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Journal of

MILK and FOOD TECHNOLOGY

58TH ANNUAL MEETING
August 16, 17, 18, 19, 1971
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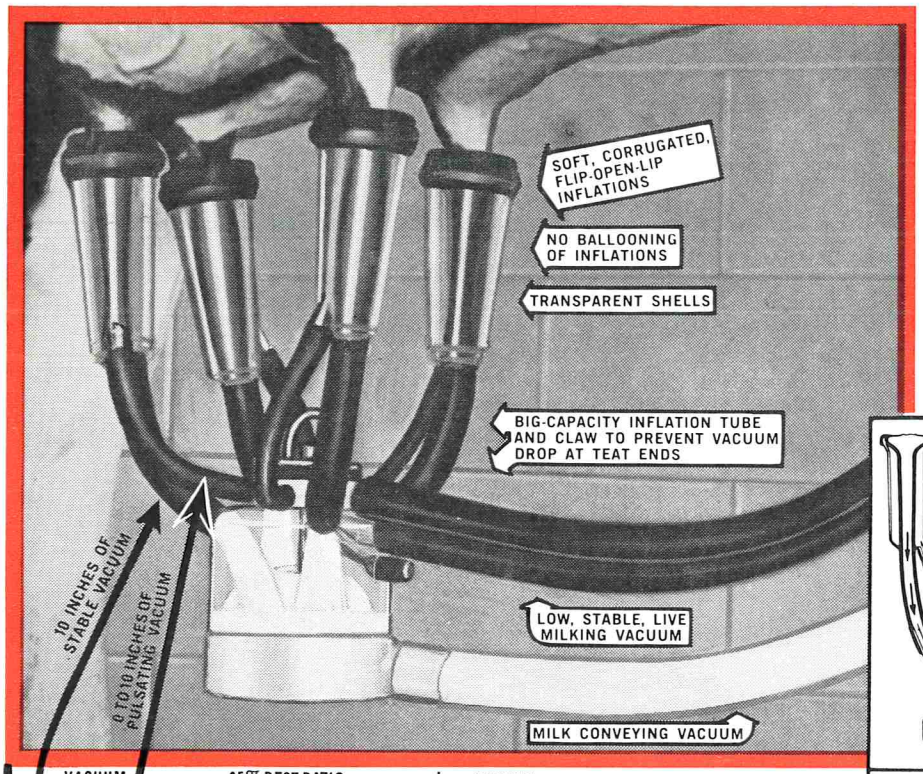
Official Publication

International Association of Milk, Food and Environmental Sanitarians, Inc.

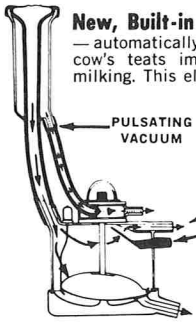
INTRODUCING THE NEW MODEL 70's Zero Concord MILKING SYSTEM....

The Only System that Provides Stable Milking Vacuum at the Teat—in Parlor or Stanchion Barn—with a Low or High, Short or Long, Pipeline—Without Injecting Air into the Milker Units to Move the Milk!



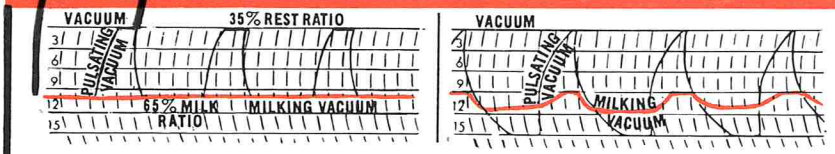
NEW MODEL 70's
Zero Concord
TWIN-VACUUM PIPELINE
MILKING SYSTEM
—WITH COW MONITOR

Only with Stable Vacuum and No Air Injection Can You Hope to Reduce Leucocyte Counts Up to 65%—Increase Milk Production as Much as 20%—and Prevent Off-Flavor and Rancid Milk

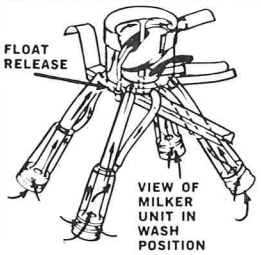


New, Built-in Vacuum Teat Release Valve — automatically releases the vacuum from the cow's teats immediately after completion of milking. This eliminates tugging and pulling to yank the milker unit off the cow. And makes possible faster, proper sanitizing of teats and teat cups — which speeds up transferring of milker unit from cow to cow.

Big Capacity Inflation Tube and Claw — prevents vacuum drop at the teat ends.



The Vacuum Stability Comparison Graphs Above Show the ZERO CONCORD's Stable Vacuum Compared with a Conventional System's Fluctuating Vacuum. Red line in above graph at right shows how milking vacuum of conventional system fluctuates — caused by milking cows and conveying milk through the same vacuum pipeline. Red line in above graph at left shows that milking vacuum of ZERO CONCORD is stable — made possible by Twin-Vacuum. Graphs made by Detco Graph Machine.



New Float Release — permits operator to quickly position float from milking cycle to washing cycle. Entire system is washed and sanitized without disassembling.

New Type Inflation — has flip-open lip that permits washing of both milk side and vacuum side of inflation.

Mastitis is costly! Total annual losses to dairymen in the U.S. attributable to mastitis have been estimated at $400 to $500 million, or $23 per cow. And more and more animal health authorities have been agreeing that many teat and udder injuries — which have contributed to the spread of mastitis — have been caused by milking with unstable vacuum.

If Mastitis is Costing YOU Money — Investigate the New Model "Seventies"- ZERO CONCORD Twin-Vacuum Pipeline Milking System. It's a combination of a revolutionary pipeline milking machine and the ZERO Completely-Automated Vacuum Bulk Milk Cooler. And was designed especially to milk with safe, stable vacuum at the teat end to prevent teat and udder injuries caused by milking with unstable vacuum.

The ZERO CONCORD's Safe, Stable Vacuum at the Teat Ends is Made Possible by ZERO's Patented Scientific Principle . . . TWIN-VACUUM . . . whereby one vacuum milks the cows — and an entirely-different vacuum moves the milk through a separate pipeline into the vacuum bulk tank. Twin-Vacuum does away with the need of injecting air into the milker units to move the milk, as is necessary with conventional milking systems.

TWIN-VACUUM Also Eliminates a Major Cause of Rancid and Off-Flavor Milk — by preventing air agitation and foaming of the milk in the pipeline. And it keeps contaminated air out of the milk.

Saves You Equipment Money, Too! TWIN-VACUUM does away with the need of a milk pump, releaser and other costly items.

Adding to the Advantages of TWIN-VACUUM is the New, Compact, Simpler, High-Capacity Milker Unit of the Model "Seventies" ZERO CONCORD. It's made of a new, light, strong, transparent material for visual milking — and a sturdy, stainless steel base. It has a revolutionary-designed claw, inflations and shells the advantages of which are pictured and described above.

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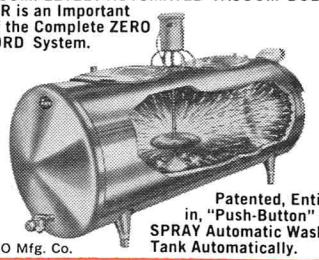

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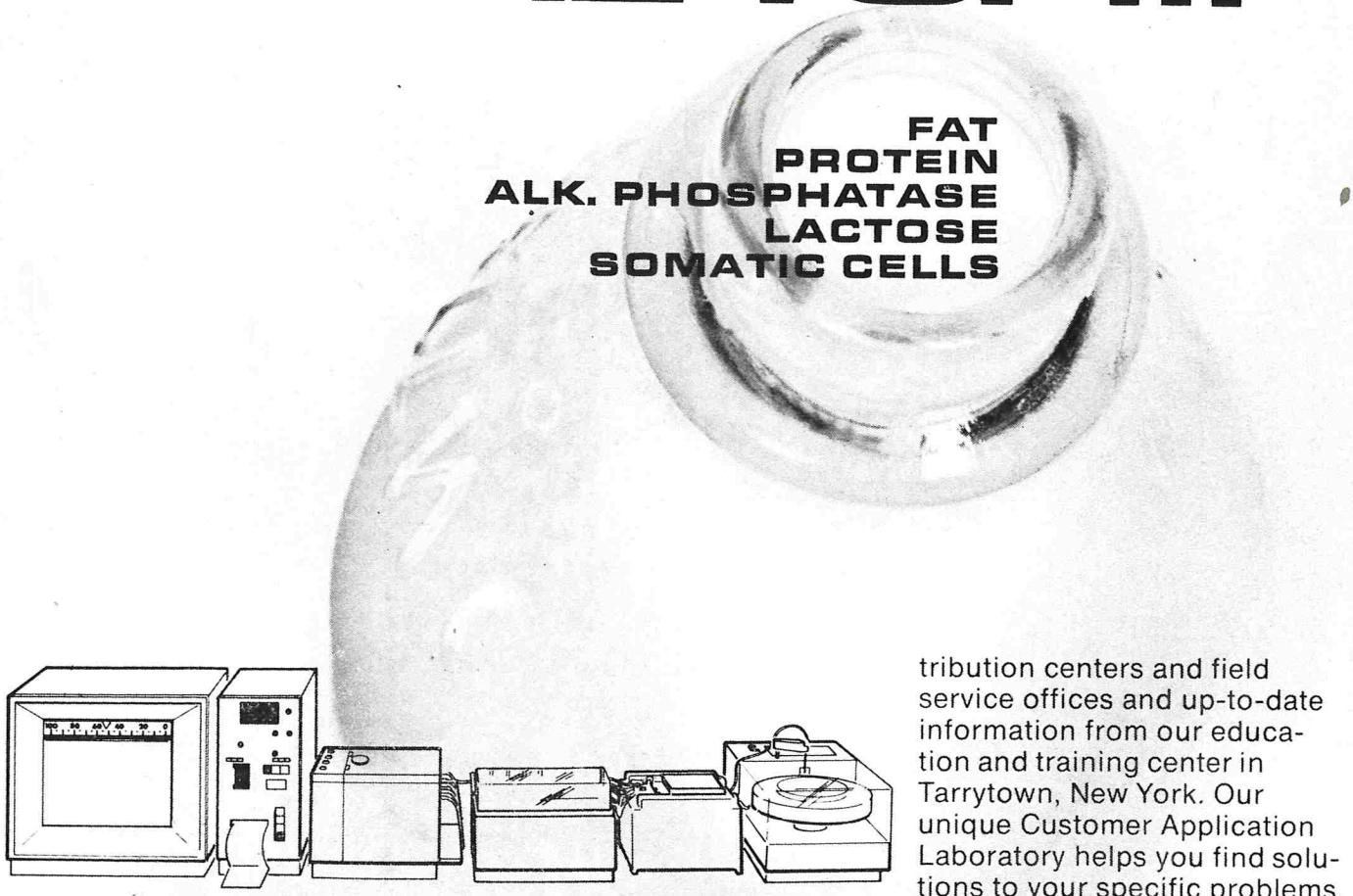

