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Journal of Milk and Food Technology

CALL FOR RESEARCH PAPERS FOR 1976 IAMFES MEETING

Contributed research papers will be an important part of the program at the 1976 Annual Meeting of IAMFES scheduled for August 8-11 at the Arlington Park Hilton, Arlington Heights, Illinois. Abstract forms and complete information about presenting papers can be found in this issue.

INTERNATIONAL ASSOCIATION MILK, FOOD & ENVIRONMENTAL SANITARIANS

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July 4th, 1975

Remember INDEPENDENCE DAY in 1776!

At Lexington and Concord, someone asked the British General, "who fired the first shot"? He said, "what does it matter, now"? **The AMERCIAN REVOLUTION was off and running** towards individual "independence and freedom" for our country!

DECLARATION of INDEPENDENCE, CONSTITUTIONAL REPUBLIC and BILL of RIGHTS for "we the people" followed, whose lawful freedoms are threatened more than ever, today, by "industrial voluntary standards" depletion of American's remaining assets, and ugly "rip offs" of individual's rights by "uniformity and conformity" none laws.

With no **Thomas Paine and his 89 page "common sense" to hot foot National and Individual lawful freedom; we as an independent Manufacturer** of COMMERCIAL REFRIGERATORS, patented Easy Swing DOORS and ECONO-COVER night cover invention of "open supermarket freezer and refrigerator cases", with a patented new building facility "fireproofed" and upgraded item and new concepts in "display refrigeration" technology coming, many tell us that our, sometimes satirical, ELIASON "Lawful Competitive Free Enterprise" NEWSLETTER could have had much to do with our direct communications and survival.

Many NAS, IAMFES, NEHA and STATE public health sanitarians have urged us since we began EXHIBITING at annual NEHA conferences, some STATE seminars and advertising in the NEHA Journal, to develop the **"MANUFACTURERS warranty/guarantee of Sanitary, Safety and Performance SPECIFICATIONS Plan"** that we published and copyrighted for FOOD RETAILERS and PUBLIC HEALTH SANITARIANS to use **for MANUFACTURERS who would make a commitment, that their FOOD, SANITATION, HEALTH and SAFETY related products would fulfill the LAW, as well as valid PUBLIC HEALTH requirements** made available through competitive upgrading.

From June 27 through July 2, we enjoyed the direct communication with delegates to the NEHA Annual Conference, in Minneapolis, Our EXHIBIT BOOTHS 1 and 2, were busily engaged in discussions on TRUTH IN SANITATION, SANITARY SPECS and VISIBLE CLEANLINESS that we advertise in the NEHA and other trade journals. Uncensored and open dialogue on all aspects of the scene, with much input towards the "viable warranty/guarantee plan" developments, were enjoyed in the Radisson Hotel's ELIASON Parlor Suite 1174.

On August 10 – 13, we hope to meet more delegates to the IAMFES "international" Annual Conference, at meetings and the ELIASON Parlor Suite "open forum", at the Royal York Hotel, in Toronto, Ontario, Canada. We also plan to bring our messages and learn a lot from IAMFES members, through ads in the IAMFES Milk Journal, whose two articles on "sanitarian's professional accountability and the common sense of sanitation performance requirements" were timely and appropriate in this frequently anticompetitive world.

Though we've EXHIBITED at industry trade shows since 1963 and at annual Sanitary Environmental national and some state conferences since 1969, **with** 20 years background in FOOD STORES, WAREHOUSES, RESTAURANTS and FARM MILK COOLING innovative upgrading, it is now time for more regional facility, product and services expansion, based on our belief that "best for the purpose high quality, long life modern designs, performance and safety in nutritive perishable foods" **can move ahead sensibly by better direct communications** between individual industry firms, users of equipment and products and municipal, county and state public health.

Yes, we are honorably commercial, but "armed with the lawful prerogatives provided by our pioneer forefathers", may we all make it with FREEDOM, way beyond next May Day and the 4th of July 1776 celebration, in our American republics, where "viable longevity is our heritage difference" between just LUCK and our TALENTS!

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Carl Eliason

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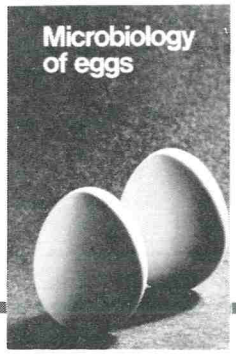

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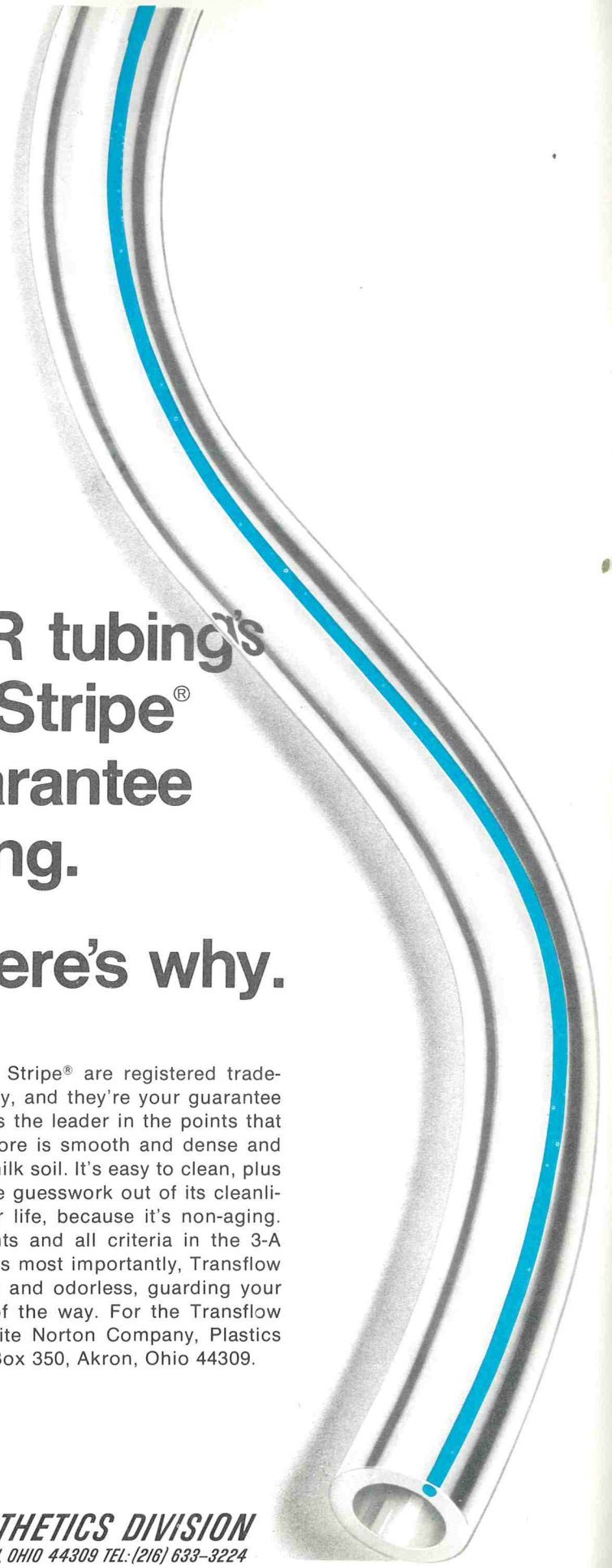

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J Milk Food Technol. Vol. 39, No. 1, Pages 4-6 (January, 1976)

Microbiology of Israeli Pickled Cheese

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(Received for publication January 2, 1975)

ABSTRACT

Bacteriological and chemical parameters of ripe Israeli pickled cheese were highly correlated in cheese and pickle (brine). Lactobacilli were found in large numbers in commercial cheese. Sodium chloride concentration appeared to control the total viable population, the lactobacilli, and the amount of lactic acid produced. Coliform bacteria and enterococci were unaffected by the sodium chloride concentration found, but were affected by lactic acid. Numerical relationships between the above findings are given.

Pickled cheese is a popular dietary article in the Balkan and the Near East countries. It is a unique cheese since it is ripened and distributed in a strong sodium chloride brine (about 10% NaCl). This cheese has a long shelf life without refrigeration which makes it a potential dairy product in developing countries.

Certain aspects of the microbiology of pickled cheese have been reported (*19*). Lactobacilli have been shown to play a role in the ripening of the cheese (*7, 9, 18*), and in a similar cheese enterococci were found to be the prime agent for ripening and maturation (*3*). The effect of coliform bacteria on the organoleptic quality and on shelf life (*1, 2, 12, 18*) has drawn some attention. Yeasts (*14*), corynebacteria (*15*), clostridia (*18*), and staphylococci (*10, 16*) have also been found in this cheese.

The present work attempts to identify some of the factors related to the stability of this cheese.

MATERIALS AND METHODS

Pickled cheese in its own brine was procured from commercial vendors, transferred to the laboratory, and examined immediately. The cheese was from several commercial dairies and was prepared from pasteurized milk using a *Streptococcus lactis* starter culture. Production of pickled cheese has been previously described in detail (*13, 17*). It involves curdling the milk with rennet and starter, draining, ladling the curd into frames equipped with cheese cloth, draining, pressing, cutting into blocks, salting, and ripening in brine.

Cheese samples were purchased from several commercial sources and 10-g samples were prepared for bacteriological examination by blending in a mechanical blender for 2 min. Blending and subsequent dilutions were carried out with 0.1% peptone water.

Plating and sample treatment were otherwise as described in *Standard Methods for the Examination of Dairy Products* (*4*). Bacteriological procedures employed are summarized in Table 1. The pH was determined by introduction of a pH electrode into the sample. Acidity (as percent of lactic acid) was determined by titration with sodium hydroxide. Chloride in the cheese was determined by titration with silver nitrate after ashing, chloride in the pickle (brine) was determined by direct titration. Dry matter was determined by drying to constant weight at 110 C. Organic acids were identified by paper chromatography (*5*). Volatile fatty acids were determined by gas chromatography on 20% Carbowax 20M (Applied Science Inc. Pa.).

Statistical treatment of the data was carried out as described by Snedecor and Cochran (*24*), on 40 cheese samples.

TABLE 1. *Bacteriological procedures employed*

Determination	Media	Plating technique	Incubation		Reference
			Temp.	Time	
Total aerobes	Plate count agar + 2% NaCl (PCAS)	Pour plate	30 C	3 days	*4*[a]
Total aerobes	Elliker agar + 1.9% NaCl (ES)	Pour plate	30 C	3 days	*11*
Lactobacilli	Acetate-Oleate agar (LBA)	Pour plate	30 C	3 days	*22*
Enterococci	Acid-TTC agar (SB)	Pour plate	37 C	48 h	*23*
Coliforms	Violet Red Bile agar (VRB)	Pour plate with overlay	37 C	24 h	*4*[b]

[a]Modified by addition of 2% NaCl.
[b]Modified by incubation at 37 C.

TABLE 2. *Chemical relationships between ripe pickled cheese and its brine*

	pH		Acidity (%)		Dry matter (%)		Sodium chloride (%)	
	Cheese	Brine	Cheese	Brine	Cheese	Brine	Cheese	Brine
Mean counts/g	4.36	4.24	1.32	1.85	43.9	21.3[a]	3.9	9.8[a]
S.D.	0.5	0.5	0.60	1.03	4.9	6.4	0.7	3.6
Correlation coefficient		0.89[b]		0.49[b]		0.31		0.49[b]
Regression coefficient[c]		1.10		0.28		—		0.10

[a]Differs significantly ($P < 0.01$) from the cheese.
[b]$P < 0.01$.
[c]Between cheese (Y) and Brine (X).

TABLE 3. *Bacteriological relationships between ripe pickled cheese and its brine*

	Microbiological medium									
	PCAS		ES		LBA		SB		VRB	
	Cheese	Brine	Cheese	Brine	Cheese	Brine	Cheese	Brine	Cheese	Brine
Mean counts/g	5.5×10^7	7.2×10^7[a]	6.0×10^7	7.0×10^7	9.7×10^6	1.3×10^7	5.6×10^5	4.7×10^4[a]	36	72
S.D.	0.8×10^7	0.6×10^7	0.8×10^7	0.6×10^7	0.8×10^6	0.8×10^7	1.3×10^5	0.9×10^4	69	200
Correlation coefficient	0.79[b]		0.73[b]		0.77[b]		0.63[b]		0.89[b]	
Regression coefficient[c]	1.10		1.07		0.79		0.91		1.06	

[a]Differs significantly ($P < 0.01$) from the cheese.
[b]$P < 0.01$.
[c]Between log bacterial counts in the cheese (Y) and log bacterial counts in the brine (X).

RESULTS

The chemical composition of the cheese and its brine together with correlation and regression coefficients are shown in Table 2. Dry matter and pH were lower, but acidity and sodium chloride concentration were higher in the brine than in the cheese. Acidity, pH, and sodium chloride concentrations correlated significantly in cheese and brine.

Bacterial counts of the cheese and the brine are shown in Table 3. Counts on all media were correlated in the cheese and brine. Counts on Plate Count agar plus 2% NaCl (PCAS) were higher and enterococci counts lower in brine than in cheese. Counts obtained on PCAS were correlated with counts on Elliker's agar plus 1.9% NaCl (ES) ($r = 0.94$) and with counts on acetate-oleate agar (LBA) ($r = 0.92$) both in cheese and brine. Counts on violet red bile (VRB) agar and on acid-TTC agar (SB) were highly correlated in both cheese and brine.

The relationship between chloride concentration and pH in the brine is shown in Table 4. Chloride concentration was also correlated with acidity ($r = 0.54$, $P<0.05$ not shown in Table 4). The relationship between sodium chloride concentration, pH, and total viable counts in PCAS and LBA are also shown in Table 4. Sodium chloride concentration was negatively correlated with log counts obtained on PCAS, ES, and LBA. No correlation was found between pH (or acidity, not shown) and the PCAS, ES, or LBA counts. However, coliform counts (VRB) and enterococcus counts (SB) were well correlated with the pH (and acidity) but not with sodium chloride concentration. Good correlations between ES and PCAS were always found, thus data for ES are not shown in the table.

TABLE 4. *Relation between NaCl and pH and bacterial counts in the brine*

Y	X	Correlation coefficient	Linear regression equation Y =
NaCl	pH	0.47[a]	$2.68 \times - 0.58$
NaCl	log PCAS	0.45[b]	$-0.78 \log \times + 8.34$
NaCl	log LBA	0.42[b]	$-0.51 \log \times + 8.99$
NaCl	log VRB	0.15	—
NaCl	log SB	0.11	—
pH	log PCAS	0.10	—
pH	log LBA	0.17	—
pH	log VRB	0.76[a]	$0.19 \log \times + 4.10$
pH	log SB	0.56[a]	$0.18 \log \times + 3.49$

[a]$P < 0.01$.
[b]$P < 0.05$.

Volatile fatty acids analysis showed only the presence of acetic acid and traces of propionic acid. Lactic acid was the only non-volatile acid detected.

DISCUSSION

In this work the chemistry and the bacteriology of commercial pickled cheese was studied and the interrelationships were examined. Even though this cheese is produced in numerous large and small dairies, and distributed under a wide range of climatic conditions, the ecology of the cheese was well defined. The brine and cheese were closely correlated in all parameters other than dry matter. Lack of correlation of the dry matter content may have resulted because cheese particles of various sizes were found in the brine. Viable bacteria were always slightly more numerous in the brine than in the cheese, and the ratio of colony forming units (plate count) to gross dry matter (3.4×10^6 in brine, 1.3×10^6 in cheese) also indicates intense biological activity in the brine. This is parallel to findings concerning the biological activities during the pickling of fish and meat (*6*). Fermentation in the brine and its contribution to the final character of the product has been little investigated previously in pickled cheese. The close correlation between almost all parameters in brine and cheese together with the indication that microbiological activity was intense in the brine led to concentration of the subsequent investigation on the brine.

Although the starter culture initially added contained predominantly *S. lactis,* lactobacilli formed a significant part of the climax stage population in market cheese (and its brine). The prime role of the lactic acid bacteria in production of this cheese is also demonstrated by the fermentation acids found. Neither the origin of the lactobacilli nor the point at which they begin to play a significant part in the fermentation are known at present. Possibly, the source may be in the dairy plant environment as has been shown in Cheddar cheese (*21*).

The correlation between the chloride ion concentration and the main bacterial population, with no correlation of this population with acidity or pH, indicates that at this steady state of the fermentation the sodium chloride concentration was the major controlling factor in this system. Chloride ion concentration in the brine was also

correlated both with the pH and the titratable acidity. Acidity was predominantly due to the lactic acid, with small amounts of acetic acid appearing concomitantly. Extrapolation of the regression equation between chloride concentration and acidity gives theoretical maximal values for acidity and chloride concentrations. For acidity, this is at about 2.5% lactic acid which corresponds closely with established final, self limiting level of acidity in other dairy products (*8, 25*). The maximal chloride concentration (28%) also corresponds with other data concerning biological activity in high salt environments (*1*).

The size of two secondary populations examined, the coliform bacteria and the enterococci, was correlated with the pH (or acidity), but not with the sodium chloride concentration. From regression equations it would appear that the limiting pH would be about 4.1 and 3.5 for growth of coliform bacteria and enterococci, respectively, and under conditions examined the sodium chloride had no demonstrable effect. The ability of both coliform bacteria and enterococci to withstand high sodium chloride levels has been previously demonstrated in meat pickling brines (*6, 20*) and in hard cheese (*3*). Due to changes in counts of these widely used indicators of poor sanitation, their use for this purpose is only valid for the fresh product, unless complete chemical and historical data of the product are well known and standardized.

The ecology of pickled cheese and its brine may be tentatively summarized as follows: Chloride controls the size of the main bacterial population and hence the metabolic product, lactic acid. Lactic acid controls the coliform and enterococcus populations which are resistant to the sodium chloride levels present. The study of this particular cheese demonstrates a well controlled ecological system with numerically definable chemical and biological relationships.

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Bacteriological Survey of Frankfurters Produced at Establishments Under Federal Inspection

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(Received for publication June 20, 1975)

ABSTRACT

At the time of manufacture, 78% of 69 sets of packaged frankfurters collected from 40 firms had aerobic plate counts of 10,000 or fewer/g and 90% had 20,000 or fewer/g (geometric means of 10 units/set), 91% were coliform-negative, and 93% were *Staphylococcus aureus*-negative. Only one of 690 packaged frankfurter units was *Escherichia coli*-positive, and all units were salmonellae-negative.

Since 1967, the Meat and Poultry Inspection Program of the U.S. Department of Agriculture has been conducting a series of bacteriological surveys to determine the bacterial levels of food products during preparation and as packaged for shipment from firms located in all regions of the nation. Because of ready access to federally inspected firms, extensive information of this nature can be gathered only by authorized personnel of the U.S.D.A.

The surveys are being conducted by personnel of the Microbiology Staff who visit firms to examine food processing equipment before use, to observe practices of personal hygiene, and to collect samples for analyses.

To date, surveys of eviscerated chickens, fresh pork sausage, frozen meat and gravy products, and raw beef patties have been completed, and the results have been published (*6-9*). This report presents the results of a survey of firms producing frankfurters.

To manufacture frankfurters, various types and species of meat trimmings are ground and then chopped in a cutter with water, spices, seasonings, curing ingredients, and in a few instances, milk solids or soy protein. Most sausage makers next pass the chopped mixture through an emulsion mill; some chop the mixture to an emulsion by means of the cutter. The emulsion is extruded into cellulose casings which are linked mechanically. The linked sausage is hung on sticks, the sticks are placed on racks, and the racks are taken to a smokehouse for cooking. The lowest internal cooking temperature observed during this survey was 66 C and the highest was 82 C; most processors cooked the frankfurters to an internal temperature of 69 to 71 C. The cooked frankfurters are cooled to an internal temperature of 4 C or below, peeled mechanically, and packaged. A few firms used a continuous processing system wherein strands of linked frankfurters are transported automatically through chambers for smoking-cooking and chilling, emerging ready for peeling and packaging. Detailed descriptions of frankfurter manufacturing and the processing equipment have been compiled by MacKensie (*4*) and Kramlich, et al. (*3*).

[1]*U.S.D.A., Eastern Laboratory, Athens, Georgia*

MATERIALS AND METHODS

Sampling

From February 1972 to June 1973, samples were collected in 40 federally inspected establishments producing frankfurters. Eight of the firms were located in the Northeast, nine in the South and Southeast, 17 in the West and Midwest, and six on the West Coast.

A total of 1,163 production line samples and 690 retail-size packages (units) were collected and analyzed. A group of samples from each plant included samples of the raw meats used for the frankfurters; samples at each stage of processing; samples of spices and extenders; a set of 10 units of packaged frankfurters related to the production line samples, and from 29 plants, a set of 10 units of packaged frankfurters produced 1 to 3 days before the date of the plant visit. Samples were frozen promptly and were shipped under dry ice to the laboratory. Generally, analysis was begun 3 to 4 weeks after collection.

Laboratory methods

Methods used for aerobic plate counts (APC), coliforms, *Escherichia coli, Staphylococcus aureus,* and salmonellae have been described (*11*). For more representative sampling, the 50-g portion from each packaged frankfurter unit was taken aseptically from cross-section portions of at least four frankfurters.

RESULTS AND DISCUSSION

Figure 1 presents the APC's of 69 sets of packaged frankfurter units (10 units/set) and of the 690 individual units. Most of the individual units (86%) had APC's of 20,000 or fewer/g. Accordingly, both the arithmetic averages and geometric means of the APC's of most of the sets (84% and 90%) were 20,000 or fewer/g.

Spices (paprika, pepper, ginger) collected from 2 firms had high APC's ranging from 4×10^6 to 1×10^7/g, and packaged spice-seasoning mixes from 5 firms had high counts ranging from 5×10^5 to 5×10^6/g. The counts of these spices and spice-seasoning mixes were the same

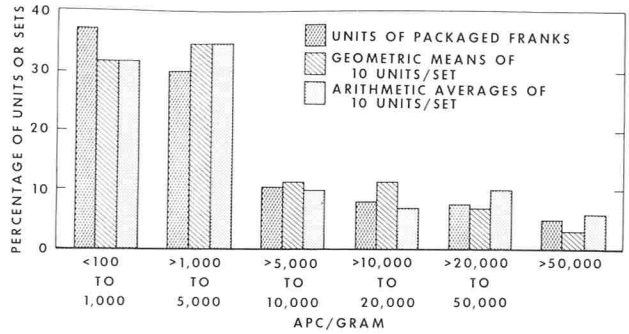

Figure 1. *Distribution of aerobic plate counts at 35 C of 69 sets of packaged frankfurter units (10 units/set) and of the 690 individual units. Each set represents a different firm or a different production date.*

after heat shocking aqueous suspensions at 71 C for 2 min, and the viable populations were identified as spore-forming bacilli. Because 0.1 to 0.5% by weight of spices or up to 3% of packaged mixes are added to the meat emulsions, these additives contributed to the APC's of 11 sets of frankfurters collected from the seven firms. The APC's of the 11 sets ranged from 4,000 to 40,000/g. By calculation, the use of "sterilized" spices by these seven firms could have reduced the counts to a range of 100 to 20,000/g.

In general, conditions of sanitation along the packaging lines were good. As required in federally inspected establishments, all equipment contacted by the cooked frankfurters was treated with a sanitizing agent after being cleaned, hand-sanitizing solutions were provided for employees, and in many establishments the employees used disposable plastic gloves. The packaging areas were maintained at 10 C or below. Accordingly, the incidence of indicator microorganisms in the packaged frankfurters was low, and the APC's of the frankfurters were nearly always the same both before and after packaging.

Only six of the 69 sets of packaged frankfurters contained coliform-positive units, and two of these sets contained only one coliform-positive unit. In all, 670 (97%) of the 690 units were coliform-negative and only one unit was *E. coli*-positive. Only five of the sets contained *S. aureus*-positive units, and three of these sets contained only one *S. aureus*-positive unit. In all, 681 (98.7%) of the 690 units were *S. aureus*-negative. Every unit was salmonellae-negative in 25-g portions.

All samples (127) of cooked frankfurters collected before peeling and packaging were negative for coliforms and *S. aureus*, however, slight contamination resulted from the peeling-packaging operation in a few firms. Two of the *S. aureus*-positive sets of packaged frankfurters, each from a different firm, contained three units with *S. aureus*. Both sets had been packaged before the plant visits, and both sets had higher APC's than the *S. aureus*-negative sets packaged during the visits. One set of packaged frankfurters contained nine coliform-positive units, and contained the only unit from which *E. coli* was isolated. In this firm, the peeled frankfurters contacted a cracked and frayed composition conveyor belt. The two sets of frankfurters with APC's over 50,000/g, each with about 100,000/g (geometric means), were collected from the same firm. Samples of cooked frankfurters collected just before packaging had APC's of 40,000/g. In this firm, two composition conveyor belts contacted by the peeled frankfurters were cracked and blistered to the degree that cleanability was impaired.

Of 842 raw meat production line samples, 56 (6.7%) were salmonellae-positive. Most of the salmonellae were isolated from pork trimmings and emulsions containing pork; only one lot of beef was salmonellae-positive. All of the 321 production line samples of cooked frankfurters and non-meat items were salmonellae-negative.

The APC's of 163 samples of raw emulsions ranged from 5×10^4 to 1×10^7/g. There was no pattern relating the APC's of cooked frankfurters to the APC's of the raw emulsions from which the frankfurters were made. As reported by Palumbo et al. (5), the normal cooking process for frankfurters is capable of destroying large numbers of bacteria.

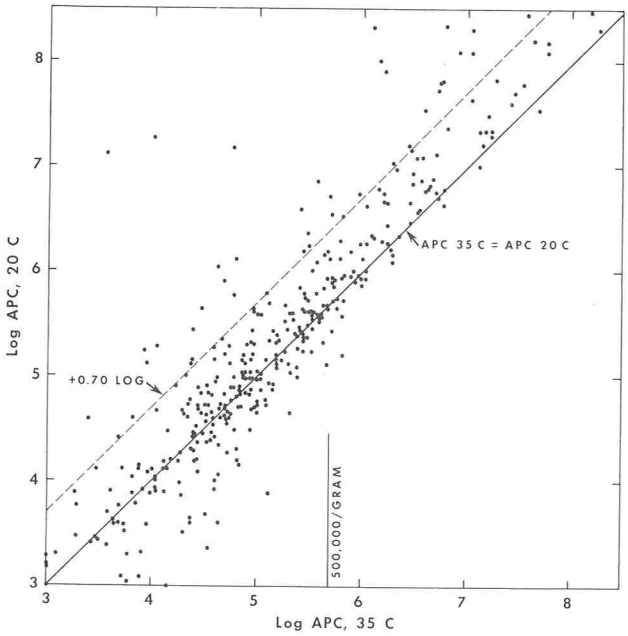

Figure 2. *Aerobic plate counts at 35 C vs. aerobic plate counts at 20 C of 382 raw meat trimmings and cuts used for frankfurters.*

Figure 2 compares APC's at 35 C (2 day incubation) with APC's at 20 C (4 day incubation) of 382 raw meat trimmings and cuts used for frankfurters. If both APC values were equal, all points would fall on the solid diagonal line. Although there is a slight shift from mesophilic to psychrotrophic flora in the raw meats with APC's of more than 500,000/g, most of the points are on or near the line, showing that the APC's of most samples were nearly the same at both incubation temperatures. However, 46 (12%) of the samples had counts at least five times greater at the 20 C incubation temperature (those points on or above the broken diagonal line), suggesting

that some of the raw meat had been under prolonged refrigerated storage.

Nearly always, cooked frankfurters collected before and after chilling had the same APC's, and in 67 of the 69 sets the APC's of the 10 units within the sets were about the same. These findings suggest that, probably because of their small diameter and the relatively wide spaces between looped strands, frankfurters usually attain proper internal temperature regardless of location in the smokehouse during the cooking process, and that cooked frankfurters are nearly always chilled rapidly.

Evidence of slow chilling of cooked frankfurters was noted in one firm. Upon removal from the smokehouse after a 10-min cold water shower, the internal temperature of the frankfurters was 32 C and the APC's of two samples of the frankfurters were 4,800 and 5,600/g. The following day, the internal temperature of the frankfurters was 8 C and the APC's of four samples of the frankfurters ranged from 5,000 to 500,000/g. Two sets of packaged frankfurters, one set of the same batch and the other of the previous day's pack, contained units with APC's ranging from 2,000 to 7,000,000/g. This condition has since been corrected in the firm; follow-up samples of packaged frankfurters had APC's ranging from 2,000 to 4,000/g.

This survey shows that at the time of manufacture and by the laboratory methods employed, 78% of 69 sets of packaged frankfurters had APC's of 10,000 or fewer/g and 90% had APC's of 20,000 or fewer/g (geometric means of 10 units/set), 91% were coliform-negative, and 93% were *S. aureus*-negative. Only one of 690 packaged frankfurter units was *E. coli*-positive, and all units were salmonellae-negative. Recent studies by other investigators (*1, 2, 5, 10*). in which much fewer numbers of frankfurters were examined, showed similar levels of bacteria in freshly prepared frankfurters.

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Effects of Light on Concentrations of Some Volatile Materials in Milk

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ABSTRACT

Gas chromatographic analyses of head-space vapors of steam distillates from milk enabled me to monitor part per billion changes in concentrations of acetaldehyde, propanal, methyl sulfide, acetone, butanone, n-pentanal, and n-hexanal before and after milks were exposed to sunlight in a clear glass flask and fluorescent light in a plastic jug. n-Pentanal and n-hexanal increased about the same under either light (19.6 to 68.5 ppb and 19.1 to 83.6 ppb, respectively). Acetaldehyde increased much more under sunlight than under fluorescent light. Methyl sulfide increased slightly in concentration under 44 h of fluorescent light (6.8 to 11.6 ppb) but did not change appreciably during exposure to 1 h of sunlight. Changes in concentrations of the same compounds, except acetone and butanone, were determined in raw; laboratory pasteurized; and plant pasteurized, homogenized, Vacu-Therm-treated milk exposed to sunlight for 20 min. Except for n-pentanal, all carbonyl compounds in pasteurized, homogenized, Vacu-Therm-treated milk changed less after exposure to 20 min sunlight than did the same compounds from either raw or laboratory pasteurized milk. n-Pentanal was relatively higher in exposed-pasteurized milk than in either raw or laboratory pasteurized milk. Differences between concentrations of volatile materials in exposed raw and in laboratory pasteurized milk were slight. Exposing skimmilk to sunlight for 20 min produced more acetaldehyde than similarly treating homogenized-pasteurized milk, indicating that nonfat fractions are precursors of acetaldhyde. Increases in all other carbonyl compounds could be attributed to the fat.

The nature, significance, and prevalance of light-activated flavors in milk have been described recently (*4, 6, 10*). Barnard (*1*) reported that 86.1% of the fluid milk samples in blow mold, plastic containers from retail stores, during a 1970 survey were oxidized. Milk in other containers was likewise defective but less so (12.7-50%). The increasing incidence of oxidized flavor recently reported is attributed to increased use of all-plastic, translucent containers. In addition to the serious flavor problem cited, researchers have demonstrated the irradiation destruction of riboflavin, ascorbic acid, and tryptophan and significant nutritional losses (*4, 5, 12*).

Lack of a specific test for the defect probably deters fundamental studies of light-activated flavors. The 2-thiobarbituric acid test which effectively measures copper-induced, oxidized flavor, is totally unsuited to test for the light-activated off-flavor (*12*). Although in most studies flavor measurements have been used to indicate the severity of the light-induced off-flavor, such measurements lack objectivity. Research reported here was designed to quantify changes in concentration of volatile materials in milk exposed to light. The simple, routine method developed can be used to study fundamental aspects of the oxidized flavor defect.

[1]*Contribution 906, Department of Dairy and Poultry Sciences, Kansas Agricultural Experiment Station, Manhattan.*

MATERIALS AND METHODS

Measuring volatile materials in milk

Milk was steam distilled in micro-Kjeldahl equipment, and the head-space vapors of the distillate analyzed by GLC. Concentration of volatile materials in ppb were calculated from peak heights (*3*). GLC peaks were identified by using a combination of subtractive techniques (*2*) and peak retention times. Aldehydes were distinguished from ketones by treating the distillates with one drop of saturated aqueous potassium permanganate to oxidize the aldehydes (*8*) before GLC analysis. Two stainless steel columns were used: one (3.05 m × 3.18 mm) packed with 20% Carbowax 20 M on 60/80 mesh, acid-washed HMDS treated Chromosorb P; the other (a 152.4-m capillary column with an ID of .76 mm) coated with OV-101. The capillary column was used to confirm the identities of n-pentanal and n-hexanal.

Concentrations of compounds reported here were calculated with the aid of regression equations previously determined by converting peak heights from chromatograms obtained from the packed Carbowax column to ppb of each component.

Exposure of milk to sunlight and fluorescent light

About 500 ml of commercial pasteurized-homogenized milk at 5 C was exposed to direct sunlight for up to 1 h in a ground-glass, stoppered, liter Erlenmeyer flask. Radiant energy during exposure ranged from 0.91 to 1.09 ly/min (1 ly = 1 g cal/cm^2) and the ambient temperature was 7-10 C. At intervals of 0, 5, 10, 20, 30, and 60 min, aliquots were removed and analyzed for volatile materials, and TBA tests for fat oxidation were conducted by King's method (*9*).

Two, two-liter aliquots of pasteurized-homogenized milk were transferred into clean, sterilized, disposable, capped plastic jugs. One jug was placed below a GE 40-watt, cool, white-fluorescent bulb in a vertical milk display case at 5 C storage, with the milk 21 cm below the light; the other jug, into a refrigerator at the same temperature. Samples were drawn from each plastic jug at 4.5, 18, and 44 h and analyzed for volatile materials as described. Milk exposed for 18 h also was analyzed for TBA.

Effects of processing on susceptibility to light-activated changes in milk

A liter of raw milk was collected from the surge tank of the De Laval Vacu-Therm pasteurizer at the University Dairy plant during routine milk processing. Another sample of about 500 ml was collected a few minutes later after pasteurization at 76.5 C, homogenization in a CP homogenizer using a single-service, conical homo valve and Vacu-Therm treatment. About half of the raw milk was transferred to a liter Erlenmeyer flask and was laboratory pasteurized at 63 C for 30 min with gentle agitation. Each was divided into two equal parts: a control and a sample at 5 C exposed in clear-glass Erlenmeyer flasks to mid-morning sunlight of 0.97-1.16 ly/min for 20 min. The ambient

temperatures of the samples were 4-7 C.
Distillations for GLC analyses were made within 3 h after exposure. GLC analyses were the same day.

Effect of sunlight on fortified skim and whole homo milk

Commercial Vacu-Therm-pasteurized, homogenized milk and commercial skimmilk (0.13% fat) fortified with 1% NFM solids and Vacu-Therm pasteurized were each exposed to mid-morning sun 20 min in a 250 ml, glass stoppered Erlenmeyer flasks. Unexposed samples of the same milks were the controls. Distillations and GLC analyses again were done within 3 h of exposure.

RESULTS AND DISCUSSION

Subtractive techniques (*2*) proved that all peaks except one were from compounds with carbonyl functional groups. The peak that was not from a carbonyl compound was eliminated with mercuric chloride and had a retention time identical to methyl sulfide. Other researchers have identified methyl sulfide in normal milk (*11*).

Acetaldehyde, propanal, acetone, and butanone are among the carbonyl compounds that have been identified as normal constituents in milk (*7*). GLC peaks with retention times close to five and six carbon chain carbonyl compounds were scrutinized with the aid of an OV-101 capillary column. Because these compounds had the same retention times on the capillary column as n-pentanal and n-hexanal (and differed from the corresponding ketones) and because their corresponding GLC peaks were eliminated when the distillates were treated with saturated permanganate, I identified them as such and calculated their concentrations from n-pentanal and n-hexanal standard curves. Standard curves obtained from the analysis of milks spiked with measured ppb of the compounds identified in milk provided regression equations to use in calculating concentrations of compounds in experimental samples.

Exposing pasteurized, homogenized milk to sunlight increased concentrations of most of the volatile materials found after only 5 min (Table 1). Acetaldehyde increased from 6.1 to 35.5 ppb during 1-h exposure, whereas methyl sulfide did not change appreciably. n-Pentanal reached a maximum of 142.8 ppb after a 20-min exposure then decreased to 110.4 ppb at 1 h. N-Hexanal increased steadily from 19.1 to 83.6 ppb throughout the 1-h exposure to sunlight. All TBA values were relatively low (Table 1). It is generally agreed that TBA does not reflect light-activated flavor defects, but TBA increased slightly from 0.020 to 0.022 for up to 30-min exposure and to 0.034 for 1-h exposure.

Changes in volatile materials in milk in a plastic jug exposed to fluorescent light are shown in Table 2. A similar pattern of changes in volatile materials occurred in milk exposed to sunlight and fluorescent light, although times of exposure and light intensities were different. The principal difference was the methyl sulfide increased slightly in milk exposed to fluorescent light but not in milk in a clear glass flask exposed to sunlight. Acetaldehyde concentrations were not so pronounced in milk that was fluorescently treated as in that exposed to sunlight.

Susceptibilities to light activation by raw, laboratory pasteurized, and plant pasteurized milks from the same milk supply are compared in Table 3. Concentrations of each chemical component in all control milks were

TABLE 1. *Changes in concentrations of indicated chemical constituents of pasteurized, homogenized milk after exposure to direct sunlight*[a]

Compounds	Times exposed (min)					
	0	5	10	20	30	60
	(conc ppb)					
Acetaldehyde	6.1	14.3	14.1	20.8	28.3	35.5
Methyl sulfide	7.2	7.4	6.8	6.4	6.2	6.5
Propanal	2.6	3.6	4.2	4.2	6.2	5.7
Acetone	311.0	351.0	346.0	355.0	390.0	365.0
Butanone	23.3	29.3	29.5	29.5	30.3	29.9
n-Pentanal	11.8	65.8	105.4	142.8	130.0	110.4
n-Hexanal	19.1	32.1	54.0	68.0	75.8	83.6
TBA values	(0.020)	(0.020)	(0.020)	(0.022)	(0.027)	(0.034)

[a]At 11 A.M. standard time (o exposure time) sun energy was 0.91 g cal/cm²/min; at noon 1.09 g cal/cm²/min.

TABLE 2. *Concentrations of indicated constituents compared in unexposed milk and in pasteurized, homogenized milk exposed to fluorescent light*

Compounds	Refrigerator			Display case[a]		
	Storage time (h)[b]			Storage time (h)[b]		
	4.5	18	44	4.5	18	44
	(conc ppb)					
Acetaldehyde	5.8	7.5	4.8	7.4	8.3	12.0
Methyl sulfide	6.8	8.2	7.4	7.2	8.3	11.6
Propanal	2.4	2.8	2.7	2.7	3.1	5.4
Acetone	390.0	406.0	363.0	365.0	398.0	414.0
Butanone	51.0	51.6	44.2	48.6	48.8	48.6
n-Pentanal	8.2	14.6	13.2	37.0	48.5	114.0
n-Hexanal	19.6	26.4	24.4	30.0	32.1	68.5

[a]Exposed to 40W fluorescent bulb at 21 cm distance.
[b]At 18 h, TBA values of both refrigerator and display case samples are .014.

TABLE 3. *Changes in concentrations of indicated constituents of raw, laboratory pasteurized, and plant pasteurized, homogenized milk exposed to sunlight*

Time exposed	Raw milk	Lab past.[a]	Plant past. milk[b]
		Acetaldehyde (ppb)	
0	6.3	6.4	6.4
20 min	14.9	19.9	13.6
		Methyl sulfide (ppb)	
0	10.0	8.8	7.4
20 min	10.2	10.2	8.2
		Propanal (ppb)	
0	2.2	2.2	2.2
20 min	3.9	3.9	3.6
		n-Pentanal (ppb)	
0	4.6	10.3	7.4
20 min	40.6	44.9	80.2
		n-Hexanal (ppb)	
0	12.9	14.4	13.9
20 min	55.5	60.2	30.6
		TBA values	
0	.019	.019	.013
20 min	.023	.024	.027

[a]63C for 30 min.
[b]Vac-Therm, HTST pasteurized, homogenized.

similar; n-pentanal varied most. Those in the exposed plant pasteurized milk (Vacu-Therm) increased least for all components, except for n-pentanal, which increased the most. Again methyl sulfide did not increase upon exposure to sunlight.

TBA values were poor measures of the volatiles in light-activated milk. Surprisingly, TBA values changed most in the plant pasteurized milk (0.013 to 0.027), despite lower concentrations of volatiles produced. The .027 TBA indicates acceptable milk.

TABLE 4. *Changes in concentrations of indicated chemical constituents of commercial skim milk and whole homo milk exposed to sunlight*

Time exposed	Skim milk[a]	Plant past. milk[b]
	Acetaldehyde (ppb)	
0	8.3	9.0
20 min	26.6	20.2
	Methyl sulfide (ppb)	
0	7.6	8.4
20 min	5.9	7.3
	Propanal (ppb)	
0	2.4	3.2
20 min	3.5	6.5
	n-Pentanal (ppb)	
0	2.4	26.2
20 min	15.4	179.5
	n-Hexanal (ppb)	
0	11.8	28.0
20 min	21.2	66.4

[a]Commercial skim milk fortified with 1% NFDM solids.
[b]Homo milk from the same source as the skim milk.

