and skim milk was subsequently confirmed on American volunteers, each consuming 2-4 liters per day. Administration of radioactive acetate to human subjects on the fermented milk diet indicated that cholesterol biosynthesis was inhibited by MF. In later studies with human volunteers, English workers demonstrated a hypocholesteremic effect of unfermented whole and skim milk when consumed at a level of about 2 liters per day per caput for 2 weeks. Although the MF apparently exists in unfermented milk, there are suggestions that its concentration is slightly higher in fermented compared to unfermented milks. A hypocholesteremic effect of milk has also been demonstrated in rats by several researchers. Even though the identity of the MF is unknown at this time, it has been suggested that it may be 3-hydroxy-3-methylglutaric acid (HMG) and/or orotic acid. It is not known whether HMG occurs in cow's milk, but HMG is known to inhibit the rate limiting enzyme in cholesterol biosynthesis, HMG-CoA-reductase. Orotic acid does occur in cow's milk (73-122 mg/liter), and it has marked effects on lipid metabolism in rats. The hypolipemic action of orotic acid in rats is accompanied by induction of a fatty liver.

In past years, milk has received some adverse publicity in terms of its effect on the blood cholesterol level of the consumer. Since it is perceived to increase the blood cholesterol level and since elevated serum cholesterol is a risk factor in the etiology of atherosclerosis, many people avoid consuming whole milk, often on the advice of their physician. Indeed, milk fat, when consumed as butter or cream, does exert a hypercholesteremic response in humans (*10*). For example, Table 1 illustrates the elevation in blood cholesterol levels of humans consuming butter and cream compared to the same person receiving polyunsaturated cottonseed oil. However, in the light of recent events data in this table are important upon consideration of the protein source in the diet. Note that in individuals receiving casein as the source of protein there was a substantial elevation in serum cholesterol of those subjects consuming cream or butter. On the other hand, in the volunteer getting whole milk or dried skim milk as the source of protein there was very little increase in serum cholesterol. Although this study includes only three subjects, it suggests that there might be components in the serum portion of milk that tend to override the hypercholesteremic effects of the milkfat. Recent research on human volunteers does indicate that there is a factor(s) in the serum of milk that exerts a hypocholesteremic effect in humans. This brief review will consider recent studies, involving humans and lower animals, on the hypocholesteremic effect of cow's milk.

STUDIES WITH HUMANS

The initial observation that cow's milk may actually be hypocholesteremic in humans was made by Mann and Spoerry (*26*) in 1974. These investigators studied a group of Maasai tribesman in Africa since the Maasai male seems to be immune to clinical forms of coronary heart disease (CHD) (*28*). The Maasai possess low levels of serum cholesterol (*29*) and a low incidence of clinical coronary disease despite a diet rich in milk and meat which is alleged to be a factor in CHD of western man. While investigating this paradox, Mann and Spoerry (*26*) serendipitously discovered that consumption of large quantities of fermented milk by the Maasai actually lowered their serum cholesterol values and overwhelmed the hypercholesteremic action of a surfactant added to the milk. At the time, these workers were examining a hypothesis that surfactant food additives (in the diet of Westerners) would facilitate absorption of cholesterol from the gut resulting in hypercholesteremia. Since the Maasai normally did not consume surfactants in the diet and since they possessed a normally low serum cholesterol value, they were selected as subjects to test this hypothesis.

A group of 24 Maasai males aged 16 to 23 years was divided randomly into two groups of 12. One group

received fermented milk containing an olive oil placebo (control) whereas the "treatment" group received Tween 20 in the fermented milk. The men were given their usual diet of fermented milk for 6 days per week and meat on the 7th. The cow's milk (produced by European breeds) was obtained from a local creamery as pasteurized milk without additives or homogenization. It was seeded with a wild culture of *Lactobacillus* and after 2-3 days in a 65-70 F room, it was stirred to disperse the curd and the additives were incorporated. Tween 20 was added at a level of 10 mg/g fat in the "treatment" milk.

Body weights, heights, skin fold, blood pressure and blood cholesterol of the subjects were measured. Blood cholesterol was determined on treatment days –3, 0 and thereafter at 7-day intervals.

Normally, each Maasai male consumes abut 4-5 liters of fermented milk daily containing approximately 660 k cal/liter. However as this study progressed the consumption of milk increased until by treatment day 4, the 24 men were consuming 200 liters/day amounting to 8.33 liters/man/day which would furnish each subject with 5,500 kcal. Despite efforts by the investigators to restrict the caloric intake of the two groups, excessive weight gain in the subjects caused the experiment to be terminated after 3 weeks.

Subsequent analysis of the serum indicated that the levels of serum cholesterol decreased in both groups of volunteers in a 3-week period despite the gain in weight and contrary to the original hypothesis (Fig. 1). In fact, there was an inverse relationship between weight gain (consumption of milk) and serum cholesterol (Fig. 2). Eight of the Maasai men increased their body weight by 6 or more pounds in 21 days (average 8 lb) but their level of serum cholesterol fell an average of 28 mg/100ml. On the other hand, sixteen men gaining 5 or less pounds exhibited a serum cholesterol decrease of 8.19 mg/100 ml. This inverse relationship between serum cholesterol and consumption of the fermented milk suggested to Mann and Spoerry (*26*) that there must be a factor in the milk that somehow regulates the serum levels of cholesterol. These workers postulated that a

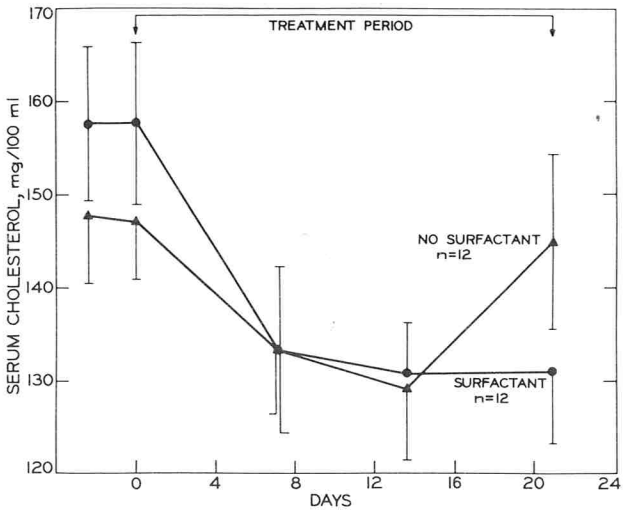

Figure 1. *Effect of fermented milk with and without added surfactant on cholesterol in serum of Maasai. Redrawn from Mann and Spoerry (26).*

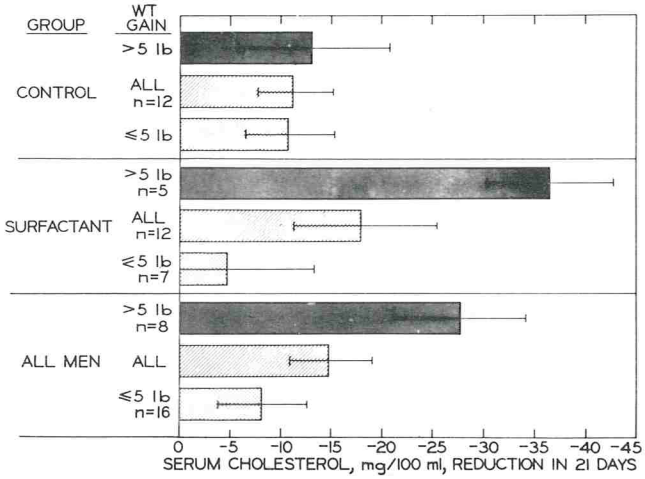

Figure 2. *Changes of cholesteremia in Maasai according to change of body weight during 21-day period of treatment with fermented milk. Redrawn from Mann and Spoerry (26).*

TABLE 1. *Effects of whole milk, cream, butter, and cottonseed oil on serum cholesterol and triglyceride concentrations.*[a]

Subject	Diet[b]		Serum lipid concentrations	
	Protein	Fat	Cholesterol mg/100 ml (Mean 3rd or 4th weeks)	Triglyceride mg/100 ml (Range)
1. L. H.	Casein	Cottonseed oil	175	
Male	Casein	Cream	245	
	Casein	Cottonseed oil	160	
2. J. G.	Casein	Cottonseed oil	220	517-703
Female	Casein	Butter	315	468-761
	Casein	Cream	300	761-1,034
	Casein	Cottonseed oil	225	585-732
3. A. H.	Whole milk	Whole milk	270	390-507
Female	Dry skim milk	Cream	275	458-526
	Dry skim milk	Butter	268	380-500
	Dry skim milk	Cottonseed oil	225	273-487

[a]From Goldsmith et al. (*10*).

[b]Fat furnished 42% of the calories, protein 12% of the calories in subjects 1 and 2 and 15% in subject 3. Carbohydrate furnished 46% of the calories in subject 1 and 2, and consisted of equal amounts of dextrose and starch. In subject 3, carbohydrate furnished 43% of the calories of which 4% was dextrose, 15% starch, and the remainder was that found in milk.

component of milk inhibits the biosynthesis of cholesterol to reduce the serum levels. The effect of this factor overwhelmed the expected serum cholterol elevation resulting from the surfactant. In addition, the factor apparently over-rode any hyper-cholesteremic effects of the fat and cholesterol in the milk.

Subsequently, Mann (*24*) confirmed and extended the aforementioned findings on American volunteers. In this latter study, 26 subjects (20 men and 6 women), aged 24-55 years, were fed a whole milk yogurt prepared by addition of Dannon's plain yogurt to fresh, homogenized and vitamin D enriched cow's milk. The mixture was fermented at 23-30 C for 24-36 h with occasional shaking, after which the pH of the yogurt became about 4. The normocholesteremic subjects consumed a prescribed amount of yogurt in 4-6 intakes throughout the day. The subjects also consumed other foods or fresh fruit daily. In addition, only meat was consumed on the 7th day to obtain iron in the Maasai fashion.

A summary of the effects of 19 treatment trials with 13 subjects given various amounts and kinds of yogurt or milk is shown in Table 2. In general, there was a reduction in serum cholesterol during the 12-day feeding period with a slow return toward normality upon cessation of the yogurt diet. Both whole and skim milk yogurt produced a statistically significant reduction of cholesteremia. However, Mann (*24*) claimed that fresh milk at an intake of 2 liters daily did not statistically affect cholesteremia and he seemed to think that the factor affecting serum cholesterol, is produced or enhanced in milk by microbial action. But, as pointed out by Howard (*16*), an inspection of Mann's data could lead to an alternative conclusion. Howard further argues that fresh milk may very well reduce serum cholesterol but the number of subjects used in Mann's study was insufficient to obtain statistical significance. Indeed, recently Howard and Marks (*17*) demonstrated a hypocholesteremic response of unfermented whole milk and skim milk in a larger group of subjects (Table 3). Eight men and eight women, aged 22-55, were divided into two equal groups. One group consumed 4 pints of whole milk daily whereas the second group received 4 pints of reconstituted dried skim milk daily. The volunteers distributed the milk throughout the three main meals, and they adjusted their caloric intake so that their weights remained constant. After an initial baseline

TABLE 2. *The effects of various intakes of milk on cholesteremia in American volunteers (serum cholesterol in mg/100 ml)*[a].