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Journal of

MILK and FOOD TECHNOLOGY

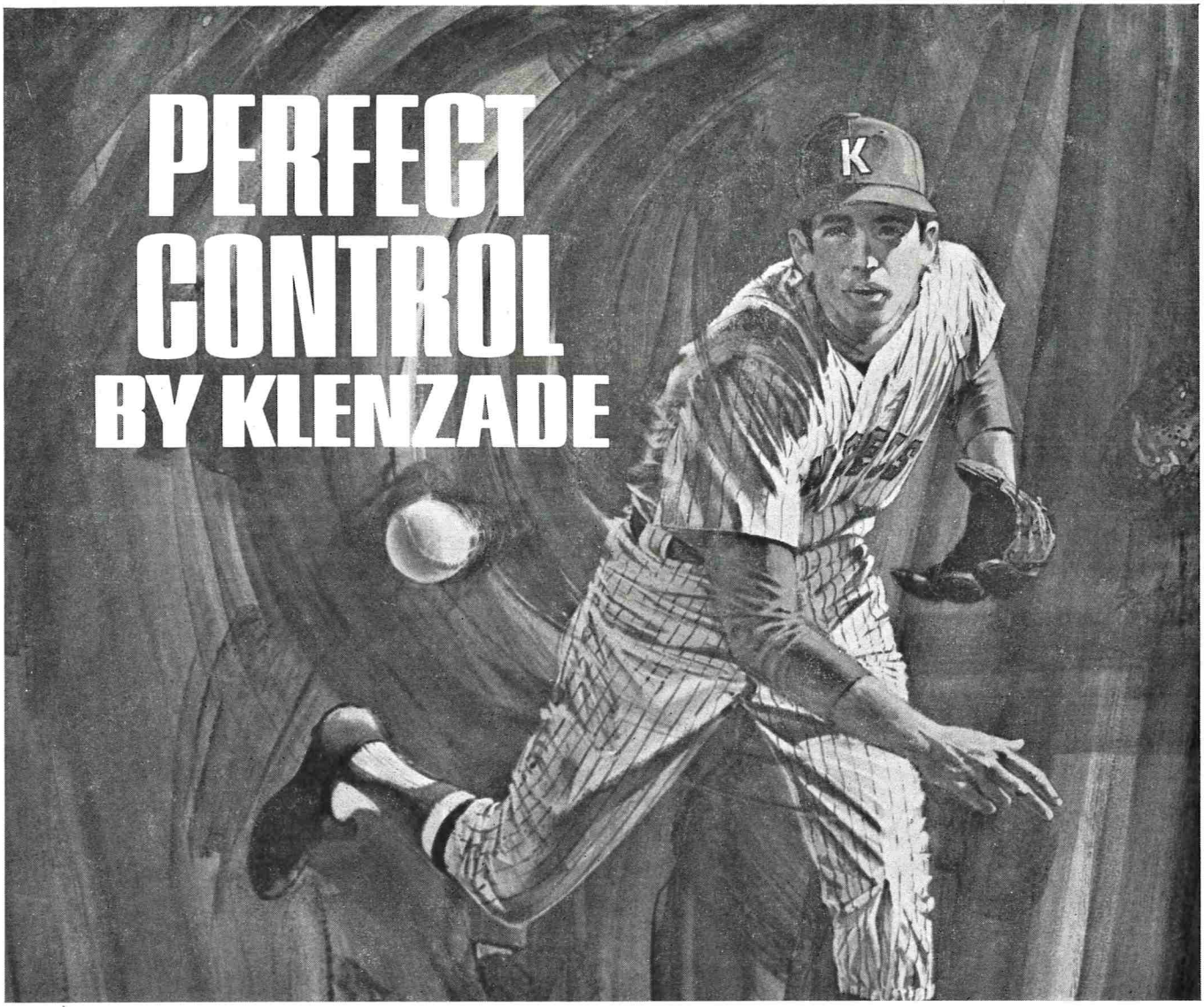
INCLUDING MILK AND FOOD SANITATION

Official Publication

International Association of Milk, Food and Environmental Sanitarians, Inc.

Reg. U. S. Pat. Off.

Vol. 34 April, 1971 No. 4



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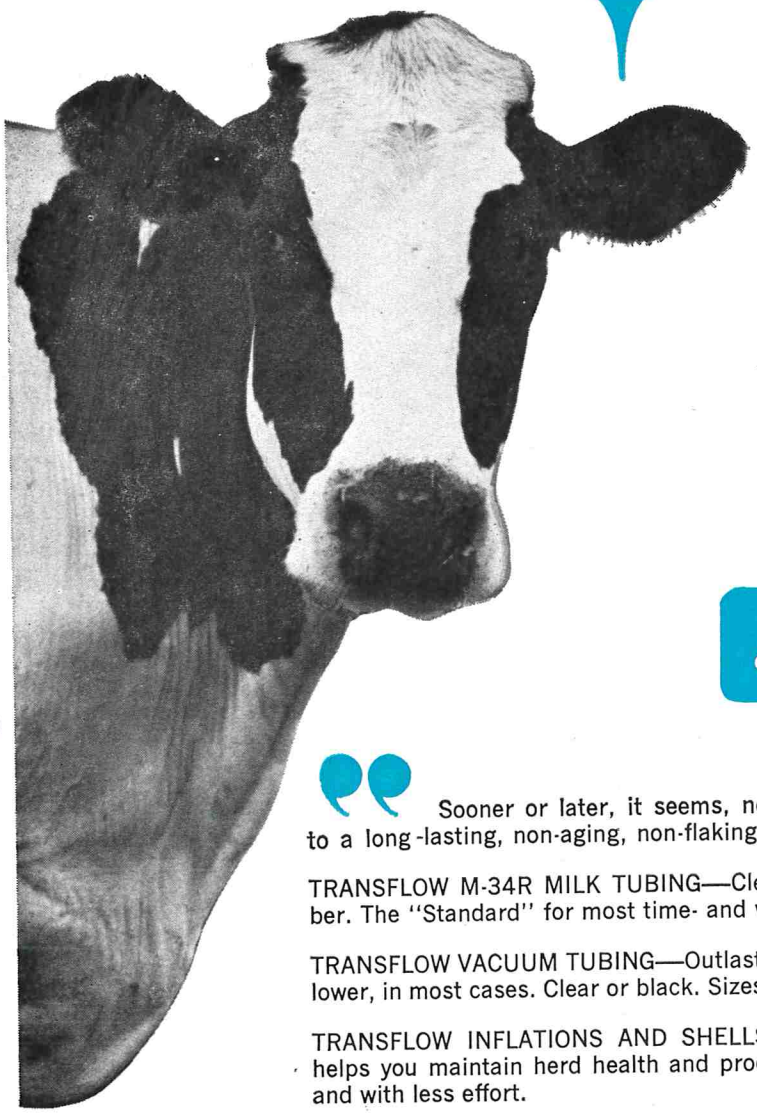

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Papers concerned with other subjects in the areas of food and dairy science, environmental control and health, and sanitation also are suitable for publication in the *Journal.* Authors who may have a question about the suitability of their manuscript for publication are invited to request an opinion from the Editor.

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All materials intended for the "Association Affairs" and "News and Events" sections of the *Journal* should be submitted in flat form by first class mail to the Managing Editor, Mr. H. L. Thomasson, Box 437, Shelbyville, Indiana 46176. Subjects suitable for inclusion in the "News and Events" section include: announcements of meetings, short courses, or other events of interest to the readership; notices of position changes and promotions; announcements of new products of interest to the readership; and notices of death and obituaries of members. Any questions on suitability of material can be answered by the Managing Editor.

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Most institutions accept the page charge as a necessary cost of conducting research and communicating the results. Nevertheless, it is realized that some authors may not have funds available for this purpose and hence exceptions can be made when necessary. Inability to pay the page charge shall not prohibit publication of an acceptable manuscript. An author will be informed of the cost of publishing a paper when he receives galley proofs.

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The *Journal* enjoys a wide readership in the United States and in foreign countries. Readers include persons at various levels in industrial, regulatory, and academic organizations. As a consequence, the *Journal* attempts to publish a variety of papers so that it is of maximum benefit to its readers,

The following types of papers are acceptable for publication in the *Journal.*

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The research paper reports results of original research which has not been published elsewhere. It usually consists of 8 to 12 double-spaced typewritten pages plus appropriate tables and figures. A research paper deals in some depth with its subject.

Research note

A research note is a short paper which describes observations made in a rather limited area of investigation. Negative results are sometimes best reported in the form of a research note. The research note should not be used as a vehicle for reporting inferior research. A research note generally consists of less than 5 double-spaced typewritten pages of text together with appropriate figures or tables. Organization of a research note is the same as that of a research paper. The designation, "A Research Note" will appear above the titles of these papers since the *Journal* does not devote a separate section to research notes.

The author should specify that his manuscript is a research note so that it can be properly evaluated during the reviewing process.

Review papers (General interest-technical)

Well written, thorough, well documented review papers on subjects of concern to the readers of the *Journal* are encouraged and will be published promptly. If a review paper substantially exceeds approximately 25 double-spaced typewritten pages, it may be necessary to subdivide the manuscript so that it will appear in several issues of the *Journal.* Authors are invited to discuss their plans for review papers with the Editor.

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The *Journal* regularly publishes some nontechnical papers as a service to those readers who are not involved with the technical aspects of dairy and food science. These "grassroots" papers might deal with such topics as working with people, organization of a sanitation program, organization of a regulatory agency, organization of an educational program, use of visual aids, and similar subjects. *Papers of this type should be well written and properly organized with appropriate subheadings.* Often talks given at meetings can be modified sufficiently to make them appropriate for publication. Authors planning to prepare general interest nontechnical papers are invited to correspond with the Editor if they have questions about the suitability of their material.