Table 4 shows changes in concentrations of chemical compounds from skim milk and whole homogenized milk exposed to sunlight 20 min. Higher concentrations of acetaldehyde from skim milk than from whole homogenized milk, both exposed to sunlight, show that acetaldehyde originates from the nonfat milk fraction. n-Pentanal also increased significantly in the exposed skimmilk, and though the increase was much less than in the exposed homogenized milk, the ratios of the increases were similar. Precursors of all other carbonyl compounds apparently were from the lipids. Their concentrations did not differ appreciably between control skim milk and homogenized milk.

Changes in concentration of several carbonyl compounds in milk exposed to sunlight or to fluorescent light are in the part-per-billion range. The same chemical compounds produced in sunlight-exposed milk were observed in milk exposed to fluorescent light, but in somewhat different proportions. Whether the observed changes cause the typical light activated flavor still needs to be investigated. The origin and mechanism of acetaldehyde formation merits additional study. Lower concentrations of n-pentanal after 20 min exposure to sunlight suggests that it may interact or be altered.

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The Effect of Salting on the Microbiology of Poultry Meat

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ABSTRACT

An investigation was carried out on the effect of salting on microbiology of chilled poultry meat, with particular emphasis on possible selective influence of salt on predominant spoilage organisms. A significant reduction in total aerobic counts at 4.5 and 20 C and *Enterobacteriaceae* counts on the skin surface of fresh broiler carcasses was achieved by salting; the mean log reductions per cm² being 1.19, 1.32, and 1.35, respectively. The two major genera found on the carcasses at the time of spoilage at 4.5 C were *Moraxella* and *Pseudomonas*. The percentage of *Moraxella* Increased from 48 in untreated to 68% in salt-treated samples, while pigmented *Pseudomonas* decreased from 31 (untreated) to 12% (salt-treated) of the total isolates. The recovery of isolates of *Pseudomonas* and *Moraxella*, in mixed populations, from heart infusion broth containing NaCl concentrations from 0.09 (a_w 0.990) to 5.22 *M* (a_w 0.814) was studied in 4 h-periods at 4.5 and 20 C. After 1 h incubation, as NaCl concentration was increased, the numbers of *Pseudomonas* were increasingly reduced both at 4.5 and 20 C, whereas numbers of *Moraxella* remained almost unchanged. The reduction of *Pseudomonas* counts, even at low NaCl concentrations, persisted after 2 h and 4 h incubation. Some reduction in *Moraxella* counts became apparent only after 4 h, and in concentrations higher than about 4.0 *M* (ca. a_w 0.85). It is concluded that the much higher salt tolerance of *Moraxella* as compared with that of *Pseudomonas*, accounts for the increase in the percentage of *Moraxella* in the bacterial population, as salt was added to the poultry meat.

In the processing of poultry meat, salt might be used either in solution as a component of curing brines, or applied dry directly to the meat. When added to foods, it appears that salt inhibits microbial growth mainly by lowering water activity of microbial environment. Dehydration and shrinkage of bacterial cells suspended in hypertonic salt solutions, and its association with plasmolysis, has been reported for both gram-negative (*3, 23*) and gram-positive (*11*) bacteria. In the processing of "kosher" poultry, freshly eviscerated carcasses are individually covered with dry salt for 1 h at ambient temperature. During this period it is probable there are relatively low water activities on the salted surfaces, and that potential spoilage and pathogenic bacteria might be inhibited or destroyed. Harrison and Morgan (*7*) found that when *Pseudomonas aeruginosa* was kept for 40 min in 4.5 *M* NaCl and then transferred into 0.14 *M* NaCl for 20 min, only 0.1% of the organisms survived this "osmotic shock" if carried out at 40 C, and 7% if at 2 C.

In the present investigation to study the effect of salting on microbiology of chilled poultry meat, particular emphasis has been placed on possible selective influence of salt upon predominant spoilage organisms.

MATERIALS AND METHODS

Initial counts

Twenty broiler carcasses, taken at random from a commercial line, were sampled for microbiological analysis both with and without salting. The carcasses were aseptically cut in half lengthwise, one half salt-treated and the other half used as control. Sampling was done by swabbing approximately 10 cm² of the breast for 30 sec, using a cotton swab. A 0.1% peptone solution was used for serial dilutions. Total aerobic counts were made on tryptone glucose extract agar (TGE, Difco) plates, incubated both at 20 C for 72 h and at 4.5 C for 14 days. To estimate the *Enterobacteriaceae* count, violet red bile agar (VRB, Difco) with 1% glucose was used (*15*). The double-poured VRB plates were incubated for 18-24 h at 35 C.

Salting

Each half of the carcass was well covered with (dry) food-grade salt ("kosher" procedure) for 1 h at 25 C. Most of the salt was then removed from the carcass by one immersion in water at 25 C for 30 sec, followed by two consecutive immersions (3 min each) at 4 C. Controls also passed through the three-step immersion process. Tap water chlorinated to 10 ppm of total available residual chlorine was used, and the bird:water ratio at immersions was approximately 1:2 (w/v).

TABLE 1. *Tests used in the screening of the Gram-negative bacterial isolates*

Characteristic	*Pseudomonas* Pigmented	*Pseudomonas* Non-pigmented	*Alcaligenes*	*Aeromonas-Vibrio*	*Acinetobacter*	*Moraxella*	*Enterobacteriaceae*
Motility		+	+	+ or −	−	−	+ or −
Flagella		polar	peritrichous	polar			peritrichous
Fluorescence	+	−	−	−	−	−	−
Oxidase		+	+	+	−	+	−
Metabolism of glucose		oxidative	oxidative or inert	fermentative	oxidative or inert	inert	fermentative
Anaerobic production of alkalinity from arginine		+	−	+ −	−		+ or −
Penicillin		resistant	variable	resistant	resistant	sensitive	resistant
Oxytetracycline	resistant	sensitive	variable	sensitive	sensitive		sensitive

Identification of spoilage organisms

Legs of both salt-treated and untreated freshly processed broilers were aseptically placed into sterile polyethylene bags (18 oz) and stored at 4.5 C. After spoilage odors developed, 100 ml of sterile 0.1% peptone solution was poured into the polyethylene bag and the contents shaken thoroughly for 1 min. From serial dilutions of this suspension to 10^{-6} in 0.1% peptone, 0.1-ml aliquots were surface-plated on heart infusion agar (HIA, Difco). The plates were incubated at 4.5 C for 14 days. All colonies (up to 10) from the highest dilution showing growth, were isolated. A statistical randomized procedure employing a numbered grid was used for selecting colonies from plates containing more than 10 colonies. A total of about 100 isolates was taken for each treatment. Isolates were grown in heart infusion broth (HIB, Difco) at 20 C and then examined for the following characteristics: gram reaction, morphology, motility (hanging drop technique), production of fluorescin on the medium of King et al. (*9*), oxidase activity (*10*), metabolism of glucose (*8*), production of alkalinity from arginine (*21*), sensitivity to penicillin (2.5 I.U.) and oxytetracycline (10 μg) by the paper disc method, growth on MacConkey agar, and flagella stain by using the modification of Fontana's method described by Rhodes (*17*). Characterisitics outlined in Table 1 were used for identification of gram-negative isolates, and for the genera *Alcaligenes, Acinetobacter,* and *Moraxella* the definitions in the 8th edition of the *Bergey's Manual for Determinative Bacteriology* (*4*) were followed.

Additional examinations for cultural and physiological characteristics of *Pseudomonas* were done as outlined in the *Manual of Microbiological Methods* (*19*), and included: reaction in litmus milk; gelatin liquefaction; casein hydrolysis; growth at 20, 37, and 42 C; indole production; and nitrate reduction.

Effect of NaCl on mixed cultures of Pseudomonas *and* Moraxella

A series of experiments was designed to follow the survival of species of *Pseudomonas* and *Moraxella* in mixed cultures in HIB, to which progressively increasing amounts of reagent grade NaCl were added. The pH of the media was measured with a glass electrode. No efforts were made to adjust the pH of the different samples, which varied from 7.54 in HIB containing 0.09 *M* NaCl to 7.46 in HIB containing 5.22 *M* NaCl. For these studies, cultures were grown in HIB, transferred twice, and the final cell concentrations adjusted to an optical density of 0.25 at 530 nm with sterile 0.1% peptone before inoculation.

The *Pseudomonas* sp. utilized in these experiments (strain 71) shows the following characteristics: motile rods, polar flagellation, fluorescent pigment, oxidative metabolism of glucose, production of alkalinity from arginine both aerobically and anaerobically, oxidase positive, growth at 20 and 37 C but not at 42 C, indole negative, gelatin liquefaction, reduction of litmus, casein hydrolysis, resistance to penicillin but sensitivity to oxytetracycline, and reduction of nitrate.

The second organism used in these trials was a non-motile short rod, oxidase positive, inert towards glucose, sensitive both to penicillin and oxytetracycline, did not produce alkalinity from arginine anaerobically, did not digest casein, grew readily at 20 C, but did not grow at 37 C or 42 C; it was defined as *Moraxella* sp. (strain 59).

Temperature-equilibrated samples of the HIB containing different amounts of NaCl were inoculated with 18-h old cultures to provide approximately 10^6 cells per milliliter, the inoculum consisting of a 1:1 ratio of *Pseudomonas* to *Moraxella.* Sets of the test samples were then incubated at the temperatures (4.5 or 20 C) at which they were previously stored.

After incubation, viable counts of *Pseudomonas* and *Moraxella* were determined by surface-plating 0.1 ml aliquots of decimal dilutions in 0.1% peptone + 0.85% NaCl onto plates of nutrient agar (Difco) to which 30% skim milk was added. The plates were incubated at 20 C for 2-4 days. In this medium, casein-digesting *Pseudomonas* 71 could easily be counted separately from the non-digesting *Moraxella* 59. Results were expressed as the logs of colony-forming units (CFU) per milliliter.

The growth rates of *Pseudomonas* 71 and *Moraxella* 59 were measured in HIB at 20 C. Viable counts were determined at selected intervals, surface-plating 0.1 ml aliquots of the cultures on HIA and incubating the plates for 2 days at 20 C. From the results obtained, growth curves were drawn and the mean generation time calculated.

Counts of bacteria stored in NaCl solutions

For the microbiological examination of cured meats, Gardner and Kitchell (*6*) recommended addition of 4% NaCl to both the plating diluent (0.1% peptone) and the plating medium (plate count agar), as a means of minimizing possible osmotic damage. Preliminary tests showed that when 0.85, 4.0 or 6.0% NaCl, was added to 0.1% peptone diluent, recovery of *Pseudomonas* 71 stored in 6.0% NaCl (3.3×10^6 organisms/ml) for 1 h and plated on HIA, was 75, 65, and 65%, respectively. However, when HIA contained either 4 or 6% NaCl, less than 0.3% of the inoculum was recovered. Based on these data, 0.1% peptone + 0.85% NaCl was used for counts of bacteria stored in NaCl solutions, and the dilutions plated on agar medium without NaCl additions.

Determination of a_w

Water activity (a_w) values for HIB containing various NaCl concentrations were calculated to allow comparison of the results presented herein with other reports dealing with the influence of water activity upon bacterial growth, in which NaCl has been used to reduce a_w. Calculations of a_w were made from experimentally determined freezing point depression values, using the equations reported by Strong et al. (*20*). Freezing point was determined at the freezing temperature plateau of the cooling temperature curve, cooling being achieved under continuous stirring, in baths of either salt-ice (from 0.09 to 3.17 *M* NaCl concentrations) or ethanol-dry ice mixtures (from 3.17 to 5.22 *M* NaCl), and measured with 24-gauge copper-constantan thermocouples connected to a Telrad model 702 recorder. The relationships of concentration of NaCl, freezing point depression, and a_w in HIB are shown in Table 2.

TABLE 2. *Freezing depression values obtained by the addition of various amounts of NaCl to HIB and the corresponding water activities*

M NaCl in medium	Freezing point depression[a]	a_w
0.09[b]	1.05	0.990
0.26	1.56	0.985
0.77	3.34	0.968
1.11	4.58	0.957
1.80	7.08	0.935
2.48	9.87	0.913
3.17	11.67	0.898
3.51	14.37	0.878
3.85	16.16	0.864
4.53	20.16	0.836
5.22	23.54	0.814

[a]Experimentally determined, measured in C
[b]Basal medium (HIB), no additional solute

RESULTS AND DISCUSSION

Effect of salting on reduction of initial counts

Commercial production of "kosher" ready-to-cook poultry includes covering of the carcasses with dry salt for 1 h at ambient temperature. Most of the salt is subsequently removed from the carcasses by rinsing with water. Fresh broiler carcasses were covered with salt for 1 h at 25 C and then rinsed with water chlorinated to a level of 10 ppm total residual chlorine. The mean logs of bacterial counts per cm^2 skin surface of salt-treated and untreated carcasses are presented in Table 3. Total

TABLE 3. *Microbial counts*[a] *on broiler breast skin surfaces as affected by salting*

	Total aerobic count at		
	20 C 72 hr	4.5 C 14 days	*Enterobacteriaceae*
Control	4.10	2.30	2.89
Salt-treated	2.78[b]	1.11[b]	1.54[b]

[a]Results are given as mean logs of counts per cm^2
[b]Population significantly different from control at 0.5% level

aerobic counts at 20 and 4.5 C and *Enterobacteriaceae* counts were significantly reduced by salting, the mean log reductions per cm^2 being 1.32, 1.19 and 1.35, respectively.

Initial total counts at 4.5 and 20 C are considered good indicators of potential shelf-life of refrigerated poultry meat, and an initial reduction in total count of more than 1 log/cm^2 skin surface has usually been followed by extra shelf-life (*1*). However, in our experiments, although an initial count reduction of more than 1 log/cm^2 was effected by salting, no significant extension of shelf-life, as determined by the evidence of spoilage odor, occurred. Mossel (*14*) recommended the use of a total *Enterobacteriaceae* count as a helpful indicator for the presence of pathogenic *Enterobacteriaceae,* which may be heterogeneously distributed in a food sample. *Enterobacteriaceae* counts were also reduced by salting, and this can be taken as an indication that presence of salmonellae or enteropathogenic *Escherichia coli* is less likely.

Spoilage organisms

The hypothesis that salting might affect some predominant spoilage organisms more than others was considered. The two major genera found in the carcasses at the time of spoilage at 4.5 C were *Moraxella* and *Pseudomonas* (Table 4). The percentage of isolates

TABLE 4. *Organisms present at time of spoilage*

Organism	Percentage of total isolates	
	Untreated	Salt-treated
Pigmented *Pseudomonas*	31	12
Nonpigmented *Pseudomonas*	2	0
Moraxella	48	68
Aeromonas-Vibrio	2	6
Alcaligenes	0	4
Unclassified	17	10

identified as *Moraxella* increased from 48% in the untreated samples to 68% in the salt-treated ones, while pigmented *Pseudomonas* decreased from 31% (untreated) to 12% (salt-treated) of total isolates.

The cultures identified in this study as *Moraxella* were sensitive both to penicillin and oxytetracycline. All the unclassified isolates were gram-negative coccoids which differed from *Moraxella* by having an oxidative metabolism of glucose, but which could not be classified as *Acinetobacter* because of their sensitivity to penicillin.

As previously stated, salting caused a significant reduction of the initial total count without however leading to a consequent extension of the storage life of the refrigerated poultry meat. This might be explained by a different composition of the bacterial population developing on the salt-treated poultry meat at storage, assuming that this population had a higher specific activity towards spoilage. This question deserves further investigation.

Pseudomonads have been reported by many authors as the predominant spoilage organisms of poultry meat at refrigeration temperatures (*2, 5, 16*). Variations, both quantitative and qualitative, in bacterial genera predominating in poultry, meat, and fish spoiled at refrigeration temperatures, may be expected due to the influence of a number of factors such as season and environment. Oxidase-positive saccharolytic strains, showing *Moraxella* morphology and conforming to other generic characteristics of both *Moraxella* and *Acinetobacter,* have been isolated from cold-stored poultry, and are reported in the 8th edition of *Bergey's Manual of Determinative Bacteriology* (*4*) under *Species incertae sedis* ("Other *Moraxella*-like Taxa") of the genus *Moraxella.*

Effect of NaCl on Pseudomonas and Moraxella

The inhibition of gram-negative and predominance of gram-positive bacteria, following the addition of salt to food products, has been widely reported. However, little is known about the selective action of salt on different Gram-negative bacteria.

The percentages of *Pseudomonas* and *Moraxella* among the population isolated from the salt-treated samples at the time of spoilage, differed considerably from those observed with the untreated samples (Table 4). The hypothesis that these two groups might differ also in their sensitivity to sodium chloride was therefore considered.

The recovery of two isolates *Pseudomonas* 71 and *Moraxella* 59, in mixed populations, from HIB containing NaCl in concentrations from 0.09 *M* (a_w 0.990) to 5.22 *M* (a_w 0.814) was studied over a 4 h-period at 4.5 and 20 C. *Pseudomonas* 71 and *Moraxella* 59 had similar generation times in HIB at 20 C : 1.45 h and 1.48 h, respectively. The number of colony-forming units, immediately after inoculation of the test media, was approximately 10^6/ml. The inhibition of *Pseudomonas* strains in response to reduction of a_w has been reported by several workers (*12, 22*), the minimal a_w value permitting growth in culture media being usually higher than 0.95. Reduced salt-tolerance of *Pseudomonas* observed at 4.5 C as compared with 20 C supports the views of other workers (*13, 22*) that greatest tolerance for low a_w occurs at the temperature which is nearly optimal for growth of the organism. The use of a_w values to interpret microbiological phenomena provides a basis for comparing the effect of adding different sugars or salts, as well as dehydration effects. Several authors claimed that the response to a particular a_w is, at least for some organisms, largely independent of the solute used to adjust the a_w of the medium (*18, 22*), while others (*12, 20*) concluded that the specific solute used to reduce a_w influences the minimal a_w required for growth.

Fig. 1 shows that after 1 h incubation, as NaCl concentration was increased, the numbers of *Pseudomonas* were increasingly reduced both at 4.5 C and at 20 C. At 4.5 C numbers of CFU were reduced much more than at 20 C. Numbers of *Moraxella,*

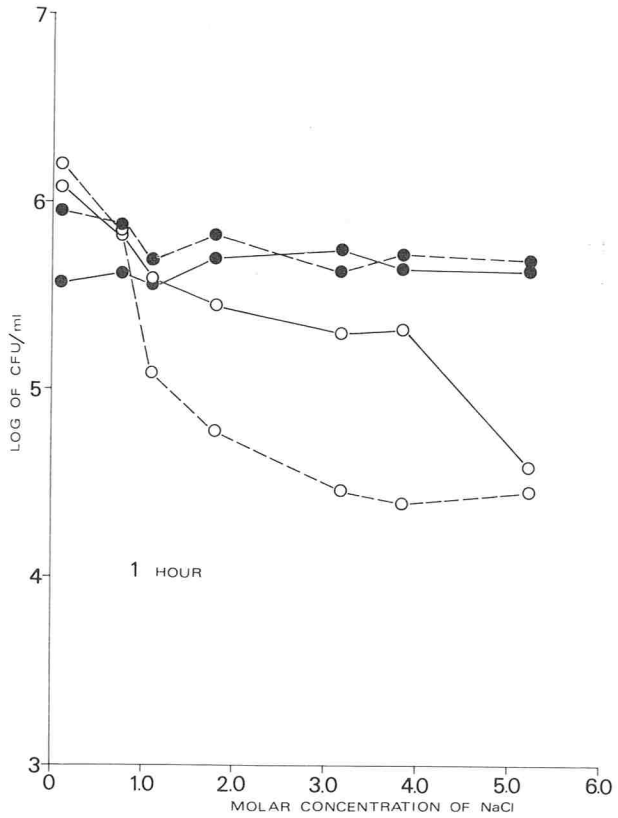

Figure 1. *Variations in counts of* Pseudomonas *71 (open circles) and* Moraxella *59 (closed circles) in mixed culture, after 1 h of incubation at 4.5 C (dashed line) and 20 C (solid line) in heart infusion broth with various NaCl concentrations.*

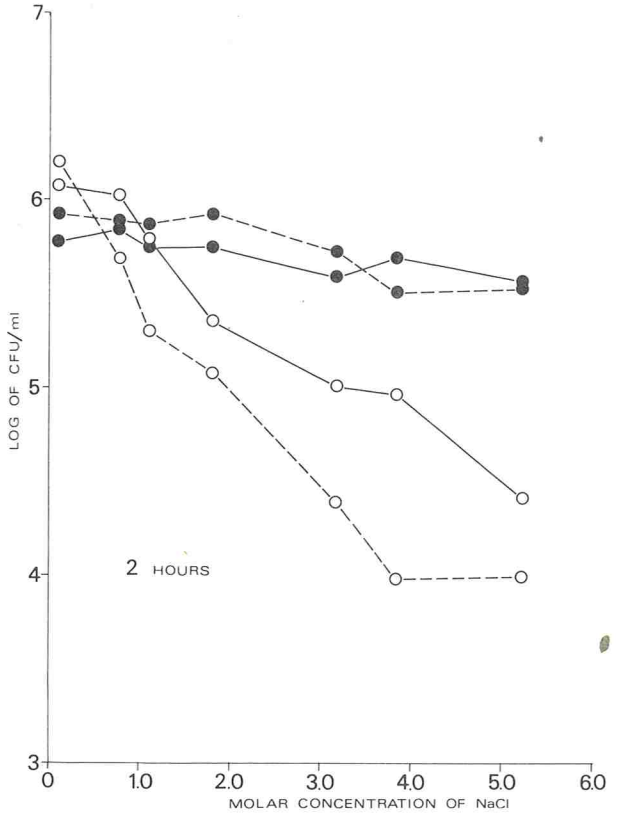

Figure 2. *Variations in counts of* Pseudomonas *71 (open circles) and* Moraxella *59 (closed circles) in mixed culture, after 2 h of incubation at 4.5 C (dashed line) and 20 C (solid line) in heart infusion broth with various NaCl concentrations.*

on the contrary, remained almost unchanged after 1 h incubation, as NaCl concentration was raised, both at 4.5 and at 20 C. The reduction of *Pseudomonas* counts, evident after 1 h incubation even at relatively low NaCl concentrations, became more pronounced after 2 h (Fig. 2) and 4 h (Fig. 3), whereas with *Moraxella* some reduction in counts became apparent only after 4 h and in concentrations higher than about 4.0 *M*.

It can, therefore be concluded that the much higher salt-tolerance of *Moraxella,* as compared with that of *Pseudomonas,* is the basis for the increase in the percentage of *Moraxella* in the bacterial population as salt was added to poultry meat.

Bacteria growing at temperatures of about 5 C, referred to as psychrotrophs, are known to play an important role in spoilage of foods stored under refrigeration. From a practical point of view, application of the data reported here to the storage of perishable food products at refrigeration temperatures, may be of importance. Although high salt concentration or low water activities in general will inhibit *Pseudomonas,* they may not necessarily inhibit other psychrotrophic gram-negative organisms such as *Moraxella* which might cause spoilage.

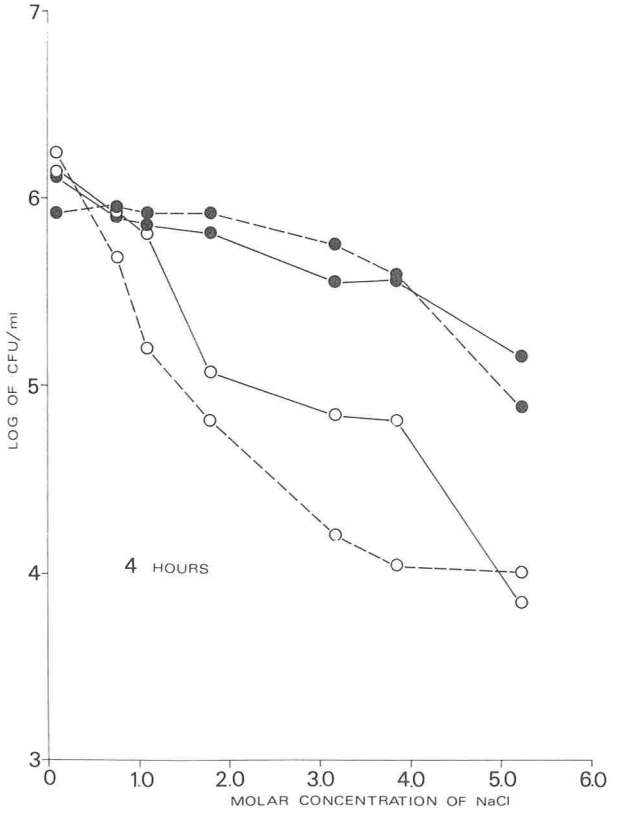

Figure 3. *Variations in counts of* Pseudomonas *71 (open circles) and* Moraxella *59 (closed circles) in mixed culture, after 4 h of incubation at 4.5 C (dashed line) and 20 C (solid line) in heart infusion broth with various NaCl concentrations.*

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Chemical and Microbial Changes in Dehulled Confectionery Sunflower Kernels during Storage Under Controlled Conditions

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ABSTRACT

Samples of freshly dehulled, confectionery sunflower kernels were adjusted to moistures of 5.2, 10.5, and 14.7%, sealed in plastic bags and stored at 35, 75, and 95 F (1.7, 23.9, and 35 C) for 12 weeks. At 2-week intervals aliquots were removed for flavor, chemical, and microbiological analyses. Acid values of oil extracted from stored kernels increased with temperature, moisture content, and storage time. At acid values of 4 or higher, kernels had a sour flavor. In general, the peroxide value decreased with increased moisture at each temperature and storage period. The initial aerobic plate count of the sunflower kernels was log 6.83/g, the *Enterobacteriaceae* count was log 6.15/g, and the yeast and mold count was log 3.65/g. From countable plates randomly selected, about 80% of the *Enterobacteriaceae* were identified as *Enterobacter agglomerans* (*Erwinia herbicola*). At 35 F microbial counts generally changed little. At 75 F, however, counts decreased rapidly; and at 95 F, yeast and mold counts of 14.7% moisture kernels increased, *Enterobacteriaceae* counts decreased, and aerobic plate counts decreased except in high moisture samples. A microbiological survey of whole sunflower seed and dehulled kernels from three dehulling operations indicated that contamination of the dehulled kernels was primarily from sunflower hulls rather than from processing equipment.

Sunflower seed are sometimes harvested at high moisture content and then stored without adequate drying. This can result in serious problems particularly for confectionery type sunflower seed for the edible food trade. Since refrigeration is costly and profit margin is small, seed are normally stored at ambient temperature. Dehulled, unprotected sunflower kernels (termed "meats" in the trade) are more susceptible to oxidative deterioration; therefore, conditions of storage are quite important. Flavor and color deterioration are inevitable with the severity depending upon the moisture content, temperature, and length of storage.

Growth of microorganisms on sunflower seed and dehulled kernels and the increase in acidity during storage have been investigated (*3, 4, 7, 13*). Poisson et al. (*13*) found that sunflower kernels of the high-oil type can be satisfactorily stored for several months if maintained below 22 C and at a moisture content below 6%. They found that even slight increases in moisture and temperature accelerated acid production particularly if the initial acidity of the seed was high.

Christensen (*3*) found that fungal invasion and decrease in germinability in seed were proportional to increase in moisture content, temperature, and time of storage. Storage fungi, especially *Aspergillus* and *Penicillium,* invaded the seed within a short time at 10-11% moisture when the temperature was high enough for fungal growth. Christensen (*4*) also reported that 6% moisture was the lower limit that permitted invasion of sunflower seed meats by storage fungi.

Since there is a stable market for confectionery sunflower seed in the edible food trade, information is needed on their quality and storage stability. We have investigated the effect of storage conditions on the chemical composition and microbiological quality of dehulled sunflower kernels.

MATERIALS AND METHODS

Storage study

Freshly dehulled confectionery sunflower kernels were obtained from Agway, Inc., Grandin, North Dakota. Initially, the kernels contained 5.2% moisture; 0.90 acid value; 48.4% total oil (dry basis); and 28.7% protein (dry basis). The lot of kernels was divided into three equal portions and placed in polyethylene bags. Known weights of water were added to two of the bagged samples to give moisture contents of 10.5 and 14.7%. After thorough mixing, moisture of kernels was allowed to equilibrate at 35 F for 48 h with periodic mixing.

For storage studies, about 150-g samples of kernels equilibrated to moisture contents of 5.2, 10.5, and 14.7% were placed into several heavy duty Kapak plastic bags and sealed. According to the manufacturer, the permeabilities of the plastic bags per 100 inches^2/24 h were: water vapor, 0.1 g at 100 F; oxygen from air, 1 ml at 1 atm at 77 F; and carbon dioxide, 27 ml at 1 atm at 77 F. The bags of dehulled sunflower kernels were stored at 35, 75, and 95 F (1.7, 23.9, and 35.0 C) for 12 weeks. At 2-week intervals, bags containing kernels at each of the moisture contents and at each of the temperatures were removed for chemical and microbiological analyses.

Kernels from the treatments were examined informally by the authors for obvious differences in odor, flavor, and color. Moisture content of kernels was determined by drying 10-g portions at 102 C in a forced draft oven for 72 h. Kernels were analyzed for peroxides and free fatty acids (acid value) by official AOCS methods (*11*). Fatty acid methyl esters were measured as previously described, by a Tracor MT 220 gas liquid chromatograph equipped with an Infotronics model CRS-101 digital integrator (*9*).

For microbiological examination, 11 g of kernels were added to sterile 4-oz jars containing 12.5 g of 6-mm solid glass beads; 99 ml of a Butterfield buffered phosphate diluent (*12*) were added; and the jars were then shaken vigorously through a 1 ft arc for 1 min.

Aerobic plate counts were made in duplicate on plate count agar (Difco); plates were incubated 72 h at 28 C (*14*).

The *Enterobacteriaceae* count was estimated by using violet red bile (VRB) agar (Difco) with 1% glucose (*10*). The double poured plates were incubated 18-24 h at 35 C. The counts were reported as logarithmic averages and expressed as microorganisms per gram of sample. *Enterobacteriaceae* were isolated from the highest dilution plated during microbiological analysis from the 4th and 6th week storage samples and identified by the R-B tube enteric differential system (Diagnostic Research, Inc.), API 20 strip (Analytab Products, Inc.) (*5*) and by the methods of Edwards and Ewing (*6*).

Yeast and mold counts were made in duplicate on potato dextrose agar (Difco) acidified to pH 3.5 with 10% tartaric acid. Plates were incubated 72 h at 28 C.

Decortication study

Whole confectionery sunflower seed and dehulled kernels prepared from the same lot of whole seed were obtained from three dehulling operations. Samples were analyzed as described above. For the microbiological examination, samples were prepared by AOAC method for tree nuts (*12*). Fifty-gram samples were aseptically weighed into sterile jars, 50 ml of diluent added, and jars agitated vigorously 60 times thru a 1 ft arc to obtain 10° dilution. Serial dilutions and counts were made as described for storage study.

RESULTS AND DISCUSSION

Effect of storage on chemical characteristics

The peroxide value of oil extracted from sunflower kernels stored at 35, 75, and 95 F is shown in Table 1.

In general, peroxide value decreased with an increase in moisture at each storage period and temperature. The peroxide value sharply increased beginning with the fourth week of storage which would indicate that some oxidative deterioration had taken place even at 35 F. At the high moisture level (14.7%), the hydroperoxide formed tended to decompose rapidly so that little accumulated at higher temperatures.

Oils extracted from dehulled sunflower kernels were also examined for changes in ultraviolet absorption at 234 and 278 nm. Absorption at 234 nm is due to the presence of conjugated dienes that are formed along with hydroperoxides during the initial oxidative deterioration of unsaturated fatty acids such as found in sunflower oil triglycerides (*1*). Beginning with the eighth week of storage, absorption at 234 nm tended to decrease with an increase in moisture content. For samples with 5.2% moisture, absorbance increased with temperature beginning with the sixth week of storage. At the high moisture level (14.7%), however, absorbance decreased with increase in temperature. The decrease in absorption of 234 nm indicated that the conjugated dienes were breaking down. In addition, absorption at 278 nm increased with storage temperature. This would be expected if conjugated dienes were being decomposed at the higher temperatures. These materials are unstable and decompose to form ketones and α, β-unsaturated aldehydes which absorb at 260-280 nm (*1*).

At 5.2 and 10.5% moisture and all temperatures, absorbance at 234 nm and peroxide value were significantly correlated ($P < .05$); but at 14.7% moisture correlation was low. Absorbance at 278 nm and peroxide value were significantly correlated ($P < .05$) only at the low moisture level (5.2%).

There was no appreciable change in the content of saturated fatty acids or linoleic acid of sunflower kernels during storage. Saturated fatty acids ranged from 10.3 to 11.0% and linoleic acid content from 61.3 to 63.0%. In addition, there was very little, if any, change in the total oil content and practically no change in the moisture content of the kernels stored at 35 and 75 F. However, the samples at each moisture level stored at 95 F gradually decreased in moisture. The decrease was greatest for the low moisture samples, from 5.2% to 4.54% during 12 weeks of storage. Although microflora or kernel respiration was indicated by gas accumulation during storage particularly at the higher temperatures, the moisture contents did not vary greatly since the kernels were confined in sealed plastic bags.

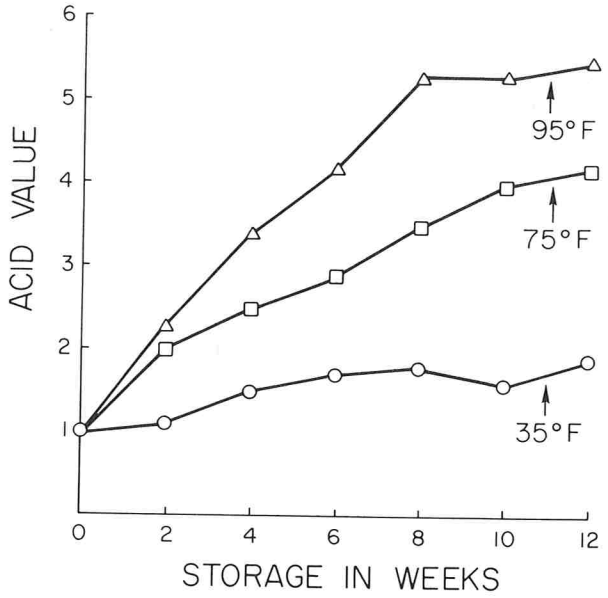

Figure 1. *Effect of storage on the acid value of the oil extracted from dehulled sunflower kernels with 5.2% moisture content. The acid value is the mg of KOH required to neutralize 1 g of oil.*

TABLE 1. *Peroxide value of the oil extracted from dehulled sunflower kernels stored at 35, 75, and 95 F*

Storage (weeks)	Peroxide value (meq/kg)								
	Storage at 35 F Moisture, %			Storage at 75 F Moisture, %			Storage at 95 F Moisture, %		
	5.2	10.5	14.7	5.2	10.5	14.7	5.2	10.5	14.7
0	1.4	—	—	1.4	—	—	1.4	—	—
4	5.7	3.9	3.7	7.2	8.9	2.7	7.2	3.8	2.2
8	9.8	7.2	5.6	10.1	9.9	2.9	9.1	4.4	1.7
12	6.6	7.2	4.7	8.4	7.5	4.9	7.3	2.7	1.7

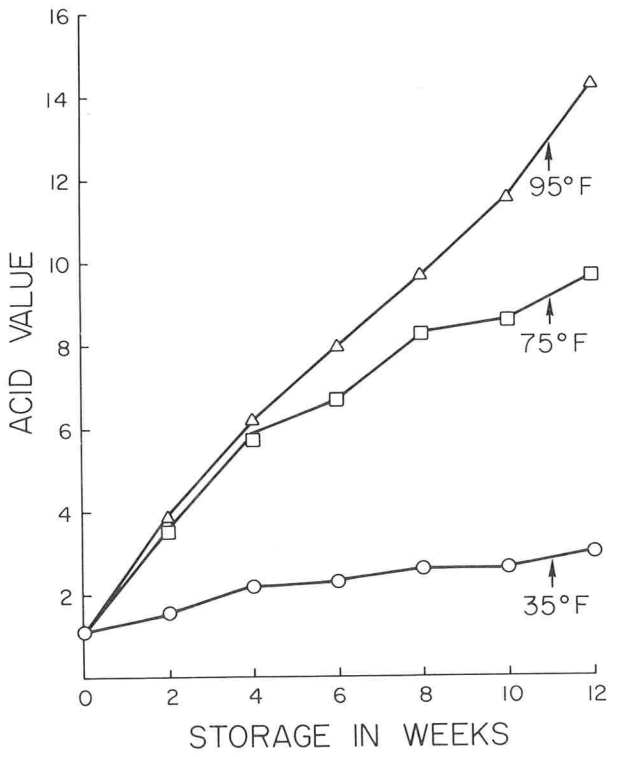

Figure 2. *Effect of storage on the acid value of the oil extracted from dehulled sunflower kernels with 10.5% moisture content.*

The effects of storage of sunflower kernels on acid value of the oil are shown in Fig. 1, 2, and 3. Acid value increased with temperature and moisture content. At 35 F, increase was small during 12 weeks storage at the three moisture levels. At 75 and 95 F, the acid value of the kernels increased substantially throughout storage and the increases generally were greater at the higher moisture levels.

Immediately after the bags were opened, the kernels were screened for obvious changes in color and flavor. At 35 F and 5.2% moisture content, the flavor and color of the sunflower kernels remained satisfactory during 12 weeks of storage. At 10.5%, slight off-color developed after 6 weeks and slight off-flavor, after 12 weeks. Samples with 14.7% moisture developed pronounced musty odor, sweet off-flavor, and dark color by the end of 6 weeks of storage.

When the acid value of the oil was about 4 or higher, kernels had a pronounced acid or sour flavor. At 35 F, acid values did not exceed 3 and pronounced acid flavor did not develop. Samples with 5.2% moisture had a sour flavor after 10 weeks at 75 F and after 4-6 weeks at 95 F. Also kernels with 10.5% moisture stored at 75 and 95 F were sour, dark in color, and unfit to eat by the end of 2 weeks of storage. Kernels stored at 95 F had a burnt flavor.

Effect of storage on the microflora

Aerobic plate counts of dehulled sunflower kernels during storage at three temperatures and moisture contents are shown in Fig. 4. The initial count was log

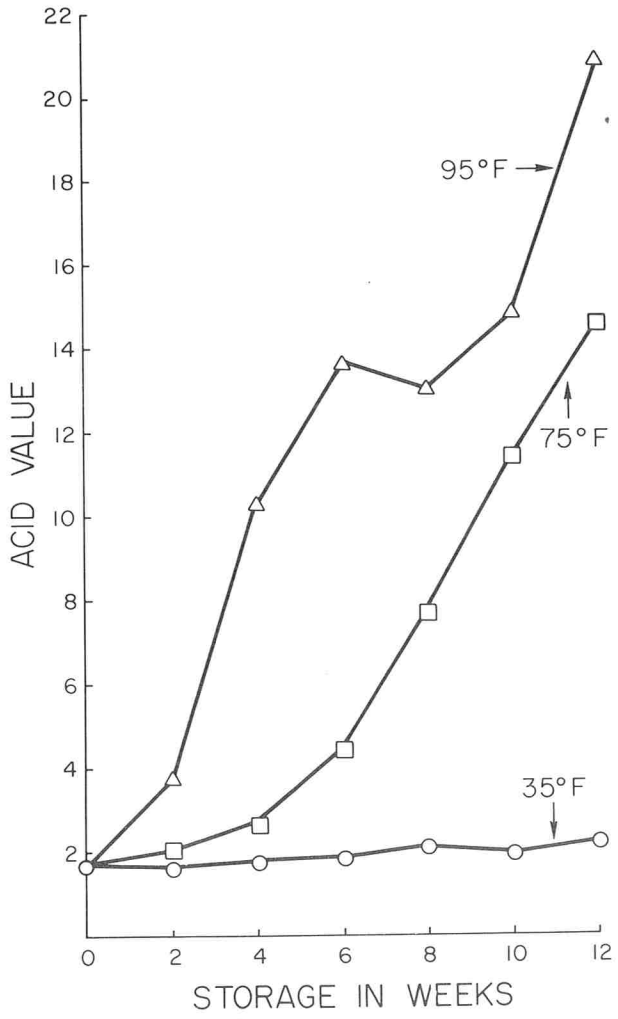

Figure 3. *Effect of storage on the acid value of the oil extracted from dehulled sunflower kernels with 14.7% moisture content.*

6.83/g. At 35 F, after a small initial decrease in count the first 2 weeks, there was no particular change in count at any of the three moisture levels. At 75 F, particularly for kernels stored at 5.2 and 10.5% moisture, counts rapidly decreased during the first 2 weeks and then more gradually with no particular difference due to moisture content. At 95 F, counts of samples of 5.2% and 10.5% moisture content decreased rapidly 2 weeks and after 12 weeks were log 3.78 and log 3.11, respectively. Counts of kernels stored at 14.7% moisture decreased during the first 2 weeks, then increased to a high of log 7.18 after 6 weeks. Data in Table 3 indicate that these microorganisms were yeast and not bacteria.