Subjects		Pre-treatment	Treatment days 4	8	12	16	20	24	28	32	36
Treatment: 4 liters whole milk yogurt daily for 12 days											
3 males + 1 female	Mean	208	220	209	186	*173*[b]	*169*	172	190	214	211
	Sem	16.6	23.2	27.6	29.1	18.7	22.7	25.8	30.6	25.5	15.1
Treatment: 2 liters whole milk yogurt daily for 12 days											
4 males + 2 females	Mean	193	199	*165*	*175*	*169*	*177*	—	196	—	198
	Sem	34.3	16.1	20.9	16.6	19.6	24.0	—	18.7	—	16.6
Treatment: 2 liters skim milk yogurt daily for 12 days											
3 males + 2 females	Mean	211	208	196	*150*	*162*	—	181	—	202	218
	Sem	21.1	18.7	11.0	40.9	32.5	—	18.3	—	13.3	13.5
Treatment: 2 liters whole fresh milk daily for 12 days											
3 males +1 female	Mean	196	206	206	177	188	200	179			
	Sem	13.6	21.0	22.5	16.5	20.3	18.7	10.6			

[a]From Mann (*24*).
[b]Values in italics: P<0.05 using paired t-test.

TABLE 3. *Effect of whole milk, skim milk and milk-fat on serum lipids of human subjects (mg/100 ml) (mean ± SEM)*[a].

Serum-lipid	Week 0[b]	1	2	3
Whole milk				
Cholesterol	213 ± 9.4	208 ± 9.3	202[c] ± 8.0	203[c] ± 8.6
Triglyceride	103 ± 9.4	95 ±14.7	95 ±14.7	71 ±11.8
Skim milk				
Cholesterol	220 ±15.3	191[c] ±16.9	188[d] ±12.0	212 ±19.4
Triglyceride	105 ±10.6	113 ±13.3	106 ±15.6	83 ±11.6
Milkfat				
Cholesterol	197 ± 5.7		231[c] ± 9.6	
Triglyceride	111 ±17.2		159 ±14.3	

[a]From Howard and Marks (*17*).
[b]Mean of two baseline values.
[c]p<0.05.
[d]p<0.001.

was established for blood cholesterol and triglyceride, further blood samples were analyzed for these lipids after 1 and 2 weeks on the milk and 1 week after a normal diet was resumed. The data in Table 3 indicate a significant (paired t test) reduction in serum cholesterol occurred in both groups on the milk supplement. The effect was significant at the second week for the whole milk group and highly significant at the first and second week for the skim milk group. After the second week, the fall in serum cholesterol was 5% for the whole milk group and 15% for those on skim milk. One week later (week 3), after a normal diet had been resumed, the mean for serum cholesterol still had not reached the pretreatment level. The observed hypocholesteremic effect of whole milk is somewhat surprising since 4 pints of whole milk would contain 84 g of fat and 600 mg of cholesterol. To illustrate the significance of this, Howard and Marks (*17*) fed seven human volunteers 113 g of butter daily, which contains the same quantity of fat and cholesterol as 4 pints of milk. The volunteers were maintained on an isocaloric diet for 14 days and their serum cholesterol levels increased dramatically as expected (Table 3). These data suggest that unfermented milk also contains the hypocholesterolemic milk factor (MF). However, Mann (*25*) claims that there is slightly more MF in fermented milk.

At present, the identity of MF is unknown, but Mann (*25*) states that it is nonprotein, dialyzable, heat and acid stable and polar. Mann (*24*) has also administered radioactive acetate to human volunteers and observed that incorporation of the acetate into serum cholesterol is inhibited during the consumption of yogurt indicating decreased cholesterol biosynthesis. Mann (*24*) postulates that 3-hydroxy-3-methylglutaric acid (HMG) in the fermented milk inhibits the rate limiting enzyme in cholesterol biosynthesis, hydroxymethyl glutaryl CoA reductase (HMG CoA reductase). However, no analytical data were presented on the HMG content of the yogurt. Howard (*16*) initially suggested that calcium might be the MF; however, in a subsequent study by Howard and Marks (*17*), when seven volunteers consumed 2.4 g of calcium daily as the gluconate for two weeks there was no decrement in their serum cholesterol. They concluded that calcium is not the MF. Helms (*15*) has suggested that MF might be lactose but without presenting any experimental data. Based on in vitro studies with rat liver preparations, Bernstein et al. (*2,3*) suggest that MF may, in part, be the orotic acid in milk. This is predicated on the observed inhibition of cholesterol biosynthesis in vitro by orotic acid and on the general effects that orotic acid has on lipid metabolism. More will be written on this subject later in the review.

STUDIES WITH LOWER ANIMALS

Malinow and McLaughlin (*23*) observed a decrease in plasma cholesterol levels of rats reared on a chow-skim milk diet (Table 4). Young Sprague-Dawley rats were weaned at 21 days after parturition and were continued on the chow-skim milk diet (24 rats) with a chow-tap water diet (25 rats) serving as a control. The data in Table 4 indicate that the male progeny that had received the skim milk had lower plasma cholesterol concentrations than those on tap water. These difference were not observed in the female progeny at 43 days, but they were present at 64 days. Rats on the skim milk regimen were initially heavier than those on water, but the weight differences disappeared at 64 days of age. These data again suggest there is a factor in milk that exerts a hypocholesteremic effect.

Additional observations by Nair and Mann (*29*) point to a hypocholesteremic factor in milk. Male, albino Sprague-Dawley rats each weighing 50-60 g were divided into groups of six animals and fed various diets ad lib. Blood cholesterol values were determined at 2-week intervals. The cholesteremic responses of the rats on diet C (97% chow, 2.5% corn oil and 0.5% cholesterol) are compared with those of rats receiving skim milk powder on diet D (72% chow, 25% skim milk powder, 2.5% corn oil and 0.5% cholesterol) in Fig. 3. The effect of dietary milk powder in lowering the cholesteremic response was apparent throughout. At the 42nd day and thereafter the cholesteremia was significantly lower in the group receiving milk in addition to cholesterol. The cholesteremia was also significantly lower at the 11th week in the milk-cholesterol fed group (diet D) than in the control group (diet A) (t =2.46) receiving only rat chow (data not shown). Thus a diet containing 25% skim milk powder was hypocholesteremic both in the presence and absence of dietary cholesterol. When Nair and Mann (*29*) included 0.1% 3-hydroxy-3-methylglutaric acid (HMG) into diets with or without cholesterol, the hypocholesteremic effects were similar to those observed for skim milk. These workers, therefore, postulated that HMG was the hypocholesteremic factor (MF) in milk.

TABLE 4. *Plasma cholesterol concentration in rats on two dietary regimens (mean ± SEM)*[a].

	Plasma cholesterol (mg/100 ml)			
	Chow-water		Chow-skin milk	
Animals	43-day-old	64-day-old	43-day-old	64-day-old
Males	95 ± 3 (10)	107 ± 3 (10)	76 ± 38[d] (13)	85 ± 4[d] (13)
Females	94 ± 3 (15)	109 ± 2 (15)	109 ± 3[c] (11)	96 ± 4[c] (11)
All rats	95 ± 2 (25)	109 ± 2 (25)	91 ± 4[b] (24)	90 ± 3[d] (24)

[a]From Malinow and McLaughlin (*23*).

[b]p (Student's t-test) Chow-water vs. Chow-skimmilk.

[b]Nonsignificant.

[c]<0.01.

[d]<0.001.

Number of rats between parentheses.

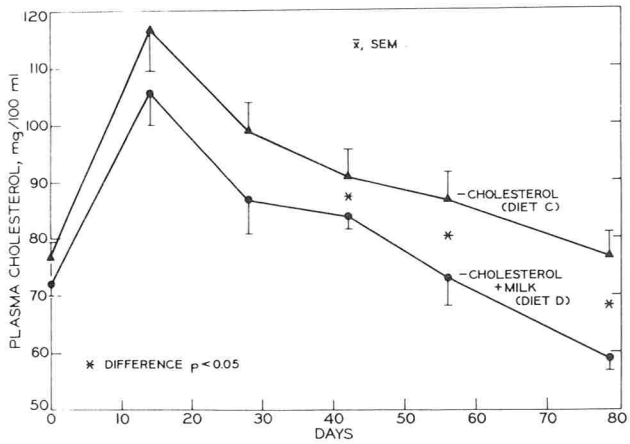

Figure 3. *The effect of adding 25% skim milk powder to a diet containing 0.5% cholesterol on cholesteremia in the rat. Each point is the mean and SEM for six animals. Redrawn from Nair and Mann (29).*

However, no analyses for HMG in the milk were presented to support this speculation.

In work that preceded the aforementioned in vivo studies on rats, Boguslawski and Wrobel (5) reported on the existence of a factor in milk that inhibited cholesterol biosynthesis in rat liver. Initially, they studied incorporation of [^{14}C]-acetate and [^{3}H]-mevalonate into choesterol using a rat liver fraction (from 5-6 week old Wistar rats) in vitro. Data in Table 5 indicate that addition of 10 μl of cow's milk (2% fat) or 25μl of human milk completely inhibited incorporation of [^{14}C]-acetate and [^{3}H]-mevalonate into digitonin-precipitable sterols by rat liver preparations. The inhibitory factor of cow's or human milk resisted 10 min boiling and was in the supernatant fluid after protein had been precipitated from cow's milk with 5% lactic acid or 5% trichloroacetic acid. Overnight dialysis completely abolished the inhibitory effect of cow's milk. These data indicate that the inhibitor is a nonprotein, low molecular weight compound.

Although the inhibitor is effective both in vivo and in vitro, it affects sterol synthesis in a different way when added to the cell free system than when fed to the intact animal. This point is illustrated by data in Table 6 which indicated that incorporation of [^{14}C]-acetate into cholesterol by rat liver slices, from animals previously fed 2% cow's milk, was inhibited whereas that of [^{3}H]-mevalonate was not. Conceivably, there could be more than one factor in the milk which differentially affects in vitro and in vivo biosynthesis. In any event, there is or are factor(s) in cow's milk that inhibit biosynthesis of cholesterol in rat liver.

Recent work by Bernstein et al. (*2,3*) suggests that orotic acid of milk may be involved in its hypocholesteremic effect. Orotic acid, a pyrimidine intermediate in nucleic acid synthesis, generally exists in milk at a concentration range of 73-122 mg per liter (Table 7). As might be expected from the concentration effect, orotic acid is extremely high in dried milk and dried whey powders (Table 7). It also exists in yogurt and cultured

TABLE 6. *Effect of dietary cow's milk on the incorporation of* [^{14}C]-*acetate and* [^{3}H]-*mevalonate into digitonin-precipitable sterols by rat liver slices*[a].

Group[b]	Diet	Specific radioactivity of sterols: ^{3}H d.p.m. mg^{-1} mevalonate	Specific radioactivity of sterols: ^{14}C d.p.m. mg^{-1} acetate
1	Solid food + drinking Water (control)	4,470 ± 580	15,400 ± 1,270
2	Solid food + milk	4,600 ± 420	7,300 ± 1,290
3	Milk only	4,600 ± 310	8,600 ± 1,100

[a]From Boguslawski and Wrobel (5).
[b]Each group consisted of three rats. The animals were kept for 3 days on the diet indicated. Daily intake of solid food in groups 1 and 2 was about 8.5 g per rat. Daily intake of milk was about 7 ml per rat in group 2 and 12 ml per rat in group 3. 0.5-g portions of liver slices obtained in duplicate from each animal were used for incorporation studies. The results of a typical experiment are presented. Values are the means from three animals ± s.d.

TABLE 7. *Orotic acid content of milk and other dairy products.*

Product	Orotic acid content mg/liter or kg	References
Milk	73-122[a]	(*2,11,14,34*)
Yogurt	34-46[a]	(*34*)
Cultured buttermilk	53[a]	(2)
Crude casein	26[b]	(*14*)
Milk powders	950-1,689[b]	(*14, 33*)
Dried delactosed whey	2,600[b]	(*14*)

[a]mg/liter.
[b]mg/kg.