Letter to the Editor

Readers are invited to submit letters to the editor to express their opinion on papers published in the *Journal* or on other matters which may be of concern to the entire readership. The letter to the editor also may be used to report limited observations made in the field or in the laboratory which cannot be published as a research note. This mechanism should be particularly valuable for the exchange of information by persons who are unable to attend annual meetings of IAMFES or by laboratory workers whose duties preclude publication of full-fledged research papers. A letter to the editor must be signed by its author(s).

Book reviews

Authors and publishers of books in the fields covered by this *Journal* (see earlier discussion of Subject Matter) are invited to submit their books to the Editor. Books will then be reviewed by a specialist in the field covered by the book and a review will be published in an early issue of the *Journal.*

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A. All manuscripts should be typed double-spaced on 8.5 by 11-inch bond paper. *Lines on each page should be numbered to facilitate review of papers.* Use of paper with prenumbered lines is satisfactory. Side margins should be one inch wide and pages should not be stapled together.

B. The Editor assumes that the senior author has received proper clearance from his organization for publication of the paper. Authors should be aware of procedures for approval within their own organization.

C. A manuscript should be read critically by someone other than the author before it is submitted. This will help to eliminate errors and to clarify statements.

D. The second edition of *Style Manual for Biological Journals* (published by the American Institute of Biological Sciences, 3900 Wisconsin Ave. N.W., Washington, D. C. 20016) has been adopted by the *Journal* and should be consulted by authors for technical details of manuscript preparation. Abbreviations for journals and for botanical, chemical, physical, mathematical, and statistical terms should conform to the *Style Manual.*

E. *Organization of research papers and research notes*

1. The *title* should appear at the top of the first page. It should be as brief as possible, contain no abbreviations, and be truly indicative of the subject matter discussed in the paper. Care should be exercised by the author in preparing the title since it is often used in information retrieval systems. *Good information can be lost through a poor title!*
2. *Name(s) of author(s) and affiliation(s)* should follow under the title. If an author has changed location since the work was completed, his new address should be given in a footnote.
3. The *Abstract* appears at the beginning of the paper. It should be brief, factual, and not exceed 200 words. The abstract should be intelligible without reading the remainder of the paper. Generally, an abstract should not contain abbreviations. Abstracts of papers are reprinted by abstracting journals and so will be disseminated beyond the readership of the *Journal* to people who often do not have access to the entire paper. This suggests that abstracts should be prepared with great care.
4. The *text* should contain: *(a)* introductory statements, objectives or reasons for research, and related literature, *(b)* materials and methods *(c)* results, *(d)* discussion (may be combined with results), *(e)* conclusions (only if needed; should not repeat the abstract), *(f)* acknowledgements, and *(g)* references.
5. *Citation of references* should follow the style of the *Style Manual for Biological Journals.* Several examples of proper citations are given below.
   a. *Paper in a journal*:
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   b. *Paper in a book*:
      Leviton, A., and E. H. Marth. 1965. Fermentations, p. 673 to 770. *In* B. H. Webb and A. H.

Johnson (ed) Fundamentals of dairy chemistry. Avi Publishing Co., Westport, Conn.

c. *Book*:
Frazier, W. C., E. H. Marth, and R. H. Deibel. 1968. Laboratory manual for food microbiology. 4th ed. Burgess Publishing Co., Minneapolis. 122 p.

d. *Patent*:
Hussong, R. V., E. H. Marth, and D. G. Vakaleris. 1964. Manufacture of cottage cheese. U. S. Pat. 3,117,870. Jan. 14.

For citation of bulletins, annual reports, publications of federal agencies, etc., see *Style Manual for Biological Journals*. References should be listed in alphabetical order and numbered. Numbers in parentheses, independently or in conjuction with last names of authors, should be used in the text for designating references.

F. *Organization of review and general interest papers*
These papers should have a title, give name(s) or author(s) and affiliation(s), and the text should begin with an abstract. See items 1, 2, and 3 under E. The remainder of the text should begin with an introductory statement and then should be subdivided into appropriate sections each with a subheading which is descriptive of the subject matter in the section. Review papers, by their very nature, utilize a large number of references. Citation of references in the text and listing of references at the end of the paper should be done as mentioned in section E-5 above.

G. *Preparation of figures*
Figures consisting of drawings, diagrams, charts, and similar material should be prepared in India ink on 8.5 by 11-inch tracing paper, white drawing paper, or blue linen. Do not use paper with green, red, or yellow lines since they cannot be removed and will appear in the final copy. A lettering guide must be used to prepare all letters which appear on figures. *Titles for all figures must be on separate sheets and not on the figures.* Use Arabic numbers for numbering of figures. Glossy prints of figures are suitable for use. They should be at least 4 by 5 inches in size. If photographs of equipment, etc. are submitted, the images should be sharp, there should be good contrast, and a minimum of distracting items should appear in the picture.

H. *Preparation of tables*
Each table should be typed on a separate sheet of 8.5 by 11-inch bond paper. *Tables should not be included in the text of the paper.* Use Arabic numbers for numbering of tables. *Titles should be as brief as possible but fully descriptive.* Headings and subheadings should be concise with columns or rows of data carefully centered below them. Use only horizontal lines to separate sections of tables. *Data in tables should not be repeated in figures.* When possible use figures instead of tables since the latter are more costly to prepare for publication.

AMENDMENT TO E-3-A SANITARY STANDARDS FOR PUMPS FOR LIQUID EGG PRODUCTS

Serial #E-0201

Formulated by
International Association of Milk, Food and Environmental Sanitarians
United States Public Health Service
United States Department of Agriculture
Institute of American Poultry Industries
Dairy and Food Industries Supply Association

The E-3-A Sanitary Standards for Pumps for Liquid Egg Products, Serial #E-0200 are amended by adding a new subsection 7 to A. *MATERIAL.*

7. Pump impellors or rotors, and cases or stators, which operate in conjunction with a metallic counterpart and the sealing faces of rotary seals may be covered with a ceramic material. Ceramic materials shall be inert, non-porous, non-toxic, non-absorbent, insoluble, resistant to scratching, scoring and distortion when exposed to the conditions encountered in the environment of intended use and in cleaning and bactericidal treatment.

This amendment is effective May 22, 1971.

BACTERIOLOGICAL TESTING OF MILK FOR REGULATORY PURPOSES—USEFULNESS OF CURRENT PROCEDURES AND RECOMMENDATIONS FOR CHANGE[1]

I. THE PROBLEM

R. B. Read, Jr.
Food and Drug Administration
1090 Tusculum Avenue
Cincinnati, Ohio 45226

(Received for publication October 22, 1970)

Testing milk for regulatory purposes began as the result of the general belief that raw milk was causing a high incidence of human diseases such as tuberculosis, brucellosis, typhoid fever, streptococcal sore throat, and diptheria. Because there was no practical way to test milk routinely for all pathogens, use of either a total count or a total viable count as an indicator of milk quality was proposed and accepted by those concerned with regulatory testing. Procedures such as the Methylene Blue and Resazurin reduction tests, the Direct Microscopic Count, and the Standard Plate Count were developed, evaluated, and used.

As the technology of Grade A milk production advanced, the bacterial count in raw milk was gradually reduced, and predominating types of bacteria in milk undoubtedly also changed. With these changes came successively greater problems in relating reduction times and Direct Microscopic Counts to number of bacteria present in milk. As a result, reduction tests and the Direct Microscopic Count were discontinued in favor of the Standard Plate Count for regulatory testing of milk.

Use of farm bulk tanks for Grade A milk is believed to have changed the bacterial flora common in raw milk so that the Standard Plate Count technique is of either limited or no value in reflecting farm conditions. Similarly, use of the Standard Plate Count on finished products has been criticized because it is generally applied to products that have just been packaged and therefore does not reflect the microbiological quality of milk as purchased by the consumer.

Certainly, these problems dictate that we pause and give extensive consideration to what we are doing when we test milk for regulatory purposes. We need to ask ourselves questions such as: (*a*) is there need to continue bacteriological testing of milk; (*b*) if so, what condition(s) are we trying to measure when we test bulk tank raw milk, storage tank raw milk, and finished products; and (*c*) once we identify what we are trying to measure, which tests shall we use?

At this point we need ideas, we need discussion, and we need research. From this, I am hopeful that a consensus will result that will be the foundation for constructive change, if this should be indicated. The purpose of this Symposium is simply to provide a vehicle for the expression of ideas, for discussion, and perhaps for the stimulation of research that will be helpful in the resolution of these problems.

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[1]The Public Health Committee of the American Dairy Science Association arranged for a Symposium on "Bacteriological Testing of Milk for Regulatory Purposes—Usefulness of Current Procedures and Recommendations for Change." The Symposium was held at the Annual Meeting of the American Dairy Science Association, University of Florida, Gainesville, Florida on June 29, 1970. Four papers and a statement of the problem were presented. Cooperation by Dr. E. O. Herreid, Editor-in-Chief of the *Journal of Dairy Science*, the Journal Management Committee of the *Journal of Dairy Science*, and the authors makes possible the publication of all the Symposium papers in the *Journal of Milk and Food Technology*. Participants in the Symposium include: Dr. R. B. Read, Jr., Dr. C. K. Johns, Dr. G. W. Reinbold, Dr. R. B. Maxcy, and Dr. H. C. Olson.

BACTERIOLOGICAL TESTING OF MILK FOR REGULATORY PURPOSES–USEFULNESS OF CURRENT PROCEDURES AND RECOMMENDATIONS FOR CHANGE

II. BACTERIOLOGICAL TESTING OF RAW MILK FOR REGULATORY PURPOSES[1]

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Abstract

While routine bacteriological tests afford virtually no protection against milk-borne disease, they should reflect insanitary production conditions. Mounting evidence suggests that current tests are doing an indifferent job here. No single test can furnish an adequate picture. We need to reflect the presence of psychrotrophs, thermodurics, mastitis organisms, lactic acid bacteria, etc.

Psychrotrophs, the most important group in refrigerated milk, come mainly from dirty udders and equipment. These deserve major attention. Usefulness of the Standard Plate Count in reflecting their presence would be enhanced by an incubation temperature lower than 32 C. The psychrotrophic count at 7 C for 10 days has obvious limitations. These can be minimized by suggested modifications. Preliminary Incubation (P. I.) at 13 C for 18 hr indirectly reflects psychrotrophs, which multiply rapidly under these conditions. Other procedures suggested include the cytochrome oxidase test, Water Agar test, catalase production, nitrate reduction at 30 C, and a test for sodium desoxycholate-tolerant bacteria. For the detection of thermodurics, a simple nitrate-formate reduction test following P. I. at 22 C for 16 hr has been proposed.