Reduction in microbial count of sunflower kernels may have been due to depletion of oxygen in sealed plastic bags in which samples were stored since most of the microorganisms were aerobes. The oxygen was removed by respiration of the microorganisms at well as by seed respiration. Respiration was obvious at 75 and 95 F storage because after 4 weeks the plastic bags of 14.7% moisture kernels were turgid with gas. Reduction in count also might have been due to the increase in free

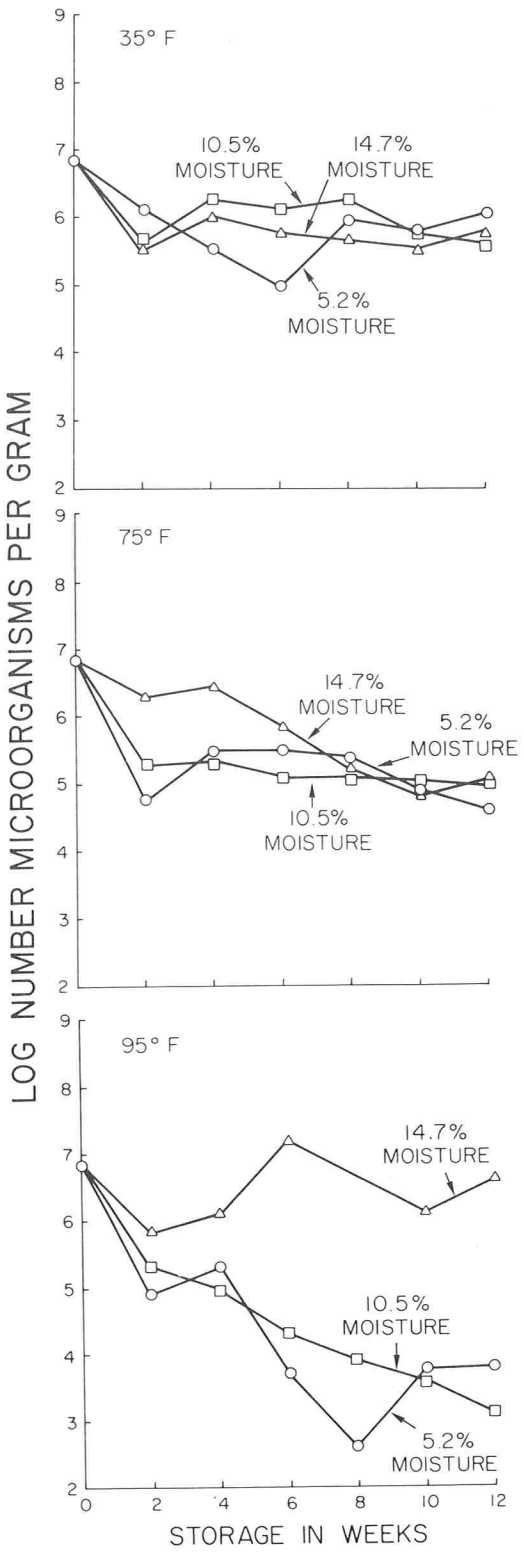

Figure 4. *Logarithms of the number of microorganisms per gram (aerobic plate count) of dehulled sunflower kernels stored at 35, 75, and 95 F at three different moisture levels.*

fatty acid. Karabinos and Ferlin (*8*) found that fatty acids in the C9 to C12 range are bactericidal to a number of organisms. They also reported that oxidation of oleic acid produced a volatile compound which was highly bactericidal to *Staphylococcus aureus.*

The reduction in microbial count is indicated by the *Enterobacteriaceae* count shown in Table 2. Counts decreased rapidly at 75 and 95 F. Kernels containing 14.7% moisture contained no *Enterobacteriaceae* after 2 weeks storage at 95 F. At 35 F, counts decreased during the first two weeks, but changed little after that and were similar for all moisture contents.

At 4 and 6 weeks of storage, VRB agar plates containing about 20 typical *Enterobacteriaceae* colonies per plate were randomly selected and isolated from all the colonies on each plate and identified. Approximately 80% of the microorganisms were identified as *Enterobacter agglomerans;* the other were of the genera *Pectobacterium* and *Klebsiella.* The species nomenclature was based on the best classification criteria available at the time the study was conducted. However, with the recent publication of the 8th edition of *Bergey's Manual of Determinative Bacteriology* (*2*), the following should be noted: *Enterobacter agglomerans* and *Pectobacterium* are now recognized as *Erwinia herbicola. Escherichia coli* was not detected. The absence of *E. coli* indicated that the kernels were not contaminated with fecal matter during the decortication of the sunflower seed in spite of the high *Enterobacteriaceae* count. However, these data suggest that good sanitary practices and principles should be followed in the handling and packaging of dehulled sunflower kernels.

Yeast and mold counts were variable (Table 3). In general, counts of low and medium moisture kernels declined during storage at 75 and 95 F. Beginning with the fourth week of storage, the yeast and mold count of the high moisture samples at 75 and 95 F began to increase particularly at 95 F. Based on observations of cultural and morphological characteristics, the number of mold colonies decreased and yeast increased. After 10 weeks at 95 F, most of the organisms on the plates were yeast. These data indicate that the high total plate count for kernels stored at 95 F and 14.7% moisture (Fig. 4-95 F) was primarily due to facultative anaerobic yeast and not to bacteria.

Chemical and microbiological data indicate that the dehulled sunflower kernels should be stored at low temperatures and low moistures to maintain their desirable flavor and color characteristics. Even storage of kernels in a confined atmosphere, probably at low oxygen level did not prevent growth of microorganisms, such as facultative anaerobic yeast, that can cause rapid deterioration of sunflower kernels at higher temperature and moisture levels.

Decortication study

Since the freshly dehulled sunflower kernels from the storage studies had a relatively high aerobic plate count (log 6.83/g) (Fig. 4), a preliminary survey was made of three sunflower dehulling operations to determine if kernels were being contaminated during the dehulling operation. Microbiological counts of whole confectionery sunflower seed and dehulled kernels from different

TABLE 2. Enterobacteriaceae *count of dehulled sunflower kernels during 12 weeks storage*[a]

Storage temp. (F)	Moisture[b] (%)	Storage (weeks) 0	2	4	6	8	10	12
35	5.2	6.15	6.08	5.36	4.23	5.36	4.77	5.42
	10.5	—	4.96	5.72	4.15	5.52	5.15	4.38
	14.7	—	4.72	5.61	5.15	5.15	4.28	5.30
75	5.2	6.15	5.18	4.48	4.72	4.69	4.15	3.90
	10.5	—	4.48	3.30	2.90	4.11	0.00	0.00
	14.7	—	4.38	2.30	0.00	0.00	0.00	0.00
95	5.2	6.15	4.40	3.95	2.70	0	2.90	0
	10.5	—	0	0	0	0	0	0
	14.7	—	0	0	0	0	0	0

[a]Duplicate count; log of numbers per gram of sample.
[b]Moisture content of kernels at beginning of storage.

TABLE 3. *Yeast and mold count of dehulled sunflower kernels during 12 weeks storage*[a]

Storage temp. (F)	Moisture[b] (%)	Storage (weeks) 0	2	4	6	8	10	12
35	5.2	3.65	3.36	3.35	2.85	3.48	3.80	3.28
	10.5	—	2.35	2.35	2.63	2.81	2.56	2.54
	14.7	—	2.95	2.65	3.15	2.74	2.93	2.35
75	5.2	3.65	3.06	3.15	3.31	3.18	3.26	2.88
	10.5	—	2.20	2.46	2.15	2.26	1.74	3.10
	14.7	—	3.49	3.97	3.18	2.56	3.71	4.21
95	5.2	3.65	3.08	2.93	2.63	2.57	2.56	2.00
	10.5	—	2.19	2.23	1.78	1.54	1.60	1.18
	14.7	—	2.42	TNC[c]	TNC[c]	4.81	6.11	6.59

[a]Duplicate count; log of numbers per gram of sample.
[b]Moisture content of kernels at beginning of storage.
[c]Too numerous to count at dilution used.

TABLE 4. *Microbial counts of whole sunflower seed and dehulled kernels from three dehulling operations*

Processor	Whole seed: Standard plate count	Whole seed: Entero-bacteriaceae count	Whole seed: Yeast & mold count	Dehulled Kernels: Standard plate count	Dehulled Kernels: Entero-bacteriaceae count	Dehulled Kernels: Yeast & mold count
	(Log of number of colonies per gram)					
A	6.84	6.05	3.40	5.40	4.54	3.10
B	7.25	6.48	3.46	6.17	5.21	2.95
C	7.69	7.21	3.50	5.98	5.47	3.67

processing operations are shown in Table 4.

The aerobic plate counts of whole seed were variable ranging from log 6.84/g to log 7.69/g. The total count of dehulled kernels ranged from log 5.40 to log 6.17/g. The dehulled kernel plate count was only about 2% to 8% of the plate count of the whole seed.

The *Enterobacteriaceae* counts of the whole seed and dehulled kernels ranged from 11.1 to 16.7% of the plate count for two of the processors and from about 31 to 33% for the other processor. The yeast and mold counts were variable but low.

In the dehulling of confectionery sunflower seed, the hull is removed from the seed in an impact dehuller where the seed is broken apart and hulls with a high surface contamination of microorganisms come in contact with the almost sterile kernel for a short period. During dehulling and subsequent screening, contamination of kernels by hull microorganisms is inevitable. Results obtained in the present study, however, indicate that contamination of kernels from seed hulls or from dehulling equipment will be low.

ACKNOWLEDGMENTS

The authors are indebted to Dr. N. A. Cox for identification of the *Enterobacteriaceae.*

Use of a company and/or product name by the Department does not imply approval or recommendation of the product exclusive of others which may also be suitable.

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Longer Shelf Life for Milk Seen from Maryland Research

Last fall (1974) the dairy science and agricultural engineering departments at the University of Maryland in College Park began research on a substitute process for pasteurization that may increase the shelf life of milk indefinitely.

This research was facilitated by a $24,000 grant from an equipment manufacturer, DASI Industries, Inc., of Silver Spring (Mongtomery county). (RADIO NOTE: DASI is pronounced as "Daisy.")

Now the research is well under way. And University scientists are satisfied that the process of ultra-high (UHT) treatment with the patented DASI FreeFalling-Film heater has definite possibilities for prolonging the shelf life of milk without affecting its taste.

Heading the Maryland research team is Dr. Joseph F. Mattick of College Park, a professor of dairy science. His principal assistant is Dr. Dennis C.Westhoff of Beltsville, an associate professor in the same department. Both are Prince Georges county residents.

Installation of the equipment last fall and winter was supervised by Dr. Andrew M. Cowan of Frederick, a former associate professor of agricultural engineering at the College Park campus. Dr. Cowan is now employed by the U.S. Department of Agriculture at Beltsville, Md.

Dr. Mattick and Dr. Westhoff have been studying microbial counts, keeping quality and flavor of the milk.

Major questions which the research team raised at the onset of the project were: Does the equipment work? What do potential consumers think of the taste? Other questions have included: How does the new process affect the protein and other nutrients in milk? How does it affect cheese produced from UHT-treated milk.

Although the research is not fully completed, it appears that the technology involved does provide tremendous potential for future dairy processing operations.

Informal taste-panel tests of the UHT-treated milk began in September at the University's dairy sales room, located along U.S. highway 1 in College Park. They are conducted with drop-in customers each Tuesday afternoon under the supervision of Steven R. Framm, a first-year graduate student in food science.

Framm hails from North Bellmore, N.Y. He is a graduate of Nassau Community College and Penn State University.

The dairy sales room, site of the taste-testing, has long been famous for its ice cream, made on the premises with milk from Holstein and Guernsey cows at the nearby dairy barn on the College Park campus.

The UHT equipment has been installed in Turner laboratory, the University of Maryland's dairy science research facility in the rear of the dairy sales room at College Park. It represents an accumulation of 20 years of research in both the U.S. and Europe.

Earlier experimental UHT sterilizers have been operated in this country at both Michigan State University and the U.S. Department of Agriculture's Eastern regional research laboratory, formerly located in Washington, D.C.

Equipment used in the other laboratories had problems in matching economic feasibility with production of milk that was both sterile and had good flavor. The Maryland equipment has changes in design which hold good promise of making the operation commercially feasible.

In recognitiion of its apparent promise, the FreeFalling Film UHT heater being tested at the University of Maryland has earned for DASI Industries, Inc., a "Top Honors" citation in the fifth biennial Putman Food Awards competition for 1973-74.

The presentation to DASI officials was made on Oct. 14 at a University of Maryland "dairy press day" at the College Park campus. Making the presentation was Roy Hlavacek, editor of *Food Processing,* a Chicago-based trade magazine of the Putman Publishing Company.

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A Collaborative Study of the Microtiter Count Method and Standard Plate Count Method for Viable Cell Count of Raw Milk

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ABSTRACT

The Microtiter Count method was compared with the Standard Plate Count (SPC) method in four different laboratories. An unamended as well as a modified procedure were used. Statistical analysis showed that at the 95% confidence limit the amended procedure was reliable compared with the SPC for making a viable cell count of raw milk. The Microtiter Count method was credited with savings of time, space, and material. However, the operator needs some practice and comparative testings before using the method for routine analysis.

A Microtiter method to enumerate viable cells in bacterial cultures and milk has been developed and tested by Fung and Kraft (*3*), and Fung and LaGrange (*4*). This procedure was also used to evaluate heat destruction of bacterial spores by Baldock et al. (*2*). Recently Casas and Leon (Abstr. Annu. Meet. Amer. Soc. Microbiol. 1974:13) reported that the procedure as outlined by Fung and LaGrange (*4*) compared favorably by statistical methods with the Standard Plate Count in evaluating viable count of mesophiles, psychrotrophs, and coliforms from raw milk samples.

To have a more objective evaluation of this technique, a collaborative study was made by two state public health laboratories and two university laboratories on viable cell counts of raw milk using the proposed Microtiter Count method and the Standard Plate Count Method. This report describes the results of the study.

MATERIALS AND METHODS

Samples

All four laboratories were asked to use the raw milk samples they routinely analyze or those obtainable from local sources. A maximum of 100 samples were examined. In most instances the tests were made by experienced technicians and researchers but in one instance an undergraduate student with minimum microbiology experience was asked to do some comparisons.

Evaluation procedures

Standard Plate Counts were made using the procedure described in *Standard Methods for the Examination of Dairy Products* (*1*). The Microtiter Count method followed procedures described previously (*3, 4*). Each milk sample was analyzed in duplicate by each method. The Microtiter Count method consists of rapid serial dilution and spot plating procedures. All microtiter equipment was obtained through the courtesy of Dynatech Laboratory Inc. (Alexandria, VA). A sterile Cornwall Syringe (Becton, Dickinson and Company, Rutherford, NJ) was used to dispense 0.225 ml of buffer solution into each of 96 wells in a pre-sterilized, plastic microtiter "flat-bottom" plate (8 × 12 wells). Small, 0.025-ml aliquots, milk samples were introduced to the first of each series of four wells by pre-calibrated sterile loops. The first row of wells gave 1:10 dilutions of the original samples. The loops were rotated rapidly in the dilution buffer 30 times to ensure homogeneity before being introduced into the next set of wells containing sterile buffered diluent. Serial dilution in this manner to the fourth row of wells gave dilutions of the original sample of 10^{-1} through 10^{-4}. Although further dilutions could be made, for testing raw milk up to 10^{-4} dilution was sufficent. The most convenient number of loops to be operated simultaneously was four; however, more or fewer loops could be operated at one time depending on the skill of the operator and the number of samples present.

Two drops of each dilution to be tested were spotted, using sterile 0.025- or 0.050-ml droppers, onto the surface of Standard Plate Count agar plates which had been previously poured and dried overnight. One sterile pipette was used for the same series by first spotting the 10^{-4} dilution then subsequently to the 10^{-1} dilution. Four to 8 drops could be spotted on the surface of one petri dish. The spotted plates, after drying for about 30 min at room temperature, were incubated at 32 C for 15 to 20 h before counting the colonies. After preliminary testing, an amendment to the original protocol was made. This included overlaying a small amount of agar after the spotted drops were absorbed into the agar plate and prolonging incubation time to 48 h.

Enumeration of cell density

For the Standard Plate Count, plates containing 30-300 colonies were used to calculate cell density. In some instances when only one dilution was plated, plates with colony numbers not within the above range were also counted. For the Microtiter Count method, spots containing an arbitrary range of 10-100 colonies were used. A stage microscope (Spencer, American Optical Co., Buffalo, NY) at a magnification of 10 times was used to facilitate counting when desired. The counts were then multiplied by the appropriate conversion factors (to per ml) and dilution factors to estimate the cell densities in the original samples. When the numbers of colonies on the spots of two consecutive dilutions fell within the range of 10-100, the number of the lower dilution was recommended to be used for calculation as a lower dilution has less inherent error.

RESULTS

Raw data from the four laboratories were collected by the coordinator and analyzed by the t-test for paired samples (*6*) using the MINITAB 2 program (*5*) and the

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computer facilities at The Pennsylvania State University. Data were tabulated in sets of 25 comparisons (in duplicate) each. A total of 12 sets was collected. Statistical manipulations of each set as well as of two large groups were made and are tabulated in Table 1.

TABLE 1. *Combined Comparative Data of Microtiter Viable Counts and Standard Plate Counts*

Set no. (duplicateof 25 samples per set)	t-value[1]	a, significant at	Reject H_0: $\mu_1 = \mu_2$ at $a = 0.05$
Unamended			
1	-1.611	0.1136	cannot
2	-4.325	0.0001	can
3	0.633	0.5298	cannot
4	-2.521	0.015	can
Sets 1-4	-2.947	0.0036	can
Amended			
5	0.354	0.7249	cannot
6	0.073	0.9419	cannot
7	-1.430	0.159	cannot
8	-1.095	0.2787	cannot
9	1.819	0.0751	cannot
10	2.203	0.0323	can
11	-0.023	0.9820	cannot
12	-0.106	0.9159	cannot
Sets 5-12	1.733	0.0839	cannot

[1]t-test for paired samples $t = \dfrac{\bar{d} - 0}{\dfrac{S_d}{\sqrt{n}}}$

$\bar{d}$ is the average of the sample differences
S_d is the sample standard deviation of the difference
n is the sample size

Sets 1-4 are data obtained by the unamended procedure (i.e. no agar overlay, overnight incubation only), while sets 5-12 were obtained by the amended procedure (i.e. agar overlay, 48-h incubation). Differences between means of treatment groups in sets 1 and 3 were not statistically significant at the 95% confidence limit. However, sets 2 and 4 as well as combined data of all four sets showed significant differences between means of treatment groups at 95% confidence limit. Under this statistical protocol these data indicate that the unamended Microtiter Count procedure was not satisfactory for milk count when compared to the Standard Plate Count procedure.

With the exception of set 10, data of all the sets obtained by the amended procedure showed no significant differences between treatment means at the 95% confidence limit. The combined t-value of the eight sets also showed no significant differences between means. These data indicate that the amended procedure is reliable in making viable cell counts when compared with the Standard Plate Count procedure.

Originally these data were analyzed by the statistical protocol suggested in the chapter "Simplified Techniques for Viable Counts of Raw Milk" in *Standard Methods for the Examination of Dairy Products* (*1*). After analysis of data it was decided that the statistical protocol should not be used for this type of study and that the conventional t-test should be used.

DISCUSSION

The primary objective of this project was to evaluate the accuracy of the Microtiter Count method compared to the SPC method on raw milk counts by four different laboratories following the same protocol and using the same equipment and supplies from one manufacturer. Most of the sets showed that results from the amended Microtiter method compare favorably with those from the SPC by the statistical criteria employed. It was found, however, that the unamended Microtiter Count method was not satisfactory. Thus the amended procedure is suggested for continued investigative efforts.

Several comments by those who actually performed the tests are summarized as follows. Positive comments concerning the Microtiter Count: (*a*) saving of space in operation and incubation; (*b*) savings of diluent, agar, and petri dishes; (*c*) savings of pre- and post-experiment manipulations, i.e. no need to make and prepare large numbers of bottles for dilution blanks and agar, and petri dishes and related sterilization and clean-up of glassware; and (*d*) savings of operational time in terms of making dilutions and plating; this is especially advantageous when high cell density is expected from samples.

Negative comments concerning the Microtiter Count method: (*a*) need some time to master the technique, more skill is involved than in the SPC method; (*b*) when a laboratory accident occurs more samples will be lost per plate compared with SPC method; and (*c*) automatic pipetting syringe must be checked frequently for accuracy, and proper gravimetric calibration is difficult and tedious.

In conclusion, the amended Microtiter Count method was found to be statistically acceptable for making viable cell counts of raw milk when compared with the SPC method. However, in using the method an operator must master the technique and do comparative tests using a t-test for paired samples (*5*) as statistical support.

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Changes in the Microflora of Bovine Colostrum During Natural Fermentation

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ABSTRACT

Colostrum from five cows was collected and pooled separately and allowed to ferment naturally for 21 days. During this time the pH decreased and was approximately 4.0 at the end of the fermentation. The titratable acidity of the colostrum at this time was 1.5% or more. Numbers of total aerobic bacteria, fecal streptococci, coliform bacteria, and gram-negative bacteria increased rapidly during the first 2-3 days of fermentation. After several days of reasonable stability, numbers of aerobic bacteria and coliform bacteria declined, whereas numbers of fecal streptococci and gram-negative bacteria remained fairly constant. The number of yeasts and molds increased much more slowly than did those of bacteria and in some instances growth of these organisms was still evident when the fermentation was terminated.

Dairymen have observed that feeding fermented colostrum is beneficial to the well being of calves (*4,5*). Aside from the obvious nutritional quality of the product, other benefits claimed for fermented colostrum include: (*a*) reducing the incidence of scours caused by enteropathogenic strains of *Escherichia coli,* and (*b*) providing the calf with a rich source of immune globulins. In addition, fermented colostrum should have an improved keeping quality so it can be stored easily and fed to a calf long after its mother's milk has become normal in composition.

In practice, colostrum obtained from four, five, or more milkings after parturition is pooled in a suitable container and then stored for up to 3 weeks during which it undergoes a natural fermentation. During the early stages of the fermentation fresh colostrum is added periodically and fermented colostrum is removed from the lot at intervals when it is fed to calves. Generally, fermented colostrum is diluted with water before it is fed; to feed it undiluted might actually serve to enhance rather than retard scouring.

Relatively little work has been done on the microbiology of fermented colostrum although results of limited tests were reported by Palmer and Mudd (*3*). Consequently, a study was initiated to learn more about the microbiology of naturally fermented bovine colostrum. Results of the work are described in this paper. A preliminary report of this work has been given (*6*).

MATERIALS AND METHODS

Bovine colostrum was collected separately from five cows. Three of the cows were Holsteins (A, B, and E) and the other two were Aryshires (C and D). Colostrum was collected for 10 milkings following parturition and stored in 20-gal. containers at ambient temperature (15-25 C) in a dairy barn. Colostrum was added to the container as cows were milked and also was removed as needed to feed calves. Storage of colostrum ended after three weeks.

Sampling

At intervals during the fermentation period, each lot of colostrum was mixed well and then a sample was taken aseptically. Samples were immediately taken to the laboratory and tested.

Microbiological examination

Serial dilutions of colostrum were made with sterile phosphate-buffered distilled water and were plated in duplicate. Pour plates were made to determine: (*a*) total plate count: Plate Count agar (Difco) and incubation at 32 C for 48 h; (*b*) gram-negative count: Plate Count agar (Difco) + 35 units of penicillin G/ml and incubation at 32 C for 48 h; (*c*) yeast and mold count: acidified (to pH 3.5 with sterile tartaric acid) Potato Dextrose agar (Difco) and incubation at 22 C for 3-5 days; (*d*) coliform count: Violet Red Bile agar and incubation at 32 C for 24 h: (*e*) streptococcus count: KF Streptococcus agar (Difco) and incubation at 37 C for 48 h: (*f*) total lactic count: APT agar + 0.04% sodium azide and incubation at 30 C for 5 days.

After plates were incubated as described, colonies were counted, and average values were calculated. Between 50 and 100 colonies each were picked from the APT agar + 0.04% sodium azide, KF Streptococcus agar, and Violet Red Bile agar. Appropriate tests were done to permit identification of the isolates as to the genus and species.

Identification of isolates

Isolates obtained from Violet Red Bile agar were tested for gram reaction, ability to ferment lactose, reaction to the IMViC series of tests, and changes produced on TSI agar slants.

Streptococci obtained from KF Streptococcus agar plates were identified on the basis of gram stain; catalase reaction; growth at 10, 45, and 50 C; survival after heating at 60 C for 30 min; and growth in 6.5% NaCl, at pH 9.6, in 0.1% methylene blue, litmus milk, ethyl violet azide broth, and 0.04% potassium tellurite.

Isolates from APT agar + 0.04% sodium azide were identified by using the same characteristics that served to identify isolates from KF Streptococcus agar.

Determination of pH and titratable acidity

The pH was determined with a Corning pH meter (Model 10). Titratable acidity was determined by titrating replicate 9-g samples with 0.1 N NaOH to pH 8.5 using the same pH meter.

RESULTS

Changes in the pH of colostrum are recorded in Table 1. The pH value of three samples dropped from about 6.4 to 4.5 or below by the end of the first 4 days of

TABLE 1. *pH Values of colostrum during fermentation*

Time (days)	Time of day	Cow A	Cow B	Cow C	Cow D	Cow E
1	A.M.[a]	6.23	6.37	6.50	6.20	6.25
	P.M.[b]	6.22	6.51	6.10	5.52	6.25
2	A.M.	5.65	5.85	—	5.50	6.20
	P.M.	5.42	5.82	5.00	5.12	4.85
3	A.M.	4.90	5.12	4.85	5.15	4.87
	P.M.	4.73	5.29	4.45	5.00	4.87
4	A.M.	4.52	5.05	4.50	4.90	4.65
	P.M.	4.08	5.20	4.39	4.88	4.48
5	A.M.	4.52	5.10	4.35	4.85	4.58
	P.M.	4.27	5.00	4.35	4.82	4.60
7	—	4.25	4.65	4.25	4.78	4.42
10	—	4.32	4.39	4.23	4.72	4.37
14	—	4.25	4.30	4.30	4.70	4.20
21	—	4.00	4.00	4.10	4.12	4.10

[a]Sample taken after colostrum obtained in the morning was added to the lot.
[b]Sample taken after colostrum obtained in the evening was added to the lot.

fermentation. The drop in pH of the other two was less abrupt during the initial stages of fermentation. At the end of storage, the pH of all samples was approximately 4.0 and the titratable acidity (data not shown) of three of the samples was 1.5% or more. The somewhat erratic changes in pH during the first 5 days resulted because colostrum was added to the lot twice each day.

The change in the aerobic plate count is documented in Fig. 1. Numbers of microorganisms in colostrum increased rapidly during the first 2 days to approximately 10^9/ml. Then the numbers tended to stabilize, with a few erratic exceptions, until about 7 days after which a decline in viable aerobic population was observed. After 21 days of storage, numbers of aerobic microorganisms in colostrum still were large—approximately 10^8/ml. Changes in microbial population were similar in colostrum from the different cows.

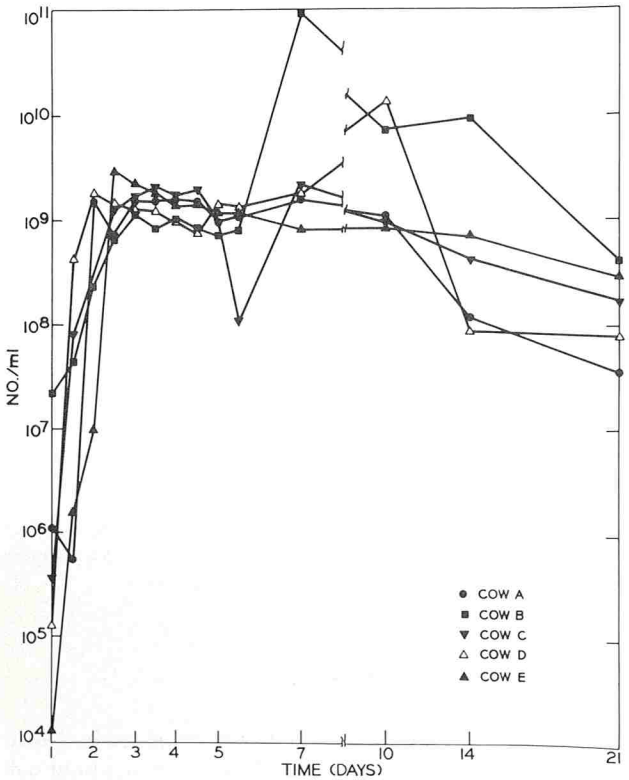

Figure 1. *The aerobic plate count of colostrum during fermentation.*

Figure 2 shows the numbers of lactic acid bacteria in colostrum during fermentation. The numbers of these bacteria increased during the first 7 days to 10^6 to 10^7/ml, then leveled off. After 21 days, the number of lactic acid bacteria still ranged between 10^5 to nearly 10^8/ml, depending on the sample. There also was considerable variation in number of lactic acid bacteria (as measured by APT agar plus azide) that developed in colostrum from different cows. Eighty seven colonies were isolated from APT agar and all were *Streptococcus faecalis.*

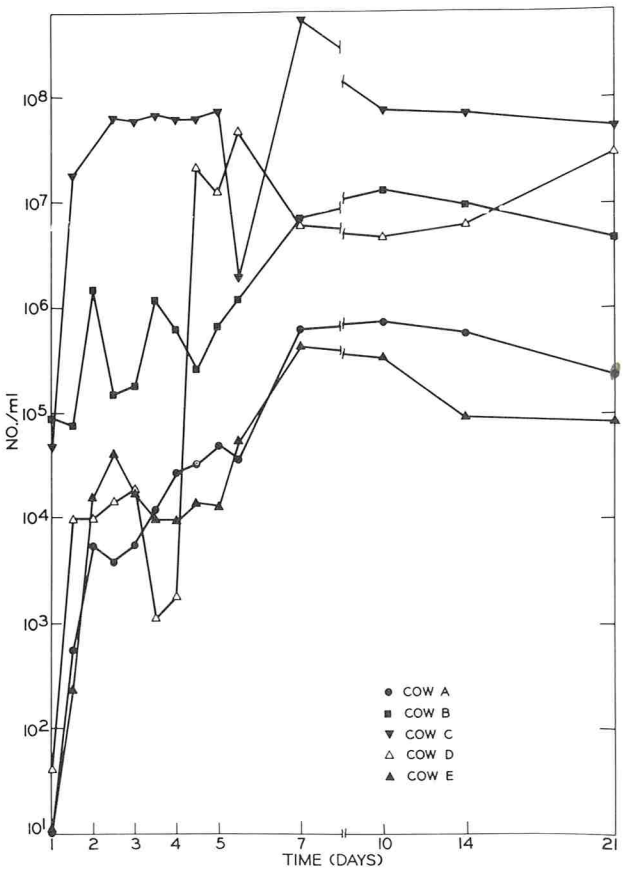

Figure 2. *Numbers of lactic acid bacteria, as determined with APT agar plus azide, in colostrum during fermentation.*

Figure 3 shows the numbers of fecal streptococci; these results parallel the data for the lactic acid bacteria. Numbers increased markedly for the first 2 days, then leveled off, but final numbers varied from 10^4 to 10^8/ml for colostrum from the five cows. All 60 colonies isolated from KF Streptococcus agar were *S. faecalis.*

Figure 4 shows the coliform count of colostrum. Numbers increased rapidly for the first 3 days to 10^7 to

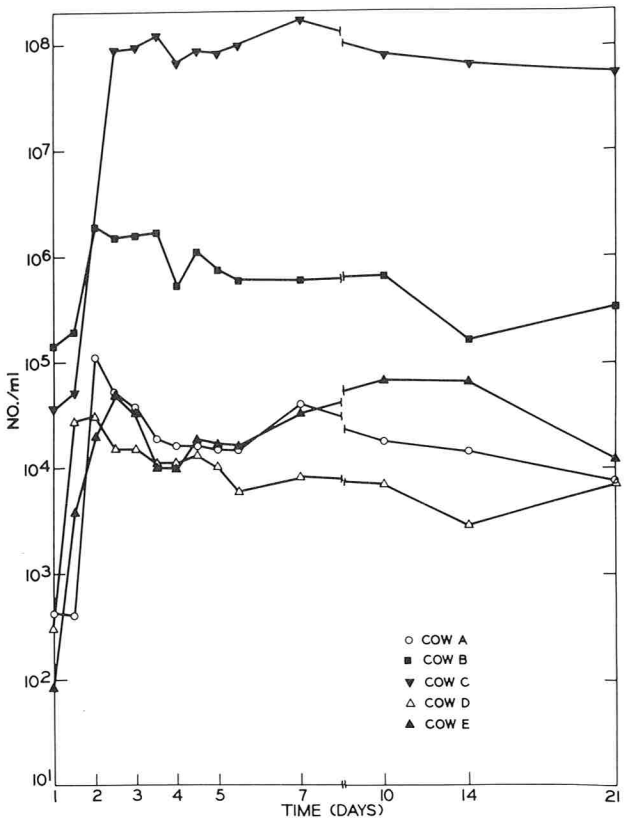

Figure 3. *Number of fecal streptococci, as determined with KF Streptococcus agar, in colostrum during fermentation.*

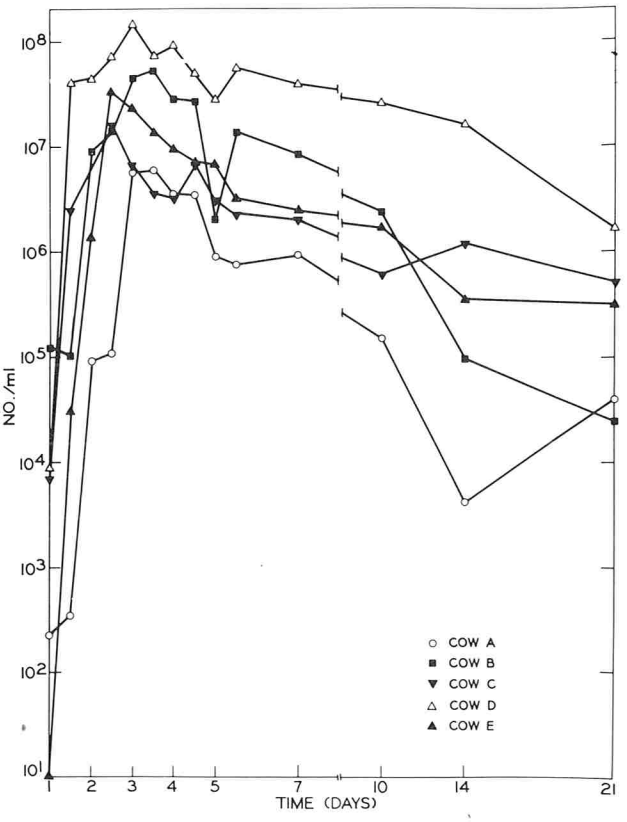

Figure 4. *Number of coliform bacteria, as determined with Violet Red Bile agar, in colostrum during fermentation.*

10^8/ml, and then declined after 5 days. Numbers of coliforms in colostrum from some cows decreased by three logs. At the end of storage, viable coliforms in colostrum ranged from 10^4 to 10^6/ml. Ninety eight percent of the 94 colonies isolated from Violet Red Bile agar were coliforms. Approximately 44% were *Escherichia coli,* 8% *Enterobacter aerogenes,* and 48% were intermediate forms.

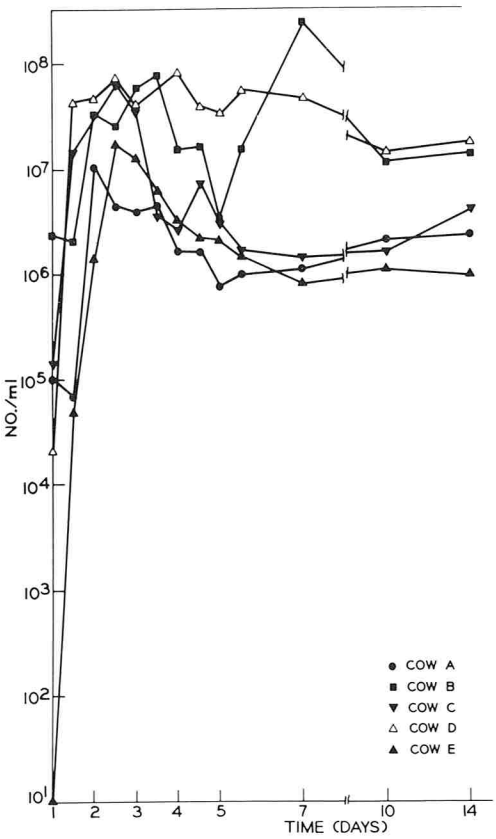

Figure 5. *Number of gram-negative bacteria, as determined with penicillin agar, in colostrum during fermentation.*

The gram-negative count is given in Fig. 5. Numbers of gram-negative bacteria increased rapidly for the first 3 days to 10^7 to 10^8/ml, then they decreased and leveled off after 5 days. At the end of the fermentation all milks contained more than 10^6 gram-negative bacteria per milliliter, as measured by this test.

Figures 6 and 7 give the yeast and mold counts of the different lots of colostrum. Numbers of yeasts (Fig. 6) increased for the first 10 days and populations of 10^5 to more than 10^6/ml were achieved after which the numbers leveled off. Thirty typical yeast colonies were isolated from Potato Dextrose agar and examined microscopically and all were yeasts. The number of molds (Fig. 7) increased gradually to 10^3 to 10^6/ml over the 21-day storage period. In some instances, further incubation probably would have resulted in more mold growth.

DISCUSSION

Palmer and Mudd (*3*) stored colostrum for three weeks

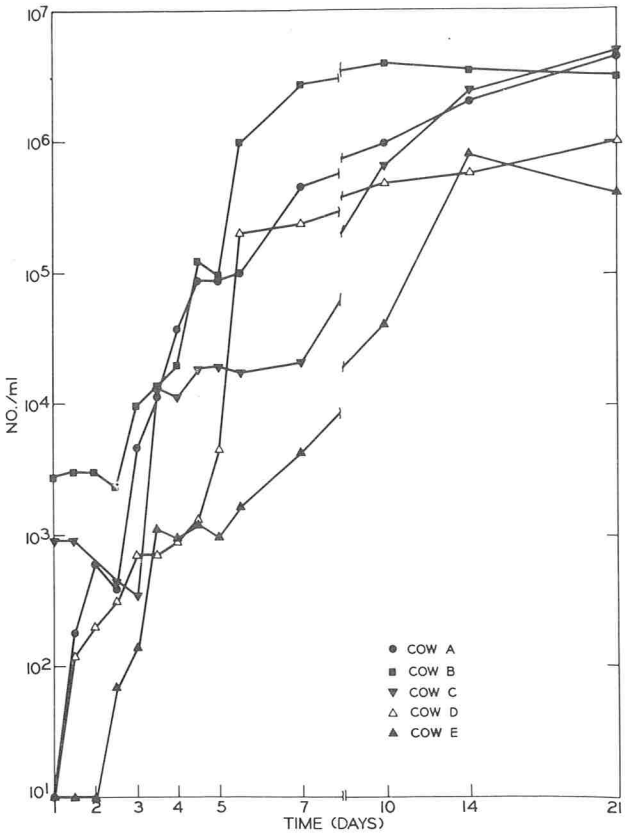

Figure 6. *Numbers of yeasts, as determined with acidified Potato Dextrose agar, in colostrum during fermentation.*

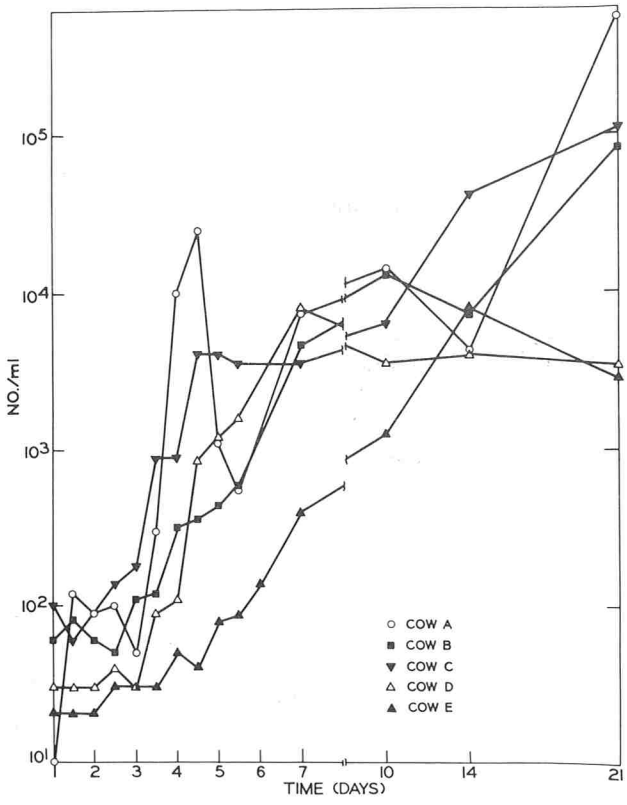

Figure 7. *Numbers of molds, as determined with acidified Potato Dextrose agar, in colostrum during fermentation.*

at 4 C, 30 C, and ambient temperature. They found an increase in total count during the first 3 days of storage followed by a leveling off or decline in the numbers. This happened at all temperatures of storage. Numbers of coliform bacteria tended to increase initially and then decreased markedly after 5 days of storage. Molds and yeasts tended to increase in numbers for the first 7 days and then leveled off. The titratable acidity of colostrum rose rapidly during the first 7 days of storage and then leveled off.

As did Palmer and Mudd, we found that the number of coliforms in colostrum increased rapidly, but declined appreciably after 5 days of fermentation. The increase in titratable acidity produced by the lactics and other bacteria may have accounted for the decrease in numbers of coliforms after initial rapid growth. However, the number of coliforms, 10^4 to 10^6/ml, was still relatively high after 21 days; therefore, the theory that the fermentation process produces sufficient lactic acid to eliminate *E. coli* from colostrum so the calf does not ingest the organisms in large numbers and hence does not develop scours may not be valid. While some dairymen find that colostrum is not effective in reducing scours unless it is a few days old, our data suggest that this time would allow development of a large population of coliforms that would persist throughout the storage period.