TABLE 5. *Effect of milk on the incorporation of* [^{14}C]-*acetate and* [^{3}H]-*mevalonate into digitonin-precipitable sterols by 600 g of supernatant fluid of rat liver.*[a]

Addition	(μl)	Specific radioactivity of sterols[b]: ^{3}H d.p.m. mg^{-1} mevalonate	% of control	^{14}C d.p.m. mg^{-1} acetate	% of control
None (control)		39,400	100	10,000	100
Cow's milk	10	780	2	—	0
Cow's milk boiled	10	800	2	—	0
Human milk	25	1,900	5	—	0
Human milk boiled	25	2,000	5	—	0
Cows milk dialyzed	25	38,800	100	9,800	100
Supernatant fluid of cow's milk deproteinised with lactic acid, final conc. 5%	25	750	2	—	0
Supernatant fluid of cow's milk deproteinised with trichloroacetic acid, final conc. 5%	25	—	0	—	0
Neutralized lactic acid 5%	50	39,000	100	10,000	100
Neutralized trichloroacetic acid 5%	50	39,000	100	10,000	100

[a]From Boguslawski and Wrobel (5).
[b]The values represent the means of typical experiments done in triplicate.

buttermilk but at reduced levels compared to the original milk (Table 7). Okonkwo and Kinsella (*34*) reported that milk contained orotic acid at levels of 83 ± 12 mg per liter whereas the orotic acid in yogurt varied from 34 to 46 mg per liter. However, Ferreira (*8*) did not observe a decrease in orotic acid content of yogurt over the same time period. This suggests there may be strain differences in the use of orotic acid by culture microorganisms. A lower orotic acid level in yogurt, of course, would be at variance with its being equated with the hypocholesteremic factor (MF) and Mann's position that the concentration of the MF is higher in yogurt than in milk (*25*). Also, orotic acid concentration is very low in human milk (0-37 mg/liter) (*11*) but human milk inhibits cholesterol biosynthesis in rat liver preparations (*15*) thus militating against the involvement of orotic acid in this aspect of the hypocholesterolemic action of milk. However, there are data which suggest that orotic acid may be one, but not necessarily the only, factor involved in the hypocholesteremic effect of milk.

Bernstein et al. (*3*) were unable to demonstrate any inhibition of purified rat liver HMG CoA reductase by milk (Table 8). This would indicate that the rate limiting enzyme for cholesterol biosynthesis in rat liver, at least, is not inhibited by the MF. Also, there is apparently insufficient quantity of HMG in milk to inhibit HMG-CoA reductase. However, incubation of rat liver preparations with various radioactive precursors along the cholesterol biosynthetic route indicated the milk and orotic acid (at the same concentration as in the milk) inhibited incorporation of [^{14}C]-acetate into cholesterol but not those precursors further along the chain (Table 9). Since incorporation of label from acetyl CoA into cholesterol was not inhibited, this would suggest that acetyl CoA synthetase was the enzyme inhibited. Indead, subsequent studies with purified yeast acetyl CoA synthetase indicated that orotic acid was a non-competitive inhibitor of this enzyme with a K_i of 6.6×10^{-5} M (Fig. 4). However, as seen from the data in Table 9, orotic acid is not quite as effective in inhibiting incorporation of acetate into cholesterol as whole milk. This might suggest the presence of other factors in milk that also inhibit acetyl CoA synthetase. It should be pointed out that Bernstein et al. (*2,3*) did not observe an inhibition of mevalonate incorporation into cholesterol by rat liver preparations in vitro as did Boguslawski and Wrobel (*5*). From the studies of Bernstein et al. (*2,3*), the suggestion was made that orotic acid may be involved in the hypocholesteremic effect of milk. Although, any effect that orotic acid, or milk for that matter, has on biosynthesis of acetyl CoA in vivo in rats is open to question. Conceivably, in the in vivo situation the ubiquitous acetyl CoA could be derived from sources other than acetyl CoA synthetase to maintain cholesterol biosynthesis. However, inhibition of acetyl-CoA synthetase by milk and by orotic acid and the inability of milk to inhibit HMG-CoA reductase, as observed by Bernstein et al. (*2,3*), have ramifications with regard to the experiments by Mann (*24*) wherein radioactive acetate was administered to human subjects. In the biosynthesis of cholesterol from acetate, the acetate would be

TABLE 8. *Effect of raw or pasteurized, homogenized bovine milk on the rate of formation of mevalonate from HMG-CoA by HMG-CoA reductase from rat liver.*[a]

Sample[b]	*Mevalonate* n moles/min
Control (no milk)	9.3
Raw milk	
12μl	9.5
50μl	9.5
Pasteurized, homogenized milk	
12 μl	9.0
50 μl	10.0

[a]From Bernstein et al. (*3*).
[b]Total reaction volume was 0.5 ml.

TABLE 9. *Effect of milk or orotic acid on incorporation of acetate, acetyl coenzyme A, 3-hydroxy-3-methylglutaryl coenzyme A, or mevalonate into cholesterol by rat liver preparations.*[a]

Compound	% Inhibition[b] Milk (50 ul)[c]	% Inhibition[b] Orotic acid (6.7 uM)
[1-^{14}C]acetate	72 ± 10 (12 replicates)	48 ± 18 (13 replicates)
[1-^{14}C]acetyl coenzyme A	8.5 ± 5 (5 replicates)	(−1.5) ± 7 (5 replicates)
3-Hydroxy-3-methyl-[3-^{14}C] glutaryl Co A	3.0 ± 5 (4 replicates)	(−20) ± 19 (4 replicates)
[5-^{3}H]mevalonic acid	1.6 ± 7 (7 replicates)	(−2.3) ± 7 (7 replicates)

[a]From Bernstein et al. (*3*).
[b]Mean ± one standard deviation.
[c]Represents a final concentration of 6.7 μM orotic acid in 3.5 ml reaction volume.

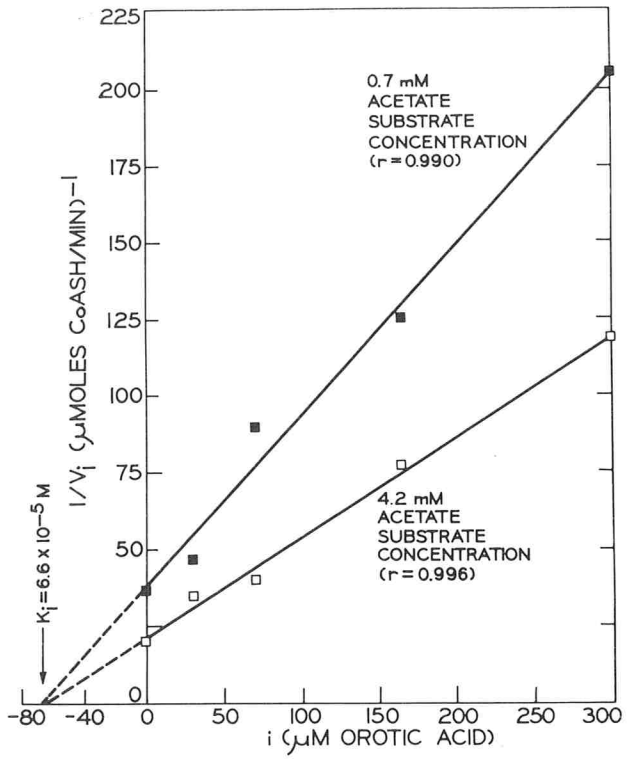

Figure 4. *Graphical determination of the K_i for the inhibition of yeast acetyl-Co A synthetase by orotic acid. From Bernstein et al. (3).*

activated by acetyl-CoA synthetase and a decrease in radioactivity of cholesterol may be observed if this enzyme is inhibited by the MF. Along these lines, it would probably prove instructive to administer radioactive glucose as a source of acetyl-CoA to human volunteers and determine whether MF inhibits incorporation of label into cholesterol. In spite of the aforementioned difficulties, orotic acid also has other effects of lipid metabolism that may also favor it as one of the active components in the hypocholesteremic effect of milk. Therefore a breif discussion of some effects of orotic acid on lipid biochemistry ensues.

OROTIC ACID

Orotic acid has a marked effect on the biochemistry of lipoproteins and on distribution of lipids between the liver and serum in rats. The hypocholesteremic effect of milk may, in part, reflect a maldistribution of lipids induced by the orotic acid content. This distribution may not be all to the good since orotic acid has been known for some time to induce fatty livers in rats fed orotic acid in the diet.

Standerfer and Handler (*39*) first observed the induction of fatty livers in young rats receiving 0.2 to 1.0% orotic acid in the diet. The liver fat almost doubled in those fats receiving 1% orotic acid compared to the control animals. Lipotropic factors such as folic acid, cobalamine, methionine and choline failed to prevent the accumulation of liver fat. Induction of fatty liver by orotic acid was confirmed by Handschumacker and coworkers (*13*).

Okonkwo and Kinsella (*35*) have recently reported development of fatty livers, within 8 days, in rats consuming skim milk powder in the diet to yield a level of 0.15% orotic acid. There are also indications that induction of fatty livers by orotic acid may be under some sort of hormonal control (*12,30*). However, there are also exogenous factors that can affect accumulation of fat in livers as precipitated by orotic acid. For example, Witting (*43*) observed that the levels of hepatic neutral lipids in the orotic acid-fed rat (1.5% in the diet) were inversely related to the dietary levels of polyunsaturated fatty acids, as in the choline-deficient rat. Clofibrate, a hypolipidemic drug, will reverse accumulation of lipid in livers of rats fed 1% orotic in the diet (*31,32*).

As summarized in Table 10, orotic acid in the diet of rats results in lowered serum lipid levels but greater quantities of lipid in the liver. There is a 25-50% reduction in serum cholesterol, a 44-64% reduction in serum triglycerides but a marked increase in the hepatic lipids. The metabolic abnormalities assoicated with orotic acid feeding are not severe and animals have been maintained on such a diet for more than 200 days without major untoward effects (*7*). However, induction of fatty liver seems to be somewhat species dependent. Bloomfield et al. (*4*) and Kruski and Narayan (*22*) did not observe fatty livers in chickens fed 1% orotic acid in the diet. Similar findings have also been reported for the mouse (*9*). It is not known what effect, if any, orotic acid has on the lipids of human liver.

TABLE 10. *Effects of orotic acid 1% in the diet on levels of lipid in liver and in serum of male rats.*[a]

Liver triglyceride mg/100 g	Liver cholesterol mg/100 g	Serum triglyceride mg/100 ml	Serum cholesterol mg/100 ml	References
21,000 (500)[b]	810 (200)[b]	25 (70)[b]	25 (50)[c]	(*31*)
4,430 (840)[d]	500 (250)[d]	20 (53)[d]	61 (81)[d]	(*6*)
4,980 (1,560)[e]	730 (250)[e]	34 (61)[e]	33 (66)[e]	(*45*)

[a]Values in parentheses are those of control animals.
[b]After 22 days on diet.
[c]After 7 days on diet.
[d]After 10 days on diet.
[e]After 14 days on diet.