Extensive collaborative testing in various areas should precede the adoption of new methods and standards, while a suitable incentive to producers can greatly encourage sanitary milk production.

In most developed countries some regulatory control of milk supplies is considered desirable. Such control was originally designed to protect consumers from milk-borne infections, with emphasis on animal diseases and farm inspection. Routine bacteriological testing of raw milk came later, using a "total" viable count [now the Standard Plate Count (SPC)], Direct Microscopic Count, or Methylene Blue Reduction Test *(1)*. There have been no major changes in tests during the last 50 years.

It was generally accepted that care in producing and handling milk was best reflected by such tests, although they do not in themselves ensure freedom from milk-borne disease. Only proper pasteurization is effective for this purpose. While care taken to protect milk from excessive bacterial contamination may have slightly reduced the chances of it containing pathogens *(1)*, what has really made raw milk safer for farm families has been the eradication of bovine tuberculosis and brucellosis. Mastitis still presents a hazard from toxigenic staphylococci. *Low count milk may actually be more dangerous*, as witness disease outbreaks from raw certified milk. Again, in Scotland, where an appreciable volume of raw milk is sold by producer-retailers, some of the premium milks have been the most dangerous *(32)*. And in California an outbreak with 147 cases of staphylococcal food poisoning *(2)* in 1967 supports the findings *(24, 36)* that these organisms grow best in low-count milk.

Since routine bacteriological tests afford virtually no protection against milk-borne disease, some may ask if there is any good reason for continuing them. I believe there is. I feel that we need to be able to assure the consumer that milk has been produced under acceptable conditions *(8)*. The ideal way to accomplish this would be through frequent milking-time inspections. But these are expensive *(31, 51)*. If we are to rely upon bacteriological tests to supplement less frequent inspections, we must ask ourselves whether those currently in use tell us what we need to know about sanitary milk production.

Problems With the Plate Count

A recent comprehensive review *(14, 17)* indicates that the commonly used tests, such as the Standard Plate Count *(1)*, have rarely been closely correlated with production conditions. With the adoption of farm bulk tanks, criticsm of the SPC has mounted *(3)*. Several factors may be responsible for this lack of correlation. Storage at lower temperatures is an obvious one. Another is the greater dilution of contaminating bacteria from equipment as the volume of milk per farm increases. "Dilution is the dairyman's best friend!" A third, rarely mentioned, is that at low ambient temperatures, limited growth takes place even on dirty equipment *(47)*. And finally, we employ an incubation temperature, 32 C, too high for

[1]Presented at the Annual Meeting of the American Dairy Science Association, University of Florida, Gainesville, Florida, June 29, 1970.

TABLE 1. INADEQUACY OF STANDARD PLATE COUNT AT 32 C IN REFLECTING NUMBERS OF PSYCHROTROPHIC BACTERIA IN RAW MILK.[1]

Producer no.	Standard plate count	Psychrotrophic count	Sodium desoxycholate tolerant count
5212	5,200	54,000	<300
2883	8,800	11,000	1,000
9398	11,000	20,000	<300
1319	13,000	160,000	1,100
1746	18,000	200,000	5,400
9693	19,000	21,000	1,600
1228	20,000	20,000	11,000
5893	29,000	400,000	<300
8317	34,000	37,000	11,000
1446	120,000	170,000	78,000

[1]Data furnished by Roy E. Ginn, Dairy Quality Control Institute, Inc. St. Paul, Minn. Selected from 56 samples.

TABLE 2. INADEQUACY OF STANDARD PLATE COUNT AT 32 C IN REFLECTING PSYCHROTROPHIC BACTERIA IN RAW MILK.[1]

Sample no.	Fresh sample		After PI at 13 C for 18 hr	
	SPC[2]	PBC[3]	SPC[2]	PBC[3]
7	2,200	100	18,000	28,000
9	2,500	40	22,000	26,000
23	3,600	30	8,100	15,000
29	5,100	250	96,000	130,000
37	6,900	230	35,000	51,000
39	7,000	60	130,000	140,000
49	10,000	36,000	1,800,000	2,200,000
55	14,000	620	910,000	920,000
77	32,000	170,000	4,300,000	6,400,000
88	72,000	190,000	8,300,000	13,000,000
89	76,000	200,000	4,000,000	5,000,000
98	140,000	41,000	5,800,000	64,000,000

[1]From Canada Dept. Agriculture Publication 1084, 1960.
[2]Standard Plate Count, 32 C for 48 hr.
[3]Psychrotrophic Bacteria Count, 5 C for 7 days.

growth of many psychrotrophs, and some thermodurics *(16)*.

If the SPC, or any other viable count procedure, is to do a more adequate job of reflecting the presence of saprophytic contaminants, I firmly believe a lower incubation temperature is essential *(2a, 17)*. Even extending the incubation period for raw milk to 72 hr in studies at three Canadian centers *(22)* gave virtually no increase in count. Various investigators have found that the psychrotrophic count (PBC) in raw milk, with plates incubated at 7 C for 10 days, often greatly exceeds the SPC. This is illustrated in Tables 1 and 2. Results in Table 2 were from incubation at 5 C for 7 days, the standard procedure at that time (1957) *(20)*. When incubation was continued another 2 days, some counts were as much as 40 times higher!) Similar higher counts at 5 C for 7 days were reported by LaGrange and Nelson *(25)* for manufacturing-grade milks. Blankenagel *(4)* has found 25 C to be the optimum temperature for 48 hr counts of sodium desoxycholate-tolerant bacteria (almost all Gram-negative rods.) (Table 3 and Fig. 1). There seems little doubt that at 32 C we are missing a significant percentage of organisms representative of insanitary conditions. This may help to explain why there are many cases of poor conditions and low SPCs. A lower temperature, even if it involves a longer incubation period, should improve any "total" viable count procedure.

Recognizing the inadequacy of 32 C incubation, what temperature should be adopted? Most countries outside North America have followed the suggestion of the International Dairy Federation, which in 1958 recommended 30 C for 72 hr *(19)*. In Britain the value of this combination of temperature and time for advisory purposes was recognized back in 1945 *(38)*, although routine dye reduction tests are still conducted at 37 C! Collaborative studies by a

TABLE 3. EFFECT OF INCUBATION TEMPERATURE ON COLONY COUNTS OF SODIUM DESOXYCHOLATE-TOLERANT ORGANISMS FROM 54 BULK-TANK SAMPLES

	Incubation temperature (C)			
	32	28	25	21
Median count/ml	160	275	290	250
Average count/ml	510	610	700	710
No. of samples with maximum counts[1]	11	20	26	24

[1]In some instances maximum counts appeared at two or more incubation temperatures. All plates counted after 48 hr. Data furnished by G. Blankenagel, 1970.

TABLE 4. EFFECT OF REFRIGERATED STORAGE ON STANDARD PLATE COUNTS OF TANKER SAMPLES.[1]

Route No.	Date (1969)	Standard plate count	
		Day collected	After 48 hr at 40 F
8	3-31	30,000	520,000
16		50,000	4,000,000
18		110,000	3,800,000
19		17,000	100,000
22		26,000	350,000
8[2]	4-1	30,000	1,100,000
15		260,000	330,000
17		33,000	130,000
20		16,000	610,000
24		31,000	740,000
15	4-7	120,000	680,000
17		14,000	240,000
20		40,000	1,200,000
34		5,000	190,000
8	4-8	19,000	48,000
16		13,000	40,000
18		15,000	130,000
22		21,000	800,000

[1]Data furnished by H. J. Barnum
[2]Sample of 3-31 replated next day.

TABLE 5. INCIDENCE OF DIFFERENT TYPES OF MICRO-ORGANISMS IN RINSES OF FARM DAIRY EQUIPMENT[1]

Type of organism	Per cent cultures isolated from rinses with colony counts of			
	<10,000/ft²		>250,000/ft²	
Streptococci	4.2		15.5	
Micrococci	72.8		19.7	
Corynebacteria	6.2		2.7	
Other asporogenous Gm+ rods	4.7		5.6	
Arthrobacter	2.6		0.5	
Coliforms	0.0		6.9	
Non-pigmented Gm- rods	2.3	4.2	15.2	47.2
Pigmented Gm- rods	1.9		24.8	

[1]Average of 16 rinses of equipment sanitized with chlorine. From Thomas et al., J. Soc. Dairy Technol. 17:210, 1964.

TABLE 6. INCIDENCE OF MICROCOCCI AND GRAM-NEGATIVE RODS IN CAN MILK SAMPLES FROM 87 FARMS[1]

Colony count (30 C, 72 hr)	Percentage	
	Micrococci	Gram-negative rods
<5,000	68.7	5.9
5-20,000	52.4	7.9
20-200,000	29.7	31.4
200-1,000,000	20.7	56.8
>1,000,000	19.0	54.0

[1]From Thomas et al., J. Applied Bacteriol. 25:108, 1962.

committee of ADSA, chaired by M. L. Speck, reported in favor of a lower temperature in 1955 *(2a)*. Huhtanen *(18)* found counts after 48 hr incubation were higher at 27 C than those at 30 C, which again were much higher than those at 33 C, while Iowa investigators *(17)* recommend 28 C for 4 days. Crawley and Twomey *(6)* found 30 C superior for plate, thermoduric, and coliform counts, as well as for nitrate and methylene blue reduction tests. We in North America appear to be dragging our feet in comparison with other countries in taking steps to adopt a more appropriate temperature.

Today there is a growing recognition that psychrotrophic bacteria are the most important type in milk, raw or pasteurized. They grow at refrigeration temperatures (Table 4). Many are proteolytic and/or lipolytic, causing spoilage *(39)*. Even more important in relation to this discussion, they come largely from neglected equipment *(38)* as shown in Tables 5 and 6. They also may come from filthy cows *(21)* (Table 7).

Many workers have emphasized the complexity of the problem of assessing the sanitary quality of milk; no one test is likely to furnish all the information needed. Unfortunately, many in the industry, as well as in regulatory agencies, regard any milk with an SPC below the legal limit as being of high sanitary quality, never stopping to ask themselves just how meaningless such counts may be. As so well stated by Fay *(11)*: "The objective of testing is not to determine how many bacteria there are in a sample but to identify the producer who is doing less than a good job with the tools with which he has to work."