Infection with *E. coli,* colibacillosis, is a major cause of death in young calves. Colostrum contains antibodies against many *E. coli* strains, but antibodies against some strains of *E. coli* which cause infection in colostrum-fed calves, have not been found (*4*). Roy (*4*) described tests in which colostrum containing 10^7 *E. coli*/ml was fed, and it had no adverse effect on calves. Colostrum contains antibodies which are not present in normal milk, but the ability of the newborn calf to absorb antibodies is highest immediately after birth, and 36 h later the calf cannot absorb antibodies.

The chemical composition of colostrum is different from that of normal milk and is influenced by breed of the cow, individual cows within a breed, length of dry period, feed intake during the dry period, etc. (*1*). A batch of colostrum representing the first 5 days of production has about 15 to 18% solids and contains 10 times more Vitamin A and three times more Vitamin D than does normal milk. Colostrum is much higher in minerals such as iron than is normal milk.

It has been indicated (*1*) that colostrum is beneficial to the intestinal tract of calves. One theory is that antibodies help resist secondary bacterial infections that develop when scours is present. Another theory is that desirable or harmless bacteria replace the harmful ones in the intestine or that bacteria from the flora found in colostrum become implanted in the intestine and prevent invasion by harmful bacteria which can cause scours.

Colostrum is highly nutritious (it contains a large amount of protein) and hence its consumption should result in healthy calves which are less likely to become ill

than are calves that suffer from dietary deficiencies. Hence, use of fermented colostrum because of its nutritional value may be more sound than because it contains few coliform bacteria. The fermentation process probably does not change its nutritional value (although more work must be done in this area), but is a method to preserve colostrum for several weeks during which time it can be fed to calves. More studies should be made to determine the factors which result in a desirable fermentation. The possibility of controlling the fermentation by adding readily available cultured buttermilk to the fresh colostrum might be investigated. Also, the fermentation when allowed to progress naturally at temperatures higher than those used in this study, might yield an unsatisfactory product unless sufficient lactic acid bacteria for rapid acid production were present initially in the colostrum. Such experiments have been described recently by Muller and Syhre (*2*).

ACKNOWLEDGMENTS

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The Natural History of Anisakiasis in Animals[1,2]

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ABSTRACT

Adult anisakid nematodes are known to be parasites in the alimentary tracts of amphibians, reptiles, birds, and mammals. The known life cycle patterns among the anisakids are reviewed and from what is known, it is not possible to predict whether those hosts in which larval forms occur are true intermediate hosts or paratenic hosts. This is especially true of marine fishes in which third- and fourth-stage larvae are common.

Among physical parameters known to influence the survival, development, and distribution of anisakids, temperature apparently is an important one. Similarly, a series of experiments has revealed that two unidentified species of *Contracaecum* from marine fish are not infective to laboratory mammals primarily because of their inability to survive mammalian body temperatures.

It has also been demonstrated that *Contracaecum* larvae normally present in the digestive tracts of marine fishes will migrate out of dead hosts or into their mesenteries and musculature. Thus, in considering the potential public health importance of larval anisakids in marine fish, those in the digestive tract should not be considered as unimportant since they will migrate into muscles.

Ever since the reports by van Thiel et al. (*86*) and Kuiper et al. (*43*) in Holland that larval nematodes can cause an acute abdominal syndrome in humans, there has been an interest in these and related worms all over the world, especially in the Scandinavian countries, Britain, and Japan. Since all evidences indicate that human infections are derived from ingestion of raw or poorly cooked marine fish, the greatest interest has been in countries where fish constitute a major part of the protein diet. In fact, subsequent to the reports by van Thiel and his associates, Japanese scientists have reexamined the situation in their country and as Oshima (*57*) has pointed out, it is now documented that sporadic reports of cases of gastrointestinal eosinophilic granuloma have appeared in various Japanese medical journals since 1940. The situation was deemed to be of sufficient public health importance that a research group was formed with financial aid from the Japanese government in 1965 to conduct a survey and to establish a research program directed at this disease.

Although the syndrome in humans is now delineated, there is still some confusion as what should be the designation of this disease. Part of the confusion is due to disagreement among taxonomists as to what are the correct designations of the nematodes causing the disease. Some of the taxonomic problems have been reviewed by Myers in this symposium. To complicate matters, according to Chitwood (*14*), species of *Phocanema, Anisakis,* and *Contracaecum* can all cause "a dangerous parasitic syndrome in man." Thus, is the disease to be designated as phocanemiasis, anisakiasis, or contracaeciasis? Perhaps the best solution to this nomenclatural problem is as follows. Since the disease first recognized in the Netherlands was believed to be caused by a larval nematode designated as *Eustoma rotundatum* (*83, 43*), which was later renamed *Anisakis marina* by van Thiel (*65*), and since the gastrointestinal eosinophilic granuloma and associated symptoms in man resulting from infection by this group of larval nematodes are very similar if not identical, for the sake of identification, the designation of anisakiasis, which is already widely employed, is recommended. On the other hand, this group of nematodes is commonly also pathogenic to their intermediate and paratenic hosts, both invertebrates and vertebrates, and may also be pathogenic to their natural definitive hosts. Consequently, the question remains what the disease in hosts other than humans should be known as. I am recommending that the use of an adjective to designate the host should resolve this problem. For example, if a larval nematode of this group should cause a disease syndrome in a fish, the disease should be designated as piscian anisakiasis. If the pathological alterations occur in a crustacean, the syndrome should be designated as crustacean anisakiasis. In following this nomenclatural procedure, the disease in humans should be designated as human anisakiasis.

The purpose of this contribution is not to consider human anisakiasis, which has been reviewed by Oshima (*57*) and also by Jackson in this symposium. Rather, the intent is to consider the normal biology of the nematodes involved, i.e., what is known about their life cycles, their relationships with their normal intermediate, paratenic, and definitive hosts, and the impact of both micro- and macroecological parameters on the biology of these parasites. However, before reviewing and contributing to

[1] *This paper is the third in a series of five developed from presentations at the symposium, "Anisakiasis: A New Disease from Raw Fish," held under the auspices of the New York Society of Tropical Medicine on 14 March 1974 at Rockefeller University in New York City.*

[2] *The original data included in the paper have resulted from research supported by a contract (No. 223-73-2225) from the Food and Drug Administration, U.S. Department of Health, Education and Welfare.*

these aspects of the overall problem it should be mentioned that according to the systematic interpretation of Skrjabin (*78*), there are 21 genera belonging to the only subfamily Anisakinae of the family Anisakidae. In most instances nothing is known about the biology of the various species except the descriptions of the adult worms and the hosts from which they were collected. Nevertheless, such information has revealed that adult anisakid nematodes are parasitic in the alimentary tracts of amphibians, reptiles, birds, and mammals. Furthermore, in the case of mammals, these nematodes occur in both marine and terrestrial species. Because of the ubiquitous nature of the anisakid nematodes, a review of the normal biology of all of the known species would require several volumes. In fact, Skrjabin's (*78*) treatise, which deals exclusively with the alpha taxonomy of the Anisakidae, is 617 pages long. Consequently, for the purposes of this contribution, only the general patterns as they apply to those genera that occur as adults in marine vertebrates are being considered.

LIFE CYCLE PATTERNS

Although well over 200 species of anisakid nematodes have been described, there is surprisingly little information relative to the life cycles of these nematodes. However, drawing upon what is known about the life cycles of anisakids of terrestrial and freshwater vertebrates, and piecing together what has been reported from marine animals, it is generally recognized that the life cycles of the marine anisakids involve at least one, more commonly two, intermediate hosts, in addition to the definitive host (*47, 57*). Furthermore, alternative developmental routes may occur, at least in the case of *Contracaecum spiculigerum,* a parasite of marine piscivorous birds (*26*).

The definitive host, as stated, may be a piscivorous reptile, fish, bird, or mammal in the case of the marine anisakids. The adult worms normally occur in either the stomach or the small intestine. With those species that parasitize birds, the nematodes usually are buried in the mucosal wall of the proventriculus.

EGG

As eggs are laid, they are passed out into seawater in feces and as is the pattern among ascaroids, the first-stage larva undergoes the first molt while still within the egg capsule. The capsule or shell of ascaroids that parasitize terrestrial animals are usually thick and resistant to dessication, low temperatures, and certain chemicals; however, the eggs of anisakids that occur in marine vertebrates are thin-shelled and are nonresistant to dessication, low temperatures, and chemicals. This difference is undoubtedly of adaptive value to each category of nematodes since those eggs that are passed into marine waters generally do not come in contact with dessication, extreme variations in temperature, or chemical irritants, while all three types of environmental stresses are commonly encountered by eggs passed onto terrestrial habitats.

Although the intracapsular development of the first-stage larva and its subsequent molt have not been studied extensively, some information is available. Scott (*71*) has examined this stage of development of *Phocanema decipiens,*[a] a parasite of seals, by monitoring eggs removed from the uteri of three gravid females taken from the stomach of a harbor seal, *Phoca vitulina.* One group of eggs was kept in the dark at 2-4 C, the second also in the dark at 13-14 C, and the third was exposed to the normal 24-h light/dark regimen at 17 to 25 C. Scott found that development was very slow at 2-4 C. The embryos did not reach the morula stage until the end of the fourth day and gastrulation did not occur until the 18th days. No movement of larvae was observed even after 27 days. On the other hand, development was much more rapid at 13-14 C. Specifically, the morula stage occurred after 3 days, gastrulae were noted by the end of the 5th day, and movement of some embryos was observed after 7 days. Moreover, some eggs hatched as early as 13 days after isolation; however, all hatched by the end of the 14th day. Scott noted that the form escaping from the capsules were ensheathed second-stage larvae and these, although active, were attached to the bottom of the glass dishes by their posterior ends. These larvae averaged 200 μm long and little anatomical detail was observable other than the presence of a small stylet (penetration tooth) at the anterior terminal, and the esophagus and ventriculus which had differentiated.

According to Scott's (*71*) report, the most rapid development occurred at 17 to 25 C. At this temperature range, the morula stage was reached after 2 days, gastrulae were observed after 3 days, and movement of some embryos was noticed after 4 days. Furthermore, hatching began as early as the 8th day and all eggs hatched by the end of the 9th day. It was noted, however, that mass mortality of the escaped larvae occurred within a few hours. From these data it can be concluded that the intracapsular development, molt, and hatching of the larva of *P. decipiens* is temperature dependent and although the rate is greatest at 17-25 C, it would appear that these higher temperatures induced mortality.

Another similar study has been reported by Kobayashi et al. (*37, 38*). These Japanese investigators examined the development and hatching of what they considered to be a species of *Anisakis,* probably *A. simplex,* from the blue white dolphin. Examination of their reports leads one to believe that they were actually working with two species of nematodes since they reported the occurrence of two morphologically distinguishable types of adult females: one being slender and with the vulva situated in the anterior half of the body, and the other being stout and

[a] *Scott* (*75*) placed this nematode in the genus *Porrocaecum* while *Margolis* (*47*), *Oshima* (*57*), *and others consider it to be a member of* Terranova; *however, in following Chitwood* (*14*), *it isbeing assigned to* Phocanema.

with the vulva located in the posterior half of the body.

According to Kobayashi and his associates, eggs removed from females were ellipsoidal and measured 45.5-58.1 × 41.3-53.2 μm. When maintained in artificial seawater at 2, 7, 17, 27, and 37 C, ensheathed second-stage larvae commonly hatched at 27 C after 3 to 9 days of incubation, although there were variations in temperature dependency. For example, they reported that one batch began to hatch after 34 days at 2 C, after 14 days at 7 C, after 5 days at 17 C, but did not develop at 27 C. On the other hand, another batch did not develop at 7 C but began to hatch after 11 days at 17 C and after 3 days at 27 C. Thus, it would appear that one type of egg was killed at 27 C while the other was not, and the second type was inhibited in its development by low temperatures (7 C). These differences may be explained by the fact that Kobayashi et al. were actually examining eggs from two distinct species of nematodes as suggested by the morphology of the adult females.

According to Huizuiga (*26*), the eggs of *Contracaecum spiculigerum,* a parasite in the proventriculus of marine piscivorous birds, especially carmorants, are slightly ovoid and adhesive. From what is known, this is quite typical of the eggs of marine anisakids (*38, 39, 49, 52, 81*). Structurally, each egg capsule is comprised of two transparent or semitransparent layers: a smooth or slightly mammilated external layer and an inner vitelline membrane. Unlike the eggs of terrestrial ascaroids, the surface of eggs of marine anisakids is not covered with a proteinaceous tunic. In the case of *C. spiculigerum* eggs, Huizinga (*26*) has reported that they are negatively buoyant in 30°/00 seawater and sink at a rate of 0.14 cm/sec. Furthermore, although newly-laid eggs will remain viable at 7 C for up to 10 months with only 10% mortality, subsequent development and hatching in 10-14 days require higher temperatures, i.e., 21-23 C.

Additional work must be done on the temperature dependency of eggs of a variety of species of anisakids relative to development and hatching before any definitive statements can be made; however, from the limited data available, it would appear that there is a correlation between the ambient temperature and intracapsular development and hatching. Relative to this point, it is of interest to note that Davey (*18*) has reported that the ambient temperature of the macroenvironment appears to influence distribution of specific species of anisakids. Specifically, he has reported that *Anisakis typica* occurs in cetaceans from warm waters and *A. simplex* from hosts from colder waters. Since cetaceans are homeotherms, this observation suggests that the geographic distribution of anisakids is dependent upon the hatchability of eggs in the macroenvironment or perhaps also on the survival of larvae in poikilothermic intermediate or paratenic hosts. It is noted, that Oshima (*57*) has reported that eggs of an unspecified species of *Anisakis* can develop and hatch in seawater at 2 C.

In addition to temperature, a variety of other ambient factors may influence or control the development, molting, and hatching of the intracapsular larvae of anisakids. The effects of such factors as pO_2, pCO_2, pH, ionic concentration, and other physical factors need to be studied.

The egg and intracapsular development of another species of marine anisakid, *Contracaecum spiculigerum,* as well as a part of its life cycle, has been studied by Huizinga (*26*). Actually, Thomas (*81, 82, 83*) was the first to investigate aspects of the life cycle of this nematode. According to him, eggs passing out of the double-crested cormorant, *Phalacrocorax auritus,* hatch to the free-living larval stage in about 5 days at room temperature in water. Furthermore, he (*81*) reported that the parasites undergo two molts within the egg capsule and hence the escaping larvae is of the third stage. However, according to Huizinga (*26*), the intracapsular larva only molts once and hence the free-living larva is of the second stage. As a result of evaluating the sequence of events subsequent to this phase of the life cycle of *C. spiculigerum* as reported by Huizinga, it is quite evident that he is correct in that only one molt occurs within the egg capsule.

As with the few other anisakid eggs that have been studied, embryonic development with the egg capsule of *C. spiculigerum* appears to be temperature dependent. According to Huizinga (*26*) when these eggs are maintained in seawater, tap water, or distilled water at 21 C, early cleavage leading to formation of the blastula occurs in 1-2 days, early gastrulation leading to formation of the second cell layer occurs by day 3, sluggishly motile late gastrula stage, i.e., the so-called "tadpole" stage, occurs in 3-4 days, a motile first-stage larva is developed by days 4-5, and the first molt occurs between days 5 and 7. The second-stage larva, within the capsule, is ensheathed and motile. If maintained at 13 C, development of larva to the second stage is prolonged to 25-30 days, and if maintained at 7 C, development does not occur.

All of the second-stage anisakids that have been studied possess a stylet or boring tooth. During the hatching process, this anteriorly situated structure is pressed against the inner surface of the egg capsule and as a result of body undulations, bores a narrow hole through the capsule through which the larva exits. It is of interest to note the occurrence of a buttonshaped structure of unknown function within the egg of *C. spiculigerum* before hatching and this structure is retained within the empty capsule after hatching (*26*).

Probably the most detailed study to date on the egg and intracapsular development of a marine anisakid is that by Markowski (*49*) on *Contracaecum aduncum,* an intestinal parasite of piscivorous marine fish. Markowski has reported that a single molt occurs within the egg capsule but the second-stage larva does not hatch. Rather, the egg is ingested by a copepod and it is only after the egg reaches the crustacean's intestine that the ensheathed second-stage larva hatches.

Based on the available information reviewed above, it

would appear that the eggs of marine anisakids all undergo an incubation period in seawater. Subsequently, with one type of egg, an ensheathed second-stage larva hatches from the capsule and is free-living, while in the second type of egg ingestion by an intermediate host is necessary to induce hatching and the escape of the ensheathed second-stage larva.

FIRST-STAGE LARVA

It has been stated that the first-stage larva of anisakids develops within the egg capsule after an incubation period in the aquatic environment. Because of the intracapsular position of anisakid larvae at this developmental stage, detailed descriptions are not available. It is known, however, that developing first-stage larvae of *Contracaecum spiculigerum* do not stain with a 1:1000 aqueous dilution of methylene blue (*26*). Moreover, since the internal anatomy of second-stage larvae are not totally differentiated, except for the esophagus and ventriculus, it may be assumed that the degree of organogenesis in first-stage larvae is rudimentary.

SECOND-STAGE LARVA

The form escaping from the pliable capsule is the second-stage larva. As stated earlier, it bears a small boring tooth or stylet. Furthermore, both the esophagus and ventriculus is distinguishable but the rest of the alimentary tract is not clearly defined. Also, the second-stage larva is ensheathed in the cuticle of the first-stage larva. Its behavior, at least in the case of the second-stage larva of *Phocanema decipiens,* has been noted. Specifically, according to Scott (*71*), it is very active, wriggling and gyrating about a vertical plane. However, once it becomes attached to the bottom by its posterior end, it remains somewhat sedentary and does not move along the substrate.

The second-stage larva of *Contracaecum spiculigerum* has been studied by Huizinga (*26*). According to him, larvae at this stage are attached to the substrate by the aid of a viscous material at their anterior ends. They also often are attached to the empty egg capsules. Only once did he observe a larvae attached by its posterior end. Thus, it would appear that the attachment ends of the second-stage larvae of *P. decipiens* and *C. spiculigerum* differ. As to whether this difference is real remains to be reexamined. Moreover, further ethological studies must be done before it can be stated conclusively as to whether this behavioral pattern, i.e., the slow gyrating motion while attached at one end, is of survival value. It would appear, however, that this type of movement could serve as a signal for the first intermediate host.

As stated, it has been reported that eggs of *C. spiculigerum* will hatch in seawater, tap water, as well as distilled water (*26*). It is noted, however, that the transfer of larvae that had hatched in seawater into tap water will cause premature death in a few days. These moribund larvae are swollen and the occurrence of an enlarged, posteriorly situated vacuole suggests inefficient osmoregulation. Second-stage larvae appear to be best adjusted to seawater (*36*). Also, second-stage larvae placed in LaPage's (*44*) exsheathing fluid do not lose their sheaths (*26*).

THIRD-STAGE LARVA

The free-living but somewhat sedentary second-stage larva must be ingested by an intermediate host, usually a first intermediate host, before further development occurs.

There is still very little experimental evidence relative to the first intermediate host of anisakids. It should be obvious why this is true since the maintenance of marine vertebrates, which are the definitive hosts, is beyond the capacity of most laboratories. Furthermore, even if third-stage larvae are encountered in nature, their developmental morphology is such that they are extremely difficult, if not impossible, to identify. In spite of these difficulties, some information is available, if not on those species known to be involved in human anisakiasis, at least on certain other species of the Anisakidae.

It appears that the first intermediate host is usually an aquatic protostomate invertebrate. Relative to marine anisakids, Wülker (*88*) has reported the occurrence of larval *Contracaecum* in *Rhinocalalanus nasutus,* Cobb (*15*) described *Paranisakis pectinis* in the visceral mass of a scallop, *Aequipecten,* collected at Beaufort, North Carolina, and Hutton et al. (*29*) found larval *Contracaecum* in several species of shrimps. It is noted that Hutton (*28*), while examining specimens of the bay scallop, *Aequipecten gibbus,* collected off the east coast of Florida, found numerous specimens of an immature nematode which he believed to represent Cobb's (*15*) species. However, his finding of the presence of a short, anteriorly projecting intestinal caecum influenced him to transfer *P. pectinis* to the genus *Porrocaecum.* The same larval worm was reported by Cheng (*12*) to occur in 2.3% of 400 specimens of the scallop *Aequipecten irradians* from off the coast of North Carolina. In this pelecypod, this parasite is commonly, although not always, found in the adductor muscle, causing it to become brownish in color.

A search of the literature has revealed that several other investigators have reported finding larvae of *Contracaecum* spp. in the coelomic cavities of marine invertebrates. Included among these is the report by Apstein (*3*) of the occurrence of these larvae in the planktonic copepods *Calanus finmarchicus, Pseudocalanus* sp., and *Euchaeta* sp. from the North Sea, and the report by Wülker (*87*), who identified Apstein's specimens, that similar larvae occur in the chaetognath *Sagitta* sp. from a collection at the Helgolander Institute in Germany. That *Contracaecum* larvae, presumably third-stage larvae, occur in *Sagitta* spp. also has been reported by others (*5, 45, 66*). Also, others (*29, 42, 48*)

have reported larval *Contracaecum* in shrimp, and in three species of mysid crustaceans (*72*).

With the finding of anisakid larvae in marine invertebrates, the question arises as to whether these are indeed the first or only intermediate hosts. It should be pointed out that since these nematode larvae are poorly developed from the standpoint of internal and external morphology, it is not possible to identify with certainty as to whether they are second- or third-stage larvae or even fourth-stage larvae. Consequently, the role of the invertebrates in the life cycles of anisakids remains unclear and can only be evaluated more critically as experimental life cycle data become available. Unfortunately, information relative to the first intermediate host resulting from experimental infections is scarce. Available reports include Markowski's (*49*) successful infection of the marine copepods *Eurytemora affinis* and *Ascartia bifilosa* with second-stage larvae of *Contracaecum aduncum,* Penner's (*62*) infection of another marine copepod, *Tigriopus californicus,* with the second-stage larvae of *Contracaecum* sp. hatched from eggs obtained from sea lions, Valter's (*64*) report that isopods can be infected with second-stage larvae of *C. aduncum,* and Huizinga's (*26*) success at infecting the copepods *Cyclops vernalis* and *Tigriopus californicus* with second-stage larvae of *C. spiculigerum.* Also, several Japanese workers have reported what are designated as Type I *Anisakis* larvae (most probably the larvae of *A. simplex*) in the musculature of the cephalopod *Todarodes pacificus* (*25, 30, 36, 39, 40, 41, 54, 59, 60, 61, 70, 79, 89*) and Type II *Anisakis* larvae (most probably the larvae of *A. physeteris*) in *T. pacificus* as well as another cephalopod, *Doryteuthis bleekeri* (*36, 40*). Although experimental proof is wanting, the general opinion held by Japanese parasitologists at this time is that the larval anisakids from cephalopods are third-stage larvae, and thus are infective to the natural definitive host or man if ingested in raw or poorly cooked squid.

More recently, Oshima et al. (*58*) and Oshima (*56*) have attempted to infect euphausiid crustaceans with second-stage larvae of *Anisakis simplex* and *A. typica* that had hatched from eggs removed from the uterus of adult worms from blue white dolphins caught off the coast of Japan. These investigators have reported that a certain percentage of both *Euphausia similis* and *E. pacifica* became infected and that the larval nematodes underwent the second molt in these experimental crustacean intermediate hosts.

Relative to the role of invertebrates, it is of interest to note that with nonmarine anisakids, Huizinga (*27*) has reported that the copepod *Cyclops vernalis* serves as the first intermediate host for *Contracaecum multipapillatum,* an intestinal parasite of birds, and Mozgovoi and Ryzhikov (*52*) have found that the amphipod *Macrohectopus branickii* serves in the same capacity for *Contracaecum osculatum baicalense* in Lake Baikal in the USSR. This nematode is a parasite of the Baikal seal, *Phoca sibirica.* Also, Mozgovoi (*51*) has reported that earthworms serve as the only intermediate host for *Porrocaecum crassum,* an intestinal parasite of ducks and other birds. Based upon reports such as those listed above and also on her own ecological studies, Myers (*53*) has listed annelids, isopods, mysids, shrimps, crabs, lobsters, molluscs, echinoderms, and even teleost fishes as possible first intermediate hosts for *Phocanema decipiens.*

It would appear from the foregoing that most commonly an invertebrate is involved as the first intermediate host of marine and nonmarine anisakids; however, the picture is far from being that simple. The uncertainty of the necessity of an invertebrate first intermediate host arises as a result of the reports by Thomas (*81, 82, 83*) that he was able to recover encapsulated but viable larvae of *Contracaecum spiculigerum* in guppies that had been fed second-stage larvae. The seeming contradiction between this finding and Penner's (*62*) report of being able to infect the copepod *Tigriopus californicus* with similar larvae of *Contracaecum* spp. caused Olsen (*55*) to comment that the life cycle of *C. spiculigerum* should be reinvestigated. This, as stated, has been carried out by Huizinga (*26*). However, instead of resolving the paradox, Huizinga has succeeded in demonstrating further complexities in the anisakid life history pattern. Specifically, although he was able to infect both *Cyclops vernalis* and *T. californicus* with second-stage larvae by permitting the copepods to feed off the bottom of petri dishes where the larvae were attached, he reported that although exsheathment and some growth ensued in the crustacean host, the second molt does not occur. Thus, technically, the copepod must be considered a paratenic host rather than a true intermediate host. Further details of Huizinga's findings are reviewed below to substantiate this interpretation.

As the experimental copepod host swims along the bottom of the dish, it encounters second-stage larvae. When this occurs, the copepod either darts to one side and seizes a larva or pauses above a larva, seizes it by one end, and by employing its ventral appendages forces the worm into its mouth. It is observations such as this that have caused this author to interpret what is known about the behavior of free-living, second-stage larvae described earlier to be of survival value. Specifically, it would appear that larvae attached at one end and gyrating could serve to attract the bottom-feeding invertebrate host.

Once within the copepod's intestine, each second-stage larva exsheaths and within 15-30 min penetrates through the host's intestinal wall by use of its boring tooth and comes to lie in the coelom. At this site it coils and uncoils continuously. In 7 days time they increase in size from 329 μm to 350μm long but further differentiation of the internal organs does not occur nor does the next molt (*26*). A single copepod may harbor 6-10 larvae. *T. californicus* harboring exsheathed larvae showed a

considerably higher mortality rate, with 58 of 60 experimentally infected copepods dying between 1 and 6 days post-infection; while only 1 out of 60 noninfected copepods died. Thus, anisakiasis is apparently a lethal disease to crustaceans and possibly other categories of invertebrate hosts. It remains to be determined what causes the death of infected crustaceans.

In addition to infecting copepods, Huizinga (*26*) also fed second-stage larvae to fish. He used both the guppy, *Lebistes reticulatus,* and the killifish, *Fundulus heteroclitus,* as experimental hosts. After feeding large numbers of second-stage larvae directly to both species of fish, the hosts were examined at time intervals. In *L. reticulatus,* some larvae found in the intestine had exsheathed between the 1st and 3rd days post-feeding, although the majority retained their sheaths, died, and were passed out in feces by day 3. Also, a few exsheathed larvae were found buried in the intestinal mucosa of six fish on day 7. These had increased to 500 μm in length and were not encapsulated. Furthermore, three larvae were recovered after digestion of the mesenteries of a single guppie on day 16 and these were 753 μm long and each showed a partially developed intestinal caecum and a ventricular appendix. Since second-stage larvae do undergo exsheathment in guppies but the second molt was not observed and assumed not to have occurred, this fish must also be considered as a paratenic host rather than a true intermediate host, at least when larvae are directly fed to it.

With *F. heteroclitus,* when second-stage larvae were fed directly, no parasites were recovered.

Huizinga (*26*) also fed copepods harboring second-stage larvae to both guppies and killifish. In both hosts, larvae were recovered; however, it is important to note that in the case of *F. heteroclitus,* larvae undergoing the second molt were found encapsulated in the intestinal mucosa, although the larvae did not exsheath but retained the cuticle closely adhered to the new cuticle. Thus, these larvae should be considered as ensheathed, third-stage larvae. Such larvae averaged 1236 μm long, had a well-developed intestinal caecum, ventriculus, and ventricular appendix. Moreover, the esophagus, lips, and boring tooth were well developed but the interlabia were not present. The reaction tissue of the fish host consisted of a thin, fibrous capsule on the periphery of which were found neutrophilic granulocytes.

The fact that the second molt occurs in *F. heteroclitus* is significant since this phenomenon qualifies this fish as a true intermediate host. Thus, of the two species of fish tested, *L. reticulatus* is considered a paratenic host and *F. heteroclitus* an intermediate host. Hence, a generalization that the true intermediate host of *C. spicudigerum* is a fish cannot be made. Also, it is now apparent why the life cycle pattern among anisakids is so perplexing since at least *C. spicudigerum* can pass from a crustacean to a fish or directly from free-living second stage larva to a fish. Huizinga (*26*) did not feed larvae from fish to experimental definitive hosts, although he assumed that ensheathed third-stage larvae, when ingested by a suitable host would undergo two additional molts and reach maturity.

It is also of interest to note that there is apparently no reaction in *L. reticulatus* and yet it is not a compatible intermediate host. On the other hand, encapsulation occurs in *F. heteroclitus,* which is apparently a compatible intermediate host. This finding appears to be an exception to the principle that there is less or no cellular reaction in hosts that are compatible (*11*). This point is discussed again later.

LARVAL ANISAKIDS IN FISH

Turning now to the interrelationship between larval anisakids and fishes, especially marine fish, numerous reports are available. In fact, anisakid nematodes are considered one of the major groups of helminth parasites of fish, with some causing serious pathological alterations. The anisakids in marine fish are either adults or larvae. The adults are limited exclusively to the alimentary tract while larvae may occur in various tissues, the coelom, as well as the alimentary tract. As a result of studies conducted at this Institute, it can be stated with certainty that third-stage larval anisakids found in the alimentary tract represent specimens that have recently arrived at that site as the result of ingestion by the fish. If the fish is a compatible host, these larvae will develop to maturity; if not, they are either passed out in feces or may penetrate the stomach or intestinal wall and survive either encapsulated or free in the liver, peritoneum, or some other tissue. If this occurs, no further development, except for minimal growth, ensues, and the fish must be considered as paratenic hosts for third-stage larvae. However, if an additional molt occurs, the third, the fish qualifies as a second intermediate host. It should be remembered, however, that Huizinga (*26*) has demonstrated that free-living second-stage larvae can infect fish directly. Even then, the fish, such as *Lebistes reticulatus,* can only serve as paratenic hosts, while others, such as *Fundulus heteroclitus,* can serve as true intermediate hosts. As to which category of host fish that harbor larvae belong can only be resolved as each life cycle is examined. A generalization pertaining to the relationship between this group of nematodes and their fish hosts is not possible.

Larval anisakids in marine fish are primarily represented by members of the genera *Contracaecum, Anisakis,* and *Porrocaecum.* The number of parasites per fish may reach several hundred and these occur in a variety of internal organs and tissues including the liver, gonads, somatic musculature, messenteries, and peritoneum. The large number of parasites is probably the result of accumulation over a period (*9, 35, 64, 73*). This condition, as most parasitologists know, differs from that of adult helminths in the alimentary tract.

Numerous species of marine fish have been reported to harbor larval anisakids. The monographs by Dollfus (*21*), Templeman (*80*), Yamaguti (*90*), and Skrjabin (*78*) as well

as the listing compiled by Oshima (*57*) for Japanese waters, are recommended to those interested in host records.

PISCIAN ANISAKIASIS

There is considerable information pertaining to the pathology of piscian anisakiasis. Although several reviews of nematodiasis in marine fish are available (*19, 20, 47*), to provide some degree of completeness, some salient aspects of the pathobiology of larval anisakids in fish are being presented herein.

The first report of the pathobiology of fish due to larval anisakid appears to be that of Agersborg (*1*) who reported that northern Norwegians regarded cod (*Gadus callarias* and *G. virens*) with livers heavily infected with roundworms to be less fat than uninfected or lightly infected fish. According to Margolis (*47*) the nematodes in cod liver are certain to be larval *Anisakis* or *Contracaecum*. A number of investigators have reported on the pathology of larval anisakids in cod liver since then (*8, 22, 63, 67, 68, 74*).

In brief, there is now ample evidence that the presence of *Contracaecum* larvae in livers of cod adversely affects absolute size or weight of this organ, weight of the liver relative to body weight, fat content of the liver, total weight of the fish, and the condition factor (coefficient of condition) of the fish. Furthermore, there is a correlation between these alterations of the fish and its liver with both number and sizes of the worms present in the liver (*2, 7, 19, 24, 65, 75, 76, 77*) although both Shulman (*74*) and Petrushevsky and Shulman (*64*) have pointed out that these relationships, especially that between the degree of hepatic anisakiasis and the absolute and relative weights of the liver need not be direct. These Russian investigators have offered the explanation that the variability in the relationships may be due to such other factors as duration of the infection, size of the parasites, location of parasites within the liver, age and physiological (including spawning) stage of the fish, and individual resistance of the fish.

Relative to pathological changes to the liver associated with larval *Contracaecum,* Petrushevsky and Shulman (*64*) have pointed out that damage is greater when the worms penetrate the parenchyma. Also, actively moving, nonencapsulated worms are more destructive than those encapsulated by the host.

Petrushevsky and Shulman (*64, 65*) have summarized reports on changes in the fat contents of livers of the cod due to the presence of *Contracaecum* larvae. The fat content may drop from as much as 57% in an uninfected liver to 14.5% if large numbers of nematodes are present; however, it is of interest to note that the vitamin A contents of infected livers are no affected by *Contracaecum.*

Although weight changes, drops in fat contents, and histopathological changes in infected cod livers have been investigated, the mechanisms responsible for such changes have not been investigated in depth. With capsules formed around larvae, it is generally agreed that these represent the host's internal defense reactions against the parasites. However, it remains unresolved why *Contracaecum* larvae are encapsulated in some fish and not in others. This is especially puzzling where the fish hosts are of the same species. It may be that in instances where encapsulation occurs, the nematodes had become moribund and consequently, chemical changes associated with impending death elicit the host's cellular response. Relative to this point, it is noted that examination by this author of cod, *Gadus callarias,* from off the coast of Provincetown, Massachusetts, have yet to reveal the encapsulation of *Contracaecum* larvae in liver. These nematodes, however, were all viable; none were dead or moribund, thus lending credence to the hypothesis that only moribund or dead worms elicit encapsulation.

Other than the occurrence of encapsulation around worms, other histopathological alterations occur in infected livers. According to Bazikalova (*8*), such changes may be due to toxins produced by the parasites. Her postulation is based upon the observation that *Contracaecum* larvae are commonly situated immediately beneath the surfacial capsule of the host's liver and yet histopathological changes occur in the parenchyma. This belief also has been expressed by Guiart (*24*). On the other hand, Bauer (*7*) and Dogiel (*19*) consider the liver atrophy in cods to be the result of mechanical damage. Clearly, an experimental approach must be taken to resolve this question.

In addition to fish belonging to the family Gadidae, larval *Contracaecum* also occur in other species of fish. For example, these larvae have been reported to be responsible for mortalities among *Labrus festivus* and *Pagellus erythrinus* in the Oceanographic Museum of Monaco in 1936. In this instance, the dying and dead fish were reported to be extremely emaciated and with partially or totally atrophied livers (*24*). It was also reported that some of the nematodes were encapsulated while others were not. The fact that this occurs within the same host strengthens the hypothesis that some worms become chemically altered and are recognized as "nonself" and are consequently encapsulated.

In addition to *Contracaecum* larvae, the larvae of *Anisakis* have also been reported to cause hepatic anisakiasis in fish. Included among such reports are those by Kahl (*33*) who found these worms in *Sebastes marinus* from northern European waters, Akhmerov (*2*) who found these larvae in *Theragara chalcogramma* from Pacific waters of the USSR, Petrushevsky and Kogteva (*63*) who found these parasites in *Myoxocephalus scorpius* from the White Sea, and Remotti (*68*) and Brian (*10*) who reported *Anisakis* larvae in *Merluccius merluccius* from the Mediterranean.

The most common site for *Anisakis* larvae is in the liver; however, this author has found such larvae in the coelom and peritoneum of porgies, *Stenotomus chrysops,* collected off the coast of Massachussetts. When found

associated with the liver, atrophy of this organ commonly occurs, even if the nematodes are superficially situated beneath the hepatic capsule (*68*). This observation, if true, would appear to support the contention that the parasites secrete a toxin which permeates the entire liver. However, Brian (*10*) has reported the absence of a general inflammatory reaction or general degenerative changes in the liver and has concluded that *Anisakis* larvae are nontoxic and that the hepatic atrophy is due to mechanical pressure exerted by the parasites. It is noted, nevertheless, that Brian (*10*) is of the opinion that if encapsulated nematodes die, they release a toxin, designated as "thanatotoxin," which causes degeneration of the surrounding hepatic tissues. As to whether this is true cannot be verified without experimental data.

Relative to the histopathological changes, it has been reported that in *Gadus morhua, Melanogrammus aeglefinus, Myoxocephalus scorpius,* and *Sebates marinus* destruction of hepatic parenchymal cells, blood vessels, and bile ducts occurs in the vicinity of the *Anisakis* larvae (*34, 50*). The parasites are encapsulated and the capsule is comprised of at least three layers: (*a*) an outer stratum consisting of loose connective tissue in which are embedded numerous blood vessels, histiocytes, large fibroblasts, and occasionally fibrocytes, leucocytes, and extravascular erythrocytes and their remnants; (*b*) a middle layer consisting of large numbers of fibroblastic elements, which usually have undergone degenerative changes; and (*c*) a thin inner layer formed of damaged cellular elements with traces of pycnosis. The capsules are being eroded continuously from within as a result of secretions and/or excretions of the parasite. Concurrently, there is a building up of the capsule on its external surface. Furthermore, as a function of time, such capsules become thinner as the connective tissue layers become more compact and these may be accompanied by a deposition of calcium. It is noted that Prusevich (*67*) has experimentally studied the development of hepatic capsules surrounding *Anisakis* larvae. He accomplished this by surgically implanting *Anisakis* larvae from the cod onto the livers of *Myoxocephalus scorpius* and found that the inflammatory reaction begins within the first few hours.

There are some data available to indicate that a parallel situation occurs as with *Contracaecum* larvae, i.e., as the number of *Anisakis* larvae increases, the condition factor of the parasitized specimens of *Myxocephalus scorpius* decreases (*63*).

Species of *Porrocaecum* larvae have also been reported to cause pathological alterations. For example, it has been reported that these larvae are more destructive to the liver of *Myoxocephalus quadricornis* from the White Sea than *Anisakis* larvae (*76*). The reason given is that the larvae of *Porrocaecum* are larger and more active and the damage inflicted on the host's liver is the result of their burrowing as well as feeding on liver cells and blood. It is noted that Mikhaylova et al. (*50*) have reported that although histopathological changes occur in the immediate vicinity of *Porrocaecum* larvae in the liver of *Myoxocephalus scorpius* from the Barents Sea, the parasites are not always encapsulated; however, if they are, the capsules are comprised of two layers; an inner one of degenerating tissue and an outer one of loose connective tissue with blood vessels and fibroblasts, leucocytes, and other phagocytes embedded therein. Once again, the question must be raised as to why some larvae are encapsulated while others are not. Furthermore, as a result of observations by this author, the question must be raised as to whether invasion of fish liver by *Porrocaecum* larvae is seasonal. The reason for this is because during July of 1972, 12 specimens of the summer flounder, *Paralichthys dentatus,* caught off the coast of Noank, Connecticut, were examined and the liver of each fish harbored at least .25 larval *Porrocaecum.* The liver of these fish were atrophied and dark brown in color. On the other hand, specimens of *P. dentatus* caught off the coast of Massachusetts early in November, 1973, were devoid of *Porrocaecum* larvae in their livers, although these larval nematodes were found in muscles and the peritoneum. One is tempted to speculate that the migration of *Porrocaecum* larvae into the fish host's liver is influenced by some physiological process that is correlated with ambient temperature; however, experiments specifically designed to prove this hypothesis remain to be done.

As with *Anisakis* and *Contracaecum* larvae, the presence of *Phocanema decipiens* larvae in the livers of fish is correlated with loss of liver and body weights (*76*).

Larval anisakids are by no means limited to the liver; as stated, this author has found larval *Porrocaecum* in the mesenteries of the summer flounder and other species of fish. Other genera of anisakid larvae have also been observed (Table 1). Before presenting my results, it appears to be of interest to review the findings of Rosenthal (*64*) relative to the relationship between *Contracaecum* larvae and the herring, *Clupea harengus.*

Rosenthal (*69*), who studied invasion of young herring by *Contracaecum* larvae in zooplankton, reported that after ingestion, the nematode larvae penetrate the gut wall and become established in the fish's body cavity where they grow and move about actively. Within 6-8 days each nematode attains a length equal to that of the fish's gut and as it continues to grow, exerts pressure on the gut. This causes the fish's intestinal lumen to become compressed and its normal functions, including peristalsis, may cease by day 10 post-infection. As the nematodes migrate anteriorly and invade the region of the heart, infected young fish begin to swim erratically and die shortly thereafter. Rosenthal is of the opinion that the rapidly growing *Contracaecum* larvae feed on the host's tissues and fluids and he also has suggested that the nematodes may produce toxic waste products, which are poisonous to the fish. It is noted that the nematode larvae usually kill young fish below 20 mm long while larger fish are not as seriously affected.