The mechanism for the abnormal lipid distribution in rats fed orotic acid apparently involves an almost complete inhibition of β-lipoprotein formation in and/or secretion from the liver (*1,36,37,38,41,42*). Windmueller (*40*) observed that plasma lipids, and particularly triglycerides, were depressed in the rat as early as 16 h after orotic acid was introduced in the diet. A minimum plateau was reached after 4 days, at which time the liver rapidly began to accumulate fat (Fig. 5). Table 11 indicates that the low density lipoprotein fraction almost disappeared from the plasma of orotic acid-fed rats. The high density fraction was reduced about 60%. Since the liver is the major source of endogenous plasma triglycerides and since the depressed plasma lipid concentrations precede liver lipid accumulation, the

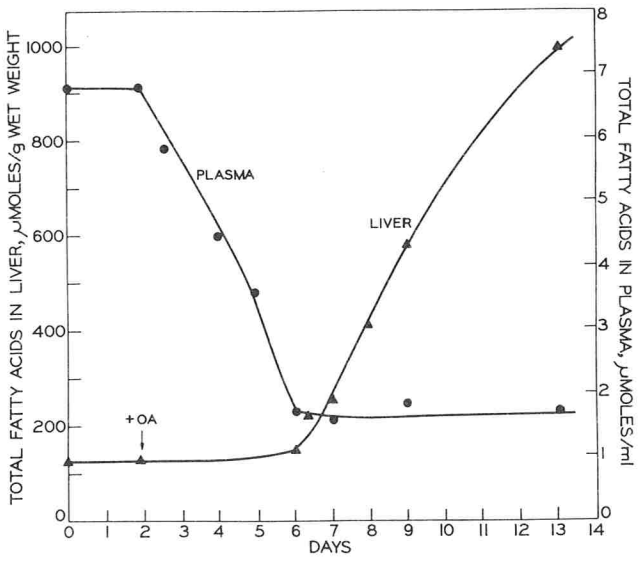

Figure 5. *Effect of dietary orotic acid on total liver and plasma fatty acids of rats. Redrawn from Windmueller (40).*

TABLE 11. *Effect of dietary orotic acid on rat plasma lipoproteins*[a]

Diet	Density < 1.063		Density > 1.63	
	Cholesterol	Phospholipid	Cholesterol	Phospholipid
	(μmoles/ml plasma × 10^2)[b]			
Control (Basal)	47	24	132	121
1% orotic acid in basal diet	5	2	54	52

[a]From Windmueller (*40*).
[b]Each value was obtained by analysis of the pooled plasma of six rats (av. wt. 170 g) fed the respective diets for 9 days. High and low density lipoproteins were obtained by ultracentrifugation for 16 h.

etiology of orotic acid-induced fatty liver was suggested to be related to a defect in the synthesis or secretion of the triglyceride-rich-low density lipoproteins by the liver (*40*). Roheim et al. (*38*) demonstrated that, in general, orotic acid did not inhibit protein biosynthesis. Their work indicated that orotic acid depressed the formation or release of very low density lipoproteins (VLDL) (d<1.019) from the liver suggesting that orotic acid interfered with synthesis of lipoprotein from lipid and apoprotein, or release of lipoprotein from liver. There are apparently no other signs of hepatic functional impairment, no necrosis, normal levels of total plasma proteins and animals treated with orotic acid grow and reproduce normally (*1,7*). However, Kinsella (*20,21*) suggests that orotic acid somehow induces peroxidation of liver lipids which ultimately results in the fatty liver.

Windmueller and Levy (*41*) observed that when rats were fed orotic acid as 1% of their diet, the plasma β-lipoprotein concentration fell within 7 days to less than 1% of normal. It rebounded to normal within 48 h following withdrawal of orotic acid from the diet. Livers, from rats fed orotic acid, perfused in situ did not release any detectable β-lipoprotein. These livers also released smaller amounts of cholesterol and phospholipid than normal livers and no triglyceride. The data suggested that orotic acid affected synthesis or release of hepatic β-lipoprotein (*41*). Pottenger and Getz (*36*) confirmed that rats fed a diet 1% in orotic acid for 7 days developed fatty livers and that there was an apparent inhibition of the secretion from the liver of low density lipoproteins without altering general liver protein synthesis. Fat droplets accumulated in the liver within rough endoplasmic reticulum vesicles. The vesicles were shown to accumulate the apolipoproteins of low and very low density lipoproteins. Subsequently, Pottenger et al. (*37*) presented data to suggest that these apoproteins accumulated in the livers of rats fed orotic acid were deficient in N-acetyl-glucosamine, galactose and sialic acid. They suggested that the missing carbohydrate is a result of the inhibition of lipoprotein secretion rather than its cause. The accumulated apoproteins were regarded as precursors of the very low density lipoproteins apoproteins.

Recently, Novikoff and co-workers (*31,32*) induced fatty livers in rats fed a diet 1% in orotic acid and observed the microscopic morphology of the livers. In livers from orotic acid-fed rats, the endoplasmic reticulum was broken into vesicles, within which lipid accumulated and by the 8th day on the diet the apoprotein B of plasma lipoproteins disappeared. At that time, all morphological signs of transport of lipid via the endoplasmic reticulum and the Golgi apparatus to the space of Disse disappeared. The Golgi elements were flattened and lacked very low density lipoprotein (VLDL) particles. The Golgi-derived secretory vacuoles were not present. Reversal of orotic acid-induced fatty liver resulted in a return of the morphorlogical features of normal lipid transport. These data indicate that transport and secretion of low density lipoproteins by the hepatocytes of orotic acid-fed rats was repressed. In addition to the interference in transport and secretion of lipoproteins in the liver, there are indications that the situation is exacerbated by increased de novo synthesis of triglycerides (*7*).

Although 1% orotic acid in the diet of chickens does not induce a fatty liver (*22*), if 1% cholesterol is added to the diet, orotic acid has a depressing effect on the elevated serum lipoproteins (Table 12). In the chicken, the serum VLDL was almost nonexistent in both the control and orotic acid-fed groups. However, when 1% cholesterol (group C) was fed in the diet, there was a substantial increase in the serum VLDL. When 1% orotic acid was added to the 1% cholesterol diet (group CO), decreases of 68, 64, 64 and 72% were found in the serum VLDL protein, phospholipids, total lipids and total cholesterol, respectively (Table 12). These data indicate that in chickens, orotic acid tends to reverse the hyperlipemic effect of dietary cholesterol.

Furuno et al. (*9*) could not induce fatty livers in mice receiving 0.25-0.75% orotic acid in the diet for 10 to 14 days. In fact, at the 0.75% level of orotic acid in the diet, there was a slight decline in the amount of liver fat. When mice were fed a diet containing 0.5% orotic acid for 2 weeks, growth was retarded, and enlargement of the kidneys was observed.

Kaidin (*18*) found that, when superimposed upon an atherogenic ration, orotic acid did not produce any hypocholesteremia in rabbits. The aforementioned data

TABLE 12. *Composition of serum lipoproteins from chickens fed control, cholesterol and orotic acid plus cholesterol diets for 8 weeks.*[a]

Lipoprotein fraction	Diet	mg/100 ml serum[b]			
		Protein	TL	PL	TC
VLDL[c]	Control	1.0 ± 0.2	5.8 ± 3.1	1.2 ± 05	2.6 ± 0.8
	C	86.6 ± 31.5	258.1 ± 136.9	49.1 ± 32.5	251.8 ± 121.2
	CO	28.0 ± 23.8	92.7 ± 81.5	17.8 ± 16.2	71.1 ± 68.0
LDL[d]	Control	27.1 ± 1.5	36.8 ± 8.6	13.7 ± 4.2	25.6 ± 6.7
	C	59.9 ± 5.2	157.7 ± 47.1	38.3 ± 8.8	152.3 ± 41.3
	CO	21.9 ± 5.5	57.8 ± 18.8	14.6 ± 3.8	41.1 ± 13.4
HDL[e]	Control	202.1 ± 21.3	136.3 ± 30.8	98.3 ± 24.4	65.5 ± 16.4
	C	167.2 ± 21.5	111.5 ± 12.8	41.4 ± 5.4	77.1 ± 13.9
	CO	127.9 ± 13.8	75.7 ± 15.4	39.3 ± 10.5	45.0 ± 7.0

[a]From Kruski and Narayan (22).
[b]Each value is the mean ± S.D. of the respective fraction from 4 chickens.
[c]Very low density lipoproteins.
[d]Low density lipoproteins.
[e]High density lipoproteins.

on rats and chickens indicate that orotic acid reduces the levels of low density serum lipoproteins, apparently interferring with their assembly and secretion by the hepatocytes in the liver. In this regard, Howard and Marks (*17*) observed that milk factor (MF) reduced the content of low density serum lipoproteins in humans consuming milk. It is tempting to speculate that the orotic acid in cow's milk may participate in its hypocholesteremic effects. Although Mann (*24*) postulates that HMG may be the milk factor, it is interesting to note that HMG does not prevent orotic acid-induced fatty liver in rats (*45*).

Perhaps some long-termed feeding studies involving humans receiving 200-400 mg of orotic acid (equivalent to about 2-4 liters of milk) per day in the diet might shed some light on this hypothesis. It should be pointed out that administration of 6 g of orotic acid/day to human patients (in equally divided doses given immediately after each meal for 6-7 days) resulted in a significant decrease in the plasma concentrations of cholesterol, triglycerides and beta and prebeta lipoproteins (*19*). Although the effects of such a massive dose of orotic acid on serum lipids of humans are significant, there may be saturation effects which prevent a proportional reduction in serum lipids above certain levels of orotic acid. Furthermore it might be more realistic to include a lower level of orotic acid mixed with the diet for longer periods (3-4 weeks). In addition, the orotic acid should be in solution to simulate the situation in milk. Also orotic acid is difficultly soluble and, if administered dry, it may not undergo complete dissolution in the gut.

CONCLUSIONS

1. A hypocholesteremic effect is observed in human volunteers consuming about 2 liters of whole milk per day for 2 weeks.
2. The hypocholesteremic effect is greater if skim milk or fermented whole or fermented skim milk is fed at a level of 2-4 liters per day.
3. The factor or factors in milk and yogurt which exert the hypocholesteremic response are, at present, unknown; however, it has been postulated that HMG and/or orotic acid may be involved.

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Walker Elected to Council on Education for Public Health

Bailus Walker, Jr., environmental health scientist, was appointed to the Council on Education for Public Health by the Executive Board of the American Public Health Association (APHA). Dr. Walker's term of membership began on January 1, 1978.

The Council on Education for Public Health is a non-profit corporation which has the responsibility for accrediting academic public health programs, i.e. schools of public health. The Council is established jointly by APHA and the Association of Schools of Public Health.

Walker, who holds a doctorate in environmental health from the University of Minnesota, is currently administrator of the Environmental Health Administration of the Government of the District of Columbia. He is also adjunct professor of environmental medicine and human ecology at Howard University.

Coming Events

April 11-13, 1978. 32nd ANNUAL MEETING OF RESEACH & DEVELOPMENT ASSOCIATES FOR MILITARY FOOD AND PACKAGING SYSTEMS. Drake Hotel, Chicago, IL. Contact: Colonel Merton Singer, R&D Associates, 90 Church St., Room 1315, New York, NY 10007.

April 17-19, 1978. DAIRY AND FOOD INDUSTRIES SUPPLY ASSN., 59th ANNUAL MEETING, Canyon Hotel Racquet & Golf Resort, Palm Springs, CA. Contact: DFISA, 5530 Wisconsin Ave., Suite 1050, Washington, DC 20015

April 26-28, 1978. 53rd ANNUAL MEETING AMERICAN DRY MILK INSTITUTE, 7th ANNUAL MEETING WHEY PRODUCTS INSTITUTE. Jointly at the Chicago Marriott O'Hare, Chicago. Contact Dr. Warren S. Clark, Jr., Executive Director, ADMI & WPI, 130 N. Franklin St., Chicago, IL 60606.

May 1-4, 1978. ADVANCE FOOD SANITATION WORKSHOP. Minneapolis, MN. Contact: Environmental Management Assn., 1701 Drew St., Clearwater, FL 33515.

June 4-7, 1978. 1978 ANNUAL MEETING OF THE INSTITUTE OF FOOD TECHNOLOGISTS, Dallas, Texas. Contact: Dan Weber, Director of Convention Services, IFT, 221 N. LaSalle St., Chicago, IL 60601.