It may be that by using a better plating medium, incubation temperature, and time, the SPC will furnish a more reliable picture of insanitary conditions on the farm. On the other hand, some workers believe that testing for specific types of contaminants would be more valuable. Unfortunately, while many tests have been compared against a reference method (usually the SPC) *there have been few attempts to*

TABLE 7. BACTERIAL COUNTS ON MILK FROM FILTHY COWS[1]

Milking	SPC[2] (×10³)	Psychrotrophs[3]	Coliforms[4]	Milking	SPC[2] (×10³)	Psychrotrophs[3]	Coliforms[4]
8 p.m.	30	100	<10	15 a.m.	43	290	1
9 a.m.	17	40	<10	p.m.	46	1,000	<1
p.m.	15	5	<10	16 a.m.	66	110	3
10 a.m.	61	5	<10	p.m.	74	3,600	<1
p.m.	23	700	21	17 a.m.	22	1,400	<1
11 a.m.	24	1,100	3	p.m.	11	190	<1
p.m.	100	10	83	18 a.m.	69	4,500	4
12 a.m.	80	10	<1	p.m.	65	21,000	<1
p.m.	81	15	1	19 a.m.	49	7,300	<1
13 a.m.	18	10	2	p.m.	70	4,300	3
p.m.	13	10	<1	20 a.m.	120	35,000	<1
14 a.m.	78	17,000	1	p.m.	15	1,200	<1
p.m.	23	80	<1	21 a.m.	33	2,900	<1

[1]Studies conducted at the Central Experimental Farm, Ottawa, in 1962. Heifers in long stalls without bedding allowed to become plastered with manure; udders not washed before milking.
[2]32 C for 48 hr.
[3]5 C for 7 days.
[4]VRB agar, 32 C, 24 hr.

TABLE 8. COMPARISON OF SPC VS. SPC-PI IN REFLECTING UNCLEAN FQUIPMENT[1]

Center	Farm no.	SPC (×10³)	SPC-PI (×10³)	Cleanliness score: Milking eqpt.[2]	Cleanliness score: Bulk tank[2]	Total score
2	141	6.8	740	21	20	41
	11	10	13,000	26	23	49
	104	14	190	21	23	44
	157	15	130	24	25	49
	178	76	14,000	22	29	51
3	135	4.6	32	35	11	46
	16	18	210	19	27	46
	102	27	120	18	28	46
	15	40	540	29	18	47

[1]From collaborative studies at Winnipeg, Man. and Guelph. Ont.
[2]Maximum score 40 points.

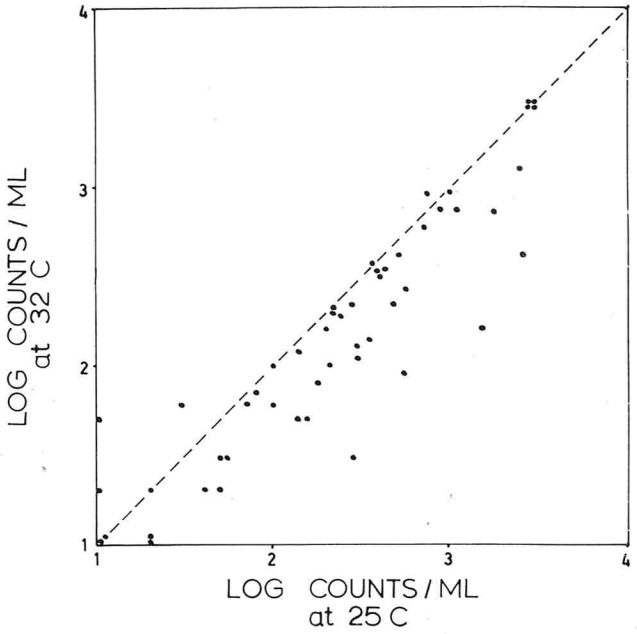

Figure 1. Effect of incubation temperature on 48 hr counts of sodium desoxycholate-tolerant bacteria. (Data by G. Blankenagel.)

discover the relative value of different tests in reflecting production conditions (7, 9, 15, 22, 29). This is admittedly more difficult, and much more expensive. The studies at Iowa State *(15)* are the most impressive; they found the psychrotrophic count (PBC) the only one of 8 tests to show a significant correlation with farm conditions ($r = -0.48$ vs -0.35 for the SPC).

OTHER TESTS NEEDED

On the assumption that even an improved version of the SPC will still not give an adequate picture, I would suggest we need two supplementary tests. One of these should reflect all or part of the psychrotrophs, which generally represent a proteinaceous film on equipment; the other should show up the thermodurics, which are usually associated with milkstone. These specific tests will not reflect such other types as mastitis organisms and lactic acid bacteria, hence the need for a "total" viable count such as the SPC. A report from Germany on automation of the "total" count *(43)* is intriguing. Such a procedure could revolutionize the bacteriological testing of milk, providing reproducible results more cheaply in less time.

While the PBC *(1)* at 7 C for 10 days is considered to reflect the psychrotrophic bacteria adequately, the need for a special incubator and the long delay in getting results have worked against adoption of this method for routine testing. These objections can be met in part through pre-incubation at 17 C for 16 hr *(27)* or by surface plating *(33)*, giving results in 5 days[1].

Various other tests have been suggested to detect all or part of the psychrotrophic content. Preliminary incubation (PI) *(10, 20)* at 13 C for 18 hr is an indirect method; udder flora fail to multiply at this temperature, while Gram-negative rods, including coliforms, often increase over 100-fold. While survey-type studies *(9, 15, 22, 29)* have not shown a statistically significant advantage for this procedure, data in Tables 8 and 9 indicate PI has some advantages in reflecting equipment uncleanliness. Those who have tried it have found it most useful in pointing out farms with low SPCs where equipment care needed to be improved. However, further standardization of technique is needed, as has recently been shown *(34)*.

The coliform count *(1)*, a statutory requirement in Scotland for many years, has been suggested by some workers. Recent studies in Iowa *(15)* have shown a low correlation with production conditions. The coliform count even fails to reflect filthy cows (Table 7) *(21)*.

The cytochrome oxidase test for pseudomonads has been suggested *(13)*. While these are a very important segment of the microflora, it is questionable whether all others should be disregarded. Also Blankenagel *(4)* reports that only a small percentage of the organisms showing up in their sodium desoxycholate-tolerant count procedure *(5)* were oxidase-positive.

As many psychrotrophs are proteolytic Gram-negative rods, Taylor *(37)* has developed a Water Agar Test as a simple method of evaluating production conditions. It should be especially useful for field

[1]Since this paper was prepared, an article appeared in the Australian Journal of Dairy Technology, Vol. 25, pp. 30-32, 1970, entitled "A Four-Day Count for Psychrotrophs" by H. S. Juffs.

TABLE 9. AVERAGE EQUIPMENT CLEANLINESS SCORES AS REFLECTED BY BACTERIAL COUNTS (286 FARMS)[1]

Center	Poor (0 - 2)			(Fair (3 - 5)			Good (6 - 9)		
	Total No.	SPC[2]	SPC-PI[3]	Total No.	SPC	SPC-PI	Total No.	SPC	SPC-PI
				Rubberware					
1	0	0	0	18	4	9	68	8	21
2	0	0	0	19	7	10	73	11	15
3	3	2	3	51	10	12	44	8	12
Combined	3	2	3	88	21	31	185	27	48
				All Equipment					
1	0	0	0	3	0	2	93	12	28
2	0	0	0	4	1	1	88	17	24
3	1	1	1	25	6	8	72	13	18
Combined	1	1	1	32	7	11	253	42	70

[1]From collaborative studies at Edmonton, Winnipeg and Guelph, summer, 1964.
[2]SPC 50,000 and over
[3]SPC-PI 200,000 and over

TABLE 10. COMPARATIVE COUNTS ON SAMPLES OF BULK TANK MILK (FRASER VALLEY MILK PRODUCERS' ASSOCIATION)

Sample no.	SPC	SPC-PI	Coliforms	LPC	SDC-tolerant	CVT count
312[1]	2,000	300,000	1,500	640	1,100	—
528[1]	4,000	220,000	90	220	1,300	—
303	5,000	>300,000	3,000	120	4,800	—
618[1]	5,000	5,000	3,200	10	3,000	—
1010[1]	9,000	280,000	1,100	110	1,200	10,000
728[1]	11,000	49,000	10	110	3,000	—
510[1]	17,000	>300,000	2,000	180	10,000	—
208[1]	21,000	>300,000	250	9,600	170	—
428[1]	20,000	40,000	350	3,300	390	—
320[1]	22,000	39,000	10	2,200	1,000	—
216[1]	35,000	250,000	70	4,800	280	—
505[1]	39,000	>300,000	10,000	6,400	2,700	—
802[1]	51,000	47,000	480	3,000	780	30,000
215[1]	65,000	190,000	220	2,500	530	—
1023	400,000	330,000	2,900	3,000	3,000	120,000

[1]Fieldman reported equipment conditions unsatisfactory. All plates incubated at 32 C.

work. The Scottish Milk Marketing Board has just completed an extensive comparison of this test with the plate count and has reported very favorably on it *(35)*. New Zealand workers *(26)* have modified the test, diluting the milk and using a buffered caseinate medium. They report a good correlation between counts so obtained and those from surface-inoculated plates.

Another indirect approach is that of Loane *(28)*. As Gram-negative rods, and some other types, produce catalase, she tests for catalase production in a sample after incubation at 32 C for 5 hr with 0.04% hydrogen peroxide. A positive correlation with the plate count is reported.

Most psychrotrophs are Gram-negative rods. They are rarely found in the udder, therefore in milk they indicate contamination. Blankenagel and Okello-Uma *(5)*, following up the work of Freeman et al. *(12)*, developed a test for these organisms, based upon their ability to tolerate 0.5% sodium desoxycholate (SDC). The standard incubation temperature (32 C) was used; subsequent studies showed maximum counts at 25 C. (Fig. 1 and Table 3). Laboratories in Denver, St. Paul, Vancouver, and Saint John, New Brunswick agreed to conduct comparative tests, while Blankenagel determined the optimum incubation temperature.