Anisakid larvae are found in parts of fish hosts other

TABLE 1. *Origin, species, and number of fish examined, and percentage of infection and genera of anisakids found.* o = Porrocaecum; * = Contracaecum; △ = Anisakis; + = Rhaphidascaris. *A total of 16 species of marine fish have been surveyed*

Origin	Fish species	No. of fish examined	% infected	Liver	Mesentery	Muscle	Intestine	Stomach	Spleen	Reprod. syst.	Gills
Massachusetts	Summer flounder (*P. dentatus*)	50	90				*	*			*
	Brown dab (*Limanda ferruginea*)	80	100		△	o	*	*			*
	Cod (*Gadus callarias*)	212	76	o		o△	*	*	o		*
	Herring (*Clupea harengus*)	76	0								
	Hake (*Merlucius merlucius*)	46	75			o	*		o		
	Gray sole (*Glyptocephalus cynoglossus*)	85	95		o		*				
	Mackeral (*Scomber scombrus*)	6	100				*		o		
	Whiting (*Merlucius bilinearis*)	83	68			o	*	*			
	Catfish (*Galeichthys felis*)	15	100		o		*				
	Ling (*Phycis regius*)	106	60			o	* +				
	Winter flounder (*Pseudopleuronectes americanus*	173	40	o	△o	o	*	*			
Georgia	Sea trout (*Lynosciom regalis*)	4	75				*				
New Jersey	Summer flounder (*Paralichthys dentatus*)	35	100	o	o	o	*	*	o		
	Sea trout (*L. regalis*)	3	33				*				
	Blue fish (*Pomatomus saltatrix*)	18	0								
	Catfish (*Galeichthys felis*)	15	100		o		*				
Maryland	Summer flounder (*Paralichthys dentatus*)	20	90	o	o	o	*	*	o		
New York	Summer flounder (*P. dentatus*)	18	100	o		o	*		o		
	Sea trout (*L. regalis*)	4	25				*				
	Sea bass (*Centropistes striatus*)	2	0								
	Mackeral (*Scomber scombrus*)	6	0								
	Striped bass (*Roccus lineatus*)	2	100				*				
Connecticut	Summer flounder (*P. dentatus*)	10	65	o	o	o	*	*	o		
	Sea bass (*C. striatus*)	2	50	o							
Massachusetts	Porgy (*Stenotomus chrysops*)	18	67				*				
Maine	Striped bass (*Roccus lineatus*)	2	50				*				

TOTAL NO. OF FISH EXAMINED: 1091

than the liver and body cavity. These larvae also have been reported from various visceral organs and muscles. Very little is known about the relationship between nematode larvae and these organs and tissues, since most of the studies have been concerned with the identification of the worms. Among what is known, Agersborg (*1*) has reported that cod, haddock, and other marine fishes with larval anisakids in their muscles become sluggish and not only can they not chase food but also readily fall prey to predators. Kahl (*33*) reported that the presence of larval nematodes in muscles is not as serious as parasitization of the stomach wall and the body cavity. The occurrence of parasites in the stomach wall may lead to perforation and subsequent infection by bacteria while invasion of the body cavity may cause visceral adhesions. Arai (*4*) has also reported partial perforation of the stomach wall by *Anisakis* larvae and that fish infected in this manner are emaciated.

The histopathology caused by anisakid larvae in fish muscles has been studied by Kahl (*33, 34*). He reported that in large fish, such as *Gadus morhau, Sebrastes marinus, Molva byrkelange,* and *M. molva, Porrocaecum* larvae are lodged in the myomeres between the connective tissue septa. Here they are coiled and are encapsulated by a tunic of connective tissue. As with larval anisakids in livers, the capsule is reported to be initially fairly thick but it gradually degenerates from within. Encapsulated worms eventually die and are resorbed and the damage to the myofibers is not severe. On the other hand, when *Porrocaecum* larvae occur in the musculature of the smelt, *Osmerus eperlanus,* the resulting damage is considerably more severe. Specifically, the worms are said not to be encapsulated but move freely through the musculature. This results in their being found in cavities filled with blood. Such cavities are the result of damaged myofibers and the

blood originates from ruptured blood vessels. This type of damage may be so severe that the host's muscular activity is impaired.

It is noted again that there is a difference between the reaction in *G. morhua, S. marinus, M. byrkelange,* and *M. molva* and in that of *O. eperlanus* to *Porrocaecum* larvae. In the first group of fish, encapsulation occurs but none occurs in the smelt. Although Kahl (*34*) is of the opinion that this difference is due to the scarcity of connective tissue in the latter fish, this explanation is not entirely satisfactory and the phenomenon requires further investigation.

When present in the stomach wall, muscle, and mesenteries, *Anisakis* larvae in *G. morhua, G. virens, S. marinus,* and *C. harengus* also cause inflammation and encapsulation. Furthermore, the capsule is thickest when the parasite is situated in the stomach wall (*33*). Such capsules also occur around the larvae of *Contracaecum* sp. and *Rhaphidascaris* sp. in the visceral tissues of *Platichthys flesus* from the Baltic Sea (*31*).

Having briefly reviewed what is known about larval anisakids in fish, the important question must be raised relative to what stage of development these anisakids are in. At first glance, it would appear that logically those larvae occurring in organs and tissues other than the alimentary tract should be third-stage larvae. Although this may be true in most instances, it cannot be stated with certainty that this is always the case. Furthermore, it cannot even be stated with certainty whether all species of fish in which larval anisakids occur are true intermediate hosts. From this author's experience and the data presented by Huizinga (*26*) it would appear that in many instances these fish are paratenic hosts. More specifically, in the marine environment, fish that serve as true intermediate hosts, i.e., those in which the ingested second-stage larvae have at least initiated the second molt, are often ingested by larger fish. If these carnivores are not suitable as hosts, the ensheathed third-stage larvae will not complete the molt but do remain infective if the host is subsequently ingested by a still larger fish.

FOURTH-STAGE LARVA

Anisakid larvae found in the stomachs of fish that serve as the definitive hosts are most commonly either fourth-stage larvae or adults. This is especially true of *Contracaecum* spp. Thus, it would appear that it is the third-stage larva that is ingested and the third and fourth molts occur in the alimentary canal of the final host. Huizinga (*26*) has postulated that with *Contracaecum spiculigerum,* the avian definitive host becomes infected when fish harboring ensheathed third-stage larvae are ingested and the larvae undergo two additional molts in the bird before attaining the adult stage. Also, Kagei et al. (*32*) have hypothesized that with *Anisakis simplex* or possibly *A. typica,* third-stage larvae undergo two molts in the first ventriculus of the blue white dolphin and in the process metamorphose and grow into adults. Thus, although experimental data are still wanting, the trend of thought, at least among certain investigators, is that it is the third-stage larva that is the infective form to the definitive host. It is noted, however, that there are published accounts to the contrary. These are discussed at a later point.

It needs to be pointed out that at least with *Contracaecum*, the occurence of fourth-stage larvae in the stomachs of marine fish need not indicate that the host had ingested an intermediate host harboring third-stage larvae. Some of this author's observations aboard a commercial fishing vessel on the Atlantic Ocean off the coast of Provincetown, Massachusetts, serve to illustrate this point. It has been observed that smaller specimens of fish which the fishermen do not choose to retain are allowed to sit on the deck in the sun for at least 3-4 h before they are thrown back into the sea. As the deck is cleared of fish before the next haul, numerous nematodes, primarily fourth-stage larvae and adults of *Contracaecum,* have been observed on the deck. These undoubtedly have migrated from dead fish as in our laboratory trials described below. More important from the standpoint of the epidemiology and/or epizootiology of anisakiasis, some of the dead fish that are thrown back into the sea undoubtedly harbor worms in their muscles and/or digestive tract, and as these fish are rapidly eaten by larger fish, it is highly possible that the parasites are passed on to the predator and if the latter is not a suitable host, it will serve as a paratenic host. This hypothesis is suggested by the finding of large numbers of fourth-stage larvae in the stomachs of large fish from the area.

PARASITE MIGRATION IN MORIBUND AND DEAD FISH

It has been observed by this author that when both the cod, *Gadus callarias,* and the summer flounder, *Paralichthys dentatus,* are maintained out of water at 30 C, there is rapid disintegration of the digestive tract. Specifically, the stomach and intestine become

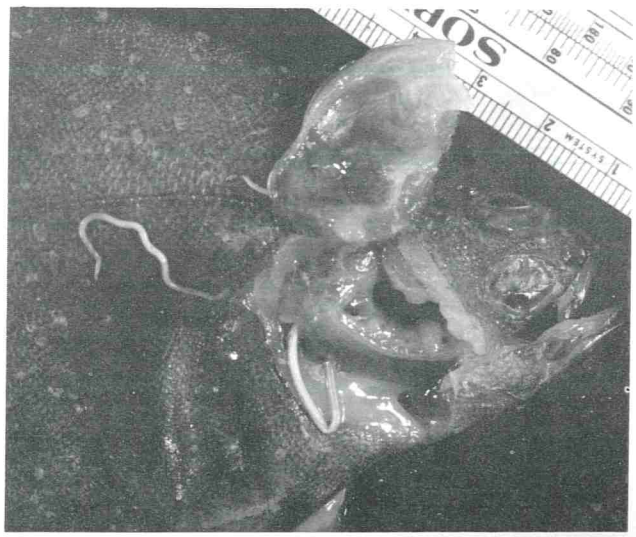

Figure 1. *Photograph showing worms,* Contracaecum *larvae, escaping from gill region of a dead summer flounder.*

gray-black in color and there is a concurrent breakdown of the cells lining these organs. Usually total disintegration occurs after 6-8 h. As this decomposition process progresses, the nematodes normally occurring in the stomach and intestine migrate via one of three routes: (*a*) Some of the migrate anteriorly; these being primarily from the stomach, and exit from the fish host via the gill filaments (Fig. 1). (*b*) Other worms, primarily those in the intestine, migrate posteriorly and exit from the anus (Fig. 2). (*c*) Lastly, still other worms, again primarily

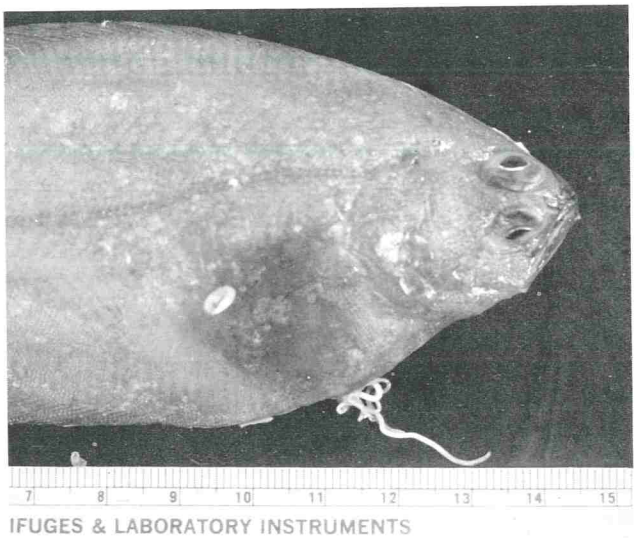

Figure 2. *Photograph showing worms,* Contracaecum *larvae, escaping from the anus of dead summer flounder. Notice dark coloration of disintegrating digestive tract.*

those from the intestine, burrow through the disintegrating intestinal wall and migrate nondirectionally in the body cavity. Some of these come to lie in the mesenteries while others enter the body musculature, where they can be detected by transmission light. Still others pass through the muscles and protrude from the body surfaces of their fish hosts (Fig. 3).

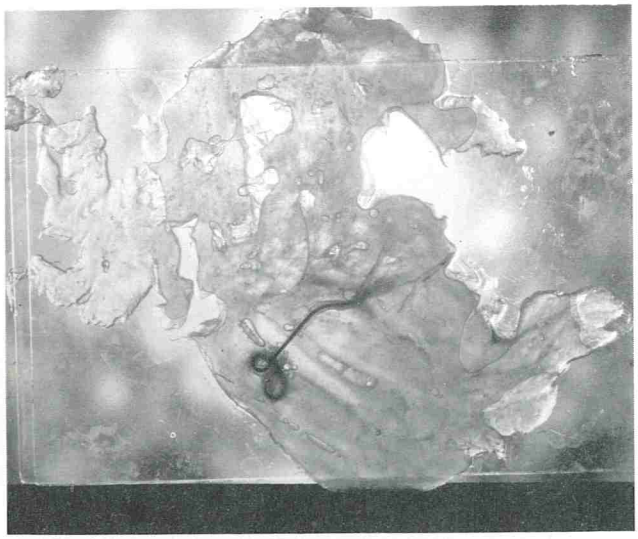

Figure 3. *Photograph of* Contracaecum *larva in muscle of summer flounder as seen in press preparation illuminated by transmission light.*

Identification of worms exiting from dead fish via the gills and anus has revealed these to be *Contracaecum* spp. Furthermore, they represent fourth-stage larvae and adults. It remains unknown, however, whether fourth-stage larvae of *Contracaecum* in the stomachs of these fish are infective when ingested by a predator. Nevertheless, it is noteworthy that evidence from this Institute suggests that these larvae from the stomachs of both *Gadus callarias* and *Paralichythys dentatus* are noninfective to either laboratory mice or rats. Furthermore, our data indicate that it is the physical parameters of the rodents' digestive tracts that are responsible for the incompatibility. The evidence that has led to these conclusions is presented at this point.

STUDIES ON THE INFECTIVITY OF CONTRACAECUM LARVAE TO RODENTS

As stated, the infectivity of fourth-stage *Contracaecum* larvae from *Gadus callarias* and *Paralichythys dentatus* to rodents has been tested at this Institute. These studies are reported below.

Materials and methods

The fish hosts were caught off the coast of Provincetown, Massachusetts, on August 19, 1973. They were immediately packed in ice and transported to this laboratory where the anisakid nematodes were removed from their stomachs and fourth-stage *Contracaecum* larvae were sorted out and placed in fish saline. Subsequently, four larvae were introduced into the stomachs of two female and three male, sexually mature laboratory mice. Each mouse was anaesthetized and the worms were introduced in water by use of a gastric tube. In addition, six smaller fourth-stage larvae of an undetermined species of *Contracaecum* were similarly introduced into the stomach of each of seven female and four male mice. The mice had been prestarved for 12 h and were returned to individual cages after the worms had been inserted. They were not fed between the time the parasites were introduced until they were autopsied. All of the mice were sacrificed at 55 h post-introduction.

In a second experiment, as indicated in Table 2, three mice were fed "large" fourth-stage *Contracaecum* larvae and three were fed "small" fourth-stage *Contracaecum* larvae. The hosts were maintained as above but were sacrified at time intervals ranging from 5 min to 6 h post-introduction of the nematodes.

Since Bacha (*6*) has shown that salts such as sodium bicarbonate enhance the successful establishment of helminth parasites in rodent hosts, it was of interest to facilitate possible establishment of the nematodes in both mice and rats by administering sodium bicarbonate before introduction of the parasites. Consequently, 14 mice were given 2.5 ml of a 5% $NaHCO_3$ solution in 0.65% NaCl saline before introduction of five fourth-stage *Contracaecum* via a gastric tube. The mice had been prestarved for 12 h before administration of the $NaHCO_3$ solution. In addition, 14 sexually mature rats

were similarly prefed 2.5 ml of the 5% $NaHCO_3$ solution before introduction of six fourth-stage *Contracaecum* larvae each. All of the nematodes were delivered into the rodents in 1 ml of the $NaHCO_3$ solution and the hosts were all examined 1 h post-introduction of the worms.

As reported below, no viable worms were recovered from any of the rodents that were fed *Contracaecum* larvae. Consequently, three other types of studies were done to determine whether the limiting factor(s) in the rodents' digestive tracts that prevent establishment of the nematodes could be (*a*) body temperature, (*b*) ambient pH, or (*c*) some other factor endogenous to the rodents' stomachs.

To ascertain the effect of temperature on the longevity of the fourth-stage larvae of *Contracaecum* sp., viable specimens removed from the stomach of the winter flounder, *Pseudopleuronectes americanus,* captured off the coast of Provincetown, Massachusetts, on January 25, 1973, were placed in 0.83% NaCl saline maintained at 12, 24, 25, 27.5, 28.5, 30.5, 32.5, 37, and 47 C. Ten worms were kept in each Stendor dish suspended in saline and maintained at each of the specified temperatures. Motility of the worms was employed as the criterion in defining the effect of the ambient temperature.

To ascertain the effect of the ambient pH on the larvae, two specimens of fourth-stage larvae of *Contracaecum* sp. removed from the stomachs and anterior portion of the small intestines of porgies, *Stenostomus chrysops,* captured off the coast of Boston, Massachusetts, were placed in 0.01 M phosphate buffer adjusted to pH 4.5, 5.5, and 6.5 with 1N HCl and maintained at 23-24 C. The motility of the worms was determined at 0, 15, 30, 45, 60, 75, 90, 105, 120, and 1620 min post-exposure.

Finally, to determine whether the incompatibility of fourth-stage *Contracaecum* larvae is due to factor(s) endogenous to the alimentary tract of laboratory rodents, 10 larvae removed from *Pseudopleuronectes americanus* were surgically implanted onto the peritoneum overlaying the small intestine of an adult rabbit. The incision on the ventrolateral abdominal wall was closed with surgical clips and was reopened 20 min later.

Results and discussion

All of the worms recovered from the stomach and intestine of mice in the first experiment, i.e., where the experimental hosts were sacrificed 55 h post-introduction, were dead and no visible pathological changes in the host were associated with their presence. In fact, only a small percentage of the inserted worms were recovered.

The results of the second experiment, i.e., where the mice were sacrificed at time intervals ranging from 5 to 60 min, are presented in Table 2. It is apparent that the larvae could not survive in the experimental host's stomach for even 5 min.

Although some of the nematodes introduced into the stomachs of rats and mice that had been pretreated with sodium bicarbonate were recovered at 1 h post-introduction, all were dead and no pathological changes were observed associated with the parasites. From this may be concluded that the preadministration of $NaHCO_3$ does not enhance the establishment of the fourth-stage larvae of *Contracaecum* sp.

Results of these studies on the effect of temperature on the longevity of the larvae are presented in Table 3. It is apparent from these that at temperatures above 27.5 C (81 F) the larvae died, or at least became nonmotile within 5 min. These data suggest that the body temperature of rodents, which is 37.4 C (97.5 F), is responsible for the incompatibility of the larval *Contracaecum.*

TABLE 3. *Effect of temperature on motility of fourth-stage larvae of* Contracaecum *sp. from fish at pH 5.3.*

Temperature (C)	Motility
12	Motile at 4 days
14	Motile at 4 days
25	Motile at 30 min
27.5	Slightly motile at 30 min
28.5	Nonmotile at 5 min
30.5	Nonmotile at 5 min
32.5	Dead in less than 5 min
37	Dead in less than 5 min
47	Dead in less than 5 min

Finally, results relative to the effect of ambient pH on survival (measured as motility) of larvae are presented in Table 4. It is evident from these that there is essentially no difference between the movement of worms maintained at pH 4.5, 5.5, and 6.5.

As a result of the original studies reported, it is concluded that the fourth-stage *Contracaecum* larvae from the species of marine fish from which they were obtained are noninfective to laboratory rodents and one reason is because the nematodes cannot survive at their body temperature. Thus, these larvae are undoubtedly parasites of some poikilotherm, most probably some piscivorous fish, in nature. We are presently testing the infectivity of the larvae of other genera of anisakids.

TABLE 2. *Protocol and results of experimental feeding of "large" and "small" fourth-stage larvae of* Contracaecum *sp. to mice*

Mouse no.	No. and species of nematode fed	Time of necropsy (time after introduction of nematodes)	Condition of worms
1	2 "large" 4th-stage *Contracaecum* sp.	5 min	dead
2	3 "large" 4th-stage *Contracaecum* sp.	1 h	dead
3	3 "large" 4th-stage *Contracaecum* sp.	6 h	dead
4	2 "small" 4th-stage *Contracaecum* sp.	5 min	dead
5	5 "small" 4th-stage *Contracaecum* sp.	30 min	dead
6	8 "small" 4th-stage *Contracaecum* sp.	1 h	dead

TABLE 4. *Effect of pH on the motility of fourth-stage larvae of* Contracaecum *from fish at 23-24 C. Each Stendor dish contained two nematodes which were in 0.01 M phosphate buffer adjusted to pH 4.5, 5.5, and 6.5 with 1N HCl. +++, rapidly motile; ++, motile; +, sluggishly motile*

Time post-initiation of experiment	Motility at pH 4.5	pH 5.5	pH 6.5
0 min	+++	+++	+++
15 min	++	+++	+++
30 min	+++	+++	+++
45 min	++	+++	++
60 min	++	+++	++
75 min	++	++	++
90 min	+	++	++
105 min	–	++	++
120 min	+	++	++
1020 min	–	++	+

By the time the parasite reaches the fourth-stage larvae, it no longer bears a boring tooth and its internal organs are clearly defined. This includes the reproductive system, although it is not yet functional.

ADULT WORM

It has been stated that according to the postulation presented by others (*26, 32*) and this author, it is the third-stage larva that is infective to the definitive host. In consideration of what is known about the life cycles of parasitic nematodes that require one or more intermediate hosts (*13, 46*) this seems reasonable. However, it must be noted that Gibson (*23*) observed only one molt for an *Anisakis* larva to attain the "pre-adult" form in the stomach of a rat and Davey (*16, 17*) reported that *Porrocaecum decipiens* only molts once in the final host. Thus, the impression is given that certain species enter the definitive host as third-stage larvae and undergo two molts therein while other species enter as fourth-stage larvae and only undergo the final or fourth molt within the definitive host. Although this may be true, only additional studies will reveal whether indeed two patterns of development occur at this stage of the life cycles of anisakid nematodes.

CONCLUSION

In conclusion, as evidenced by this review, although some information is available to the biology of anisakid nematodes, much important information remains to be discovered pertaining to many aspects of the ecology, morphology, physiology, biochemistry, infectivity, and even the life cycles of these parasites. Furthermore, the interrelationship between these nematodes and their natural hosts requires considerably more investigation and the immunological and pathological responses of their invertebrate and vertebrate hosts may well serve as models for understanding human anisakiasis.

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Edward F. Baer 1924-1975

Edward F. Baer, a microbiologist with the Food and Drug Administration for 20 years, died suddenly at his home in Silver Spring, Md., April 10, 1975. He was 50. Mr. Baer joined the Food and Drug Administration in 1955, serving first as a microbiologist in the Division of Microbiology in Washington, D.C. then in the Chicago District, and after 2 years returned to Division of Microbiology where he spent the rest of his career with the Food and Drug Administration.

Mr. Baer was born in 1924 and was a native of Peoria, Illinois. He received his B.S. in bacteriology from the University of Illinois at Champaign-Urbana in 1951. He was a member of the American Society for Microbiology, Institute of Food Technologists, Association of Official Analytical Chemists, International Association of Milk, Food, and Environmental Sanitarians and was an affiliate member of the Division of Microbial Chemistry of the American Chemical Society. He also served on the editorial board of the *Journal of Milk and Food Technology.*

Mr. Baer served in the Medical Department of the U.S. Navy from 1943 to 1952. He was attached to the Fleet Marine Force at Camp Pendleton, California was served in the South Pacific as a member of an epidemiology team during World War II. He also, during his tour of duty with the Navy, served at the Supply Depot in Albany, California, San Diego Naval Hospital, Oakland Naval Hospital, Fleet Marine Headquarters, Great Lakes Naval Training Center, and finally served in Korea until October 1952.

Following military service, Mr. Baer joined the Western Regional Research Laboratory of the United States Department of Agriculture where he served as a microbiologist from 1952 until he joined the Food and Drug Administration in 1955. While at the Food and Drug Administration, he was the author of numerous papers on *Staphylococcus aureus* and its role in food poisoning, particularly with reference to methods of detection and identification. His work was also concerned with standardization and the collaborative study of methods. Most recently the emphasis of his research activities involved the establishment of microbiological standards for foods.

He was Associate Referee for *Staphylococcus* of the Association of Official Analytical Chemists. He made several important contributions on this subject to the association which were published in the Journal and the *Official Methods of Analysis.*

He is survived by his wife Shirley of Silver Spring, his daughter Cheryl of Vienna, Virginia and 4 grandchildren. He is also survived by his mother and father, of Peoria, Illinois, a sister and a brother, also of Peoria, Illinois, another sister in San Jose, Ill., and a sister in the Order of Saint Benedict in Moline, Ill.

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The Public Health Implications of Larval *Thynnascaris* Nematodes from Shellfish[1,2]

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ABSTRACT

Nematodes of the genus *Thynnascaris,* which as adults parasitize fishes and as larvae occur in fishes and invertebrates, are reviewed with emphasis on their possible public health implications. Reported invertebrate hosts include a variety of species in seven phyla; eight new host records are reported here. At least three types of larval *Thynnascaris* occur in shrimp of North and Central America and may infect as many as 80% of some size-groups of commercial penaeid shrimp. Reviewed and presented information indicate that larvae of *Thynnascaris,* like those of anisakine ascaridoids, are killed by usual methods of food processing and preparation. Unlike anisakine larvae, most types of larval *Thynnascaris* tested fail to infect experimentally inoculated laboratory mammals. However, larvae of *Thynnascaris aduncum* have been implicated, but not confirmed, as agents of human gastrointestinal eospinophilic granulomata in France, and data are presented showing that a type of larval *Thynnascaris* from the cutlassfish, *Trichiuris lepturus,* possibly identical to a type from the brown shrimp, infects and produces gastrointestinal lesions in the mouse.

The raphidascarine genus *Thynnascaris* is of practical interest because it has been implicated in the pathogenesis of eosinophilic granulomata in man. Benatre et al. (*3*) stated that approximately 15 cases of this type of gastrointestinal lesion had been reported during the last two decades from the Brittany region of France. Although neither worms nor worm fragments were found either grossly or in serial microsections of resected lesions, those authors inferred that a larval ascaridoid in fish might be the causative agent, since people frequently consume raw sardines in that region of France. Petter (*42, 43*) surveyed the nematode parasites present in sardines of waters of the Nantes region of Brittany, found that all possessed a single type of ascaridoid identified as third and early fourth stage larvae of *Thynnascaris aduncum,* and concluded that the larvae "presumably" caused the eosinophilic granulomata.

We have concerned ourselves with members of this genus, since several larval species infect commercial shrimps and other seafood products from the Gulf of Mexico and elsewhere. Because the taxonomy and life cycles of species of this genus have been inadequately studied, it is presently impossible to assign individual larvae to any given species. Before the public health significance, if any, of species of this genus can be evaluated, research is necessary to determine both the ability of these parasites to infect model mammalian hosts and the probability of their being ingested alive following preparation of infected seafoods for consumption.

This paper briefly reviews general information about the genus *Thynnascaris,* presents data concerning known invertebrate hosts, with special emphasis on the incidence and intensity of infection of species in commercial shrimps from the Gulf of Mexico, and reports work we have done relating to the possible public health importance of larvae from seafood.

THYNNASCARIS IN GENERAL

The genus *Thynnascaris* was erected by Dollfus (*11*), who later (*12*) amended it and assigned it as a subgenus to *Contracaecum.* Hartwich (*18*) raised *Thynnascaris* to generic status to accommodate *Contracaecum*-like raphidascarines which parasitize fishes exclusively as definitive hosts. Species of *Contracaecum* in the strict sense utilize either birds or mammals as definitive hosts.

Both larval and adult *Thynnascaris* species can be clearly distinguished from those of *Contracaecum* by having an excretory pore opening near the level of the nerve ring, rather than between the subventral lips, and a ventricular appendix usually longer than the intestinal caecum, rather than shorter. Several species of *Thynnascaris* have small spinous processes projecting from the tip of the tail.

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[1] *This paper is the fourth in a series of five developed from presentations at the symposium, "Anisakiasis: A New Disease from Raw Fish," held under the auspices of the New York Society of Tropical Medicine on 14 March 1974 at Rockefeller University in New York City.*

[2] *The study was conducted in cooperation with the National Marine Fisheries Service, NOAA, U.S. Department of Commerce under PL 88-309, Project No. 2-174-R; National Fishery Products Inspection and Safety Division, under contract No. N-042-61-72(N); and Office of Sea Grant, under Grant No. 04-3-158-53. The U.S. Government is authorized to produce and distribute reprints for governmental purposes notwithstanding any copyright notation that may appear hereon.*

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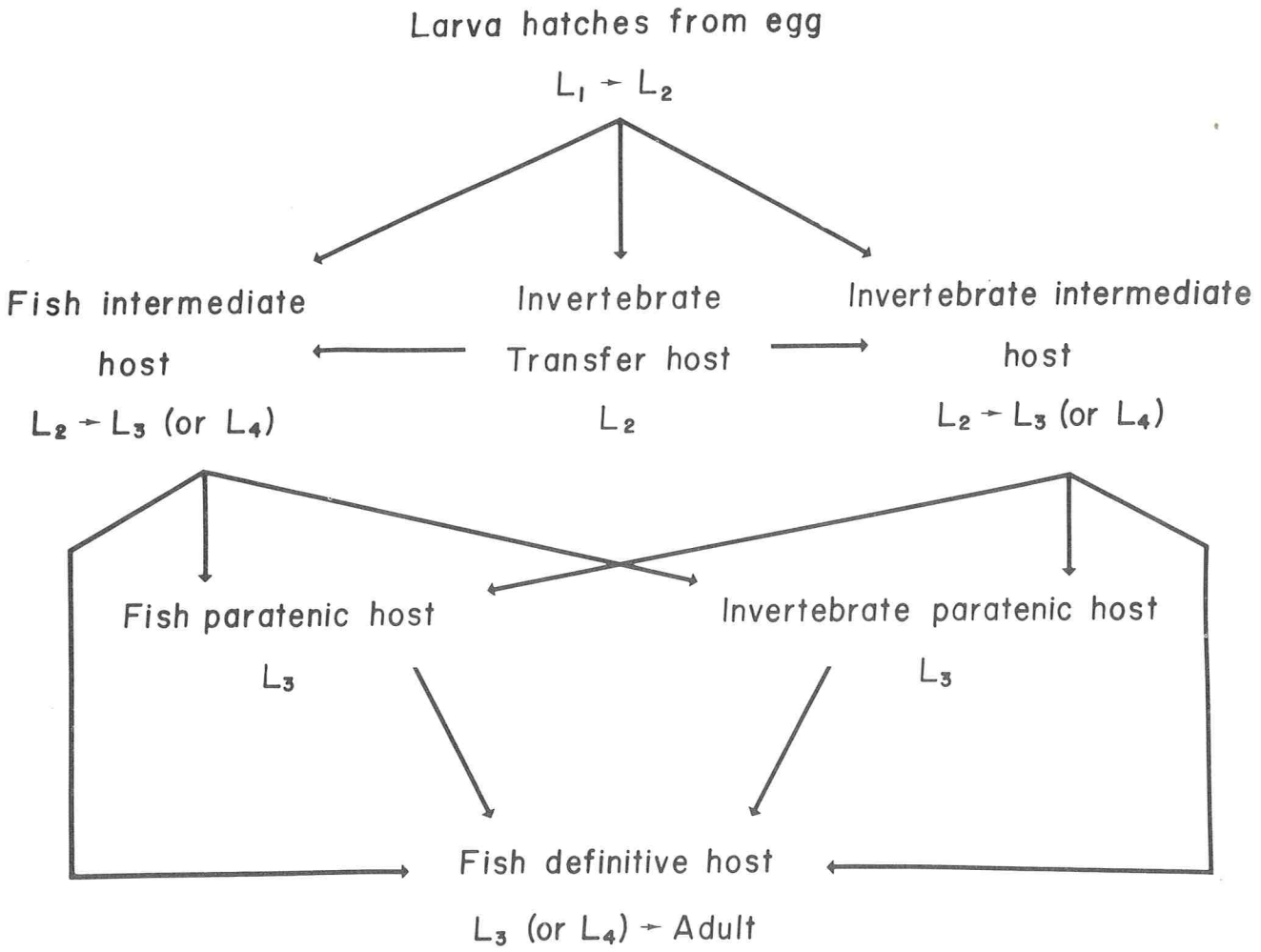

Figure 1. *Schematic life cycle for species of* Thynnascaris.

Life cycles of members of the genus are incompletely known. Even that of *T. aduncum,* a common nematode of teleosts on which numerous studies have been conducted, has promoted debate among workers (*4*). From the available literature and from our observations and inferences, the following events in a typical life cycle seem most likely (Fig. 1): Eggs passed from the definitive host settle to a substratum and embryonate. Larvae develop to the second stage, hatch, and are free-living. This second stage larva is eaten by either an invertebrate or a fish. Within an acceptable intermediate host, development proceeds to the third, and occasionally fourth, stage, either of which can infect the definitive host. In certain other invertebrates, apparently unsatisfactory as intermediate hosts, the second stage larva may simply be transported for a period without development and is infective for a true intermediate host. These transfer hosts, often copepods, provide the necessary link for infection of plankton-feeders such as herrings and anchovies. After feeding on intermediate hosts, either fishes or invertebrates may serve as paratenic hosts, which maintain infective larvae without further development, and in which larvae typically occupy specific areas such as the mesentery, hepatopancreas, liver, or muscle, depending on the species of larva and host. Third or, occasionally, fourth stage larvae develop to maturity in the alimentary tract of acceptable definitive hosts.

The presence of non-developing second stage larvae within a variety of invertebrate hosts has been reported for related species in different definitive hosts: *Contracaecum osculatum* from a mammal (*7*), *C. spiculigerum* and *C. multipapillatum* from birds (*19, 20*), and *Raphidascaris acus* from a fish (*38*). The latter two authors demonstrated that only a single fish-intermediate-host was essential for the respective cycles and that the experimentally-infected invertebrates acted as transport hosts only.

THYNNASCARIS IN INVERTEBRATES

Larval *Thynnascaris* spp. commonly occur both in fishes and invertebrates in the northern Gulf of Mexico and elsewhere. Host records from the literature and our own studies of fishes and marine invertebrates indicate that the larvae are more prevalent in fishes. Invertebrates, however, act as an important source of larvae in seafoods, since they include several edible species. Knowledge of infected invertebrates also

TABLE 1. *Invertebrate hosts of* Thynnascaris *spp.*

Host	Parasite	Locality	Authority
COELENTERATA			
Hydrozoa			
Phialidium sp.	*Contracaecum* sp.	Not stated	*68*
Polyorchis penicillatus	*Contracaecum* sp.	California	*32*
Scyphozoa			
Ceriantharia	*Contracaecum* sp.[a]	North of Congo River	*53*
CTENOPHORA			
Pleurobrachia pileus	*Contracaecum* spp.[a]	New Zealand	*5*
	Contracaecum sp.[b]	North Sea	*2*
MOLLUSCA			
Gastropoda			
Cantharus cancellarius	*Thynnascaris* sp.	Mississippi	This study
Cyclonassa neritea	*C. aduncum*	Black Sea	*10*
Margarites groenlandicus	*C. aduncum*	Experimental	*62*
Nassa reticulata	*C. aduncum*	Black Sea	*10*
Thais haemastoma floridana	*Thynnascaris* sp.	Mississippi	This study
Cephalopoda			
Lolliguncula brevis	*Thynnascaris* sp.	Mississippi	This study
Todarodes pacificus	*Contracaecum* sp.[a,d]	Japan	*56, 26, 25*
ANNELIDA			
Polychaeta			
Enoe nodosa	*Contracaecum* sp.[b]	White Sea	*47*
Gattiana cirrosa	*C. aduncum*	White Sea	*46, 47*
Gattiana sp.	*C. aduncum*	White Sea	*45*
Harmothoe imbricata	*Ascaris* sp.[b]	Great Britain	*37*
		Baltic Sea	*52*
	C. aduncum	White Sea	*60, 46, 64, 62, 47*
		Experimental	*62*
Lepidonotus sp.	*C. aduncum*	White Sea and Experimental	*45*
L. squamatus	*C. aduncum*	White Sea	*46, 47*
		Experimental	*62*
Nereis helgolandica	*C. aduncum*	White Sea	*47*
N. pelagica	*Contracaecum* sp.[b]	White Sea	*47*
Nereis sp.	*C. aduncum*	White Sea	*45*
Tomopterus helgolandica	*Contracaecum* sp.	North Sea	*48*
ARTHROPODA			
Copepoda			
Acartia biflosa	*C. aduncum*	Experimental	*58*
A. longiremis	*C. aduncum*	Experimental	*62, 46*
Calanus finmarchicus	*C. aduncum*	Barents Sea	*60*
	Contracaecum sp.[b]	North Sea	*2, 48*
Calanus sp.	Nematode[b]	North Sea	*1*
	Contracaecum sp.	North Sea	*68*
Euchoeta sp.	Nematode[b]	North Sea	*1*
	Contracaecum sp.	North Sea	*68*
Eurytemora affinis	*C. aduncum*	Experimental	*58*
Microsetella norvegica	*C. aduncum*	Experimental	*62*
Pseudocalanus elongatus	*C. aduncum*	Experimental	*46, 62*
Pseudocalanus sp.	Nematode[b]	North Sea	*1*
Pseudocalanus sp.	*Contracaecum* sp.	North Sea	*68*
Temora longicornis	*C. aduncum*	Experimental	*46*
Malacostraca			
Mysidacea			
Erythrops erythrophthalma	*Contracaecum* sp.	Nova Scotia	*54*
Meganyctiphanes norvegica	*Contracaecum* sp.	North Sea	*48*
Mysis mixta	*Contracaecum* sp.	Nova Scotia	*54*
Neomysis americana	*Contracaecum* sp.	Nova Scotia	*54*
N. vulgaris	*Contracaecum* sp.[b]	North Sea	*2*
Isopoda			
Iaera albifrons ischiosetosa	*C. aduncum*	White Sea	*61*
		Experimental	*61,64*
Amphipoda			
Caprella septendrionalis	*C. aduncum*	White Sea	*63, 64*
Unidentified	*T. bidentatum*	Experimental	*16*
Euphausiacea			
Euphausiid	*Contracaecum* sp.[a]	Northern North Pacific Ocean	*55*
Nyctiphanes couchii	*Contracaecum* sp.	North Sea	*57*
Thysanoessa raschii	*Contracaecum* sp.	North Sea	*57*
Decapoda			
Clibanarius vittatus	*Thynnascaris* sp.	Mississippi	This study
Emerita talpoida	*Thynnascaris* sp.	Mississippi	This study
Pandalus borealis	*Contracaecum* sp.[a,c]	British Columbia	*35*
	C. aduncum	Barents Sea	*60*
Penaeus aztecus	*Contracaecum* sp.[a]	Florida	*27, 23, 22*

	C. habena	Florida	*21*
	C. habena	Texas	*6*
	Thynnascaris sp.	Northern Gulf of Mexico	*41*
	Thynnascaris spp.	Mississippi	This study
P. brasiliensis	*Contracaecum* sp.[a]	Florida	*22*
	Thynnascaris sp.	Florida	*14*
P. californiensis	*Contracaecum* sp.	Mexico, Pacific Coast	*29*
	Nematode[b]	Dry Tortugas	*67*
P. duorarum	*Contracaecum* sp.[a]	Florida, North Carolina	*27, 36*
		to Campeche Banks	*23, 22*
	C. habena	Florida	*21, 65*
P. setiferus	Nematode	Florida	*67*
	Contracaecum sp.[a]	Florida	*27, 23, 22*
	C. habena	Florida	*21*
	Thynnascaris sp.	Northern Gulf of Mexico	*41*
	Thynnascaris sp.	Mississippi	This study
P. stylirostris	*Contracaecum* sp.[a]	El Salvador, Pacific Coast	*22*
P. vannamei	*Contracaecum* sp.	Mexico, Pacific Coast	*29*
	Thynnascaris sp.	Mexico, Pacific Coast	*14*
Sea crabs	*T. inquies*	India	*24*
Sicyonia dorsalis	*Contracaecum* sp.[a]	Dry Tortugas	*22, 21*
S. typica	*Contracaecum* sp.[a]	Dry Tortugas	*22, 21*
Solenocera atlantidis	*Contracaecum* sp.[a]	Dry Tortugas	*22, 21*
Trachypenaeus constrictus	*Contracaecum* sp.[a]	Florida	*22, 21*
T. similis	*Contracaecum* sp.[a]	Dry Tortugas	*22, 21*
Xiphopeneus kroyeri	*Contracaecum* sp.[a]	Florida or adjacent water	*22*
ECHINODERMATA			
Asteroidea			
Lucidia clathrata	*Thynnascaris* sp.	Mississippi	This study
CHAETOGNATHA			
Sagitta bipunctata	*Ascaris* sp.[a]	English Channel	*30*
	Contracaecum sp.[a]	New Zealand	*5*
S. elegans	*Contracaecum* sp.	White Sea	*28*
		Scotland	*57*
		North Sea	*48*
S. euxina	*Contracaecum*	Black Sea	*13*
S. friderici	Ascarid larva[d]	Moroccan Atlantic Coast	*15*
S. hispida	*Thynnascaris* sp.	Mississippi	This study
S. inflata	*Ascaris* sp.[d]	Mediterranean area	*17*
S. setosa	*Thynnascaris* sp.	France	*66*
	Nematode[b]	English Channel	*51*
	Contracaecum sp.[b]	North Sea	*48*
Sagitta sp.	*Ascaris* sp.[b]	Mediterranean area	*44*
	Contracaecum sp.	Florida	*21*
	Contracaecum sp.[a,b]	North Sea	*68, 2*
S. tennis	*Thynnascaris* sp.	Mississippi	This study

Contracaecum aduncum belongs in the genus *Thynnascaris*.
[a]An illustration or description indicates this species belongs to the genus *Thynnascaris*.
[b]Considered by other authors as a species we consider belonging to *Thynnascaris*.
[c]Adult egg-bearing worms were present, but infection considered by authors as atypical.
[d]Considered by that author to be the same as a species we consider belonging to *Thynnascaris*.

provides indications for sources of infection for fishes. Records of these hosts for species of *Thynnascaris,* or for nematodes reported as *Contracaecum* but which are probably *Thynnascaris* (Table 1), include a wide range of taxonomic groups in at least eight phyla; seven new host records are reported here.