May 7-12, 1978 SCHOOL OF ENVIRONMENTAL SANITATION MANAGEMENT. University of Illinois, Champaign, IL. Contact: Environmental Management Assn., 1701 Drew St., Clearwater, FL 33515.

May 9-11, 1978. 33rd ANNUAL PURDUE INDUSTRIAL WASTE CONFERENCE. Stewart Center, Purdue University, West Lafayette, IN. Contact: J. E. Etzel, Purdue Industrial Waste Conference, Civil Engineering Bldg., Purdue University, West Lafayette, IN 47907.

May 16-18, 1978. 3rd ANNUAL POWDER AND BULK SOLIDS CONFERENCE / EXHIBITION, O'Hare International Trade and Exhibition Center, Chicago. Contact: A. Kozlov, ISCM, Inc., 222 West Adams St., Chicago, IL 60606.

July 17-21, 1978. ADVANCES IN FOOD AND APPLIED MICROBIOLOGY. Massachusetts Institute of Technology, Cambridge, MA. Contact: Director of Summer Session, Rm. E19-356, Massachusetts Institute of Technology, Cambridge, MA 02139.

July 24-28, 1978. FOOD PROCESSORS BASIC MICROBIOLOGY SHORT COURSE. Cruess Hall, University of California, Davis. Contact: Dr. Robert J. Price, Food Science and Tech. Dept., University of California, Davis, CA 95616.

June 25-28, 1978. CANADIAN INSTITUTE OF FOOD SCIENCE AND TECHNOLOGY 21st ANNUAL CONFERENCE, Edmonton, Alberta. Contact: P. Jelen, Dept. of Food Science, University of Alberta, Edmonton, Alta. T6G 2N2.

Study Will Assess Future of United States Allied Health Education

A nation-wide study to assess the future of allied health education in the United States will be conducted by a National Commission on Allied Health Education. A recent $429,200 grant from the W. K. Kellogg Foundation of Battle Creek, Michigan to the American Society of Allied Health Professions (ASAHP) will underwrite the Commission.

The Commission will be composed of members of such national organizations as the American Medical Association, American Council on Education, National League for Nursing, American Hospital Association, allied health groups and the public.

Dr. Frank G. Dickey has been selected as chairman of the Commission. Dr. Dickey has served as Provost at the University of North Carolina, Charlotte, Executive Director of the National Commission on Accrediting, and as President of the University of Kentucky.

Dr. Dickey noted that the concept of allied health began just a decade ago, in response to a review of national health manpower needs by the U.S. Surgeon General. The allied health professions represent the therapists, environmentalists and technicians who assist or support physicians and dentists in delivery of health care.

Since that time, the number of collegiate programs offering allied health education programs has expanded to 3,953 at the basic preparation level. The programs are being offered at 1,428 institutions of higher education in the United States.

"The phenomenal growth of allied health education during the past decade has reflected a direct response by institutions of higher education to the crisis in health care and manpower shortages," Dr. Dickey said. "Such programs have often been developed, however, without a clear mandate of specific need and with insufficient curriculum guidance."

The Commission will be charged with preparing recommendations for guidance of allied health education in the 1980s. Commission exploration will include current educational models in the areas of interdisciplinary programs, curricula, teacher preparation, and career development of allied health professionals.

Dr. Ralph Boatman, ASAHP president and director of Allied Health Sciences at the University of North Carolina at Chapel Hill, said the study could have a profound effect on both the future of allied health education and the delivery of health care nationally.

"We are now in a fortunate position to examine the allied health concept's successes and failures, and to make necessary changes," Dr. Boatman said.

Named director of the study is Dr. Engin Inel Holmstrom, a health manpower education researcher. Dr. Holmstrom is currently a policy associate for the American Council on Education in Washington, D.C.

The American Society of Allied Health Professions, which will provide the secretariat for the Commission, was established in 1967 with Kellogg Foundation assistance. The Society is an educational association composed of the nation's schools and colleges with allied health educational programs. It also includes other national organizations and individuals with interest in the field.

The grant to support the Commission's work follows more than ten years of Kellogg Foundation aid for innovations in the allied health professions. In 1968, the Foundation helped create a national network of eight centers to train allied health educators.

Founded by the breakfast cereal pioneer W. K. Kellogg in 1930, the Foundation is among the five largest private philanthropic organizations in the United States. It supports programs in the areas of health, education and agriculture on four continents, including the United States and Canada, Europe, Latin America, and Australia.

Price-Support Commodities Improve School Lunches

Schools will be able to obtain increased quantities of rice, flour and other grain products and dairy and peanut products from the U.S. Department of Agriculture (USDA) to improve their school lunch programs, according to Assistant Secretary Carol Tucker Foreman.

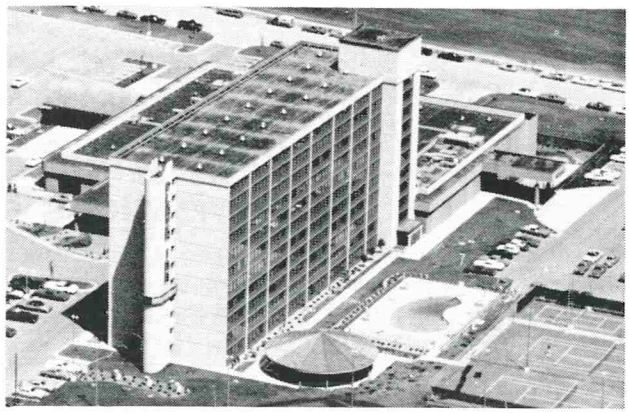
The foods were acquired by USDA under its price-support programs and will be free and over-and-above the 12.75 cents per meal that schools currently receive in cash or commodities under the National School Lunch Act.

"The department's stocks of price-support commodities have increased substantially in recent months, and this gives us an opportunity to make them available to schools so they improve their child nutrition programs. We hope the schools will move quickly to take advantage of this opportunity to use these commodities to serve better meals to children," Ms. Foreman said.

The department will continue its policy, she said, of offering states their fair share of price-support commodities against the mandated per lunch entitlement, based on traditional rates of usage and the commodities available.

Ms. Foreman said the department will continue to assess the effects of additional distribution of price-support commodities. The program will continue while commodities remain in abundant supply, and while additional distribution proves effective and efficient in the operation of both the school lunch and price-support programs.

If you care about professional improvement



Plan to attend the 65th Annual Meeting of IAMFES on August 13-17, 1978 at the

Hilton Airport Plaza, Kansas City, Missouri

For years IAMFES Annual Meetings have offered informative group sessions and discussions in a relaxed and enjoyable atmosphere.

This year promises that much and more. A wide variety of general and technical issues will be presented in a format that lets you plunge right in and tackle current problem issues in your field.

And while your pondering events of the day the evenings offer nothing but relaxation. As in years past, there's the Annual Awards banquet. But this year the Missouri and Kansas Associations have teamed up to present special evening events you, and even your family, won't want to miss.

Also, you'll have a chance to see Kansas City and all it has to offer, from the Kansas City Royals to the finest restaurants in the Midwest.

So make plans now to meet in Kansas City for a professional program and an enjoyable meeting.

1978 ANNUAL MEETING I.A.M.F.E.S.

Advance Registration Form
Kansas City, Missouri

65th Annual Meeting
August 13-17, 1978

MAIL TO: **Mr. Vernon Cupps, Co-Chairman of Registration**
IAMFES
Milk Control Service
Division of Health
City of Saint Louis
P.O. Box 14702
Saint Louis, Missouri 63178

Please Check (If Applicable):

Affiliate Delegate	☐	Executive Board	☐
Past President	☐	Speaker	☐
Executive Board	☐	Host	☐

Make Checks Payable to, *IAMFES - 1978 Meeting Fund*

Advance Register and Save - Refundable if you don't attend

REGULAR REGISTRATION FEE		*Spouse*	ADVANCE REGISTRATION FEE **(If Registered prior to August 1)**		*Spouse*
REGISTRATION	$23.00	$10.00	REGISTRATION	$20.00	$ 8.00
BANQUET	17.00	17.00	BANQUET	15.00	15.00
EVENING ON THE FARM	2.00	2.00	EVENING ON THE FARM	2.00	2.00
TOTAL	$42.00	$29.00	TOTAL	$37.00	$25.00

Name _____ Name _____
Childrens First Names _____
Affiliate or Company _____
Address _____
City _____ State _____ Zip _____

HILTON AIRPORT PLAZA INN
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Kansas City, MO 64195
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Reservations must be received by July 13, 1978.
Reservations will be held until 6:00 p.m.,
unless a later hour is specified
Check out time is 1:00 p.m.

Arrival Date _____ Departure Date _____
Arrival Time _____ Means of Transportation _____
Name _____ Name _____
Address _____
City _____ State _____ Zip _____

Please check type of accommodation required _____ Single (one person) $30 Free Parking.
_____ Double (two persons) $36 Send directly to: Reservation Manager,
Hilton Airport Plaza Inn.

Family Plan: There is no charge for children when in same room with parents.
Limited roll-a-ways available at $8.00 each.

Holders of 3-A Symbol Council Authorizations on February 20, 1978

Questions or statements concerning any of the holders of authorizations listed below, or the equipment fabricated, should be addressed to Earl O. Wright, Sec'y.-Treas., P.O. Box 701, Ames, Iowa 50010.

01-06 Storage Tanks for Milk and Milk Products

28 Cherry-Burrell Corporation (10/ 3/56)
575 E. Mill St.
Little Falls, New York 13365

102 Chester-Jensen Company, Inc. (6/ 6/58)
5th & Tilgham Streets
Chester, Pennsylvania 19013

2 CREPACO, Inc. (5/ 1/56)
100 C.P. Avenue
Lake Mills, Wisconsin 53551

117 Dairy Craft, Inc. (10/28/59)
St. Cloud Industrial Park
St. Cloud, Minnesota 56301

76 Damrow Company (10/31/57)
196 Western Avenue
Fond du Lac, Wisconsin 54935

115 DeLaval Company, Ltd. (9/28 59)
113 Park Street South
Peterborough, Ontario, Canada

109 Girton Manufacturing Company (9/30/58)
State Street
Millville, Pennsylvania 17846

114 C. E. Howard Corporation (9/21/59)
P.O. Box 2507
City of Industry, California 91746

127 Paul Mueller Company (6/29/60)
P.O. Box 828
Springfield, Missouri 65801

31 Walker Stainless Equipment Co. (10/ 4/56)
Elroy, Wisconsin 53929

02-06 Pumps for Milk and Milk Products

214R Ben H. Anderson Manufacturers (5/20/70)
Morrisonville, Wisconsin 53571

212R Babson Bros. Co. (2/20/70)
2100 S. York Rd.
Oak Brook, Illinois 60621

29R Cherry-Burrell Corporation (10/ 3/56)
2400 Sixth St., Southwest
Cedar Rapids, Iowa 52406

63R CREPACO, Inc. (4/29/57)
100 CP Avenue
Lake Mills, Wisconsin 53551

205R Dairy Equipment Company (5/22/69)
1919 South Stoughton Road
Madison, Wisconsin 53716

65R G & H Products, Inc. (5/22/57)
5718 52nd Street
Kenosha, Wisconsin 53140

145R ITT Jabsco, Incorporated (11/20/63)
1485 Dale Way
Costa Mesa, California 92626

26R Ladish Co., Tri-Clover Division (9/29/56)
9201 Wilmot Road
Kenosha, Wisconsin 53140