While, in most instances there was good agreement between the SDC count (1,000/ml limit) and other tests, in others this was not true. As these latter are of greatest interest, they are shown in Tables 1, 10, and 11. In Table 1 it is evident that the SDC counts were much lower than the PBCs. The same is true in Table 10, where a few crystal violet-tetrazolium (CVT) counts were available for comparison. Nevertheless, it appears that a significant number of sam-

TABLE 11. COMPARATIVE COUNTS ON SAMPLES OF BULK-TANK MILK[1]

Sample no.	SPC (32 C)	LPC (32 C)	SDC-tolerant bacteria at incubation temperature of			
			32 C	28 C	25 C	21 C
54	8,000	300	1,200	1,100	1,100	960
21	9,000	100	740	1,300	1,800	2,200
52	13,000	400	950	1,000	1,000	1,000
24	14,000	300	1,300	1,900	2,500	2,400
13	18,000	200	760	840	1,100	890
25	18,000	2,200	160	450	1,500	1,900
10	26,000	200	1,100	1,200	1,200	960
22	28,000	700	420	1,300	2,600	>3,000
29	41,000	200	600	810	720	990
44	63,000	400	60	70	70	80
28	83,000	4,000	<10	<10	20	<10
8	100,000	1,200	>3,000	>3,000	>3,000	>3,000
40	120,000	100	1,800	2,500	>3,000	>3,000

[1]Data furnished by G. Blankenagel. All plates counted after 48 hr incubation.

ples with low SPCs and LPCs have over 1,000/ml SDC, even at 32 C.

Table 10 also reflects the value of PI. Twelve of the 15 samples show SPCs of <40,000/ml, yet 8 of these exceed 200,000/ml after PI. And for 11 of the 12 farms the fieldman reported unsatisfactory conditions! In Table 11, only 4 of 13 samples exceed 50,000/ml SPC, while the SDC fails 7 at 32 C and 11 at 25 C.

In Saint John, N.B. the Provincial Health Laboratory made 594 comparisons between the SDC-tolerant count and the SPC-PI. They reported that: (*a*) the SDC test was very simple and rapidly performed; (*b*) when compared with the provincial standard of <300,000/ml SPC after PI, counts of <1,000/ml were easily obtained; (*c*) when the SPC-PI was >300,000 /ml the SDC count always exceeded 1,000/ml.

The Denver studies led to the conclusion that, "according to the Gram-negative (SDC) test we do need to improve some of our management practices" *(3a)*. Disadvantages noted were greater labor and equipment requirements.

Data collected at St. Paul, where farm equipment was carefully scored, are being analysed by computer. Such an analysis should be most helpful in determining the usefulness of the SDC test. Casual inspection of the data from these preliminary studies suggests it has enough merit to warrant extensive collaborative trials along with some of the other new procedures in various regions *(23)*, preferably at 25 C.

Harking back to the need for a test to reflect milkstone, the Laboratory Pasteurization Count *(1)* appears to be reasonably satisfactory, but would be improved by using a better medium and a lower incubation temperature *(42)*. Twomey and Crawley *(47)* have recently developed a nitrate-formate reduction test which detected 92% of samples containing >5,000 /ml thermodurics within 6 hr at 30 C following PI at 22 C for 16 hr. Fortunately, in most areas of North America thermoduric bacteria are not the problem they were a generation ago, probably because of new tools and better use of them on the farm. Consequently, less frequent testing may be necessary.

Just what standards should be set for any of the tests we have mentioned must await the accumulation of more extensive data from many geographical regions during all seasons. However, it is obvious that the SPC limit *(49)* of 100,000/ml is far too lenient for bulk-tank milk. Basically the problem is to persuade producers to follow recommended practices. Payment according to quality has been shown to be very effective in stimulating sanitary milk production *(30, 31a, 50)*. This would greatly reduce the need for regulatory testing.

In summary, if bacteriological tests are to better reflect production conditions, they must be conducted at temperatures where the most important contaminants can grow. "Total" viable counts should be supplemented by tests which more specifically reflect such contaminants. Extensive collaborative testing is necessary to establish the relative merits of such tests, and to indicate suitable standards. Finally, a suitable incentive to producers can greatly encourage sanitary milk production.

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SUPPLEMENT NO. 1 TO 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

Serial #0811

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 lever-operated compression type valves 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, Serial #0809." (Reference 3-A Drawings No. 3A-100-31 and No. 3A-100-32.)

These valves shall comply with the applicable provisions of this standard and the following:

E.6.1
The valve assembly shall consist of a lever, bearing housing, needle, needle seat and a body.

E.6.2
All product contact surfaces shall be of materials conforming to the criteria in sections C.1 or C.1.4 or C.1.6 of this standard.

E.6.3
The valve body and needle shall be of materials conforming to the criteria in section C.1 of this standard.

E.6.4
All product contact surfaces shall be readily accessible for cleaning and inspection, either in an assembled position or when removed. Removable parts shall be readily disassembled.

E.6.5
All internal angles of 135° or less on product contact surfaces shall have minimum radii of 1/4 inch except those where for space or functional reasons it is impossible to have a radius of 1/4 inch. When the radius is less than 1/4 inch, the product contact surface of this angle, must be readily accessible for cleaning and inspection. In no case shall the radius be less than 1/32 inch.

E.6.6
Coil springs having product contact surfaces shall have openings between the coils including the ends when the spring is in a free position. Coil springs shall be readily accessible for cleaning and inspection.

E.6.7
There shall be no threads on product contact surfaces.

E.6.8
There shall be no stuffing boxes.

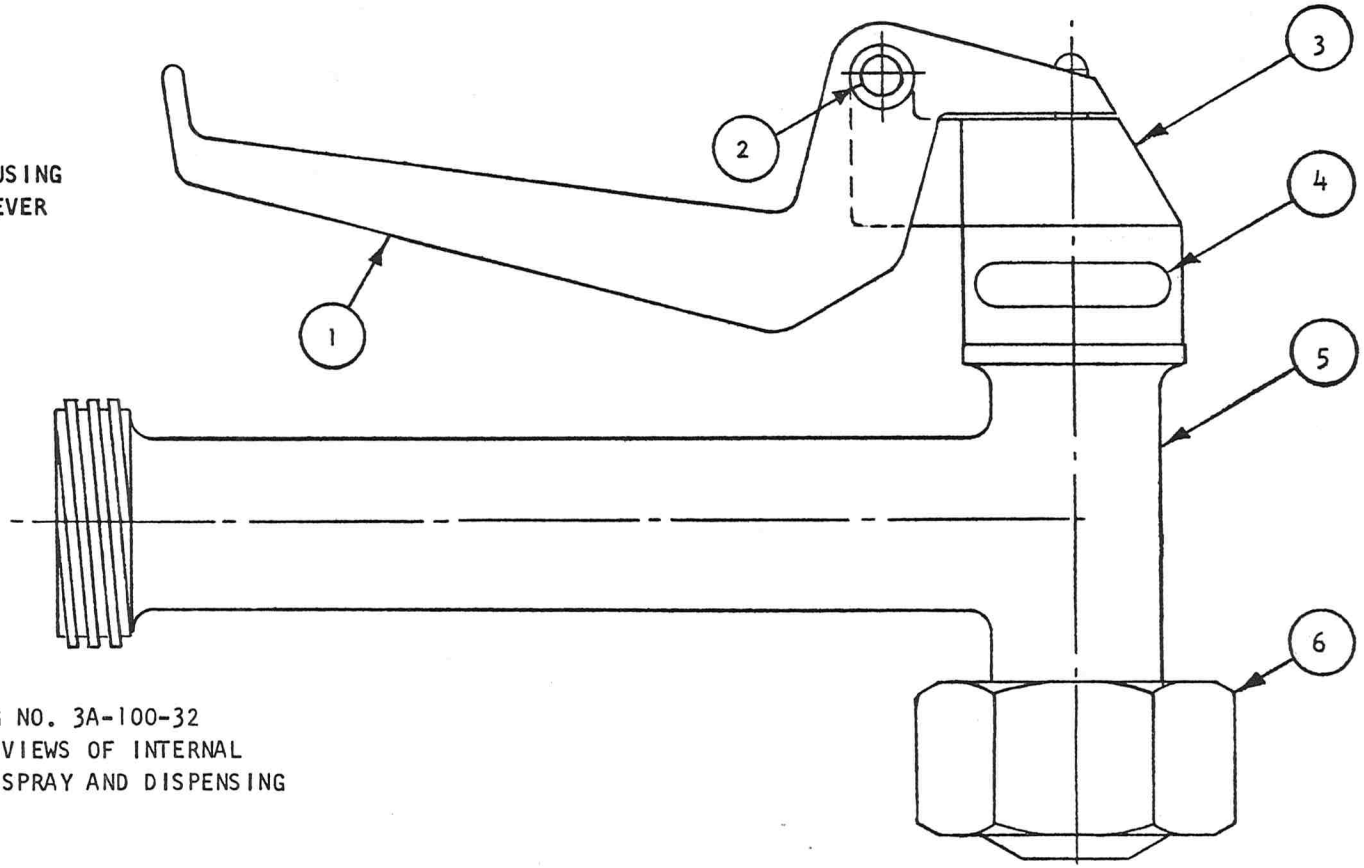
E.6.9
Retaining grooves for removable rubber or rubber-like parts and/or plastic parts shall be readily cleanable.

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

Fitting Name	*Page No.*	*3A Drawing No.*
Lever-Operated Compression	25	3A-100-31
Type Valve	26	3A-100-32

This amendment is effective July 24, 1971.

1. LEVER
2. PIVOT PIN
3. BEARING HOUSING
4. CAM LOCK LEVER
5. BODY
6. NUT



SEE 3A DRAWING NO. 3A-100-32 FOR SECTIONAL VIEWS OF INTERNAL ASSEMBLIES OF SPRAY AND DISPENSING VALVES.

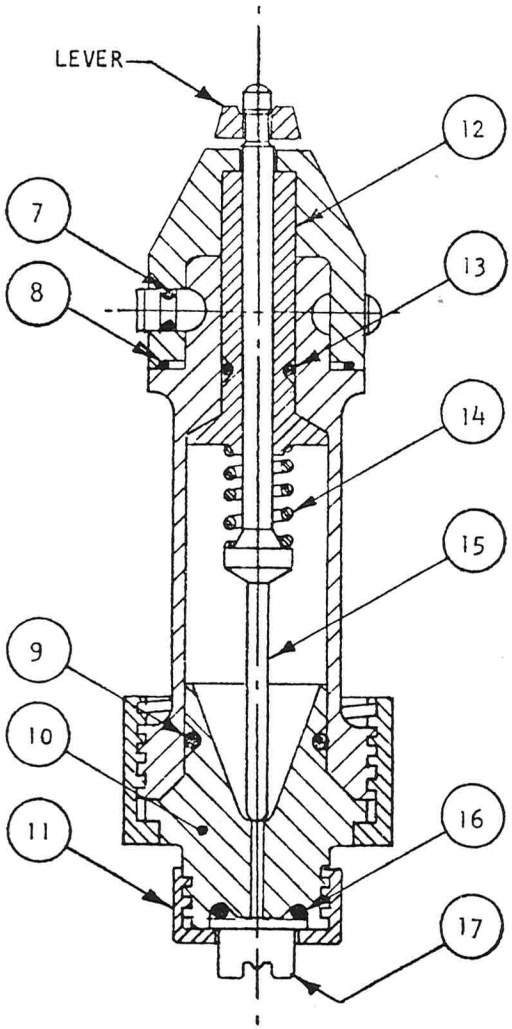
LEVER OPERATED COMPRESSION TYPE VALVES

NOTE:

THE INTERNAL DESIGN SHOWN IS INTENDED TO DEMONSTRATE GENERAL PRINCIPLES ONLY, AND IS NOT INTENDED TO LIMIT INDIVIDUAL INGENUITY. THE DESIGN USED SHALL CONFORM WITH THE GENERAL SANITARY REQUIREMENTS SET FORTH IN THIS 3-A SANITARY STANDARD AND SPECIFIC REQUIREMENTS FOR LEVER OPERATED COMPRESSION TYPE VALVES.