Penaeid shrimps comprise a leading seafood fishery in the United States, and because these crustaceans harbor different types of *Thynnascaris* larvae, special attention is directed to them. Woodburn et al. (*67*) first reported a larval nematode from shrimp in the Gulf of Mexico, and it was later identified as a juvenile *Contracaecum* sp. (*23*). Overstreet (*41*) pointed out that the species belongs to and should be placed in the genus *Thynnascaris.*

Several workers (cf. Table 1) have investigated the prevalence and intensity of infection of *Thynnascaris* spp. in shrimps. These larvae occur unencysted within the cephalothorax, primarily within the hepatopancreas, the dorsal "gland," and the cephalothoracic musculature; the gonads and serosal surface of the gut are sometimes parasitized. Based on several surveys of the three primary commercial shrimps (the brown shrimp, *Penaeus aztecus;* the white shrimp, *P. setiferus;* and the pink shrimp, *P. duorarum*) of Florida and the Gulf of Mexico and adjacent waters (*6, 22, 23, 27*), the incidence of infection varied from 2 to 16% and the intensity of infection ranged between 1 and 37 larvae per shrimp.

We present in Table 2 collection data for larval *Thynnascaris* from 663 brown and 63 white shrimp from Mississippi Sound examined during the summer of 1972. This information, together with data given by Corkern (*6*) on larval *Thynnascaris* in brown shrimp, shows that for some size-groups of shrimp both the prevalence and

TABLE 2. *Distribution of larvae of* Thynnascaris *in* Penaeus Setiferus *and* P. aztecus *from Mississippi sound and adjacent water during the summer of 1972*

Range of total length in mm	No. of shrimp examined		Per cent infected		Average no. per infected shrimp		Maximum no. per shrimp	
	White	Brown	White	Brown	White	Brown	White	Brown
100	0	70	—	19	—	1.6	—	6
100-129	1	321	0	26	—	2.0	—	17
130-159	21	256	57	31	3.8	1.4	20	5
159	41	16	68	56	9.1[a]	2.2	31[a]	4
Total	63	663	Mean 64	28	7.5[a]	1.8		

[a]Excluding single femal shrimp with 178 larvae

intensity of infection may be higher than that reported by others (*22, 27*). Corkern reported nearly 80% of brown shrimp with a carapace length near 25 mm that he examined in Texas to be infected, and we found 178 larvae in one large white shrimp in Mississippi. Generally, the incidence of infection increased with the size of shrimp, but the intensity remained comparatively stable. White shrimp had a higher average intensity of infection than brown shrimp of the corresponding size during that summer. We found no obvious differences by sex in either incidence or intensity of infection.

Our best conclusion concerning the prevalence and intensity of infection, based on both published and unpublished data collected over the last 8 years from shrimps from the northern Gulf of Mexico and Florida, is that these parameters vary widely within and among populations, age groups, and species, as well as seasonally and geographically. It appears impossible even to predict with certainty whether shrimp caught on consecutive days in a given locality will have similar levels of infection.

Three, or possibly four, types of larval *Thynnascaris* have been found in shrimps. Several workers (*6, 14, 22, 23, 27*) reported commonly finding in commercial shrimps what appears to be one larval type, which for our specimens is 3 to 5 mm long, has an esophagus approximately 15% the length of the body, and a ventricular appendix approximately 3.5 times as long as the intestinal caecum. Judging from morphometric data, from records for definitive hosts of adult worms, and from food preferences of definitive hosts, Hutton et al. (*22*) suggested that the type of larva they reported was probably *C. habena.* However, since undescribed species of *Thynnascaris* occur in fishes from the northern Gulf of Mexico, the data necessary to validate their inference are lacking.

More than 99% of the *Thynnascaris* larvae we found in 1029 brown and white shrimp collected during one season from Mississippi Sound correspond morphologically to those described above, except that they are characterized by minute spinous projections on the tip of the pointed tail. For convenience, and until these larvae are directly compared to others or can be assigned a specific name, we designate them as Mississippi Type A (MA).

Feigenbaum (*14*) found in *P. vannemei* from the Pacific coast of Mexico a second type of larval *Thynnascaris,* which clearly differs from the type already discussed. It is 1 to 2 mm long, with an esophagus approximately 11% of the body length, a ventricular appendix nearly 13 times as long as the intestinal caecum, and a spineless, bluntly-rounded tail.

We found what may be a third distinct type, designated Type MB, only in a few brown shrimp from Mississippi Sound. It is approximately 2 mm long, has an esophagus approximately 8% of the body length, a ventricular appendix at least 20 times the length of the abbreviated intestinal caecum, and a spineless, bluntly-rounded tail.

THE PUBLIC HEALTH IMPORT OF THYNNASCARIS

To reach man alive, larval nematodes in seafoods obviously must survive preparation of the food for consumption. Tolgay (*59*) destroyed larvae of *T. aduncum* in the flesh of anchovies by methods used in home cooking or salting of these fishes in Turkey (frying in oil, cooking in water, or salting in coarse salt). Individual larvae and larvae in anchovies in 20% solutions of NaCl were killed within 30 and 60 min, respectively.

At the request of the National Fishery Products Inspection and Safety Division, NOAA, we investigated the tolerance of larvae of *Thynnascaris* (Type MA) from brown and white shrimp to temperatures encountered in shrimp frozen, refrigerated, maintained at room temperature, or cooked.

The cephalothoracic viscera, removed from shrimps caught by trawling in Mississippi Sound and maintained alive in the laboratory until necropsy, were placed in 0.85% saline and teased apart, allowing larvae to emerge from the tissues. Less than 8 h post-necropsy, groups of five larvae were placed in 0.85% NaCl in small capped vials and maintained at temperatures of –20, 0, 25, or 50 C for various periods. Larvae that were maintained at 0 C were examined either periodically during periods of up to 8 weeks or following periods of 10 to 30 days; those examined periodically were brought to 25 C during a period of up to 2 h before examination. Larvae that were maintained at 25 C were examined periodically during periods of up to 8 weeks, and those at –20 C and 50 C at the termination of the various periods of maintenance at those temperatures. We determined larval viability by examining larvae in vials under 30 × stereoscopic magnification for several minutes; those failing to move following agitation of the vial were judged dead, and those failing to move during two consecutive examination

periods were removed from the vial. Ten replicates were conducted for each experiment at 0 C and 25 C with periodic examinations; five replicates were usually performed for all other series.

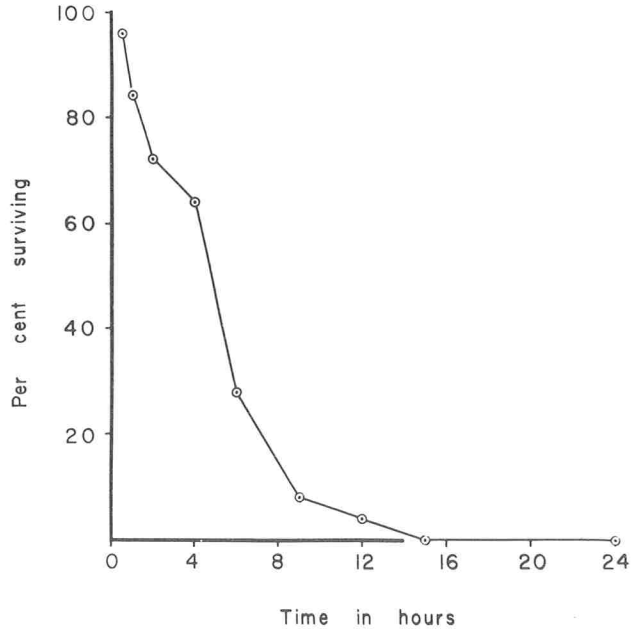

Figure 2. *Percentage of* Thynnascaris *Type MA larvae surviving maintenance at –20 C for various periods from 0.5 through 24 h. Each point represents the average of five replicates of five larvae each.*

At –20 C (Fig. 2) most larvae were alive after 4 h, but none survived after 12 h. At 0 C (Fig. 3) most larvae examined periodically remained alive for 2 weeks, and in certain replicates relatively high proportions of larvae survived for nearly a month, but none lived longer than 7 weeks. Only 25% of the larvae, however, survived continuous maintenance at 0 C for 20 days, and none survived continuous maintenance at this temperature for 30 days. At 25 C (Fig. 3) most larvae died before the fourth day, but a small proportion were alive for longer

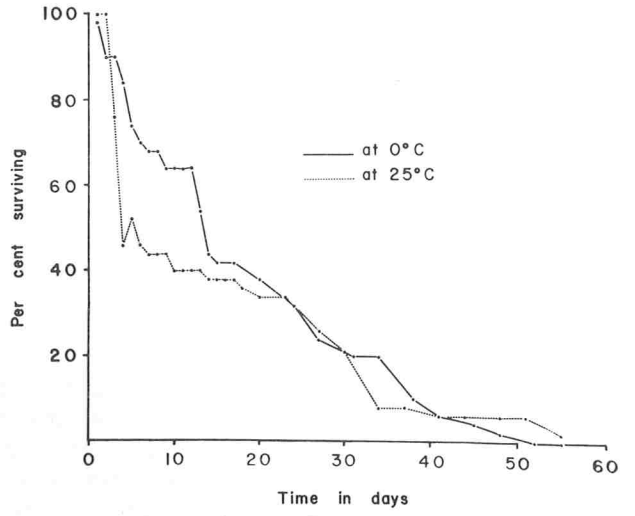

Figure 3. *Percentage of* Thynnascaris *Type MA larvae surviving following maintenance at 0 C and 25 C for periods of up to 8 weeks. Each point represents the average of 10 replicates of five larvae each.*

than 5 weeks. At 50 C no larva lived for 30 sec.

Our results for tolerance of larvae to various temperatures roughly correspond with those collected for larvae of other ascaridoids from marine fishes reported or summarized by others (*8, 31, 40, 49, 50*). They show that *Thynnascaris* larvae cannot survive the normal methods of food processing and preparation of shrimp.

Knowledge of the behavior of larval *Thynnascaris* in mammals is requisite to assessing its possible public health importance. Tolgay (*59*) and Lüleci et al. (*34*) inoculated mice, rats, guinea pigs, rabbits, and cats per os with larvae of *C. aduncum* or *"Contracaecum* sp." from anchovies and reported that at 10-20 or at 45 days post-inoculation no larvae were recovered from and no lesions were seen in any experimental host. Shiraki (*56,* summarized in *39*) found that a species of *Contracaecum* (= *Thynnascaris*) from a squid was quickly digested without any sign of penetration following inoculation per os of rabbits and dogs.

We report here initial studies of the behavior of two types of larval *Thynnascaris* in mice. During the summer of 1973, abnormally low salinity of Mississippi Sound reduced the shrimp population and prohibited us from collecting enough *Thynnascaris* larvae from shrimp for inoculating experimental mammalian hosts. Therefore, we collected for this purpose two types of *Thynnascaris* larvae, closely resembling and possibly identical to types MA and MB, from the cutlassfish, *Trichiuris lepturus.* The cutlassfish is important in the petfood industry in the northern Gulf of Mexico and is a foodfish in parts of the Caribbean Area, the Orient, and elsewhere (*9*). We found over several seasons that the fish feeds primarily on anchovies, penaeid shrimps, and mysids, all intermediate or paratenic hosts for larval *Thynnascaris,* and it may act as a paratenic host for the encountered nematodes.

We caught and iced cutlassfish from Mississippi Sound, removing nematode larvae within 8 h. The gastrointestinal tract distal to the esophagus was removed and placed in 0.85% saline to allow larvae to emerge from the serosal tissues. These larvae (usually 25 Type MA or 50 Type MB) were immediately introduced into the stomach of 30-35-g white Swiss mice, with a 15 gauge cannula fitted to a 0.25-ml syringe. We examined groups of two or four mice inoculated with Type MA larvae 1, 2, 4, 12, and 24 h post-inoculation and groups of two or three mice inoculated with Type MB larvae at 12 h and 1, 2, 3, and 5 days post-inoculation. Mice were killed with ether. The abdominal cavity and organs were washed with a jet of saline and the washings examined for larvae; the abdominal and thoracic viscera were removed, placed in 0.85% saline, opened, and examined grossly for larvae and associated lesions. We examined microscopically several larvae within tissue between two 50 × 75 mm glass slides, and we fixed tissue containing gross lesions or larvae in buffered 10% formalin for later microsectioning.

Type MA larvae died within the lumen of the gastrointestinal tract by 2 h post-inoculation, and we recovered none at later periods. No lesions were found.

Twelve hours post-inoculation Type MB larvae adhered to the mucosa of the small intestine and occurred within the stomach wall and liver and in the abdominal cavity. Presence of larvae in the wall of the stomach was associated with areas of diffuse hemorrhage; these larvae, observed in situ, moved within what appeared to be tunnel-like excavations at the level of the muscularis, and by the first day of infection an inflammatory exudate filled these excavations. Aggregations of inflammatory cells surrounded larvae washed from the abdominal cavity at that time.

Beginning the second day of infection larvae in the gastrointestinal tract persisted near the serosal surface of the stomach and small intestine or in the mesentery, in closely-adhering extensions of tissue. Larvae within the abdominal cavity ceased movement by the second day, and those within tissue after the fifth. By the fifth day of infection, areas of hemorrhage had resolved; the only grossly visible lesions, papillate elevations of the serosa, projected from the surface of the stomach and small intestine.

From the information reviewed and presented in this paper, larvae of *Thynnascaris* in seafood appear less important as a consumer hazard than those of anisakine ascaridoids. Evidence suggests, however, that larval *T. aduncum* off France has caused human disease, and that the *Thynnascaris* Type MB larva in the Gulf of Mexico could be potentially significant to those eating certain raw fishes or crustaceans. The fact that the Type MB larva in shrimp limits itself to the cephalothorax of its host may be insignificant, because the hepatopancreas rapidly lyses following the shrimp's death (*33*), and contained larvae from there and from the dorsal "gland" disperse; if such larvae subsequently enter the abdominal musculature, they could be a threat to consumers indulging in uncooked fresh shrimp.

A final point to consider is the possible effect of frozen larvae ingested by individuals sensitized by previous infection. Experimental studies by several investigators (summarized in *39, 40*) present evidence suggesting that even dead anisakine larvae or their products can elicit in sensitized animals with existing lesions an allergic reaction, which in some cases resulted in pathological changes similar to those in human anisakiasis.

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Microbiological Criteria for Food in Military and Federal Specifications

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ABSTRACT

Microbiological criteria in military and federal food specifications are presented. Approximately 10% of more than 600 specifications for food contain microbiological requirements which are applied in procurement of food for military and federal agencies. Foods were grouped into four categories which include dehydrated foods, milk and milk products, miscellaneous dairy products, and frozen foods. Microbiological requirements vary with the food item and include criteria for the aerobic plate count, coliforms, *Escherichia coli*, salmonellae, yeast and mold, proteolytic bacteria, lipolytic bacteria, psychrotrophs and direct microscopic count. Specifications may be obtained by writing to the U.S. Naval Publications and Forms Center, NPFC Code 1032, 5801 Tabor Avenue, Philadelphia, Pennsylvania 19130.

Microbiological specifications for foods purchased by Military and Federal Agencies for their own use specify the maximum acceptable number of microorganisms, or of specific types of microorganisms as determined by prescribed methods. These specifications are used by food manufacturers, procurement agencies, and testing laboratories to determine compliance with military and federal requirements for food items and to improve the food supply by standardizing the quality and assuring the safety of the food. The cheif purposes of microbiological specifications are to give assurance that: (*a*) the foods will not be responsible for spread of infectious disease, or for food poisoning; (*b*) the foods consist of high quality materials that have not deteriorated or become unduly contaminated during processing, packaging, storage and handling; (*c*) filth has not been introduced into the food; and (*d*) the foods have the keeping quality expected of the product (*7*).

Of the approximately 600 military and federal food specifications, 59 contain microbiological requirements. Attempts are being made to standardize methodology and criteria in these specifications so that the best possible method is used to isolate a specific organism or group of organisms. In addition, indices are chosen which can be used to evaluate health hazards or poor sanitary practices. It is recognized that specifications must be attainable under conditions of good commercial practice and must be easily administered and technically feasible. Microbiological requirements in these specifications are based on research data or information gathered from surveys of foods purchased by military or federal agencies whenever possible.

The microbiological criteria in Military and Federal Specifications for food reported here were compiled to inform industry and other Government agencies of the current military standards used for the examination of foods. Specifications for food and Technical Reports may be obtained by writing to the U.S. Naval Publications and Forms Center, NPFC Code 1032, 5801 Tabor Avenue, Philadelphia, PA 19120.

MICROBIOLOGICAL CRITERIA FOR DIFFERENT FOODS

Microbiological examination of food in the Military and Federal Subsistence system is made in accordance with standard procedures recommended by leading authorities (1-6, 9-11). The methods are presented in military and federal specifications for each product. Methods and criteria for all products are presented in U.S. Army Technical Report 73-33-FL (*8*). Sampling plans and procedures for inspection were in accordance with Military Standard 105D, entitled *Sampling Procedures and Tables for Inspection by Attributes.*

The microbiological criteria for cooked, dehydrated foods are presented in Table 1. The aerobic plate count (APC) limits range from 10,000/g to 200,000/g depending on the food item and the processing it receives. The limitation for coliforms is 40/g or less and *Escherichia coli* must be negative per gram. Because three of the food items are high risk items, they are required to be negative for salmonellae in 25 g.

The microbiological criteria for milk and milk products comply with Public Health Service requirements and are presented in Table 2. These products were separated from other miscellaneous dairy products listed in Table 3 for convenience and ease of presentation. The APC lmitations per ml(g) range from 5,000 to 50,000. Coliforms are limited to 10/ml(g), except for dry whole milk and filled milk (90/g). Dry, flavored dairy drinks and nonfat dry milk must be negative for salmonellae in 100 g and dry filled milk must be negative for salmonellae in 25 g. Only milk fat has a yeast and mold limitation ($\leq$30/ml or g).

Table 3 presents the microbiological criteria for miscellaneous dairy products. The APC limitations range from 10,000/g to 300,000/g. The type of product is

TABLE 1. *Microbiological criteria for dehydrated food*

Dehydrated food item	Spec. No.	Maximum count per gram				Method ref.
		APC[a]	Coliform	E. coli	Salmonellae[b]	
Beef, cooked	MIL-B-4344A	150,000	40	—	—	*3*
Beef stew, cooked	MIL-B-43404B	75,000	—	Neg	—	*5*
Beef with rice, cooked	MIL-B-43750A	75,000	—	Neg	—	*5*
Chicken and chicken products, cooked	MIL-C-0043135D	75,000	—	Neg	—	*5*
Chicken with rice, cooked	MIL-C-43289B	75,000	—	Neg	—	*5*
Chili con carne, cooked	MIL-C-43287C	75,000	—	Neg	—	*5*
Egg mix	MIL-E-43377B	25,000	10	—	Neg[b]	*2,3,11*
Escalloped potatoes with pork, cooked	MIL-E-43749A	75,000	—	Neg	—	*5*
Hash, beef, cooked	MIL-H-43224B	75,000	—	Neg	—	*5*
Macaroni, instant	MIL-M-35067A	50,000	10	—	—	*3*
Meat balls and meat ball products, cooked	MIL-M-43506	150,000	40	—	—	*3*
Pork sausage, cooked	MIL-P-43383A	200,000	40	—	—	*3*
Pork slices, cooked	MIL-P-43629	110,000	20	—	—	*3*
Potato and cheese bar, survival	MIL-P-35087C	—	—	—	Neg[b]	*6*
Spaghetti with meat sauce	MIL-S-43275B	75,000	—	Neg	—	*5*
Topping, dessert and bakery products	MIL-T-35038C	10,000	10	—	Neg[b]	*3,6*
Tuna, cooked	MIL-T-43443	200,000	40	—	—	*3*
Turkey, cooked	MIL-T-43451	200,000	40	—	—	*3*

[a]Aerobic plate count (APC).
[b]Negative per 25 g.

TABLE 2. *Microbiological criteria for milk and milk products*

Finished milk product	Spec. no.	Maximum count per ml (g)					Method ref.
		APC[a]	Coliforms	Salmonellae	Yeast and mold	DMC[d]	
Cream substitute, dry or liquid non-dairy	MIL-C-43338C	20,000	10	—	—	—	*3*
Flavored dairy drink, dry chocolate-coffee flavored	MIL-F-35100B	20,000	10	Neg[b]	—	—	*3,6*
Malted milk	C-M-50A	30,000	10	—	—	—	*3*
Milk and milk products, fresh, fluid, concentrated and frozen	C-M-1678	20,000	10	—	—	—	*2,3*
Milk (plain or chocolate flavored), cream, half and half, filled and cheese, cottage and filled	MIL-M-35082B	20,000	10	—	—	—	*3*
Milk fat	MIL-M-1036E	5,000	10	—	30	—	*3*
Milk, nonfat, dry	C-M-3050B	50,000	—	Neg[b]	—	—	*3,5*
Milk: milk, skim, half and half							
Cream: reconstituted or recombined	MIL-M-1022D	20,000	10	—	—	—	*3*
Milk, whole, dry	C-M-355a	30,000	90	—	—	40,000,000	*3*
		Premium 50,000					
		Extra grade	90	—	—	75,000,000	*3*
Milk, filled, dry, plain or chocolate, fortified	MIL-M-43241	30,000	90	Neg[c]	—	—	*3,11*

[a]Aerobic Plate Count (APC).
[b]Neg per 100 g.
[c]Neg per 25 g.
[d]DMC-Direct Microscopic Count.

indicated by Roman numerals in parenthesis. Butter is analyzed for preoteolytic and lipolytic bacteria which may not exceed 100/g and cottage cheese is analyzed for psychrotrophic bacteria which may not exceed 100/g. The coliform limitation ranges from 10/g to 90/g. Ice cream mix is the only item which must be analyzed for salmonellae (neg. per 25 g). The yeast and mold count for four products is limited to 20/g or less.

Table 4 presents the microbiological crtieria for precooked frozen foods. The limitations for precooked frozen foods are: APC, 100,000/g; coliforms, 100/g; *E. coli,* neg/g; and Salmonellae, neg/25 g. The APC for uncooked frozen foods range from 30,000/g to 500,000/g and the coliform limit ranges from 10/g to 230/g. Testing for *E. coli* and salmonellae is not required for uncooked frozen food.

These criteria are current and are presented as they appear in the specifications. However, revision and

TABLE 3. *Microbiological criteria for miscellaneous dairy products*

Finished dairy product	Spec. no.	Maximum count per gram (ml) APC[a]	Coliforms	Salmonellae	Yeast and mold	Method ref.
Butter	C-B-801G	b	10	—	20	*3*
Buttermilk, Fluid and milk, whole, fresh cultured	C-B-816F	—	10	—	—	*3*
Buttermilk solids; dry cultured and uncultured	C-B-825a	50,000(I-1)[c]	90	—	—	*3*
		300,000(I-2,II)[c]	90	—	—	*3*
Cheese, cottage	C-C-281E	e	10	—	10	*3*
Cheese, processed, American, dehydrated	MIL-C-35053B	50,000	90	—	—	*3*
Cream, sour; cultured	C-C-678a	—	10	—	10	*3*
Ice cream, ice milk and sherbert imitation; ices and novelties	MIL-I-35027B	50,000(I-IV)[c]	20	—	—	*3*
		10,000(V)[c]	—	—	—	*3*
Frozen fudge bar	MIL-I-35027B	50,000	20	—	—	*3*
Ice cream mixes, dehydrated	MIL-I-00705D	30,000	10	Neg[d]	20	*3,6*
Ice cream, sherberts, ices	EE-I-116B	50,000(I,II,III)[c]	10	—	—	*3*
		10,000(IV)[c]	10	—	—	*3*

[a]Aerobic Plate Count (APC).
[b]Proteolytic and lipolytic count $\leq$ 100 per gram.
[c](I-V) Type of product.
[d]Neg per 25 g.
[e]Psychrotrophs $\leq$ 100 per gram.

TABLE 4. *Microbiological criteria for frozen foods*

Food Item	Spec. No.	Maximum count per gram APC[a]	Coliforms	*E. coli*	Salmonellae[b]	Method ref.
		Precooked Frozen				
Chicken a la king	LP/P DES 12-70	100,000	100	Neg	Neg	*2,3,10*
Chicken cacciatore	LP/P DES 20-70	100,000	100	Neg	Neg	*2,3,4,10*
Macaroni and cheese	LP/P DES 15-70	100,000	100	Neg	Neg	*2,3,4*
Meal, precooked	MIL-M-13966D	100,000	100	Neg	—	*2,3,4*
Pork and beef chop suey	LP/P DES 34-70	100,000	100	Neg	Neg	*2,3*
Pork loin, sliced with gravy	LP/P DES 27-70	100,000	100	Neg	Neg	*2*
Shrimp creole	LP/P DES 37-30	100,000	100	Neg	Neg	*2*
Swiss steak w/gravy	LP/P DES 21-70	100,000	100	Neg	Neg	*2,3,4,10*
Turkey w/gravy	LP/P DES 22-70	100,000	100	Neg	Neg	*2,3,10*
		Frozen Uncooked				
Eggs and egg products[d]	C-E-230C	50,000[c]	—	—	Neg	*2*
Oysters, fresh, shucked	PP-0-956F	500,000	230(MPN)[e]	—	—	*1*
Shrimp, raw, breaded	PP-S-315C	500,000	50(MPN)	—	—	*5*
Topping, dessert and bakery products	MIL-T-35024A	30,000	10	—	—	*3*

[a]Aerobic Plate Count (APC).
[b]Per 25 g.
[c]Table grade, 20,000 per gram.
[d]Yeast and mold 50 per gram.
[e]Fecal coliforms per 100 g at 44.5 C.

updating of some specifications is currently underway and new specifications are being developed. Since only the microbiological criteria are presented, the original specification should be consulted for information regarding quality assurance, inspection, sampling, packaging, microbiological and chemical analysis, storage and material requirements. Specifications may be obtained by writing to the U.S. Naval Publications and Forms Center, NPFC Code 1032, 5801 Tabor Avenue, Philadelphia, Pennsylvania 19130.

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Some 25 members of the Automatic Merchandising Health-Industry Council (AMHIC) participated in the annual meeting held in October in connection with the national convention and exhibit of the vending and foodservice management industry in New Orleans, sponsored by the National Automatic Merchandising Association (NAMA). Shown presiding at the meeting (center of photo) are from left, David E. Hartley, NAMA public health and safety counsel who is AMHIC secretary, and Justin Diercks, Omaha-Douglas County Health Department, AMHIC co-chairman.

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Recent Trends in Vending

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ABSTRACT

The recent evolution in design and construction of food and beverage vending machines is discussed, with particular emphasis on machine features having health and safety importance. New types of equipment and future innovations are explored on the basis of presently available technology.

At any trade show or exhibit of the vending industry, an often-heard comment is that "there's nothing new this year!" Of course, the statement is true if it means that no one has yet introduced a machine that tosses pizzas in the air or makes crepe suzette. By 1975 the range of products that can effectively and economically be vended has fairly well been met.

But an industry whose sales have spiralled from $600 million in 1946 to $9 billion in 1975 has obviously not stood pat—there has been a steady evolution in equipment, vendible products and services, particularly in the food vending segment of the industry. To look at some new and recent industry trends, the logical apporach is on a product-by-product basis since each machine is designed to accommodate a generic product category.

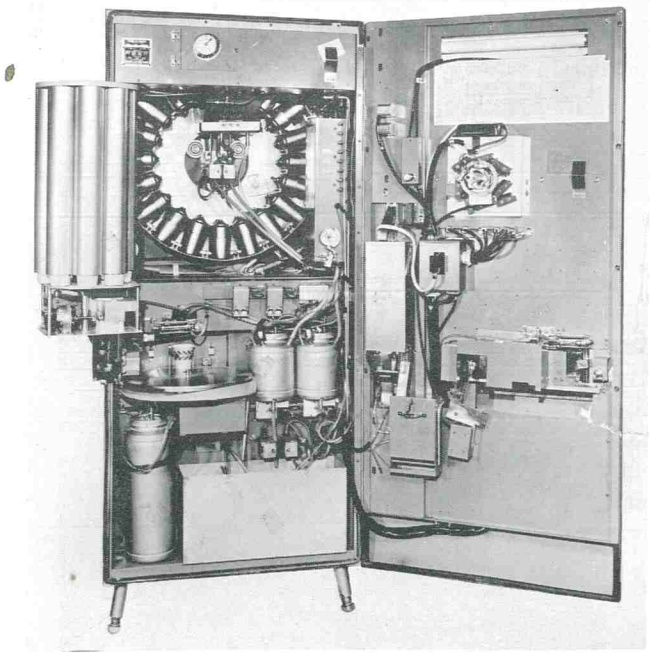

Figure 1. *First brewed coffee vender—1950's. Each of the 21 cylindrical brewers held eight cups of dry ground coffee. Note rotating cup platform for rear filling, front delivery. (Square Mfg. Co.)*

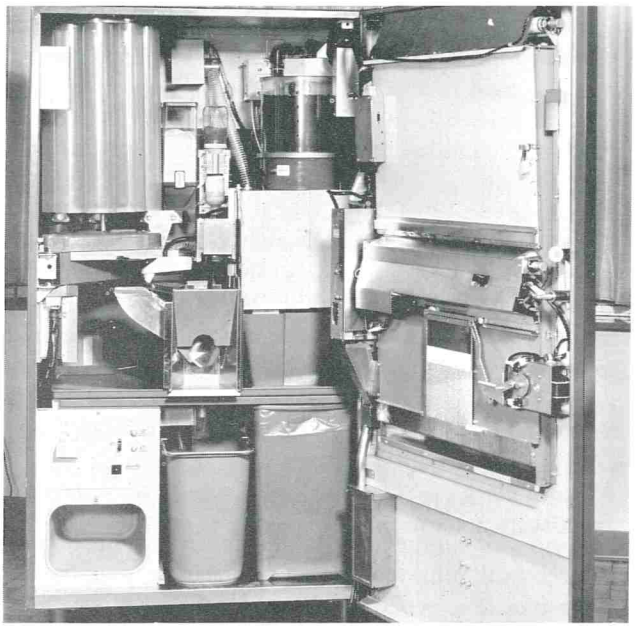

Figure 2. *Vender for individually brewed coffee, tea, soup, and chocolate—1975. Plastic shelf, lower left, removable for use as sanitation pail. (National Vendors, Inc.)*

HOT BEVERAGE VENDING

The term "coffee vending machine" is, in most instances, a misnomer. For many years, hot beverage venders have dispensed coffee and hot chocolate as a *minimum* offering (Fig. 1). Present venders may offer, additionally, one or two soup options and hot tea—with extra cream and sugar in the coffee and tea cycles.

Most present hot beverage venders use finely ground coffee brewed one cup at a time in 12 to 15 sec. The remainder use instant, or, increasingly, freeze-dried coffee. Refrigerated liquid concentrates (coffee, cream, sugar, chocolate, soup, and tea) have been entirely phased out in favor of dehydrated ingredients, with *no reduction in quality* and great advantages in equipment costs and sanitation (Fig. 2).

In this equipment category two health and safety improvements loom largest—development of 200 F hot water flushing systems or programmed push-button

cleaning systems; and, use of non-pressurized water heaters to safely generate hotter water. Of equal health significance has been the steady simplification of components and systems to promote ease of cleaning and shorten the service time.

CUP SOFT DRINK VENDORS

The advent, in the early 1960's, of compact in-machine ice-makers was one of vending's notable breakthroughs in that decade. More recently, perfection of automatic, recirculating devices for cleaning the icemaker-reservoir-tubing system has contributed greatly to the ease of machine maintenance and ice quality.

In the post-mix machine category, all machines with icemakers are now completely free of copper tubing, a change aimed at *absolute elimination of any potential for copper poisoning.* Practically all machines in the field which still contain copper tubing are equipped not only with the double check-valves required by the Public Health Service *Vending Code* but also with an added vented valve required by the National Automatic Merchandising Association's (NAMA) standards as developed by the Automatic Merchandising Health-Industry Council (AMHIC). Pre-mix soft drink machines are rapidly being phased out, largely because of the labor costs involved in carrying product canisters to the machine as opposed to piping in the water ingredient.

DAIRY PRODUCT MACHINES

For a while in the early 1960's, there were at least half a dozen bulk milk vending machines in production or at proto-type. For economic reasons, bulk milk was found to be impractical as a vended product and the machines were phased out or converted to vend fruit juices.

Supplementing the traditional carton milk and ice cream bar venders are the new fixed-shelf "sandwich machines" which are now available, optionally, for frozen products. These machines permit the industry to offer everything from parfaits to banana splits, a particularly welcome selection in schools, colleges, and public locations.

SANDWICH VENDERS

Again a misnomer. The refrigerated all-purpose or perishable food vending machines accommodate sandwiches, entrees, casseroles, salads, desserts, juices, fruit, and about anything else that will fit into the shelf space. At one time, many perishable food venders were of the constantly-heated type, holding products at 140 F or higher.

The advent of microwave ovens has reduced the use of heated perishable food machines and increased the use of refrigerated units. A sandwich or an entree has a much longer palatability shelf life when held cold and heated at the time of sale. Of interest to health officials and consumers should be the recent improvement in the automatic cut-off controls which inactivate perishable food venders when safe temperatures are lost for any reason. Controls now have improved (reduced) tolerances and NAMA's standards have been upgraded to make field testing and access by sanitarians much easier..

HOT CANNED FOOD MACHINES

Consisting of nothing more than a heated cabinet with internal, first-in first-out storage racks, there has really been no need to modify these machines over the years other than to increase the capacity or the number of product selections. But there has, indeed, occurred a sanitation breakthrough—not in the equipment *but in the cans.* The same industry that brought the can opener out into public view (and use) has solved the can opener sanitation problem by introducing self-opening hot canned foods. Public acceptance is splendid.

CANDY-CRACKER-SNACK VENDERS

While of only casual interest to most public health professionals, this equipment category has seen several changes and improvements, some affecting product freshness and insect control.

An entirely new breed of glass-front machines capable

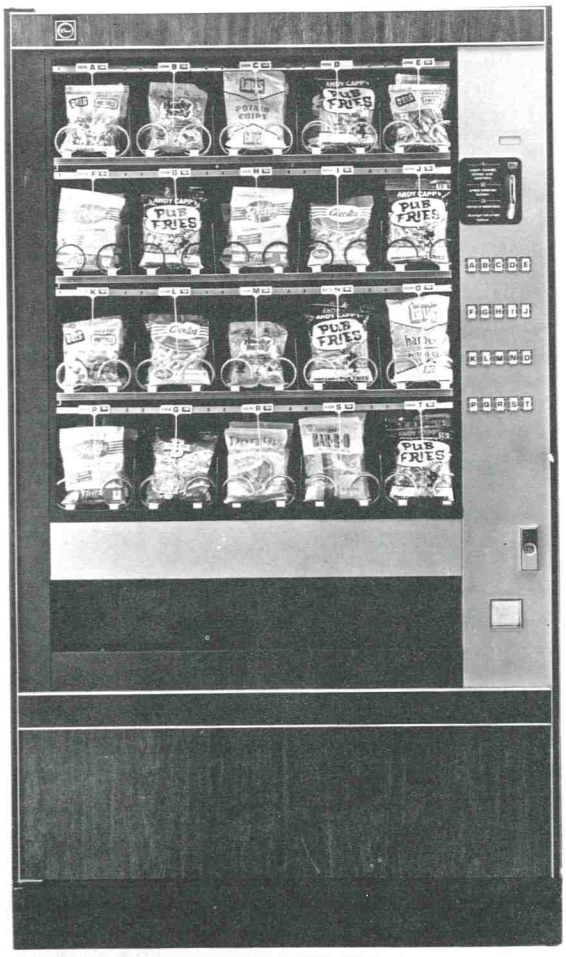

Figure 3. *New type vender for bagged snacks. Some models refrigerated for perishable food packets.* (Rowe International)

of vending bagged snacks has been introduced (Fig. 3). Utilizing spiral-wire vend systems, these machines vend first-in, first-out (FIFO), allow customer scrutiny of up to 28 product selections, and can be optionally refrigerated for use with packets of cheese, luncheon meat, bread, and other sandwich ingredients for the working person to pick up on the way home.

The same FIFO concept has been introduced into traditional candy-cookie-cracker-pastry venders of the drop-shelf variety with one added feature—*the customer now gets the item showing in the display window;* it is no longer a static display (Fig. 4).

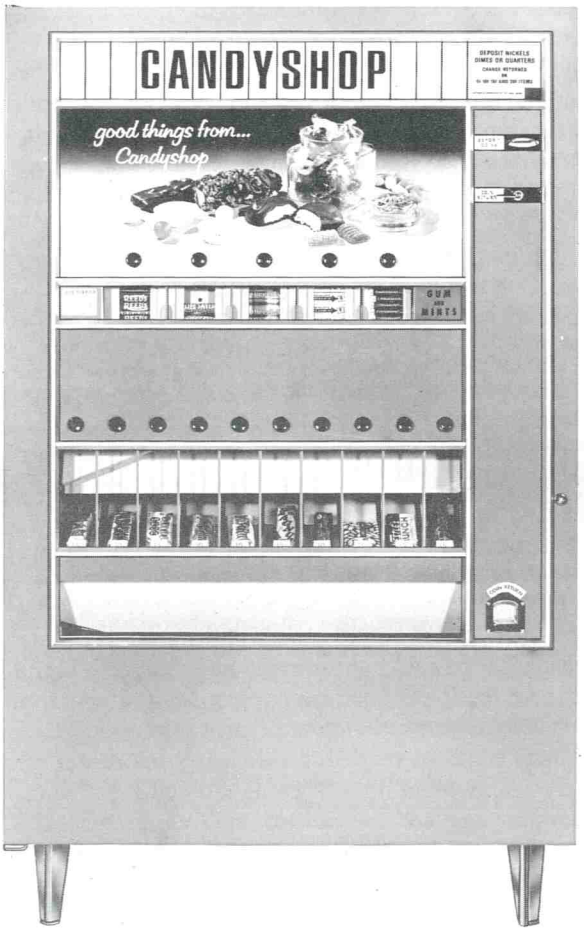

Figure 4. *Confection vender with "first-in, first-out" feature. Product on display is next to vend. (Automatic Products Co.)*

The FIFO procedure has two significant public health and consumer relations aspects—the elimination of the ages-old problem of insects, (such as the saw-tooth grain beetle) developing in products which the vending routeman might have forgotten to move downward into the next-to-vend position, and customer dissatisfaction when the display item and the items in that vend column were not the same.

OFFICE-TYPE VENDERS

Within the past few years a new off-shoot of the vending industry, office coffee service, has appeared (Fig. 5). Office coffee service (OCS) dispensers are increasingly being coin-adapted and designed for a wide range of products.

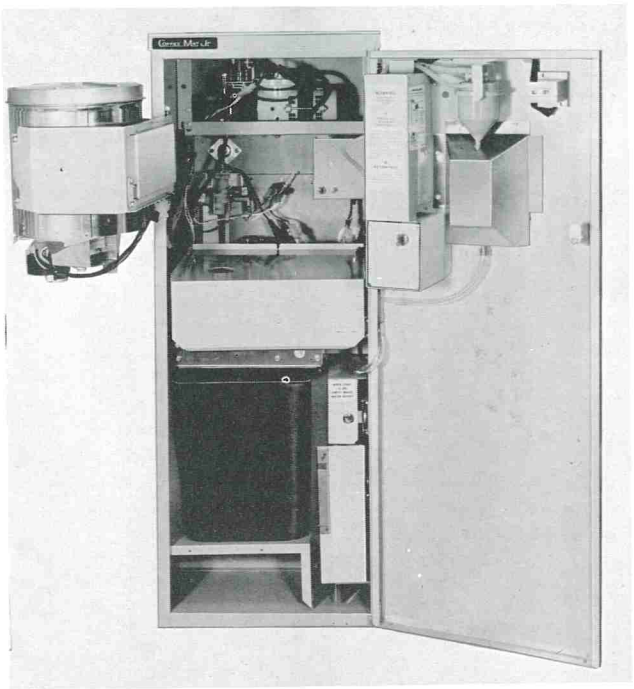

Figure 5. *New type "Mini-vender" for small locations. Vends brewed coffee only. Equipped with auto-cleaning system. (Coffee-Mat Corp.)*

This new industry provides the dispensers, products, and equipment repair services for small business establishments *but without the traditional route service of vending companies.* The equipment is sufficiently uncomplicated to allow employees at the location to do their own filling, servicing, and cleaning.

NAMA's AMHIC organization and the National Sanitation Foundation (NSF) have both developed new standards for this "mini equipment" pending inclusion of such equipment in the revised Public Health Service Vending Code, planned for 1975.