236 Megator Corporation (5/ 2/72)
125 Gamma Drive
Pittsburgh, Pennsylvania 15238

280 Stamp Corp. (9/10/76)
1021 Hampshire Place
Madison, WI 53711

241 Puriti S. A. (9/12/72)
Alfredo Noble #39, Industrial Pte. de Vigas
Tlalnepantla, Mexico

148 Robbins & Myers, Inc. (4/22/64)
Moyno Pump Division
1345 Lagonda Avenue
Springfield, Ohio 45501

72R L. C. Thomsen & Sons, Inc. (8/15/57)
1303 43rd Street
Kenosha, Wisconsin 53140

219 Tri-Canada Cherry-Burrell Ltd. (2/15/71)
6500 Northwest Drive
Mississauga, Ontario, Canada

175R Universal Milking Machine Div. (10/26/65)
National Cooperatives, Inc.
First Avenue at College
Albert Lea, Minnesota 56007

52R Viking Pump Div. (12/31/56)
Houdaille Industries, Inc.
406 State Street
Cedar Falls, Iowa 50613

5R Waukesha Foundry Company (7/ 6/56)
Waukesha, Wisconsin 53186

282 Knudsen Corporation (11/ 8/76)
715 N. Divisadero Street
Visalia, California 93277

04-03 Homogenizers and High Pressure Pumps of the Plunger Type

247 Bran and Lubbe, Inc. (4/14/73)
2508 Gross Point Road
Evanston, Illinois 60201

87 Cherry-Burrell Company (12/20/57)
2400 Sixth Street, Southwest
Cedar Rapids, Iowa 52404

37 CREPACO, Inc. (10/19/56)
100 CP Avenue
Lake Mills, Wisconsin 53538

75 Gaulin, Inc. (9/26/57)
44 Garden Street
Everett, Massachusetts 02149

237 Graco Inc. (6/ 3/72)
P.O. Box 1441
Minneapolis, Minnesota 55440

256 Hercules, Inc. (1/23/74)
2285 University Avenue
St. Paul, Minnesota 55114

282 Knudsen Corporation (11/ 8/76)
715 N. Divisadero Street
Visalia, California 93277

05-13 Stainless Steel Automotive Milk Transportation Tanks for Bulk Delivery and/or Farm Pick-up Service

131R Almont Welding Works, Inc. (9/ 3/60)
4091 Van Dyke Road
Almont, Michigan 48003

70R Brenner Tank, Inc. (8/ 5/57)
450 Arlington,
Fond du Lac, Wisconsin 54935

40 Butler Manufacturing Co. (10/20/56)
900 Sixth Ave., Southeast
Minneapolis, Minnesota 55114

66 Dairy Equipment Company (5/29/57)
1919 South Stoughton Road
Madison, Wisconsin 53716

45 The Heil Company (10/26/56)
3000 W. Montana Street
Milwaukee, Wisconsin 53235

201 Paul Krohnert Mfg., Ltd. (4/ 1/68)
811 Steeles Avenue
Milton, Ontario, Canada L9T 2Y3

85 Polar Manufacturing Company (12/20/57)
Holdingford, Minnesota 56340

121 Technova Inc. Gosselin Division (12/ 9/59)
1450 Hebert c.p. 758
Drummondville, Quebec, Canada

189 A. & L. Tougas, Ltee (10/ 3/66)
1 Tougas St.
Iberville, Quebec, Canada

47 Trailmobile, Div. of Pullman, Inc. (11/ 2/56)
701 East 16th Avenue
North Kansas City, Missouri 64116

25 Walker Stainless Equipment Co. (9/28/56)
New Lisbon, Wisconsin 53950

08-17 Fittings Used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Milk and Milk Products

291 Accurate Metering Systems, Inc. (6/22/77)
1731 Carmen Drive
Elk Grove Village, IL 60007

79R Alloy Products Corporation (11/23/57)
1045 Perkins Avenue
Waukesha, Wisconsin 53186

138R APV-CREPACO of Canada Limited (12/17/62)
1250 Ormont Dr.
Weston, Ontario, Canada M9L 2V4

245 Babson Brothers Company (2/12/73)
2100 South York Road
Oak Brook, Illinois 60521

284 Bristol Engineering Company (11/18/76)
210 Beaver Street
Yorkville, Illinois 60560

301 Brown Equip. Co., Inc. (12/ 6/77)
9955-9¼ Ave.
Hanford, California 93230

82R Cherry-Burrell Company (12/11/57)
2400 Sixth Street, Southwest
Cedar Rapids, Iowa 52406

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

271 The Foxboro Company (3/ 8/76)
Neponset Street
Foxboro, Massachusetts 02035

67R G & H Products, Inc. (6/10/57)
5718 52nd Street,
Kenosha, Wisconsin 53140

199R Graco, Inc. (12/ 8/67)
P.O. Box 1441
Minneapolis, Minnesota 55440

203R ITT-Grinnell Company, Inc. (11/ 7/68)
DIA-FLO Div
33 Centerville Rd.
Lancaster, Pennsylvania 17603

34R Ladish Co., Tri-Clover Division (10/15/56)
9201 Wilmot Road
Kenosha, Wisconsin 53140

287 Koltek OY (1/14/77)
Kotinummentieiz
SF-00700 Helsinki 70
Finland

239 LUMACO (6/30/72)
Box 688,
Teaneck, New Jersey 07666

200R Paul Mueller Co. (3/ 5/68)
P.O. Box 828
Springfield, Missouri 65801

295 Precision Stainless Products (8/11/77)
5636 Shull St.
Bell Gardens, CA 90201

242 Puriti, S. A. (9/12/72)
Alfredo Nobel #39 Industrial Pte. de Vigas
Tlalnepantla, Mexico

149R Q Controls (5/18/64)
Occidental, California 95465

73R L. C. Thomsen & Sons, Inc. (8/31/57)
1303 43rd Street
Kenosha, Wisconsin 53140

300 Superior Stainless, Inc. (11/22/77)
211 Sugar Creek Rd.
P.O. Box 622
Delavan, Wisconsin 53115

191R Tri-Canada Cherry-Burrell, Ltd. (11/23/66)
6500 Northwest Drive
Mississauga, Ontario, Canda L4V 1K4

278 Valex Products (8/30/76)
9421 Winnetka
Chatsworth, California 91311

86R Waukesha Specialty Company, Inc. (12/20/57)
Darien, Wisconsin 53114

Inlet and Outlet Leak Protector Plug Valves for Batch Pasteurizers

09-07 Instrument Fittings and Connections Used on Milk and Milk Products Equipment

269 Babson Bros. Company (1/23/76)
2100 South York Road
Oak Brook, Illinois 60521

206 The Foxboro Company (8/11/69)
Neponset Avenue
Foxboro, Massachusetts 02035

285 Tank Mate Company (12/ 7/76)
1815 Eleanor
St. Paul, Minnesota 55116

32 Taylor Instrument Process Control (10/ 4/56)
Div. Sybron Corporation
95 Ames Street
Rochester, New York 14601

246 United Electric Controls (3/24/73)
85 School Street
Watertown, Massachusetts 02172

10-00 Milk and Milk Products Filters Using Disposable Filter Media, As Amended

35 Ladish Co., Tri-Clover Division (10/15/56)
9201 Wilmot Road
Kenosha, Wisconsin 53140

11-03 Plate-type Heat Exchangers for Milk and Milk Products

20 A.P.V. Company, Inc. (9/ 4/56)
395 Fillmore Avenue
Tonawanda, New York 14150

30 Cherry-Burrell Corporation (10/ 1/56)
2400 Sixth Street, Southwest
Cedar Rapids, Iowa 52404

14 Chester-Jensen Co., Inc. (8/15/56)
5th & Tilgham Streets
Chester, Pennsylvania 19013

38 CREPACO, Inc. (10/19/56)
100 CP Avenue
Lake Mills, Wisconsin 53551

267 De Danske Mejeriers Maskinfabrik (10/15/75)
The Danish Dairies' Machine Factory
P.O. Box 66, 6000 Kolding, Denmark

120 DeLaval Company, Ltd. (12/ 3/59)
113 Park Street
South Peterborough, Ontario, Canada

279 The Schluter Co. (8/29/76)
112 E. Centerway
Janesville, WI 53545

17 The DeLaval Separator Company (8/30/56)
350 Dutchess Turnpike
Poughkeepsie, New York 12602

15 Kusel Dairy Equipment Company (8/15/56)
100 W. Milwaukee Street
Watertown, Wisconsin 53094

12-04 Internal Return Tubular Heat Exchangers, for Milk and Milk Products

248 Allegheny Bradford Corporation (4/16/73)
P.O. Box 264
Bradford, Pennsylvania 16701

243 Babson Brothers Company (10/31/72)
2100 S. York Road
Oak Brook, Illinois 60521

103 Chester-Jensen Company, Inc. (6/ 6/58)
5th & Tilgham Street
Chester, Pennsylvania 19013

152 The DeLaval Separator Co. (11/18/69)
350 Dutchess Turnpike
Poughkeepsie, New York 12602

217 Girton Manufacturing Co. (1/23/71)
Millville, Pennsylvania 17846

252 Ernest Laffranchi (12/27/73)
P.O. Box 455
Ferndale, California 95536

238 Paul Mueller Company (6/28/72)
P.O. Box 828
Springfield, Missouri 65801

96 C. E. Rogers Company (3/31/64)
P.O. Box 188
Mora, Minnesota 55051

298 Sanitary Processing Equip. Corp. (11/ 3/77)
Butternut Drive
East Syracuse, New York 13213

13-06 Farm Milk Cooling and Holding Tanks

240 Babson Brothers Company (9/ 5/72)
2100 S. York Road
Oak Brook, Illinois 60521

11R CREPACO, Inc. (7/25/56)
100 CP Ave.
Lake Mills, Wisconsin 53551

119R Dairy Craft, Inc. (10/28/59)
St. Cloud Industrial Park
St. Cloud, Minnesota 56301

4R Dairy Equipment Company (6/15/56)
1919 South Stoughton Road
Madison, Wisconsin 53716

92R DeLaval Company, Ltd. (12/27/57)
113 Park Street
South Peterborough, Ontario, Canada

49R The DeLaval Separator Company (12/ 5/56)
Dutchess Turnpike
Poughkeepsie, New York 12602

10R Girton Manufacturing Company (7/25/56)
Millville, Pennsylvania 17846

95R Globe Fabricators, Inc. (3/14/58)
3350 North Gilman Rd.
El Monte, California 91732

179R Heavy Duty Products (Preston), Ltd. (3/ 8/66)
1261 Industrial Road
Preston, Ontario, Canada

12R Paul Mueller Company (7/31/56)
P.O. Box 828
Springfield, Missouri 65801

249 Sunset Equipment Co. (4/16/73)
3765 North Dunlap Street
St. Paul, Minnesota 55112

42R VanVetter, Inc. (10/22/56)
4 South Idaho Street
Seattle, Washington 98134

16R Zero Manufacturing Company (8/27/56)
Washington, Missouri 63090

16-04 Evaporators and Vacuum Pans for Milk and Milk Products

164R Anderson IBEC (4/25/65)
19609 Progress Drive
Strongsville, Ohio 44136

254 Anhydro, Inc. (1/ 7/74)
165 John Dietsch Square
Attleboro Falls, Massachusetts 02763

132R A.P.V. Company, Inc. (10/26/60)
137 Arthur Street
Buffalo, New York 14207

263 C. E. Howard Corporation (12/21/74)
240 N. Orange Avenue
City of Industry, California 91746

107R C. E. Rogers Company (8/ 1/58)
P.O. Box 118
Mora, Minnesota 55051

277 ConTherm Corp. (8/19/76)
P.O. Box 352
Newbury Port, MA 01950

294 DeLaval Separator Co. (7/19/77)
1600 County Rd. F.
Hudson, WI 54016

186R Marriott Walker Corporation (9/ 6/66)
925 East Maple Road
Birmingham, Michigan 48010