THREADED INLET CONNECTIONS SHALL CONFORM WITH THE DIMENSIONS FOR THREADS SHOWN ON THE DRAWINGS FOR OTHER FITTINGS INCLUDED IN THIS 3-A SANITARY STANDARD. CENTER TO FACE DIMENSIONS WILL VARY WITH VALVES MADE BY DIFFERENT MANUFACTURERS.

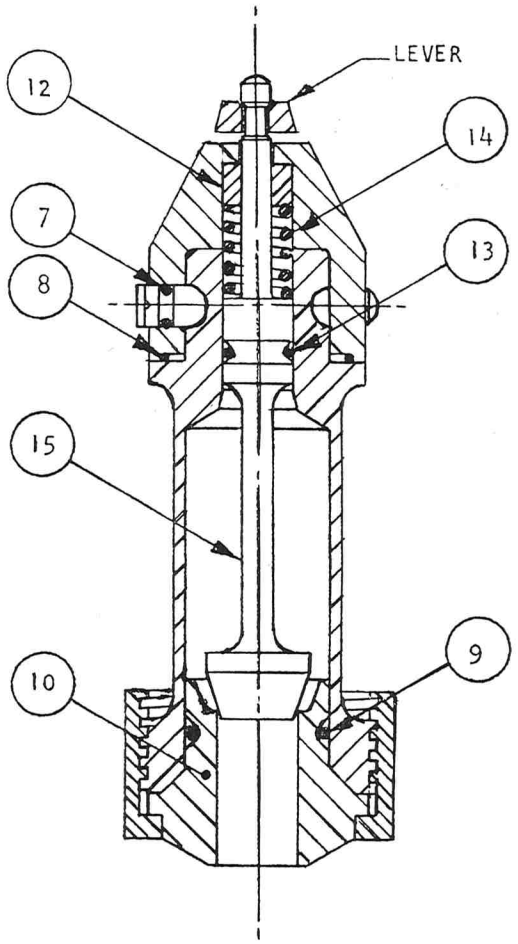
3 A STANDARD
SANITARY FITTINGS
3A-100-31



SECTIONAL VIEW OF
INTERNAL ASSEMBLY
OF SPRAY VALVE

7. LEVER RETAINER
8. GASKET
9. SEAL
10. NEEDLE SEAT
11. NUT
12. SLEEVE BEARING
13. SEAL
14. SPRING
15. NEEDLE
16. SEAL
17. SPRAY TIP

SPRAY/DISPENSING VALVES
INTERNAL ASSEMBLIES ARE
INTERCHANGEABLE



SECTIONAL VIEW OF
INTERNAL ASSEMBLY
OF DISPENSING VALVE

LEVER OPERATED COMPRESSION TYPE VALVES

3 A STANDARD
SANITARY FITTINGS
3 A-100-32

AMENDENT TO THE 3-A SANITARY STANDARDS FOR FARM MILK COOLING AND HOLDING TANKS, REVISED

Serial #1305

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

The "3-A Sanitary Standards for Farm Milk Cooling and Holding Tanks, Revised, Serial #1301," are hereby further amended in the sections indicated below:

Replace the last sentence of B-5(c) with the following:

In such tank, the maximum and the minimum vertical dimensions of the tank interior shall be 96 inches and 36 inches, respectively.

Substitute the following for subsections 3 and 4 of C – COOLING:

3. *Cooling Information*

The tank shall have an information or data plate permanently attached to it giving the following information or the information shall appear on the name plate.

(a) The maximum rate at which milk can enter the tank and comply with the cooling requirements of C.1 and C.2 above.

(b) The minimum condensing unit capacity required when the milk enters the tank at the maximum rate.

This tank is designed for {every-day / or / every-other day} pick-up.

Maximum rate at which milk can enter this tank and meet the cooling requirements of the 3-A Sanitary Standards for Farm Milk Cooling and Holding Tanks, Revised, Serial #1301, is _____ U. S. Gallons per hour. When milk enters the tank at the maximum rate, the minimum condensing unit capacity is *_____ BTU/hr. at *_____F saturated suction temperature.

(c) Whether the tank is designed for every-day or every-other-day pickup.

4. *Cooling System*

(a) In determining cooling unit capacity, the ambient temperature shall be 90°F and when water cooled condensers are used, the refrigerant condensing temperature shall be assumed to be not less than 103°F.

(b) The tank shall be provided with an automatic refrigeration control capable of functioning on a change in product temperature of not more than plus or minus 2°F at 37°F.

Add a new Section E to the Appendix to follow Section D, as follows:

E. To determine the capability of a farm cooling tank to meet the cooling requirements specified in C.1 and C.2 at the maximum rate at which milk can enter the tank given on the information plate

(a) 90°F water may be substituted for milk, and

(b) before the addition of the second and subsequent milkings,

(1) the water or milk in the tank shall be cooled to 37°F and

(2) the condensing unit shall be allowed to operate and automatically shut off.

*The BTU capacity specified is to be at the saturated suction temperature designated by the manufacturer.

This amendment is effective June 1, 1971.

FECAL CONTAMINATION OF FRUITS AND VEGETABLES DURING CULTIVATION AND PROCESSING FOR MARKET. A REVIEW[1]

EDWIN E. GELDREICH[2] AND ROBERT H. BORDNER[3]

(Received for publication November 6, 1970)

ABSTRACT

Bacteriological data collected from various field studies involving irrigation water, field crops, and soils were studied with respect to sources and magnitude of fecal contamination associated with cultivation, harvesting, and marketing of fruits and vegetables. Other reports concerned with contamination during agricultural activities were reviewed. Fecal coliform densities proved to be a better measurement of the probable occurrence of waterborne pathogens than any single test for a specific pathogenic group. When the fecal coliform density per 100 ml was above 1,000 organisms in various stream waters, *Salmonella* occurrence reached almost 100 per cent frequency. These data support the establishment of the proposed limit of 1,000 fecal coliforms per 100 ml of irrigation water, but approved sanitation practices must accompany (or supplement) use of this bacteriological standard. A concept of multiple safeguards to limit the public health hazard associated with poor quality irrigation water, irrigation farming practices, and market preparation of produce is discussed from the microbiological viewpoint.

The bacteriological quality of farm produce is frequently degraded by fecal contamination. This contamination results from various unsanitary cultivation and marketing processes which take place before the products reach the consumer. Primary among these procedures is the practice of irrigating or fertilizing with inadequately treated municipal, animal, or food processing wastes which may contain pathogenic organisms. In addition, careless handling of the crops during the growing season or after harvesting may occur. When fruits and vegetables raised or processed under these conditions are eaten raw, the transmission of disease is a matter for concern.

The expanding world population calls for a greater awareness of the public health problems related to agricultural practices. Irrigation will play an important role in providing the higher food production necessary *(54)*. As more acreage is developed for irrigation the demand for water will increase, but the quality of water available will often be poor because of greater reuse. There are no widely recognized quality criteria for farm produce or for the water with which it is irrigated.

The objectives of this paper are to provide further insight into the potential public health problems by: *(a)* a review of current information reported in the literature characterizing the sources, significance, and methods for detection of fecal contamination and *(b)* a study of the data from extensive field studies involving irrigation practices. The intent is to evaluate proposed standards for irrigation water quality in the light of these results and to suggest measures for cultivation and processing of farm crops whereby the danger to health may be reduced.

PATHOGENIC MICROORGANISMS

It is well established that disease-causing bacteria, viruses, protozoa, worms, and fungi are found in fecal material, sewage, and sewage-polluted water; consequently they may contaminate the soil and crops with which they come in contact. Animal as well as human wastes are implicated because many species of pathogens can infect both man and animals. Consumption of uncooked foods contaminated with fecal material may cause the spread of disease in livestock as well as human beings. Microorganisms known to be pathogenic for plants also can be isolated from polluted irrigation water, but the role that water and sewage play in plant disease transmission is not yet completely understood.

The disease most frequently linked with fecal contamination are typhoid and parathyphoid fevers, *Salmonella* gastroenteritis, bacillary dysentery, cholera, leptospirosis, infectious hepatitis, viral gastroenteritis, and amoebic dysentery. Although typhoid fever, cholera, and amoebic dysentery are now practically nonexistent in this country because of effective sanitation and water treatment practices, they do present health problems in other countries, particularly in Europe, Asia, and South America. Less common diseases associated with irrigation agriculture are brucellosis, tuberculosis, tularemia, swine erysipelas, coccidiosis, ascariasis, cysticercosis, fascioliasis, schistosomiasis, and hookworm and tapeworm infections. Although the route of infection is usually by ingestion, larvae of hookworms and flukes can enter the body directly through the skin. The significance of pathogenic fungi in irrigated areas awaits further study. The ability of all these organisms to cause

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[1]Presented at the American Society for Microbiology symposium "Spoilage Bacteria, Indicator Organisms and Pathogens in Raw Plant Foods" at the annual meeting, April 26-May 1, 1970, Boston, Mass.

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infection is directly related to their virulence, their presence in sufficient numbers, and the chances of ingestion, inhalation, or absorption by a susceptible host.

Epidemiological evidence

The true extent of disease infection from raw produce is unknown. Diseases associated with ingestion of such foods range from serious illnesses to low-grade infections of several days' duration or mild intestinal upsets lasting only a few hours. Since most of the latter do not require medical treatment, their number is never accurately reported.