NEW PRODUCT VENDERS

Through the years there has been an almost steady stream of new-type vending machines which inventors—often without proper market research—felt would revolutionize the vending industry. In the 1950's there were machines which: (*a*) used electrodes to heat hot dogs vended in a box; (*b*) cut and squeezed oranges while the customer waited and watched; (*c*) sliced ice cream, applied sandwich wafers on both sides and vended (the mess) on a napkin; and (*d*) the bulk milk and milkshake venders mentioned earlier.

In the 1960's the industry saw: (*a*) a machine that contained an infra-red oven to heat sandwiches; (*b*) a dispenser *in the shape of a hen* which dispensed (without refrigeration) hard-cooked eggs for the tavern trade; and, (*c*) a vender which reconstituted from dry dairy mixes a variety of "milkshakes" and associated products, perishable and non-perishable.

Figure 6. *Vender for small bags of ice. Capacity: 93 three-pound or 192 one and one-half pound bags. (The Vendo Co.)*

So far, in the 1970's, the inventive spirit in the U.S. and abroad seems not to have diminished. We have seen: (*a*) a sandwich machine with an integral microwave oven; (*b*) an orange juice vender which reconstituted frozen concentrate through something akin to the Venturi valve principle; and, (*c*) a snack vender operated on the "honor system" which triggered a 100 decibel alarm if the customer forgot to insert proper coinage.

These are merely examples—and certainly not as "far-out" in a growing industry as some of them appear. At the same time, there are other new vending concepts that seem capable of finding a place in the vending industry or in related industries experienced in the use of coin-operated, non-attended equipment. For example:

Ice vending machines

The sanitation problems which attend the manufacture and dispensing of ice from bin-type icemakers in motels and other establishments are well-known and legion. Vending machines which manufacture, store, dispense, and even inflate and heat-seal the plastic bag are now available (Fig. 6). Other icemaker-dispensers for cafeteria counters, waitress' stations, and hospital patient service areas are in production, with product contamination virtually impossible.

French-fired potato venders

An interesting innovation from Canada and Great Britain, these so-called "chip" venders store pre-blanched, frozen French fries at ambient temperature and cook in 30-40 sec on individual order.

Purified water vending machines

For many years, some areas of the U.S. have had "problem water" conditions, involving off-tastes and sediment more than potability. The recent publicity concerning the New Orleans water supply, in particular, and the safety of chlorinated municipal water supplies, in general, suggests an increasing public demand—at least in some areas—for drinking water *not taken from the tap.* Again, there is new vending equipment available to meet the need of those who demand drinking water which has been dechlorinated, filtered, and rendered as free as possible of off-tastes and off-odors.

CONCLUSION

Years ago, some vending machines were equipped to utter an almost-fogotten expression, "thank you", after each vend. With electronic sensors, future machines can be programmed to detect malfunctions, apologize, and instruct the customer to make his selection again. In a nutrition-conscious society, a pastry vender that asks "have you had your milk today" might be right on target.

It should be noted that vending machines do not presently offer every conceivable product that can reasonably be sold by automatic methods. The limitation at this time is product sales price. Many items ranging from cut-flowers and indoor plants to frozen lobster/ filet dinners could be vended if the average consumer carried more coins than most people do (currency and bill changers are not the answer, for many reasons). The vending industry's product offering could be diversified much further if new U.S. coins of higher values (beginning with the "silver dollar") are reduced in size sufficiently to become *portable.*

Whatever direction the vending industry takes in the future, its favorable and rewarding experience with nationally-recognized sanitary and safety standards guarantees that each future innovation will be carefully evaluated for consumer health protection and safety before it passes the prototype stage.

ACKNOWLEDGMENT

This paper was presented at the 62nd Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Toronto, Ontario, Canada, August 10-13, 1975.

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Report of Committee on Dairy Farm Methods, 1973-1975

M. W. JEFFERSON[1], DALE TERMUNDE[2], J. C. FLAKE[3], and JAMES B. SMATHERS[4]

SUBCOMMITTEE ON ANTIBIOTICS, PESTICIDES, AND OTHER ADULTERANTS

Adulteration of milk supplies continues to be of concern to the milk industry and regulatory agencies. A continued surveillance program must be maintained to assure the general public an unadulterated milk supply.

The prime area of study by the subcommittee these past 2 years has centered around laboratory tests being routinely used for milk and milk products throughout the United States. Through the Interstate Milk Shippers Program, state regulatory and official industry laboratories are presently under a laboratory surveillance and certification program.

This subcommittee received input from 44 states regarding the need for an expanded laboratory certification program to include milkfat, excessive water, and pesticides for those state and/or industry laboratories performing these tests for regulatory purposes. Thirty-four states were in favor of an expanded FDA-IMS-State Laboratory Evaluation Program. Milkfat testing procedures should be certified not from a pay purpose standpoint, but as a regulatory test for misbranding of packaged milk and milk products.

Based on information received from state regulatory personnel and the study conducted by the members of this subcommittee, we strongly recommend that milkfat, excessive water, and pesticide analyses used by states as they pertain to their regulatory milk program be included as a part of the FDA-IMS-State Laboratory Evaluation Program.

This expanded Laboratory Evaluation Program would insure nationwide uniformity on all tests and analyses used by the regulatory agencies in administration of a milk regulatory program.

Presently, most regulatory laboratories are using the Disc Assay Method for raw milk while FDA and others are using the *Sarcina lutea* Cyclinder Plate Method for testing finished products. The differences in these test procedures have created concern with the industry and regulatory agencies.

This subcommittee should continue to assess the magnitude of this problem and the relationship between the different tests being used.

SUBCOMMITTEE MEMBERS: *A. R. Brazis, B. Heineman, J. Boosinger, P. Dolan, V. Packard, K. Whaley, and L. Townsend,* Chairman.

SUBCOMMITTEE ON CLEANING AND SANITIZING OF FARM MILK EQUIPMENT

I. *Cooling and cleaning temperatures*
   A. Installation of a temperature recording device on the bulk tank is advantageous in observing both cooling and cleaning cycles. Both have a direct effect on milk quality.
   B. Recording temperature of pipeline washing solutions provides accurate records of the entire cycle, as well as frequency of cleaning.
   C. Experience continues to prove that to obtain best results in circulation type cleaning, starting temperature should be 160 F and ending temperature (10-min cycle) 150 F minimum. This type of temperature maintenance will provide clean equipment with a minimum amount of detergent.

II. *Water heating facilities*
   A. Estimated heater requirements for milkhouse operations:

Type	*Heater Size*
Bulk tank—Milking machines	50 gal.
Bulk tank—Mechanically cleaned and milking machines	80 gal.
Bulk tank—Pipeline or transfer system	120 gal.
Bulk tank—Pipeline (2-inch or more)	150 gal.

   B. In milking parlor operations, it is recommended that two water heaters be provided. One heater should be installed to provide water to udder washing stations. Capacity should be based on 30 gal. per 100 cows. One heater should be provided for washing equipment in milkhouse. Capacity of this heater should be based on the above estimated requirements.

III. *Maintenance and installation of vacuum systems*
   A. Vacuum pumps and regulators should be installed for easy access to observe operation, for ease of maintenance, positioned near floor drain and away from feed stuffs, and be properly ventilated for cooling and to eliminate obnoxious odors.
   B. Cleaning of entire system should be done on a once-a-month basis or when foreign matter has been known to enter the system. Follow the manufacturer's recommendations.

IV. *Cleaning of elliptical tanks*
   A. When single manhole tanks are used, it is imperative that a mechanical washer be provided. This washer should include facilities for pre-rinsing, washing, and post-rinsing. Mechanical sanitizing is optional with the producer.
   B. Spray device provided with washer should be designed for easy removal and cleaning.

V. *Cleaning of plastic milk tubing.* Preliminary investigation has shown that pre-rinsing plastic tubing with 115 F water (versus lower temperatures) results in less fogging and/or discoloration. It should be noted, however, that rinsing with temperatures over 115 F would be detrimental to cleaning. It is recommended that further investigation be implemented to evaluate the effect of rinsing temperatures on both cleaning and life of plastic tubing.

VI. *Mechanical cleaning devices.* It is recommended that when mechanical devices are installed for C.I.P. cleaning of either pipelines or bulk tanks, that identification be printed on the equipment in a conspicuous place noting the gallons of water required for each cycle. This will enable the user to provide proper concentrations of cleaning solutions based on product label recommendations.

VII. *Cleaning instructions.* Cleaning instruction cards should be posted in a conspicuous location indicating water test results and step-by-step cleaning and sanitizing instructions listing volumes of water, detergents, and sanitizers for each cycle. The producer can then use these instructions for reference eliminating possible error or misunderstanding.

SUBCOMMITTEE MEMBERS: *J. Burkett, C. C. Gehrman, C. Flack, F. Copenhaver, B. Scheib, and J. Welch,* Chairman.

[1]*Chairman of the Committee; Virginia Department of Agriculture and Commerce, 1444 East Main Street, Richmond, Virginia 23219*
[2]*Assistant Chairman of the Committee; Babson Bros. Company, 2100 South York Road, Oak Brook, Illinois 60521*
[3]*Consultant and Editor for the Committee; Evaporated Milk Association, 910 Seventeenth Street, N.W., Washington, D.C. 20006*
[4]*Consultant to the Committee; Maryland and Virginia Milk Producers Association, Inc., 1530 Wilson Boulevard, Arlington, Virginia 22209*

SUBCOMMITTEE ON EDUCATION

The Education Subcommittee has continued to assemble educational material for periodic publication of availability in the *Journal of Milk and Food Technology.*

A complete listing of this material has been assembled for inclusion in this 1973-1975 Farm Methods Committee Report.

1. Hand Book, 1973 Edition, Cost $1.00. *Milk Flavor Handbook,* Tri-State Milk Flavor Program, Prepared by: David K. Bandler, Cornell University, Sidney E. Barnard, Pennsylvania State University, and Dick H. Kleyn, Rutgers University. Write to: Cooperative Extension Service, Pennsylvania State University, 213 Borland Laboratory, University Park, Pennsylvania 16802.
2. Special Report 29, 1973 Edition, Cost $1.00. *Dairy Plant Fieldman-Hauler-Grader and Tester's Manual,* By: V. S. Packard, Extension Division, University of Minnesota. Write to: Agriculture Extension Service, University of Minnesota, St. Paul, Minnesota 55101.
3. Bulletin M. P. 232, Cost $1.00. *The Missouri Approach to Animal Waste Management,* (Guideline to Planning and Designing Animal Waste Management Systems in Missouri), Published by: Missouri Water Pollution Board and Extension Division-University of Missouri, Columbia, Missouri. Write to: Agriculture Engineering Department, Extension Division, University of Missouri, Columbia, Missouri.
4. Hand Book, 1973 Edition, Cost $1.00. *Milk Quality Assurance Handbook,* Prepared by: David K. Bandler-Cornell University, and Sidney E. Barnard-Pennsylvania State University. Write to: Cooperative Extension Service, Pennsylvania State University, 213 Borland Laboratory, University Park, Pennsylvania 16802.
5. Color Slides or 35 mm Film Strip, Cost: Free For Use, *The Story of Vacuum* (As Utilized in Milking Systems On The Dairy Farm), 137 colored slides With tape cassette-time-25 min OR 35 mm film strip with record-time-25 min. Write to: Babson Bros. Co., Dairy Research Service, 2100 South York Road, Oak Brook, Illinois 60521.
6. 35 mm Film Strip With Record (Color), Cost: Free For Use, *Garget-Mastitis With Relation To Milking Machine Operation, Time-38 min.* A vividly illustrated story of work of Dr. Kermit J. Peterson and of the Department of Veterinary Medicine, Oregon State University. Stresses the role played by milking machine operators in preventing mastitis. Write to: Babson Bros. Co., Dairy Research Service, 2100 South York Road, Oak Brook, Illinois 60521.
7. Booklet. Cost $1.00 per copy. *The Way Cows Will Be Milked On Your Dairy Tomorrow* (New 8th Edition available in early 1976). Write to: Babson Bros. Co., Dairy Research Service, 2100 South York Road, Oak Brook, Illinois 60521.
8. Mastitis Handbook, Cost is Free. *Modern Mastitis Management.* Write to: UpJohn Co., Kalamazoo, Michigan 49601
9. Book, 2nd Edition, Cost 1st Copy $1.00, Additional $.30. *The Modern Way To Efficient Milking,* Published by The Milking Machine Manufacturers Council of the Industrial Equipment Institute. Write to: Farm and Industrial Institute, 410 North Michigan Avenue, Chicago, Illinois 60611.
9. Information Bulletin 26, Cost $0.25 each. *Bulk Hauler's Guide-Lines For Proper Collection of Milk,* By: Prof. R. P. March-Cornell University. Write to: College of Agriculture, Cornell University, Ithaca, New York.
10. Colored Slide Films, Cost Free for Use. *Milkhouse and Parlor Cleaning and Sanitizing,* 70 slides (No trade names used in slides). Write to: George W. Towers, Brach Manager, West Agro Chemical, Inc., 501 Santa Fe Street, Kansas City, Missouri 64105.
11. Colored Slide Films, Cost $25 per set. *Milk Quality Tests,* 60 colored slides with 20-min script. Write to: Sidney E. Barnard, 213 Borland Laboratory, Pennsylvania State University, University Park, Pennsylvania 16802.
12. Colored Slide Films, Cost $25 per set. *Cleaning and Sanitizing Farm Milking Equipment,* 65 colored slides with 20 min script. Write to: Sidney E. Barnard, 213 Borland Laboratory, Pennsylvania State University, University Park, Pennsylvania 16802.

SUBCOMMITTEE MEMBERS: *S. Barnard, P. J. Dolan, B. Luce, V. S. Packard, R. Richter, and V. D. Nickel,* Chairman.

SUBCOMMITTEE ON PLASTICS

It has been proposed that fogging of clear, flexible plastic tubing might be markedly reduced or eliminated by using a chemical pre-rinse instead of the usual water pre-rinse. This proposal was based on scattered reports where it appeared that such a procedure was successful. Upon evaluating these reports, it appeared that other variables might have contributed to the improved clarity, so test farms were set up to investigate the effect of pre-rinse composition and temperature on clarity.

Washing, post-rinse, and sanitizing procedures were controlled within manufacturers' recommended concentrations, temperatures, and times with only type and temperature of pre-rinse varied. It has been found that the normal use of a water pre-rinse at proper temperature, immediately after milking, provides rinsing approximately equal to any chemical treatment evaluated. We say "approximately" because measurement of retained film and clarity loss were within experimental error.

The melting point of milk is about 105 F and pre-rinse temperature recommendations have been based on easier soil removal when the fat portion is melted. In this series of tests we found no major difference in tubing clarity between water pre-rinse temperatures of 90 F and 115 F. Circulation velocity in the lines was very good and it is felt that although milkfat is not melted at 90 F, it is quite soft and the major amount was removed due to the good physical fluid action in the cleaning systems. At temperatures below 90 F, the increase in retained soil was easily seen. This soil also contained moisture which contributes to a foggy appearance. It is supposed, that in a system where circulation is not as vigorous and/or where water is hard, 90 F pre-rinse would be too low and soil removal would be inefficient.

By these experimental results, we do not imply that present recommendations should be changed if circulation is good and soft water is available, but feel that this work gives further proof for presently recommended good cleaning practice which should be: (*a*) pre-rinse immediately after milking with water at 100-110 F, (*b*) dump water—do not re-circulate, (*c*) rinse until dumped water runs clear, (*d*) follow with cleaning solution at temperature recommended by manufacturer for 10 min, (*e*) post-rinse with acid according to manufacturer's recommendation, and (*f*) sanitize just before milking.

It is possible that the detergency of a chemical pre-rinse may compensate for a slightly lower pre-rinse temperature, but it appears that the additional cost would be economically impractical where a simple water pre-rinse is satisfactory.

SUBCOMMITTEE MEMBERS: *O. L. Majerus, S. B. Spencer, J. Smucker, J. Malone, R. Page, and B. M. Saffian,* Chairman.

SUBCOMMITTEE ON TESTING FOR CLEANLINESS OF MILK PRODUCTION

A major goal for this Subcommittee was to establish if a relationship exists between sediment testing and abnormal milk detection. It is a fact that when a group of properly warmed universal one-ounce milk samples are subjected to sediment testing, some will vacuum rapidly, some very slowly, and some will resist passage by fouling the sediment test disc.

Reports have been received from the Subcommittee members covering 1240 tests of one-ounce samples through a 0.1-inch diameter test disc. The findings are as follows:

(*a*) Approximately 20% of the samples resisted filtration and thus the full one-ounce sample could not be tested.
(*b*) Over two-thirds of the samples resisting filtration showed DMSCC levels in excess of 1,000,000/ml.
(*c*) Approximately one-third of the samples resisting filtration showed DMSCC levels in excess of 1,500,000/ml.
(*d*) Samples resisting filtration generally showed high MWR or WMT reaction.
(*e*) The samples that filtered most rapidly in each group of four

samples tested simultaneoulsy generally showed low DMSCC levels and low MWR and WMT values.

(*f*) Sediment level in a milk sample has to be extremely high before it fouls the disc. High DMSCC levels are more likely to be the cause of fouling.

(*g*) Test discs foul and samples tend to resist filtration when the DMSCC value approaches 1,000,000/ml.

These findings indicate the one-ounce sediment test is a useful means for twofold milk quality determination. The test determines the degree of milk cleanliness and instances of abnormal milk as evident by disc fouling.

The universal sample system fouling makes such testing and retesting practical. Repeated fouling of a sediment test disc should be a valid reason for a fieldman or sanitarian to give a producer preliminary warning of this fact, and to take steps to determine and correct the cause.

SUBCOMMITTEE MEMBERS: *P. Bergner, G. Cavin, H. Eastman, H. D. Gleason, M. Neff, R. J. Weaver, D. Webber, and M. H. Roman,* Chairman.

SUBCOMMITTEE ON C.I.P. CLEANING OF MILKER UNITS IN THE PARLOR OF A MILKING BARN

A. *Objectives*
1. Reduce handling time of milker units before and after milking.
2. Permit operators to attach milker units directly to wash manifold at each stall.
3. Teat cups are in a sealed position on washing unit (manifold) so foreign matter cannot enter inflation during washing and storage between milking.
4. Milker units, milk lines, wash lines, and manifold to self-drain.
5. C.I.P. wash manifold provided with self-closing dust and fly protective cover.
6. Physical cleaning of external parts of milking units.

B. *Procedures*
1. After milking, teat cups of units are attached to self-draining manifolds (when manual cleaning is necessary, cleaning should be done in the milk house.
2. When vacuum pulsation is used, pulsate inflations during washing.
3. Washing solutions are circulated from wash tank in the milk room, through wash line, milker units and manifold to return line, back to the receiver and tank for recirculation.

C. *Additional recommendations*
1. Before installation, draw a sketch of the system including equipment specifications and diagrams denoting water flow.
2. Submit this sketch to the following for approval before installation:
   a. The regulatory agency responsible for the milk supply.
   b. The milk producer's fieldman.

SUBCOMMITTEE MEMBERS: *R. L. Appleby, R. Ayres, C. Luchterhand, J. Reeder, A. E. Tesdal, A. Wisdom, and K. Harrington,* Chairman.

SUBCOMMITTEE ON CLEANING AND SANITIZING OF FARM PICKUP TANKS AND TRANSPORTS— SILO TANKS

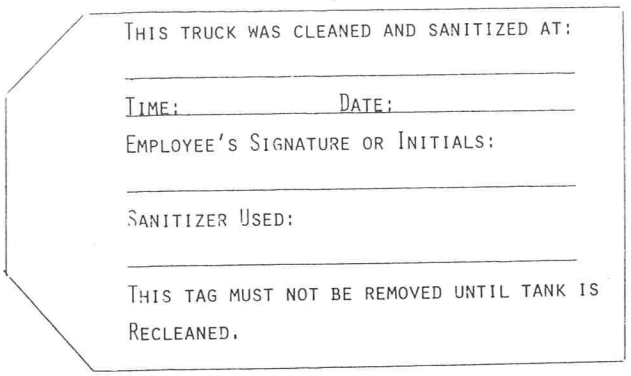
FARM MILK PICKUP TANKERS AND TRANSPORTS

The farm bulk pickup tanker, backbone of the milk collection system in the United Sates, is the vital link in the quality chain from produce to processor. This tank must be cleaned, sanitized, and maintained in a condition where it cannot cause any product deterioration during pumping or transporting. The responsibility for these actions must be clearly defined to reach this objective.

The Subcommittee attempted to determine preferred procedures and methods for cleaning and sanitizing the pickup tanker. The driver has the direct responsibility to be certain the pump, tank, and other milk contact surfaces are cleaned and sanitized before pumping milk into the truck. The Subcommittee is aware that numerous opportunities for failure are present due to use of relief drivers, deliveries at various times of day and night, and various cleaning facilities provided at different dairies. Sanitarians have found the following procedures have worked:

I. *Driver responsibility before loading*
   A. The driver must be certain the tank is completely clean and sanitized before departure for milk pickup.
   B. If the unit is found to be deficient in cleaning, remedial action should be taken to clean, sanitize and properly drain the unit before loading.
   C. At times units are not used for several days. If a tank has been out of use for over two days, it should be rewashed and sanitized before use.
   D. The driver should keep the outside of the unit as clean as practical. Driver appearance should be clean and neat.
   E. If the farm provides pumps or additional piping beyond that carried on the truck, the driver should check for cleanliness of this equipment prior to loading and work with the farmer to correct any deficiencies found.

II. *Responsibility of the driver and/or plant personnel following receipt of the load*
   A. The Subcommittee finds that most successful programs clearly define the responsibility for the cleanliness of the tank interior, manhole area, pump, hose, and pump compartments. Plant personnel normally are assigned responsibility for the cleanliness of the tank.
      1. Plant personnel should clean the interior of the tank by hand washing or C.I.P. cleaning. Care must be given to proper sizing of pumps, solution volumes, and piping to do an adequate cleaning job on tanks of various sizes and dimensions. Built-in nozzles, spray heads, drop-in units, or bottom mounted "Roto-Turn" cleaning systems should be designed, installed, and maintained so the spray pattern will contact all interior surfaces. Preliminary results of a Florida study indicate that spray head spacing, gallons per minute, and pounds per square inch pressure are critical in a satisfactory C.I.P. system.
      2. Plant personnel should be required to hand wash the C.I.P. ports, manhole covers, gaskets and other manhole accessories and inspect the tank interior to determine acceptable cleaning.
      3. The regulatory authority should establish a tagging procedure calling for the plant employee to sign that he has cleaned and sanitized his area of the truck. An example of the tag used in one market is shown below:



   The regulatory authority may adopt other means of accomplishing this procedure. Example, signing or initiating the C.I.P. system recording chart.

   B. The driver should be required to be certain the pump compartment, hoses, and pump are thoroughly cleaned and sanitized following each delivery.
      1. The proper wrenches, brushes, mat, or soft container neces-

sary to disassemble and wash the pump, valves, hoses, and other accessories should be available at each delivery location.

2. The plant should provide a method of cleaning the pump hose by recirculation within a tank containing a hot cleaning solution of proper strength and temperature. This method of cleaning assures internal and external cleanliness of the hose and tends to increase the life of the hose. Where pump hoses are hand washed, the length should be restricted to a length that could be properly cleaned with a brush and stiff handle. A recording device should be installed to assure proper time and solution temperature. Stainless steel baskets may be provided to suspend pump parts in the solution tank to allow cleaning by circulation.
3. Following cleaning, the pump parts should be assembled and sanitized by spray or circulation of a sanitizing solution. The responsibility for seeing that this is accomplished rests with the driver even though the requirement to do the work in some markets may be assigned to plant personnel.
4. The driver should be required to sign the truck cleaning tag indicating that the truck and equipment have been cleaned.
5. The driver must be certain that the sanitizing solution is completely drained.

III. *Responsibility of the dairy plant, reload station, transfer station or receiving station*

A. The station should furnish the necessary material and facilities to allow the proper cleaning and sanitizing of all tankers.

B. The station should establish a routine quality control check to assure that the cleaning solutions are in compliance with the manufacturer's recommendations and that personnel are completing the required work. Charts from temperature-pressure recorders on C.I.P. lines may be used to compare cleaning cycles to the load receiving reports as a means of determining compliance with the plant cleaning procedures. Routine laboratory checks of cleaner and sanitizer strength should be a requirement. Swab tests or other tests for microorganisms may also be used to determine satisfactory compliance.

C. When milk is to be loaded from the plant storage into transport tankers it would be an added safeguard if all transport tankers are washed and sanitized before loading due to out of service time, travel time, and the fact that products other than milk might have been hauled on the last trip.

D. The responsible authority, cooperative, or receiving plant should institute a routine tanker inspection program to assure the cleaning and sanitizing program is obtaining the desired results.

IV. *Responsibility of the regulatory authority*

A. The regulatory authorities should meet with plant management and haulers to establish equipment criteria and procedures that will minimize the problems in the market:

1. Standardize C.I.P. systems in the area to be compatible with all truck units and adequate to handle the largest unit in operation.
2. Establish location requirements for truck C.I.P. ports so the equipment at various plants will properly wash all truck units.
3. Establish a spot check procedure to determine compliance by all personnel involved to include the checking of pressure-temperature recording charts.
4. Meet with plant management and truck operators to procedures to meet new problems with miniumum inconvenience and expense to the industry.
5. Work with all segments of the industry to establish solution concentrations, line pressures, and facility requirements to make the best use of today's technology.
6. Require that detailed cleaning-sanitizing regimen be posted adjacent to clean-up pump or make-up tank at each cleaning location and require that pressure-temperature recording charts be checked on a regular basis.
7. Require that pump compartment door gaskets and dust cover gaskets be maintained in good repair.

B. The Subcommittee recommends that C.I.P. cleaning systems include the following:

1. Tank valve sizes should be standardized in each marketing area.
   Proper size C.I.P. pumps for adequate coverage of tanker walls with cleaning solution. Manufacturer's recommendations should be followed to insure proper volumes and pressures in the cleaning system. A pressure-temperature recording device should be installed in the system.
3. Initial rinse water should be discarded.
4. Proper positioning of the spray ball or tear drop spray head within the tank is critical for complete coverage. Openings in spray balls or tear drop spray heads must be kept to prevent deflection of spray jets.
5. Burst wash and rinse cycles are recommended to allow the return pump to keep flooding of long tankers to a minimum and to increase turbulence of the cleaning and sanitizing solutions.

V. Pump *cleaning procedure.* The following cleaning procedure for the tanker pump and pump parts is recommended:

A. Disassemble and rinse the pump and parts.

B. Clean all parts using suitable brushes and cleaning solution by hand or by recirculation.

C. Rinse with potable water.

D. Brush pump housing with an acceptable sanitizing solution.

E. Hand dip pump parts in an acceptable sanitizing solution and assemble the pump.

F. Clean and sanitize the entire pump compartment.

FARM SILO TANKS—STORAGE TANKS

With the movement to larger farm milk storage tanks, the Subcommittee was asked to investigate cleaning and sanitizing procedures that would assure clean, sanitized tanks.

In view of the size of these storage tanks, it is the consensus of the Subcommittee that a properly designed C.I.P. system is a must for uniform cleaning and sanitizing. Due to the various shapes and sizes of tanks available, it is imperative that the C.I.P. system be designed to assure complete coverage of the interior surface of the tank. With the larger surface area being subjected to the rapid changes in temperature and the corresponding expansion and contraction of the surface and air in the tank, all tanks must be properly vented during the cleaning process to avoid possible collapse of the walls. Recommended vent sizes are published in the 3-A Sanitary Standards for Silo-Type Storage Tanks, Serial #22-03.

When washing farm storage tanks that are refrigerated by direct expansion, it is mandatory that solution temperatures be in compliance with manufacturer's recommendations to avoid developing high refrigerant pressures and possible buckling of the interior surface.

The cleaning procedure for farm storage tanks should start immediately after the tank has been emptied. A cleaning regimen developed by the tank manufacturer and the cleaner supplier should be followed. A suggested procedure should include the following:

(*a*) Rinse with cold or tepid water to remove most of the milk residue from the tank. This rinse solution should not be recycled. If the tank is not scheduled for cleaning immediately, the tank driver should close the tank to minimize the amount of milk film drying before the complete cleaning procedure.

(*b*) An approved cleaning solution of sufficient volume, concentration, temperature, and under sufficient pressure should be circulated to wash the interior surfaces of the tank. Any parts requiring hand washing should be cleaned at this time. The temperature of the solution and the circulation time should be controlled to avoid the possibility of redepositing soil on milk contact surfaces. Installation of a pressure-temperature recording device would aid materially in the control of the cleaning regimen.

(*c*) Following washing, the solution should be allowed to drain from the tank.

(*d*) Following draining, the tank should be rinsed with cold water

and allowed to dry. An acidified rinse can be substituted for this final cold water rinse provided it is compatible with the other cleaning materials.

(*e*) Before the start of filling, the tank should be checked for cleanliness and then sanitized with an approved sanitizer.

(*f*) Filling pipes or other equipment inserted in the tank during the milking process must be removed following each milking, cleaned and sanitized before use at subsequent milkings.

(*g*) Manhole gaskets, sampling devices, or other equipment requiring hand cleaning must be removed, cleaned, and sanitized when the tank is washed.

(*h*) Spray balls or other cleaning devices that may trap foreign matter should be checked on a regular basis to avoid blockage of the spray pattern.

The National Association of Dairy Equipment Manufacturers Recommends the following:

(*a*) Don't use steel wool to clean stainless steel.

(*b*) Don't use water with high concentrations of iron, salt, or sulfur.

(*c*) Don't allow bactericides or cleaners to remain on surfaces for over 20 min.

(*d*) Don't allow tools, parts, fittings, or other items to rest on tank surfaces.

(*e*) Don't use cleaners or bactericides in excess of manufacturer's recommendation to avoid surface damage or discoloration.

SUBCOMMITTEE MEMBERS: *G. Briody, M. R. Cooper, B. DeYoung, Jr., C. R. Gilman, H. Munns, B. J. Scheib, H. B. Ellison,* and *B. M. Cook,* Chairman.

SUBCOMMITTEE ON SAMPLING OF MILK IN TRANSPORT TANKS

Collection of a representative sample of milk from a transport tank is easier said than done. The basic problem is achieving proper and thorough agitation of the milk in the tank.

Certain factors must be considered relative to the agitation and sampling of milk in transport tankers. These include the following:

1. Immediately before taking a sample, the contents should be homogeneously mixed.
2. The time interval required for mixing large tanks will vary according to the following conditions:
   a. the shape and size of the tank
   b. the volume of product held
   c. the type, location, and force of agitation
   d. the creaming interval before starting agitation

Considering these variables, it is necessary to determine the minimum time of agitation required to achieve homogeneity of the milk in the tanker.

A review of previous Farm Methods Committee reports and other sources of information indicates that the following methods of agitation of transport tankers are being used:

1. None.
2. Agitation using hand stirring rod designed for the 10 gal. can. Hand stirring rods range in length from 36 inches up to 6-8 ft.
3. Thief sample made of a long stainless steel rod is used to obtain a core sample.
4. Air agitation
   a. Sanitary air hose placed in tanker using air velocity to roll the milk for agitation.
   b. Air driven motor to activate a small propeller or agitator placed into the tanker through the manhole.
   c. Air injected through a quick coupling in rear of tanker in an attempt to circulate the milk.
   d. Air line built into the bottom of the tanker where air is blown through stainless steel tube throughout the length of the bottom of the tanker to roll the milk for agitation. The same device is used for C.I.P. cleaning of the tanker. This is the Roto-Twin system discussed later in this report.
5. Mechanical agitation
   a. Propeller or agitator driven by an electric motor. The unit is placed on the manhole with the propeller down in the milk.
   b. Mechanical agitator built into tanker driven by electric motor mounted on underside of tanker.
6. Circulation of milk by use of unloading pump to pump milk out of the outlet valve and back into the tanker through the manhole. A plastic hose is usually used for this hook-up.
7. Pumping the milk into a plant storage tank and then sampling the milk through the storage tank pet cock.
8. Drip automatic sampling as milk is unloaded.

During the Fall of 1973, the Northeast Dairy Practices Committee published Bulletin 7 titled *1973 Guidelines for Sampling Fluid Milk.* These published guidelines included a section on tank truck sampling that offers procedures and ideas that could be adopted by milk receiving and shipping stations and plants that are involved in sampling.

The tank truck sampling procedures as outlined in the *1973 Guidelines for Sampling Fluid Milk* are as follows:

1. Collect a sample through manhole by aseptic means immediately after loading the milk at the last pickup for that load.
2. Drop a high speed agitator (air driven preferred) into the manhole and run for at least 10 min on tanks up to 3,000-gal. capacity. For larger tanks the minimal agitation time should be established by interval sampling and testing for the fat content until it is constant.
3. Connect the end of bulk pickup hose to a clean sanitary pipe in the manhole extending below the level of the milk and circulate the milk on the truck for at least 15 min.
4. Install attachment for air agitation which will agitate the milk for 15 min without causing rancid flavors.
5. Unload the entire tank of milk in an empty storage tank and collect a sample through the clean, sterilized sampling valve.

"The milk in trailer tank trucks that have set for a few hours is difficult to agitate and sample. For tanks of 5,000 gal. and more, practices 1, 3, and 5 seem more satisfactory."

"The use of automatic sample of all milk through a line holds the most promise. The system includes a reciprocal pump and plastic hose which forces a drop of milk into a refrigerated sample container in proportion to the quantity of milk. Adjustment for pumping capacity and size of sample is essential."

"The same precautions are exercised in collecting samples from tank trucks as for farm milk tanks. This includes identification of sample as to date, time, truck and sampler. Bacterial tests should be performed prior to flavor or compositional testing."

Another milk agitation system that may hold promise involves the Roto-Twin system designed to be installed in large tankers for a C.I.P. system. This system also can be used as an air agitation system and may work well as a method of agitating the milk in large tank trucks. It has been reported that a tank full of milk held overnight needs 10 to 15 min of air agitation by the Roto-Twin system and only 3-5 min for a fresh load of milk.

Present information indicates that odor-free, pressurized, filtered air (see 3-A Standards) or mechanically driven agitators provide the best means of milking milk in transport tanks.

SUBCOMMITTEE MEMBERS: *F. Ahalt, W. Arledge, R. A. Belknap, H. Uhlman, J. K. Webb, and W. LaGrange,* Chairman.

SUBCOMMITTEE ON WATER TREATMENT AND PROTECTION

Water treatment and protection continues to play a very important role in the Dairy Industry. Governmental regulations, industry trends towards more and more mechanization, coupled with environmental water shortages in various areas of our country, have placed an increased complexity on the problem.

Bacteriological testing of dairy farm water supplies and individual water supply systems, have not been revised for a considerable length of time. The *Grade A Pasteurized Milk Ordinance-1965 Recommendations of the U.S. Public Health Service* continues to establish the standards for testing which are only the minimum for frequency of testing. This committee, as well as many other reputable agencies, in past reports, have gone on record to increase the frequency of testing.

The Food and Drug Administration (Public Health Service) is presently preparing an update of these water requirements when used in the processing of milk and milk products. Publication is scheduled for the latter part of 1974 in the *Federal Register.*

During the past year, the U.S. Environmental Protection Agency, Water Supply Division, has published two manuals that are updates from former Public Health Service publications. They are as follows: *Cross-Connection Control Manual,* Publication No. 430/9-73-002, and *Individual Water Supply systems,* Publication No. EPA-430/0-73-003.

Publication No. EPA-430/9-73-002 is a revision of the *Water Supply and Plumbing Cross-Connections* (PHS Publication No. 957, dated 1963). Many of the original illustrations, and much of the original text has been retained. Each chapter has been reviewed for clarity and updating to be in accordance with the latest information. Also extraneous information has been omitted to simplify the reading. Chapter 2 has been updated to reflect the latest incidents of major cross-connections that have been reported.

Publication No. EPA-430/9-73-003 succeeds Public Health Service, Publication No. 24,1962. Many important contributions from the original manual have been retained. Many Federal, State, and other private agencies participated in preparation of this revised manual. The manual constitutes the latest improvement in the field from both printed and actual practice. Appendix D is based on the U.S. Public Health Service Publication No. 1451 *Recommended State Legislation and Regulation,* July, 1965. The Food and Drug Administration upon request did reevaluate the effective date that all well seals must terminate above ground level.

They did recind the July 1, 1974 date, and required information that proof of installation was before the adoption of the 1965 Pasteurized Milk Ordinance. In addition, that the bacteriological requirements were met and also that provisions of Items 8r and 7p were approved except when any changes in the system were necessary; and then the well seal must terminate above ground level.

The Subcommittee on Water Treatment and Protection recommends that the above materials should be obtained and reviewed to eliminate misinformation on the construction, installation, and operation of water supplies on dairy farm installations.

SUBCOMMITTEE MEMBERS: *J. A. Black, K. Harvey, G. Ronald, R. Ryan, C. Flack, and H. Faig,* Chairman.

SUBCOMMITTEE ON ANIMAL WASTE MANAGEMENT

The Farm Methods Committee, Task Committee on Animal Waste Management, requests the assistance of environmental sanitarians and health officials, as well as other regulatory personnel who are concerned with the high standards of sanitation in the production of Grade "A" milk to provide the Environmental Protection Agency with up-to-date information and recommendations to bring about development of reasonable and flexible national Animal Waste Management guidelines. Because of a recent court decision, this is a particularly critical time for development and refinement of regulations for feedlot runoff by EPA under the National Pollutant Discharge Elimination System (NPDES) authorized under Section 402 of the Federal Water Pollution Control Act Amendments of 1972.

It is the primary recommendation of the Task Committee that the imposition of such guidelines and regulations by the EPA be based on the need to adopt solutions to dairy farm animal waste runoff which reflect different regional, state, and local conditions. The Task Committee is convinced that broad effluent runoff control guidelines administered under a common Federal permit program will not recognize the vast differences in topography, soil, and climatic conditions which will be peculiar to the various dairy regions of the country.

Our responsibility as an association representing professional sanitarians with long experience in protecting public health should be to establish regular lines of communications with newly-established state pollution control agencies charged with administering a federally approved state feedlot permit program. This will enable the coordination of recommendations to minimize the possibility of conflicting provisions and unnecessary burdens on the dairyman. To assist in this endeavor, Section 514 of the Federal Water Pollution Control Act of 1972 provides that "the agency issuing a permit under Section 402 shall assist the applicant for the permit in coordinating the requirements of this Act with the requirements of the appropriate public health agencies."

In addition, we recommend the following:

(*a*) Animal waste be considered as a resource in the production of food and fiber and not as a waste by-product.

(*b*) Appropriate cost-sharing assistance be provided to dairymen and cattlemen who are now required to meet new legal limitations on runoff. Legal requirements now vary from state to state. The Congress has rejected administration requests to curtail the Agricultural Conservation Program (ACP) for the current year by $156 million. This action means that the ACP funds will have to be made available for use by farmers this year, even though the administration has repeatedly asked for curtailment of the program as part of budget recessions.

(*c*) All feedlot areas where animals are confined should be kept clean and free of unsanitary conditions or nuisance odors.

(*d*) Animal wastes in storage areas be managed in such a way so as not to create a potential pollution hazard to land, air, or water. Protection of ground water is an essential goal in planning a storage area. Proper planning should include measures to restrict seepage, percolation, or other movement of animal wastes to ground water. Such an area should be located at least 100 ft from water sources.

(*e*) Surface runoff from outside areas should be diverted to storage areas properly designed as in (*d*) above.

(*f*) Storage of animal wastes and/or milkhouse wastes where permitted to be stored together in a slurry form should be disposed of within a period of 1 year and be transported in leak-proof vehicles to minimize flies, other insects, and rodents.

(*g*) Liquid tank openings should be isolated by covers or fencing to prevent access by either humans or animals.

(*h*) Stacks of animal waste should not be located too close to the milkhouse but should be convenient for loading and hauling without detracting from farmstead.

(*i*) Animal waste handling systems should be practical, economical, and convenient for the dairyman to permit flexibility in fully utilizing nutrient value in land applications while minimizing overhead cost of milk production.

(*j*) Additional Federal and state funding for research should be made available to determine the optimum levels and frequency of application of animal manure on various forages, including corn and sorghum. Traditional methods of disposal need re-evaluation. Alternative methods of manure management have been suggested, but research on cost and effectiveness is lacking. Systems need to be developed that will remove animal wastes from sites of animal confinement and effectively utilize this resource material for productive purposes, such as feed or fertilizer, without causing pollution.

SUBCOMMITTEE MEMBERS: *R. Dawson, R. Ebbert, B. Greene, R. Lock, L. H. Lockhart, and J. Adams,* Chairman.

SUBCOMMITTEE ON UNIFORM AND INFORMATIVE TEAT DIP LABELING

The activities of the Uniform and Informative Teat Dip Labeling Subcommittee is as follows: In an effort to accomplish our goals, the committee agreed we should establish recommendations for labeling to be presented to the Farm Methods Committee. On October 25, 1973, a letter was sent to all members of the Task Committee asking for recommendations. These were compiled and returned to the members on December 22, 1973, for their review. On February 4, 1974, the committee met in St. Louis, and the following recommendations were agreed upon to be submitted.

(*a*) The color of the label; predominantly uniform pink background with contrasting letters.

(*b*) Illustration of a teat dipper super-imposed on a label, or a *sticker* for larger packaging.

On June 6, 1975, the art work was forwarded to Dr. Eberhart of the National Mastitis Council Teat Dip Committee.

On December 18, 1974, information was sent to all committee members asking for their suggestions and to look into the methods of applying teat dips. They were asked to acquire brochures and information that describe this equipment for our evaluation and recommendation. This information has not been completed as of this date. In addition, a request was made that our committee could be of service to the International by putting together information which might help in the uniformity of the labeling of all farm chemicals dealing with cleaning and sanitizing. Our committee will continue to work to gather this information for the Farm Methods Committee and for the respective NMC committee.