273 Niro Atomizer Inc. (5/20/76)
9165 Rumsey Road
Columbia, Maryland 21044

299 Stork-Bowen Engr. Co. (11/16/77)
P.O. Box 898
Somerville, New Jersey 08876

17-04 Fillers and Sealers of Single Service Containers For Milk and Milk Products

192 Cherry-Burrell Corporation (1/ 3/67)
a unit of AMCA International Corp.
2400 Sixth St., Southwest
Cedar Rapids, IA 52404

137 Ex-Cell-O Corporation (10/17/62)
2855 Coolidge,
Troy, Michigan 48084

220 Hercules, Inc., Package Equipment Div. (4/24/71)
2285 University Ave.
St. Paul, Minnesota 55114

281 Purity Packaging Corporation (11/ 8/76)
4190 Fisher Road
Columbus, Ohio 43228

211 Steel & Cohen (Twin-Pak, Inc.) (2/ 4/70)
745 Fifth Avenue
New York, New York 10022

19-02 Batch and Continuous Freezers, For Ice Cream, Ices and Similarly Frozen Dairy Foods, As Amended

286 Alfa-Hoyer (12/ 8/76)
Soren Nymarksvei 13
DK-8270 Hojbjerb, Denmark

146 Cherry-Burrell Company (12/10/63)
2400 Sixth Street, Southwest
Cedar Rapids, Iowa 52404

141 CREPACO, Inc. (4/15/63)
100 CP Avenue
Lake Mills, Wisconsin 53551

22-04 Silo-Type Storage Tanks for Milk and Milk Products

168 Cherry-Burrell Corporation (6/16/65)
575 E. Mill St.
Little Falls, New York 13365

154 CREPACO, Inc. (2/10/65)
100 CP Avenue
Lake Mills, Wisconsin 53551

160 Dairy Craft, Inc. (4/ 5/65)
St. Cloud Industrial Park
St. Cloud, Minnesota 56301

181 Damrow Company, Division of DEC (5/18/66)
International, Inc., 196 Western Ave.
Fond du Lac, Wisconsin 54935

262 DeLaval Company Limited (11/11/74)
113 Park Street
South, Peterborough, Ontario, Canada

156 C. E. Howard Corporation (3/ 9/65)
240 N. Orange Ave., Box 2507
City of Industry, CA 91746

276 Letsch Corporation (8/17/76)
501 N. Belcrest
Springfield, Missouri 65802

155 Paul Mueller Co. (2/10/65)
P.O. Box 828
Springfield, Missouri 65801

165 Walker Stainless Equipment Co. (4/26/65)
Elroy, Wisconsin 53929

23-01 Equipment for Packaging Frozen Desserts, Cottage Cheese and Milk Products Similar to Cottage Cheese in Single Service Containers

174 Anderson Bros. Mfg. Co. (9/28/65)
1303 Samuelson Road
Rockford, Illinois 61109

209 Doboy Packaging Machinery (7/23/69)
Domain Industries, Inc., 869 S. Knowles Ave.
New Richmond, Wisconsin 54017

302 Eskimo Pie Corp. (1/27/78)
530 E. Main St.
Richmond, Virginia 23219

258 Hercules, Inc. (2/ 8/74)
2285 University Ave.
St. Paul, Minnesota 55114

222 Maryland Cup Corporation (11/15/71)
10100 Reisterstown Road
Owings Mills, Maryland 21117

193 Triangle Package Machinery Co. (1/31/67)
6655 West Diversey Ave.
Chicago, Illinois 60635

24-00 Non-Coil Type Batch Pasteurizers

161 Cherry-Burrell Corporation (4/ 5/65)
575 E. Mill St.
Little Falls, New York 13365

158 CREPACO, Inc. (3/24/65)
100 CP Avenue
Lake Mills, Wisconsin 53551

187 Dairy Craft, Inc. (9/26/66)
St. Cloud Industrial Park
St. Cloud, Minnesota 56301

177 Girton Manufacturing Co. (2/18/66)
Millville, Pennsylvania 17846

166 Paul Mueller Co. (4/26/65)
P.O. Box 828
Springfield, Missouri 65601

25-00 Non-Coil Type Batch Processors for Milk and Milk Products

275 Bepex Corporation (7/12/76)
150 Todd Road
Santa Rosa, California 95402

162 Cherry-Burrell Corporation (4/ 5/65)
575 E. Mill St.
Little Falls, New York 13365

159 CREPACO, Inc. (3/24/65)
100 CP Avenue
Lake Mills, Wisconsin 53551

188 Dairy Craft, Inc. (9/26/66)
St. Cloud Industrial Park
St. Cloud, Minnesota 56301

283 Letsch Corporation (11/10/76)
501 N. Belcrest
Springfield, Missouri 65802

167 Paul Mueller Co. (4/26/65)
Box 828
Springfield, Missouri 65801

202 Walker Stainless Equipment Co. (9/24/68)
New Lisbon, Wisconsin 53950

26-00 Sifters for Dry Milk and Dry Milk Products

228 Day Mixing, Div. LeBlond, Inc. (2/28/72)
4932 Beech Street
Cincinnati, Ohio 45202

229 Russell Finex Inc. (3/15/72)
156 W. Sandford Boulevard
Mt. Vernon, New York 10550

173 B. F. Gump Division (9/20/65)
Blaw-Knox Food & Chem. Equip. Inc.
750 E. Ferry St., P.O. Box 1041
Buffalo, NY 14211

185 Rotex, Inc. (8/10/66)
1230 Knowlton St.
Cincinnati, Ohio 45223

176 Koppers Company, Inc. (1/ 4/66)
Metal Products Division
Sprout-Waldron Operation
Munsy, Pennsylvania 17756

172 SWECO, Inc. (9/ 1/65)
6033 E. Bandini Blvd.
Los Angeles, California 90051

28-00 Flow Meters for Milk and Liquid Milk Products

272 Accurate Metering Systems, Inc. (4/ 2/76)
1731 Carmen Drive
Elk Grove Village, Illinois 60007

253 Badger Meter, Inc. (1/ 2/74)
4545 W. Brown Deer Road
Milwaukee, Wisconsin 53223

223 C-E IN-VAL-CO, Division of Combustion (11/15/71)
Engineering, Inc.
P.O. Box 556, 3102 Charles Page Blvd.
Tulsa, Oklahoma 74101

265 Electronic Flo-Meters, Inc. (3/10/75)
P.O. Box 38269
Dallas, Texas 75238

226 Fischer & Porter Company (12/ 9/71)
County Line Road
Warminster, Pennsylvania 18974

224 The Foxboro Company (11/16/71)
Neponset Avenue
Foxboro, Massachusetts 02035

270 Taylor Instrument Process Control (2/ 9/76)
Sybron Corporation, 95 Ames Street
Rochester, New York 14601

29-00 Air Eliminators for Milk and Fluid Milk Products

30-00 Farm Milk Storage Tanks

257 Babson Bros. Co. (2/ 7/74)
2100 S. York Road
Oak Brook, Illinois 60521

31-00 Scraped Surface Heat Exchangers

274 Contherm Corporation (6/25/76)
P.O. Box 352
Newburyport, Massachusetts 01950

290 Crepaco, Inc. (6/15/77)
100 So. CP Ave.
Lake Mills, WI 53551

32-00 Uninsulated Tanks for Milk and Milk Products

264 Cherry-Burrell Company, (1/27/75)
a unit of AMCA International Corp.
575 E. Mill St.
Little Falls, NY 13365

268 Dairy Craft, Inc. (11/21/75)
P.O. Box 1227
St. Cloud, Minnesota 56301

33-00 Polished Metal Tubing for Dairy Products

289 Ladish Co., Tri-Clover Division (1/21/77)
9201 Wilmot Road
Kenosha, Wisconsin 53140

35-00 Continuous Blenders

292 Waukesha Foundry Div. ABEX Corp. (8/24/77)
1300 Lincoln Ave.
Waukesha, WI 53186

36-00 Colloid Mills

293 Waukesha Foundry Div., ABEX Corp. (8/24/77)
1300 Lincoln Ave.
Waukesha, WI 53186

3-A Revises Standards; Sifters, Fillers, Pumps

Revisions of 3-A Sanitary Standards for dry milk sifters and dry milk fillers have been signed by representatives of the 3-A Committees.

First published in 1965 and 1968, respectively, the new documents have been updated technologically to make their use more current and effective. One correction adds a provision amendment for the use of carbon seals on sanitary pumps.

They will be published in the *Journal of Food Protection* in September 1978 and will become effective in January 1979, or one year after signing.

The Standards are designated 3-A Sanitary Standards for Sifters for Dry Milk and Dry Milk Products, No. 26-01, and 3-A Sanitary Standards for Equipment for Packaging Dry Milk and Dry Milk Products, No. 27-01.

Signing the new standards were officials of the Dairy Industry Committee, representing dairy processors; U.S. Public Health Service and International Association of Milk, Food and Environmental Sanitarians; and Dairy and Food Industries Supply Association, representing equipment manufacturers.

The 3-A program safeguards the public health through standards and practices for the cleanability of dairy processing equipment to protect the product against contamination from the equipment itself or foreign elements of dust, dirt or liquids. The program is conducted through the voluntary participation of dairy processors, equipment manufacturers, public health officials and sanitarians and their trade and professional associations. In general, 3-A standards and practices are accepted in most public health jurisdictions at the federal, state and local level. They are cited in the recommended Grade "A" Pasteurized Milk Ordinance of the U.S. Public Health Service.

Environmental Sources Directory Revised

The Second Edition of the United States Directory of Environmental Sources is now available, according to the United States Environmental Protection Agency.

The cost of the directory is $21.50 for hard copy and $3.00 for microfiche copy. Copies may be ordered from: National Technical Information Service, U.S. Dept. of Commerce, 5285 Port Royal Rd., Springfield, VA 22161. The order number is PB 274-110/AS.

The directory includes 1,114 sources registered with the United States International Environmental Referral Center as of July 1, 1977. The complete document is 749 pages.

CLASSIFIED AD

For Sale

Single Service milk sample tubes. For further information and a catalogue please write, Dairy Technology, Inc., P.O. Box 101, Eugene, Oregon 97401.

Index to Advertisers

Minnesota Sanitarians Assn.

THURSDAY, APRIL 13, 1978-ALEXANDRIA, MINNESOTA

5:00 P.M.-Social Hour-Holiday Inn
6:00 P.M.-Dinner-Holiday Inn

TUESDAY, APRIL 18, 1978-ALBERT LEA, MINNESOTA

4:00 P.M.-Universal Milking Machine Company Plant Tour
5:30 P.M.-Social Hour-Universal Milking Machine Company
7:00 P.M.-Dinner-Skyline Supper Club

Position Available
Leprino Cheese Company

The Leprino Cheese Co., Denver, Colorado, solicits applications from candidates for a position in its Research and Development Laboratory. Applicants should hold an advanced degree in Dairy or Food Technology, or equivalent, with definite strength in chemistry or process engineering. Commercial experience in some combination of dairy or food processing, cheesemaking, use of processing equipment, food product development, whey utilization and/or whey processing is required. Demonstration of some significant contribution to industry in any of these fields will be requested. The successful applicant will be expected to assist in direction of food research, participate in pilot plant activities, and work effectively in interdisciplinary manufacturing plant research and production situations. Salary is negotiable. Fringe benefits are outstanding. Applicants should submit resume, publication list if applicable, references, and other pertinent information to George W. Reinbold, Leprino Cheese Co., 1830 West 38th Avenue, Denver, Colorado, 80211.

Position Available
Kansas City Health Dept.

The Kansas City Health Department, Environmental Health Services Division is seeking a professional food specialist with management experience to head the food inspection program for this city. This position offers a challenge to a person with the ability to innovate and implement an effective food sanitation program. It offers a unique opportunity to work with a young progressive management team of health professionals devoted to the expansion of quality services for the community.

This is a Sanitarian III position requiring a degree in public health, chemistry, bacteriology, sanitary sciences or related field, with graduate work in any of the foregoing, and four years of progressive responsibility in public health environmental sanitation.

Excellent employee benefits include promotional opportunities, paid vacation, holidays, sick leave, employee hospitalization plan, major medical and life insurance; City retirement program and Credit Union. Salary range-$14,448 to $20,532. Starting salary commensurate with experience. An equal opportunity employer.

Qualified professionals are asked to reply prior to April 14, 1978.

Send resume to: Mr. Earl Unell
Personnel Department
City Hall, 12th Floor
Kansas City, Missouri 64106

THE ONLY *Approved* SANITARY METHOD OF APPLYING A U. S. P. LUBRICANT TO DAIRY & FOOD PROCESSING EQUIPMENT

Haynes Spray

U. S. P. LIQUID PETROLATUM SPRAY

U.S.P. UNITED STATES PHARMACEUTICAL STANDARDS

CONTAINS NO ANIMAL OR VEGETABLE FATS. ABSOLUTELY NEUTRAL. WILL NOT TURN RANCID—CONTAMINATE OR TAINT WHEN IN CONTACT WITH FOOD PRODUCTS.

SANITARY—PURE

ODORLESS—TASTELESS

NON-TOXIC

This Fine Mist-like **HAYNES-SPRAY** *should be used to lubricate:*

SANITARY VALVES
HOMOGENIZER PISTONS — RINGS
SANITARY SEALS & PARTS
CAPPER SLIDES & PARTS
POSITIVE PUMP PARTS
GLASS & PAPER FILLING MACHINE PARTS
and for ALL OTHER SANITARY MACHINE PARTS which are cleaned daily.

The Modern **HAYNES-SPRAY** *Method of Lubrication Conforms with the Milk Ordinance and Code Recommended by the U. S. Public Health Service*

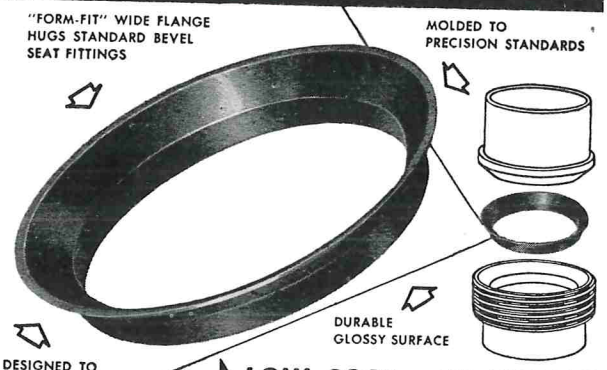
The Haynes-Spray eliminates the danger of contamination which is possible by old fashioned lubricating methods. Spreading lubricants by the use of the finger method may entirely destroy previous bactericidal treatment of equipment.

PACKED 6-12 oz. CANS PER CARTON
SHIPPING WEIGHT—7 LBS.

THE HAYNES MANUFACTURING CO.
4180 Lorain Ave. • Cleveland, Ohio 44113

HAYNES-SPRAY INGREDIENTS ARE APPROVED ADDITIVES AND CAN BE SAFELY USED AS A LUBRICANT FOR FOOD PROCESSING EQUIPMENT WHEN USED IN COMPLIANCE WITH EXISTING FOOD ADDITIVES REGULATIONS.

HAYNES SNAP-TITE GASKETS



LOW COST...RE-USABLE

LEAK-PREVENTING

NEOPRENE GASKET for Sanitary Fittings

Check these SNAP-TITE *Advantages*

- Tight joints, no leaks, no shrinkage
- Sanitary, unaffected by heat or fats
- Non-porous, no seams or crevices
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- Withstand sterilization
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- Eliminate line blocks
- Help overcome line vibrations
- Long life, use over and over

Available for 1", 1½", 2", 2½" and 3" fittings.
Packed 100 to the box. Order through your dairy supply house.

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Haynes SELF-CENTERING SNAP-TITE® Gaskets

SIZES 1" - 1½" 2" - 2½" - 3" - 4"

*MADE FROM **TEFLON®**

"The Sophisticated Gasket"

THE IDEAL UNION SEAL FOR BOTH VACUUM AND PRESSURE LINES

Gasket Color . . . slightly off-white

SNAP-TITE self-centering gaskets of TEFLON are designed for all standard bevel seat sanitary fittings. They SNAP into place providing self-alignment and ease of assembly and disassembly.
HAYNES SNAP-TITES of TEFLON are unaffected by cleaning solutions, steam and solvents. They will not embrittle at temperatures as low as minus 200° F. and are impervious to heat up to 500° F.

FOR A FITTING GASKET THAT WILL OUT-PERFORM ALL OTHERS...

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- *TEFLON ACCEPTED SAFE FOR USE ON FOOD & PROCESSING EQUIPMENT BY U. S. FOOD AND DRUG ADMINISTRATION*

*Gaskets made of DuPont TEFLON® TFE-FLUOROCARBON RESINS

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Available in both

SPRAY AND TUBE

All Lubri-Film ingredients are approved additives and can be safely utilized as a lubricant for food processing equipment when used in compliance with existing food additive regulations.

ESPECIALLY DEVELOPED FOR LUBRICATION OF FOOD PROCESSING AND PACKAGING EQUIPMENT

For Use in Dairies — Ice Cream Plants — Breweries — Beverage Plants — Bakeries — Canneries — Packing Plants

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SPRAY — PACKED 6 — 16 OZ. CANS PER CARTON
TUBES — PACKED 12 — 4 OZ. TUBES PER CARTON

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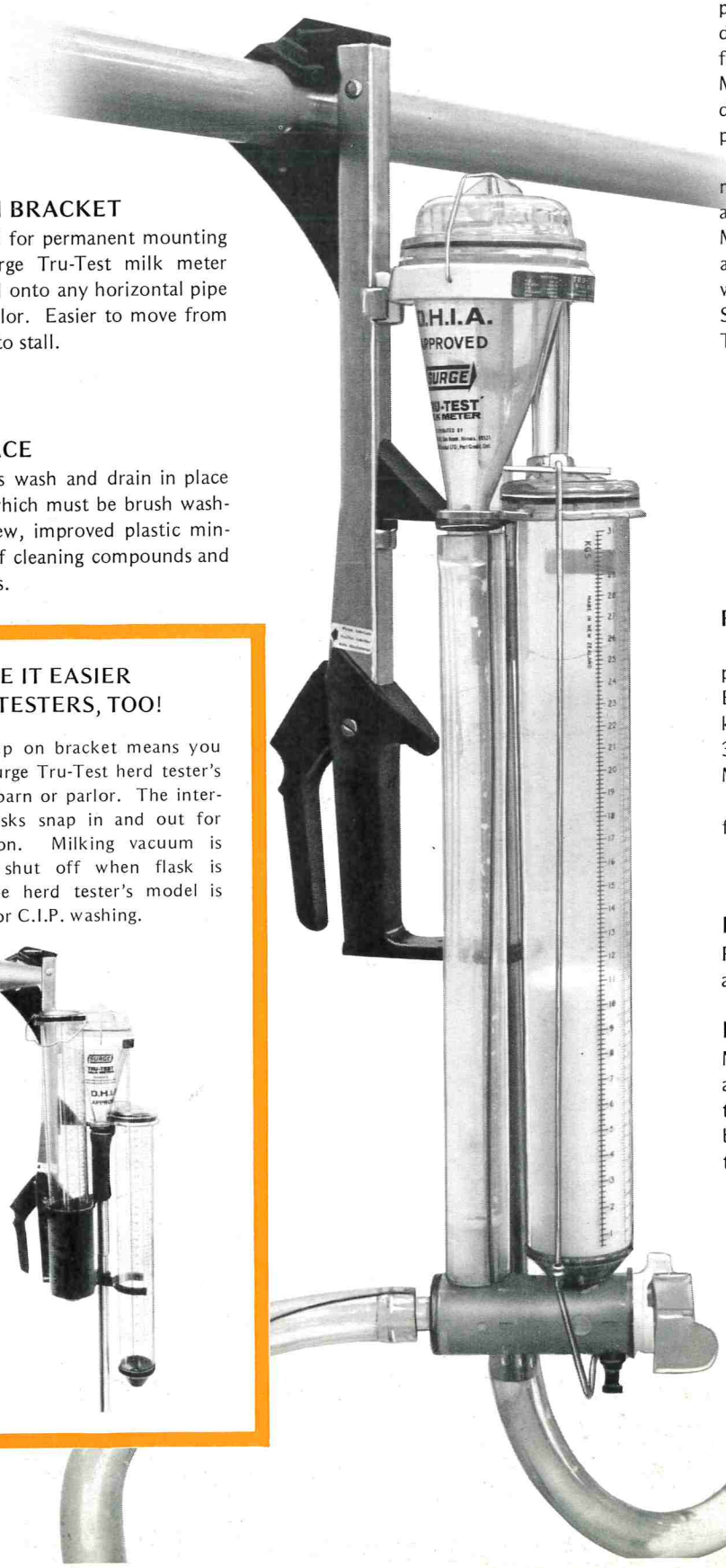
CLEVELAND, OHIO 44113

THE SURGE TRU-TEST MILK METER makes it easier to know how your cows are doing.

Many dairymen want to make routine production checks on their cows. A sudden drop in production can indicate a health or feed problem. The Surge Tru-Test Milk Meter makes it easy to get accurate, individual production readings - with either parlor or stanchion pipelines.

Whether you are on a D.H.I.A. or R.O.P. record program, or do your own sampling and recording - The Surge Tru-Test Milk Meter provides a simple way to obtain accurate production information, where you want it, and when you want it. Ask your Surge dealer to demonstrate the Surge Tru-Test Milk Meter on your dairy.



D.H.I.A. and R.O.P. APPROVED

NEW CLAMP-ON BRACKET

Eliminates the need for permanent mounting brackets. The Surge Tru-Test milk meter can be easily locked onto any horizontal pipe in your barn or parlor. Easier to move from cow to cow or stall to stall.

CLEANS-IN-PLACE

All milk surfaces wash and drain in place (except for valve, which must be brush washed separately). New, improved plastic minimizes the effects of cleaning compounds and temperature changes.

WE'VE MADE IT EASIER FOR HERD TESTERS, TOO!

The new clamp on bracket means you can use the Surge Tru-Test herd tester's model in any barn or parlor. The interchangeable flasks snap in and out for easier operation. Milking vacuum is automatically shut off when flask is removed. The herd tester's model is not designed for C.I.P. washing.

PRECISE MEASUREMENT

Metering area has no moving parts, for simplicity of operation and maximum accuracy. Easy-to-read calibrations in both pounds and kilograms, with quarter lb. increments up to 30 lbs. and kilograms in 1/5 increments. Meter capacity is 70 lbs. or 31 kgms.

The Surge Tru-Test milk meter is approved for D.H.I.A. and R.O.P. testing.

IMPROVED VALVE

Positive seal in all positions, yet easier to turn and select milk, wash, stir or sample positions.

RAPID, ONE HAND SAMPLING

Milk is agitated in the metering flask for accurate butterfat samples. Just hold the container against the sampling port and an air bleed at the top of the meter opens to break the vacuum.

We make your cows worth more.

SURGE

Sustaining Member