At our last meeting, it was suggested that our Teat Dip Committee work closer with the National Mastitis Council Teat Dip Committee. I made a suggestion to Dr. Eberhart that the Chairman of the Farm Practices Teat Dip Committee, whoever it might be, be a member of the National Mastitis Teat Dip Committee so our efforts can be correlated.

SUBCOMMITTEE MEMBERS: *Jay Boosinger, F. P. Godfredson, R. Hellensmith, L. H. Lockhart, R. Page, R. Stucky, and L. A. Skeate,* Chairman.

FARM METHODS COMMITTEE MEMBERS, 1973-1975

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J. Milk Food Technol. Vol. 39, No. 1, Pages 71-72 (January, 1976)

Report of the Editor
Journal of Milk and Food Technology, 1974-1975

REVIEW OF VOLUME THIRTY-SEVEN

Publication of the December, 1974 issue of the *Journal of Milk and Food Technology* completed Volume 37. This also was the last issue that was put together by H. L. Thomasson and printed by Roeder's Franklin Printing Service of Franklin, Indiana.

Volume 37 was among the largest to be published. It contained as many pages as did Volume 36 and was exceeded in number of pages only by Volume 35 that appeared in 1972. In 1974 we published 102 papers compared to 108 in 1973 and 64 in 1967. Of the 102 papers, approximately 70% reported research findings, 20% were technical general interest papers, and 10% discussed non-technical topics of general interest. Somewhat more pages were devoted to 3-A equipment standards in 1974 than in 1973; however, no E-3-A standards were published in 1974. Complete details about Volume 37 and other recent volumes are in Table 1.

Papers in Volume 37 again dealt with a variety of subjects. In this volume approximately 48% of the papers considered non-dairy foods and related topics, whereas 41% dealt with dairy foods and related subjects. The remaining 11% discussed environmental or other topics. This was the second year in which the number of papers dealing with non-dairy foods exceeded the number on dairy foods. Although considerably more papers on dairy foods were published in 1974 (41% of the total) than in 1973 (32%), papers on non-dairy foods continued to predominate in 1974. As indicated in the 1973 report, this trend is likely to continue in the future because of a greater research interest in non-dairy foods and because funds to support dairy research are diminishing.

The Editor wishes to express his appreciation to Mr. H. L. Thomasson for the help, advice, and cooperation that were so freely given from 1967 to 1974. Thanks also go to Roeder's Frankling Printing Service for the help and cooperation that were given by that organization.

PRESENT STATUS OF VOLUME THIRTY-EIGHT

The first six issues of Volume 38 consisted of 400 pages, including covers. This compares with 376 pages for the first six issues of Volume 37 and 264 pages for similar issues of Volume 30 (1967). The first six issues of Volume 38 contained 50 research papers, 11 technical papers of general interest, and 4 non-technical papers of general interest. This compares with 32, 17, and 7 papers in the same categories for the first six issues of Volume 37.

On July 1, 1975 there was a backlog of 42 research papers, 16 technical general interest papers, and 3 non-technical general interest papers awaiting publication. In addition, there were 26 research papers and 2 general interest papers being reviewed or revised. The backlog of papers is somewhat greater than existed at this time in 1973 or 1974. It has resulted from a heavier flow of papers and from the change in printer. In spite of the backlog we are still striving to assure prompt (usually within 6 months of submission) publication of research papers.

Effective with the first issue of Volume 38, the *Journal* has been

TABLE 1. *Summary of contents of the* Journal of Milk and Food *Technology, 1967-1974*

Item	Volume 30 (1967)	Volume 31 (1968)	Volume 32 (1969)	Volume 33 (1970)	Volume 34 (1971)	Volume 35 (1972)	Volume 36 (1973)	Volume 37 (1974)
1. Total pages, including covers	512	540	624	688	728	832	752	752
2. Total papers published	64	62	87	104	102	132	108	102
3. Research papers								
a. Number	30	32	47	66	67	78	65	72
b. Pages	137	142	205	280	288	317	284	330
c. Percent of total pages	26.7	26.3	32.9	40.7	39.5	38.1	37.7	43.9
4. General interest papers-Technical								
a. Number	11	16	14	18	24	35	31	21
b. Pages	47	74	87	99	150	242	208	160
c. Percent of total pages	9.2	13.7	12.2	14.3	20.6	29.1	27.7	21.2
5. Equipment standards								
a. 3-A, pages	9	22	12	44	40	23	17	41
b. E-3-A, pages	—	—	7[a]	16	30	15[b]	—	—
c. Percent of total pages-all standards	1.7	4.1	3.0	8.7	9.6	4.5	2.2	5.4
6. General interest papers-Nontechnical								
a. Number	23	14	26	20	11	19	12	9
b. Pages	72	65	91	64	46	76	49	29
c. Percent of total pages	14.1	12.0	14.6	9.3	6.3	9.1	6.5	3.8
7. Association affairs								
a. Pages	64	68	62	49	45	47	84	75
b. Percent of total pages	12.5	12.6	9.9	7.2	6.3	9.1	11.2	9.9
8. News and events								
a. Pages	51	42	36	23	17	7	4	0
b. Percent of total pages	9.9	7.8	5.8	3.4	2.3	0.8	0.5	0.0
Percent of pages-technical material, including standards	37.6	44.1	48.1	63.7	69.7	71.7	67.6	70.5
Percent of pages-nontechnical material	36.5	32.4	30.3	19.9	14.9	15.5	18.2	13.7
Percent of pages—covers, adds, index, etc.	25.9	23.5	21.6	16.4	15.4	12.8	14.2	15.8

[a]These were Baking Industry Equipment Standards.
[b]Includes 1 page of Baking Industry Equipment Standards.

printed by Heuss Printing and Signs, Inc. of Ames, Iowa. Composition of the *Journal* is by a cold-type computer process which has made possible changes in type face, use of bold face type, and others. The major changes were detailed in the 1973-1974 report of the Editor. Changes in appearance of the *Journal* have been well received by readers and authors. It also has been possible to print the *Journal* somewhat earlier in a given month than occurred with previous volumes. This means that an issue is in the hands of readers during the month that the *Journal* is dated.

REVIEW PAPERS

The *Journal* continues to publish review articles on timely subjects. Volume 37 contained review papers on: flavor and microbiology of Swiss cheese, radappertization of meats, bacterial spores in milk, frozen concentrated lactic starter cultures, the process cheese industry, liquid composting, enteropathogenic *Escherichia coli,* food as a source of pathogens in hospitals, and processing of stainless steel.

Thus far, Volume 38 has presented reviews on: milking machine design and udder health, components of wheat and triticale flour, grapes and wine technology, methods to detect salmonellae, and exposure of man to mercury. Awaiting publication are reviews on filth in foods, volatile compounds in milk, *Vibrio parahaemolyticus,* patulin, anisakiasis (a series of five papers), energy conservation, and water content in gaseous systems.

Authors are encouraged to prepare review papers. Such papers will be published promptly and without a page charge. Prospective authors are encouraged to contact the Editor if they have questions about the suitability of their material for publication.

EDITORIAL BOARD

The Editorial Board now consists of 41 scientists in industrial, governmental, or university laboratories. One member of the Board, E. F. Baer, died early in 1975.

Many persons on the Editorial Board continue to devote countless hours to the review of papers that appear in the *Journal.* Their continued help is acknowledged and appreciated.

The Editor frequently invites scientists who are not on the Editorial Board to review papers. Thus far in 1975 the following persons have served as ad hoc reviewers: J. C. Acton, S. E. Barnard, R. L. Bradley, Jr., D. O. Cliver, P. R. Elliker, I. E. Erdman, J. Harwig, D. H. Kropf, R. C. Lindsay, D. W. Mather, D. B. Nelson, N. F. Olson, V. S. Packard, G. S. Torrey, J. H. von Elbe, D. F. Wessley, and W. C. Winder. Their help is acknowledged and appreciated.

Respectfully submitted,

ELMER H. MARTH
Editor
Journal of Milk and Food Technology

Journal of Milk and Food Technology is Scheduled to Become *Journal of Food Protection*

At its last meeting (August, 1975, Toronto), the Journal Management Committee recommended to the IAMFES Executive Board that the name of the journal be changed from *Journal of Milk and Food Technology* to the *Journal of Food Protection.* The Executive Board then appointed Drs. E. H. Marth and K. G. Weckel as a committee to further explore this proposal and prepare a report for consideration by the Board at its meeting late in November, 1975.

Marth and Weckel prepared a report and submitted it to the Executive Board. A portion of the report follows.

"Last August we were requested to consider the recommendation of the Journal Management Committee that the name of *JMFT* be changed to the *Journal of Food Protection.* Specifically, we were instructed to summarize the advantages and disadvantages of this proposed name change and to submit this information to the members of the Executive Board before the November meeting. As we see it, the advantages and disadvantages are as follows.

Advantages

1. The proposed name more accurately reflects the content of the journal than does the present name.
2. The proposed name more accurately reflects the major purpose of IAMFES than does the present name.
3. The redundancy (milk-food) of the present name would be eliminated.
4. The term "technology," which is overused in titles of journals, would be eliminated.
5. The proposed name would give the journal a different image and hence might serve to attract papers that now are published elsewhere.

Disadvantages

1. Cost to libraries (Mr. David Oyler, librarian at Steenbock Library, University of Wisconsin-Madison, claims a one-time cost of about $150 to make all the needed changes in a given library system; he also indicated that this probably should not be a major concern; apparently each year there are a substantial number of scientific journals that change their names and libraries accept this).
2. Cost to IAMFES (redesigning the cover, changes in the patent office, and perhaps some others such as straightening out confusion among subscribers; see item 3).
3. Confusion would result, especially among subscribers. (Mr. Oyler indicated this can be minimized by sending a notice to subscribers in which they would be informed about the name change; subscription agencies would be included. This notice should go out 3-4 months ahead of the renewal notice that bears the new name of the journal; in fact, this first renewal notice probably should carry the old and new name of the journal. Also, both names should appear on the cover of the journal for perhaps 6 months before and 6 months after the name change becomes effective. The volume number could continue or we could start with volume No. 1.)
4. It is possible that some papers we now get might go elsewhere (our guess: there would be few, if any, of these).
5. The present name is well known after being used for 25 years; the new one would have to get established (this, however, is not all bad)."

The Executive Board considered this report at its meeting in November and voted to adopt the new name for the journal. Mr. E. O. Wright and Dr. Marth were instructed to proceed with implementation of this action so that the change can become effective in 1977.

Any members of IAMFES or readers of the *Journal* who care to comment on this action are invited to contact Mr. E. O. Wright, Box 701, Ames, Iowa 50010.

J. Milk Food Technol. Vol. 39, No. 1, Page 73 (January, 1976)

Supplement No. 3
3-A Sanitary Standards for Fittings Used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Milk and Milk Products, Revised

Number 08-14

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

This supplement adds the criteria for air operated positive displacement samplers to Section E. SPECIAL CONSIDERATIONS of the "3-A Sanitary Standards for Fittings Used on Milk and Milk Products Equipment and used on Sanitary Lines Conducting Milk and Milk Products, Revised, Number 08-09." (Reference 3-A Drawings No. 3A-100-36, No. 3A-100-37 and 3A-100-38.)

Add the following to Section B. DEFINITIONS:

B.3

Engineering Plating: Shall mean plated to specific dimensions or processed to specified dimensions after plating; and for these standards, the minimum thickness shall be 0.0002 inch for all product contact parts.[5]

Add the following to Section E. SPECIAL CONSIDERATIONS:

E.9

These air operated positive displacement samplers shall comply with the applicable provisions of this standard and the following:

E.9.1

The sampler assembly shall consist of a body, plunger, head, O-Rings, seals and an air operating mechanism. It shall also include a closure plug or sample bottle port closure suitable for sealing the sample bottle opening when the sampler is not in use.

E.9.2

All product contact surfaces shall be of materials conforming to the criteria in subsection C.1 of this standard, except that:

E.9.2.1

Plungers made of materials provided for in C.1 may be covered with an engineering plating of chromium.

E.9.2.2

Gaskets, seals and O-Rings may be made of materials conforming to the criteria in C.1.4 and C.1.6.

E.9.3

The sample shall be capable of being automatically controlled in a manner that will prevent overfilling of the sample bottle.

Add the following to the list of drawings in subsection F.1 of this standard:

Fitting Name	Page No.	3A Drawing No.
Air Operated Positive Displacement Sampler	30	3A-100-36
Air Operated Positive Displacement Sampler	31	3A-100-37
Air Operated Positive Displacement Sampler	32	3A-100-38

This supplement is effective Feb. 8, 1976.
Drawings in this standard not published.

[5] *QQ-C-320a Federal Specification for Chromium Plating (Electrodeposited) July 26, 1954. (Available from General Services Administration, Seventh and D Streets, NW, Room 1643, Washington, D.C.)*

News and Events

Profit from Cheese Whey . . . A New Bulletin from Tri-Clover

Tri-Clover Fractionators for protein recovery from cheese whey are decribed in a new bulletin from Ladish Co., Tri-Clover Division.

Answering the "challenge to profitability" posed by today's restrictive processing environment, the Fractionator is cited as a way to open up new profit opportunities for the cheese manufacturer—while providing a means to dispose of whey.

Making use of a unique spiral membrane filter, the Fractionator is designed to fractionate the components of whey by permeating low molecular weight materials—leaving a concentrate of lactalbumin. This valuable protein can then be further processed by evaporation, dried and bagged for sale.

Bulletin UF-76 includes a detailed flow diagram of the recovery process along with a case history of the cost factors involved with a Fractionator installation at Lynn Proteins, Inc. . . . where 252 lbs. of quality lactalbumin per 50,000 lbs. of whey are being recovered and utilized.

For details ask for Bulletin UF-76, Ladish Co., Tri-Clover Division, Kenosha, Wisconsin 53140.

1976 Dairy Marketing Forum Program

The future of government regulation and the effect of promoting and merchandising dairy products will be examined at the 1976 Dairy Marketing Forum, Tuesday and Wednesday, March 16-17, at the Ramada Inn, Champaign, Illinois.

Major topics include:

— What structural changes are ahead for the dairy industry
— Is there a future for the federal order system
— The future of government regulation from the viewpoints of retailers, milk dealers, and dairy cooperatives
— Low cost distribution of milk through covenience store
— Effect of the advertising program in New York State
— Factors affecting consumers decisions to purchase dairy products
— What can be done to increase the effectiveness in merchandising milk

Speakers for the Forum include: Dawson Ahalt, Staff Economist, Washington, D.C.; Dr. Emerson Babb, Purdue University; Harold Cope, Economist, Kroger Foods, Cincinnati, Ohio; Dr. Olan Forker, Cornell University; Ed Gelsthrope, President, H. P. Hood & Sons, Boston, Massachusetts; Dr. Truman Graf, University of Wisconsin; Robert Lowe, Becker Milk Company, Toronto; Dr. Dan Padberg, University of Illinois; Jim Reeves, Mid-America Dairymen, Inc., Springfield, Missouri; W. E. Ritmuller, Dean Foods Company; John York, General Manager, Eastern Milk Producers Cooperative, Syracuse, New York; and several others.

There will be taste of dairy products at the 1976 Forum.

The two-day Forum is sponsored and administered by the University of Illinois Department of Agricultural Economics and the Cooperative Extension Service.

For program details, please send the coupon below to: James W. Gruebele, 1976 Dairy Marketing Forum, 305 Mumford Hall, Urbana, Illinois 61801.

District of Columbia Certifies Food Service Managers

Some 3,000 food service managers have been certified as proficient in food service hygiene and sanitation by the Government of the District of Columbia.

These food service workers are employed in approximately 2,000 food establishments in Washington, D.C.

Under District of Columbia General Food Regulations all supervisory food service personnel must be trained and certified as knowledgeable in the basic principles of food protection.

Certification of food service personnel is accomplished through a written examination following the completion of a course of instruction approved by the director of environmental health for the District of Columbia.

The course, which is taught in English, Chinese, German, French, Italian and Spanish is offered in seven different locations and at various times during the day for the convenience of food service workers in the Washington area.

"The food industry's response to these efforts has been overwhelming," says Dr. Bailus Walker, Jr., director of the DC Environmental Health Administration. "In fact we have seen substantial changes in management's attitude, which is reflected in the day to day operation of the food service establishment. This in turn translates into added food protection for the consumer," Dr. Walker added.

The District of Columbia was the first municipality to require by law the certification of food service personnel.

"This requirement evolved from our recognition of the fact that enforcement alone is not sufficient to ensure adequate food protection. Regulatory agency personnel cannot be constantly present in all food-service situations and no system of inspection can ever be completely successful. Therefore the education of food service personnel is the foundation for good food sanitation practices," Dr. Walker said.

For more information: D C Environmental Health Administration, 801 North Capitol Street, N.E., Room 733, Washington, D.C. 20002, Phone (202) 629-3013 or 3014.

News and Events

Langdon, Pet Dairy Division Head, Chosen President of National Group

J. Lloyd Langdon, president of Pet Dairy Division, Johnson City, Tennessee, has been elected for a second term as president of the International Association of Ice Cream Manufacturers. The organization is the representative of the ice cream and other frozen desserts manufacturing and distributing companies in the United States and in more than 20 other countries.

Election of Mr. Langdon took place October 20 during the International's annual convention held this year in Montreal, Canada. He had served as president the prior year and was vice president from 1972 to 1974.

Active in dairy organizations for many years, Mr. Langdon is a native of Raleigh, North Carolina and a graduate of North Carolina State University. He has been with Pet since 1959, beginning as director of marketing. He subsequently advanced to the position of vice president, marketing; vice president, operations; and executive vice president and general manager. In 1970 he was named president of the Division.

Prior to joining Pet, Mr. Langdon was executive vice president of North Carolina Dairy Products Association, and also served as part owner and general manager of a farm supply company in Asheville, NC.

Other officers reelected by the IAICM were James T. Thompson, Thompson Dairy Company, Seymour, Indiana, vice president; W. Fred Atkinson, Ideal Pure Milk Company, Evansville, Indiana, treasurer; and Robert J. Darrow, Creamland Dairies, Inc., Albuquerque, New Mexico, secretary.

Albert Geiss Reelected President of the Milk Industry Foundation

Albert E. Geiss of Birmingham, Alabama has been reelected president of the Milk Industry Foundation. The Foundation is the national organization of more than 500 dairy product processing and distributing firms in the United States, Canada and approximately twenty other nations.

Mr. Geiss, who is president of the Barber Pure Milk Company, is past president and currently a director of the Alabama Dairy Products Association. Prior to being elected president of the Foundation last year, he had served for two years as vice president of the association. His election took place at the group's recent annual meeting held in Montreal, Canada.

Before joining the Barber Company, Mr. Geiss was vice president and a director of the Bowman Dairy Company in Chicago. The dairy leader was named executvie vice president and general manager of Barber in 1967.

He is a graduate in industrial engineering of Northwestern University, and served in the Pacific as an ensign in the U.S. Navy during World War II.

Also reelected as Milk Industry Foundation officers were Dwight N. Holcombe, Clover Leaf Creamery Co., Minneapolis, Minnesota, vice president; Iver Erickson, Anderson-Erickson Dairy, Des Moines, Iowa, treasurer; and Antone Larsen, Sunshine Dairy, Portland, Oregon, secretary.

Bailus Walker Appointed to EPA Advisory Board

Dr. Bailus Walker, Jr., director of the Washington, D.C. Environmental Health Administration, has been appointed to the newly created Environmental Health Advisory Committee of the U.S. Environmental Protection Agency (EPA).

The Committee, an entity of EPA's Science Advisory Board, provides to the EPA Administrator expert and independent advice on the problems and issues relating to scientific and technical aspects of the effects of environmental pollutants on public health.

Problems to be addressed by the Committee include those associated with biological, chemical and physical agents such as toxic chemicals, pesticides, air and water pollutants, solid waste, radiation and noise.

Walker is a graduate of Kentucky State University; he holds a masters degree in environmental health from the University of Michigan and a doctorate from the University of Minnesota.

Data Bank on Viruses in Food

The WHO food virology programme has established a data bank on viruses in foods and the effect of food-borne viruses on public health. Reports in the collection concern:

(a) properties of viruses that affect their transmissibility;
(b) instances of virus occurrence in foods, as evidenced by laboratory detection of the virus or by an outbreak of human disease;
(c) other evidence of virus occurrence in foods;
(d) methods for detecting food-borne viruses; and
(e) studies of virus stability or inactivation in foods.

Each report is based on one or more sources of information (usually journal articles) from all over the world and has been prepared by a laboratory virologist.

The retrieval system is now in service and precise information on specific subjects can be made available to food control authorities, epidemiologists dealing with food-borne disease outbreaks, research and laboratory workers in the field of food hygiene, and research programme planners. So that information may be given as promptly and precisely as possible, users of the system will be supplied with "Request for information" forms which direct questions in a way that is compatible with the retrieval system. These forms and additional information on the programme may be obtained from Veterinary Public Health, Division of Communicable Diseases, World Health Organization, 1211 Geneva 27, Switzerland.

Milton E. Powell, 1902-1975

Milton E. Powell was born in St. Joseph, Missouri on May 10, 1902. He was raised in Nebraska and Illinois and graduated from the University of Illinois with a B.S. in Agriculture in 1926. After a brief period as a dairy Inspector in Chicago, he went to the University of Minnesota where be obtained a M.A. Degree in Dairy Products in 1930. He received his Ph.D. Degree in Agricultural Biochemistry at the University of Missouri in 1935.

His first job after obtaining his doctorate was to establish research and control laboratories for Abbotts' Dairies, Inc. in Cameron, Wisconsin. Dr. Powell came to Knudsen Corporation in 1940 as a chemist. On his return from the military service in World War II, he became Director of Research, a position he held until his retirement in 1967. Powell joined the Corps of Engineers in 1941 as a Captain and left active duty in the army as a Lieutenant Colonel in 1946.

Dr. Powell was a member of the American Dairy Science Association, the Institute of Food Technologists, and the American Chemical Society. Powell was very active in civic and church affairs. He was a Rotarian, Scout Master, and he served on the Executive Board of his Scout Council for seven years.

Dr. Powell leaves his wife DeEtta (Greer) whom he married in 1926, and their son, Ned. Mrs. Powell and Ned Powell and his family reside in Southern California at this time.

Dr. Powell died in his sleep without prior knowledge of illness on August 28, 1975.

Gerald Cohen of Culinary Institute to Chair State Sanitarians' Food Committee

Gerald Cohen, Sanitarian and Safety Coordinator for The Culinary Institute of America, has been chosen Chairman of the Food Protection Committee of the New York State Association of Milk and Food Sanitarians.

A resident of Florida, N.Y., he is co-author of a forthcoming book on Food and Environmental Sanitation. He is a member of many food protection organizations, including the National Environment & Health Association, International Association of Milk, Food and Health and The Environmental Sanitarians.

Winter Dairy Day Programs Set for State

University of Minnesota dairy specialists will present dairy day programs at 11 locations this winter.

The educational programs, open to the public without charge, are sponsored by the University Agricultural Extension Service.

Extension Dairyman J. William Mudge will discuss milking equipment; Robert Appleman, another extension dairyman, will discuss using milking equipment; and Extension Veterinarian Dr. Ralph Farnsworth will discuss the effects of using milking equipment.

Locations and dates include: Braham, Dec. 8; Hutchinson, Dec. 9; Rosemount, Dec. 10; St. Cloud, Dec. 11; Winona, Dec. 12; Waseca, March 16; Jeffers, March 17; Morris, March 18; Crookston, March 23; Grand Rapids, March 24; and Sioux Falls, S.D., March 25.

3-A Adopts New Tubing, Bin Standards

The new 3-A sanitary standards and three revisions of existing standards were officially adopted by the 3-A Sanitary Standards Committees at their fall meeting in St. Louis Oct. 7-9, 1975.

The news standards cover sanitary tubing and dry product bins. Revised were the standards for ice cream freezers, ice cream and cottage cheese fillers and leak protector valves.

The newly approved documents will be printed in the *Journal of Food and Milk Technology* and will be effective approximately one year from now, after which manufacturers of the pertinent equipment may be authorized by the 3-A Symbol Council to affix the 3-A Symbol on equipment that complies with the standards.

For the first time sanitary tubing, formerly partially covered in the 3-A fittings standards, and dry product bins will be able to carry the 3-A Symbol visibly if authorized by the Symbol Council.

Other tentative proposals considered at St. Louis were reviewed by industry and regulatory groups and re-drafting requested. Included in this group were amendments to 3-A standards for silo tanks, transportation tanks, fittings, dryer practices, and milking practices, and proposed new standards for cottage cheese vats and dry product conveyors. These projects will be on the agenda of the 1976 spring meeting.

The 3-A program safeguards the public health through the development of 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.

Association Affairs

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Association Affairs

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Letters to the Editor

Errors in the MPN table

DEAR SIR:

In *J. Milk Food Technol.* Vol. 38, pg. 540-545, Sept. 1975, on page 544 Table 3 has a few errors in the column marked MPN (org/gram).

I will point out how apparent the error is. Example f with first three positive dilutions occur at 10^{-1} dilution. Final MPN is 39. However, in example d the first three positive dilutions occur at a 10^{-2} dilution, and the final MPN is 3,900. Cannot be because with a 10-fold increase in dilution one cannot mathematically convert numerial value 100 times.

At a time when instructing and using the MPN technique is, as I have witnessed, coming into increased use, this is a poor time to have incorrect numbers appear. It's difficult enough getting the rationale across let alone fight errors.

Assuming that in Table 3-1.0 ml. of each of listed dilutions (10^{-1} through 10^{-5} were done each in triplicate, then the following examples have incorrect MPN values: b, d, and h. Numbers should read instead 4,300; 390; and 2,900, respectively. By the way e has the sign in front going the wrong way. It should be greater than (> 110,000).

The authors also should be more specific in their examples, as I have assumed that 1.0 ml of each dilution was added to each tube in triplicate. There would be differences in values if 0.1 ml was added per tube.

MICHAEL JOGAN
Research and Analytical Laboratories
The Kroger Company
1240 State Avenue
Cincinnati, Ohio 45204

A corrected table is available

DEAR SIR:

Mr. Jogan's letter is indeed correct. It is unfortunate that these errors were included in the final proofs and we hope that the intent of the paper will remain intact.

Below, we have included a revised Tabie 3 as it should have read. Copies of the revised table are available upon request from the authors. Our thanks to Mr. Jogan for his alertness.

TABLE 3. *Examples of determining MPN estimates (1 ml sample aliquot/tube)*

Example	Dilution of sample 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	Reported positive tubes	MPN (org/gram)
a	3/3*	2/3	0/3	0/3	0/3	3-2-0	93
b	3/3	3/3	3/3	1/3	0/3	3-1-0	4300
c	3/3	2/3	2/3	0/3	0/3	3-2-2	210
d	3/3	3/3	0/3	1/3	0/3	3-0-1	390
e	3/3	3/3	3/3	3/3	3/3	3-3-3	> 110,000
f	3/3	0/3	1/3	0/3	0/3	3-0-1	39
g	3/3	2/3	1/3	1/3	0/3	3-2-2	210
h	3/3	3/3	2/3	2/3	1/3	3-2-3	2900
i	2/3	2/3	2/3	2/3	0/3	2-2-2	350
j	0/3	1/3	0/3	0/3	0/3	0-1-0	3

* $\frac{\text{Numerator}}{\text{Denominator}} = \frac{\text{Number of positive tubes}}{\text{Number of tubes inoculated}}$

J. L. OBLINGER
J. A. KOBURGER
Departmentof Food Science
University of Florida
Gainesville, Florida 32611

Association Affairs

California Affiliate Meets

The following report and attached pictures are from the 1975 annual California Association of Dairy and Milk Sanitarians and Fieldman's Conference:

The 57th Annual Association of Dairy & Milk Sanitarians and Fieldman's Conference was held at Long Beach, California on October 20-22, 1975, with more than 150 persons participating.

Harold Heiskell was presented with a copy of the IAMFES Resolution commending his 38 years total service and the last three years as chairman of the membership committee.

Eight association members received life membership. They were Richard Arnold, Lyle Beck, Lee Biggs, John McCready, Chester Topham Jr., J. C. Urquart, Ernest Waldee and Wendell Weaver.

Thirty persons presented talks and participated in panel discussions. Topics included Sanitation, Warehousing waste disposal, Laboratory programs, Marketing, Research, Antibiotics-use, testing and regulations, Automated equipment, Constructions, single service containers, product labeling, Brucellosis, ovum transplants and Interstate milk shippers program.

Harold Heiskell was elected president of the California Association of Dairy & Milk Sanitarians, The First Vice President is Richard Ayers, Second Vice President is Wayne Baragry and Secretary-Treasurer is Manual Abeyta.

Walter Wilson presents Harold Heiskell with a copy of the IAMFES Resolution commending his 38 years total service and the last three years as chairman of the membership committee.

Harold Heiskell receives presidential gavel from past-president Hugh Bement as California Dairy Princess Carolyn Veenendahl gives royal approval.

Affiliate Annual Meetings

California—October 20-22, 1975, Queensway Hilton, Long Beach.

Connecticut—January 1976.

Florida—March 16-18, 1976, Langford Hotel, Winter-Park.

Illinois—December 1, 1975, Blue Moon Restaurant, Elgin.

Indiana—October 7-9, 1975, Holiday Inn, Merrillville,

Iowa—March 22, 1976, Ramada Inn, Ames.

Kansas—October 1-3, 1975, Holiday Inn, Manhattan.

Kentucky—February 24-25, 1976, Stouffer's Inn, Louisville.

Michigan—March 1976.

New York—September 17-19, 1975, Granit Hotel, Kerhonkson.

Ontario—Eastern, November 1975, Kemptville.

Oregon—November 17, 1975, Oregon Department of Agriculture & Kings Table of International Restaurant, Salem.

South Dakota—May 11-14, 1976, Holiday Inn, Aberdeen.

Washington—September 9, 1975, Sheraton-Renton Inn, Renton.

Wisconsin—September 25-26, 1975, Holiday Inn, Tomah, Wisconsin.

Reprinted from Vol. 38, No. 11, November, 1975

Sixty-Third Annual Meeting of IAMFES

Arlington Park Hilton Hotel, Arlington Heights, Illinois
August 8-11, 1976

Instructions to Prepare Abstracts of Contributed Papers

Procedure

1. Use the printed Abstract form that appears on the other side of this page. Complete the form using a typewriter equipped with a reasonably dark ribbon.
2. Type in the title, capitalize the first letter of the first work and of any proper nouns.
3. List authors and institution(s). Capitalize first letters and initials. Indicate with an asterisk the author who will present the paper. Give complete mailing address of the author who will present the paper.
4. Type the Abstract *double-spaced,* in the space provided on the Abstract form.
5. Mail *two* copies of the Abstract before February 15, 1976 to:

   Mr. E. O. Wright
   Executive Secretary, IAMFES
   P.O. Box 701
   Ames, Iowa 50010

6. Enclose *two* self-addressed standard post cards. One will be used to acknowledge receipt of the Abstract and the other to notify the speaker about the scheduling of the paper. Two cards must be included with *each* Abstract that is submitted.

Content of the Abstract

The Abstract should describe briefly: (*a*) the problem that was studied, (*b*) methods used in the study, (*c*) essential results obtained, and (*d*) conclusions. Statements such as "results will be discussed" should not appear in an Abstract.

Oral Presentations

Papers will be scheduled so a speaker has a maximum of 15 minutes, including discussion. Hence the actual presentation should be no more than 11-13 minutes so that time for discussion will be available. Projectors for 2 × 2 inch slides will be available. If the speaker needs other projection equipment, Mr. E. O. Wright (address given earlier) should be contacted as soon as possible.

Subject Matter for Papers

Papers should report results of applied research in such areas as: food, dairy, and environmental sanitation and hygiene; foodborne disease hazards; food and dairy microbiology; food and dairy engineering; food and dairy chemistry; food additives; food and dairy technology; food service and food administration; food and dairy fermentations; quality control; mastitis; environmental health; waste disposal, pollution, and water quality.

Additional Abstract Forms

Extra copies of the Abstract forms may be obtained from Mr. E. O. Wright (address given earlier).

Membership in IAMFES

Membership in IAMFES is NOT necessary for presenting a paper at the annual meeting.

(OVER)

Annual Meeting

INTERNATIONAL ASSOCIATION OF MILK,
FOOD, AND ENVIRONMENTAL SANITARIANS, INC.

ABSTRACT FORM

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Please type abstract, double-spaced, in the space provided above.

NATIONAL MASTITIS COUNCIL ANNUAL MEETING

February 16-18, 1976

EXECUTIVE INN — LOUISVILLE, KENTUCKY

Everyone interested in prevention and control of bovine mastitis is cordially invited to attend the 15th Annual Meeting of the National Mastitis Council.

Vice President and Program Chairman Dr. R. D. Mochrie has planned an outstanding program for this meeting. Subject matter of interest to all segments will be presented, as indicated in this program preview:

Dr. F. H. S. Newbould, University of Guelph, Canada, will up-date international concepts of dealing with mastitis problems with his report on the International Dairy Federation Seminar on Mastitis Control held in Reading, England in April 1975.

Mastitis-induced changes in milk composition will be discussed by Dr. L. H. Schultz, University of Wisconsin. The effects of mastitis on processing properties in milk will be presented by Dr. R. L. Richter, University of Florida. Mr. D. C. Jordan, Colorado State University, will discuss incentive payments for high quality milk.

Teat dip formulations will be reviewed by Mr. C. R. McDuff, Economics Laboratories. Iodine in milk resulting from feed or from udder applications will be discussed by Dr. R. W. Hemken, University of Kentucky. An evaluation of Coulter Counter-Chemical method, WMT and DMSCC will be presented by Dr. D. R. Thompson, University of Minnesota. The regulatory aspects of teat dips and udder washes will be discussed by Dr. Howard Meyers, Bureau of Veterinary Medicine, Food and Drug Administration.

Milking parlor performance will be discussed by Dr. W. G. Bickert, Michigan State University. Milking machine problems will be reviewed by Mr. K. C. Kirby, Hi-Life Rubber.

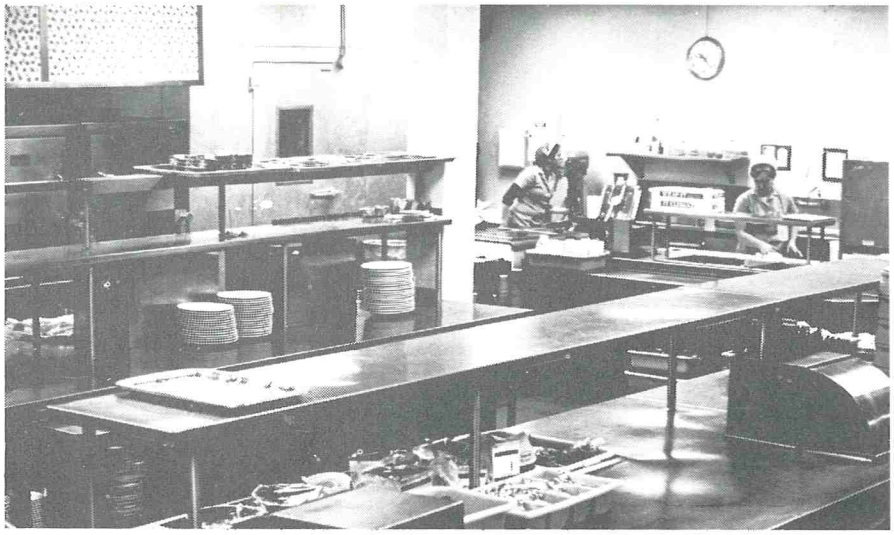
Antibiotic Problems will be dealt with in a panel discussion at the evening session. Dr. J. J. Jarrett, veterinarian, Rome, Georgia will serve as moderator. Panelists include: Dr. H. M. Trabosh, U.S. Department of Agriculture, Mr. J. R. Quayle, Wake County (NC) Health Department, Mr. J. B. Smathers, Maryland and Virginia Milk Producers Association, and a number of dairymen who will present farm practice views.

A panel discussion: Implementation of a Mastitis Management Program will conclude the annual program. Dr. J. H. Nicolai, University of Kentucky will moderate. Panel participants will be: Dr. J. R. Kunckel, veterinarian, St. Michael, Minnesota, Mr. J. W. Barnes, Michigan MPA, Mr. J. K. Webb, AMPI, and Dr. A. N. Bringe, Extension Service, University of Wisconsin.

Make your plans to attend this excellent meeting. It will start at 8:45 a.m. on February 17 and will adjourn at noon on February 18. Request advance registration form from the National Mastitis Council, 910-17th Street, NW, Washington, DC 20006.

Send request for room reservation directly to the Executive Inn, Watterson Expressway at Fairgrounds, Louisville, KY 40213.

Burdet Heinemann, *President*
National Mastitis Council



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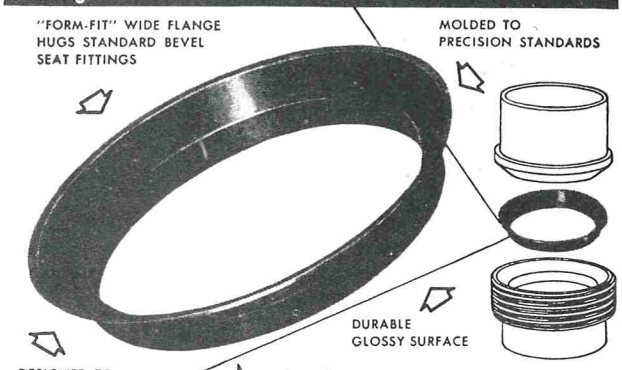

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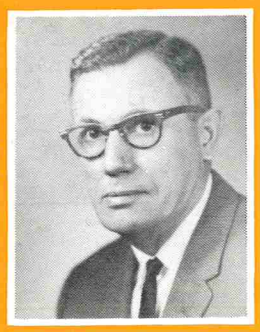
Dairy authorities speak out on better cow milking



Ralph Bonewitz, Extension Specialist
Department of Dairy Science
Kansas State University

What questions should a dairyman ask before expanding or modernizing?

Here are some of the most basic questions I believe dairymen should ask themselves before they begin a program of modernization or expansion:

What about my future in dairying? Will the changes I make be profitable in the long run? Will the changes be feasible in the short run?

Long run profitability refers to a period of eight to ten years or more. It is usually studied through the use of budgets. In the budgeting process, capital expenditures are prorated over the life of the assets by arbitrary depreciation methods.

Short run feasibility refers to the income-generating ability of a business in a short period of time. It is usually studied through the use of a projected cash flow.

Watch your cash flow

Projected cash inflow and outflow during the period are compared, reflecting payment requirements from credit agencies as well as all normal expenditures. Some business changes are capable of being profitable in the long run *but they are not capable of meeting short run demands for cash, particularly when payment requirements for capital expenditures are concentrated in the early years of an investment.*

Management must expand as herds get larger. More failures are due to lack of management than any other one thing. Hours spent thinking and getting facts will pay off and the manager or owner must assume this responsibility. A manager must have the ability to get the right things done at the right time.

Here are some more questions:

Will my expansion or modernization plan improve the chances and ease of producing a higher quality product? Will it increase the ease of the key jobs associated with dairying? Will it increase the efficiency of labor, investment and/or equipment? Will the modernized set-up provide the minimum movement of men, animals and material? Can this modern dairy farm compete with nonfarm occupations for good labor or manpower?

Do you as a manager or owner have a concern for production, people, quality milk and a desire to make the dairy more profitable? Will you develop a good milking program? One that obtains high production per cow, controls mastitis, produces clean excellent-flavored milk and uses a minimum amount of labor?

Check this planning list

Tomorrow's profitable dairy farm can be expected to be a planned operation. Here are some of the things you will want to consider during the planning phase: 1) Size of herd to be accommodated; 2) Dairy breed; 3) Climate conditions; 4) Topograpy of farmstead, slope, drainage and exposure; 5) Size and productivity of the farm; 6) Feeding program as related to feed storage and equipment for feeding; 7) Existing buildings and equipment that will fit into long range plans; 8) Availability of labor; 9) Capital available for investment; 10) Sanitary regulations or codes or present and potential milk markets; 11) Personal preference of the owner; 12) Water and air pollution regulations.

Planning with pencil and paper prior to the start of construction permits you to analyze combinations of equipment and building arrangements without any expenditure. Visit as many farms as possible and incorporate the good points seen at these farms into your facilities. Use university people and private consulting people.

Unless a dairyman regularly tests production, a pipeline milker can be the best friend that a cull cow ever had. The cull cow strolls in with the good producers, gives only enough milk to color the line, eats almost as much feed as the best cows, and then goes her merry way. A dairyman needs profitable production from every cow. You will call the correct signals and build a profit-winning dairy team if you use production records.

Feeding practices important

Constant attention to feeding practices, breeding for production and herd health during expansion are very important. A well designed, properly installed milking system is essential to proper milking. You can't afford an inadequate or poorly maintained milking system.

Productivity must be the key

The productivity of a resource depends on the amount and kind of other resources with which it is combined. As an example, when capital is substituted for labor, it requires fewer hours of labor per cow. However, unless production increases or more cows can be handled, the machinery and equipment cost will offset the savings in labor. Productivity of a resource then depends on the kind and quality of the resource with which it is combined. Modern technology is closely related then to the substitution of capital for labor and land. It increases the demands on management, emphasizes financial management and increases technical requirements of hired labor.

Finally, all of this modernization should help make dairying more pleasant, as well as more productive for you and your family.