There are however, a number of epidemiological reports which show that transmission of enteric disease or parasitic infestations does occur when raw sewage or sewage effluents are used in crop cultivation. Disease outbreaks are reported to be caused by *Salmonella* strains on raw celery *(37)*, watercress *(36)*, watermelon *(19)*, lettuce *(29, 30)*, cabbage *(18)*, endive and raw salad vegetables *(25, 26)*, and fruits *(9)*. Heavy infestations of roundworms *(Ascaris lumbricoides)* found in some European cities are related to the use of night soil on family gardens and small produce farms *(29)*. In the Orient, use of night soil on vegetables and small fruits during cultivation is estimated to cause approximately 20% of recurrent infections of amoebiasis, bacillary dysentery, enteric fevers, cholera, and hookworm *(43, 50, 73)*.

Microbiological examinations of water used to irrigate vegetable gardens in Brazil provide evidence of fecal pollution and the presence of polio and Coxackie viruses *(6, 7)*. These reports refer to previous epidemics and stress the necessity for controlling the quality of the irrigation water.

Pathogen survival and detection on farm produce

Salmonella, Shigella, enteropathogenic *Escherichia coli,* amoebic cysts, *Ascaris* ova, and enteroviruses have been detected on garden produce growing in soil contaminated or irrigated with sewage effluents *(24, 34, 68)*. Investigators using artificial contamination procedures have made numerous studies of pathogen survival on various vegetables, fruits, and animal fodder *(2, 16, 28, 35, 48)*. Previous reviews of these findings *(11, 47, 51)* are summarized in Table 1 to show survival ranges for specific pathogenic groups.

Detection of pathogens on farm produce growing in contact with polluted water and soils treated with animal or human manure is infrequent, unless the plant samples are grossly contaminated with sewage or are observed to have fecal particles adhering to them. The difficulty of pathogen recovery is caused by such environmental factors as the incidence of waterborne disease in the pollution discharge, the numbers of pathogens in the inoculum, type of soil, soil moisture retention, soil pH, availability of nutrients, and antagonistic effects of other organisms which comprise the flora. Adverse climatic effects include high temperatures, low humidity, and prolonged exposure to sunlight.

TABLE 1. PATHOGEN AND TOTAL COLIFORM SURVIVAL ON CONTAMINATED FARM PRODUCE AND FODDER[1]

Organism	Produce or fodder	Survival time
Salmonella	Fodder	12 - >42 days
	Root Crops	10 - 53 days
	Leaf vegetables	1 - 40 days
	Berries	6 hrs. - 5 days
	Orchard crops	18 hrs. - >2 days
Shigella	Fodder	<2 days
	Leaf vegetables	2 - 7 days
	Orchard crops	6 days
Enterovirus	Root crops	15 - 60 days
	Leaf vegetables	15 - 60 days
Ascaris eggs	Leaf vegetables	27 - 35 days
		<2-3 days
Endamoeba histolytica	Leaf vegetables	
Total coliforms	Fodder	12 - 34 days
	Leaf vegetables	35 days

[1]Data summarized from Rudolfs, et al. *(47)*, Sepp *(51)*, and Dunlop *(11)*.

Methodology

Next to the controlling influences of the environment, probably the most important single cause of low pathogen recoveries is the lack of sensitive laboratory methodology. Contamination of produce is localized on the outer surfaces that include the natural breaks, folds, and crevices of the plant. Laboratory examination, therefore, should be limited to the outer surfaces of those plant portions which are eaten raw. Selected plant parts are blended to form a homogeneous mix in sterile buffered water. The blending must be limited to 30 sec to reduce possible pathogen loss through mechanical or heat injury. Other methods of inoculation directly from a plant sample into selective enrichment media include the use of sample plugs removed with a flamed metal cork borer or a sterile scalpel *(40)*.

The key to successful recovery is the development of a suitable medium that will stimulate pathogen growth while suppressing the growth of organisms common to the plant epidermal layers, soil, and polluted water. Recent research developments on pathogen detection in polluted water have been directed toward improved methodology for *Salmonella* and

have produced simplified, rapid, qualitative techniques with improved sensitivity *(1)*. These newer procedures should yield more meaningful results in future studies concerned with frequency of *Salmonella* occurrence on farm produce. Qualitative methods for other waterborne pathogens, however, remain complex or lack the necessary selectivity for use with water samples or farm produce. The goal in pathogen detection is the development of quantitative methods. However, quantitation is currently attainable only by cumbersome techniques that limit the statistical significance of the data.

The occurrance and density of pathogens in polluted water and animal feces are highly variable. This variability reflects the intestinal diseases that are prevalent at a given time in the human or other animal populations which contribute their wastes to a particular effluent or watershed. To detect all disease-producing organisms the microbiologist would have to perform a variety of complex, time-consuming, and often tentative procedures for each sample analyzed. A more logical approach is the use of bacterial indicator system that will detect and measure fecal pollution from all warm blooded animals.

FECAL COLIFORMS AS POLLUTION INDICATORS

The fecal coliform group, a sub-group of the total coliform population, has high positive correlation with fecal contamination from warm blooded animals *(20)*. The principle biochemical characteristic used to identify fecal coliforms is their ability to ferment lactose with gas production at 44.5 C. Data show that 96.4% of the coliforms in human feces were positive by this test (Table 2). Examination of the excrements from other warm-blooded animals, including livestock, poultry, cats, dogs, and rodents, indicates that the fecal coliforms contributed 93.0 to 98.7% of the total coliform population.

The most numerous fecal coliform IMViC type is *E. coli* (++−−). However, other coliform IMViC types may predominate for periods of several months before a shift in type distribution occurs. For this reason it is best to measure all coliforms common to the intestinal tract. Man, particularly, has diverse fecal coliform flora. Determination of fecal coliforms present offers a more accurate measurement than the use of *E. coli* by the traditional IMViC biochemical reactions. The fecal coliform procedure is simple, yields quantative data, and is more rapid. The multiple dilution test requires 24 hr as a confirmatory procedure and the membrane filter method takes only 24 hr for final results. These distinct advantages are not provided by the IMViC identification tests.

TABLE 2. FECAL COLIFORM VERSUS *E. coli* CORRELATIONS IN ANIMAL FECES

Fecal source	Total strains examined	Percentage of total coliform population	
		Fecal coliform	*E. coli* (++− −)
Human	4,512	96.4	87.2
Livestock	2,339	98.7	95.6
Poultry	1,896	93.0	97.9
Cats, dogs, rodents	2,635	95.3	89.8

Laboratory methods

The intensive field studies which provided bacteriological data from irrigation water and vegetation to describe the magnitude and sources of fecal contamination used the following laboratory techniques in addition to those described in *Standard Methods for the Examination of Water and Wastewater*, 13th Edition *(1)*. Edible portions of vegetables were removed aseptically and weighed. Non-edible portions, such as the outer skins of green onions, blemished outer leaves of cabbage and lettuce, and the fine root structure of onions and radishes, were not used. The loose soil clinging to root crops was scraped off aseptically. The remaining outer surfaces were tested because they were considered more likely to have been in direct contact with the handlers, marketing processors, or the immediate environment. Twenty-gram samples of each vegetable and soil were blended in a Waring blender with 180 ml of buffered sterile water at high speed for 30 sec. These blended samples and irrigation water samples were immediately inoculated into the appropriate media and examined by the multiple tube procedure in *Standard Methods*.

Fecal coliform counts were performed on all samples using the multiple tube EC confirmation procedure incubated at 44.5 C for 24 hr *(1)*. All EC tubes with gas formation at the elevated temperature were recorded as positive and used in the Most Probable Number (MPN) calculations. Densities of fecal streptococci in irrigation waters were obtained by a pour plate serial dilution technique employing KF streptococcus agar. Then a fecal coliform to fecal streptoccus ratio was determined from the resulting median densities for each series of stream samples.

The tests for *Salmonella* were performed on portions of gauze strips which had been suspended in irrigation streams or ditches for five days, soil samples in cultivated fields, and the mud zone of an irrigation canal. The flasks contained 300 ml of enrichment media for enteric pathogens (Tetrathionate and SBG Sulfa Enrichment Broths). The inoculated enrichment media were incubated at 41.5 C for 24 hr. Growth from these flasks was then streaked on four plating media selective for enteric pathogens (Brillant Green Agar, Bismuth Sulfite Agar, Salmonella-Shigella Agar, and the less selective Mac-

TABLE 3. PERCENTILE DISTRIBUTION OF FECAL COLIFORM MPN VALUES PER 100 GRAMS OF FARM PRODUCE, ORNAMENTALS, AND WILD FLOWERS GROWN UNDER NON-IRRIGATON CULTIVATION

Vegetation group	No. of samples	Fecal coliform density per 100 g	
		50th Percentile	90th Percentile
Ornamental foliage			
House plants	10	<20	<20
Wild flowers	18	<20	<20
Garden plants shrubs, and trees	23	<20	790
Farm crops			
Small grain	6	<20	140
Leaf, bean, berry	24	<20	13,000
Roots and tubers	11	20	170

Conkey Agar) incubated for 18 hr at 41.5 C. Isolated colonies suspected of being enteric pathogens because of their biochemical characteristics and colony morphology were picked and inoculated into a series of media for biochemical and serological reactions to verify that they were *Salmonella.*

Magnitude of fecal contamination

Significant fecal contamination is seldom found on non-irrigated crops. In a baseline study of 92 specimens of ornamental foliage and farm crops (Table 3), more than one-half had 20 or less fecal coliforms per 100 g of vegetation sample *(22).* As might be expected, the density of fecal coliforms on house plants and foliage from wild flowers was less than 20 per 100 g in 90% of the samples. Approximately 10% of the ornamentals, shrubs, trees, and garden and farm crops examined were contaminated with fecal bacteria. The highest values were found on leafy vegetables, beans, and berry plants. In this instance the fecal contamination could have been derived only from insects, birds, rodents, or field laborers since neither irrigation water nor animal fertilizers were applied to the fields.

Fecal contamination of plants does show a small but significant increase when stream water quality is degraded by sewage effluents. Data presented in Table 4 resulted from a study of vegetables furrow-irrigated with sewage-polluted water in the South Platte River Basin *(64).* Bacteriological quality of farm produce was related to two arbitrary levels of irrigation water quality based upon fecal coliform densities. Irrigation water with a fecal coliform mean density of 630 organisms per 100 ml did not significantly alter the levels of fecal contamination for either root crops or leafy vegetables. Application of irrigation water containing a fecal coliform mean density of 58,000 organisms per 100 ml, however, resulted in greater numbers of fecal organisms on root crops. No significant increase in fecal contamination was observed in the 80 leafy vegetables examined from irrigated fields using the two grades of water quality. The difference in the bacteriological quality of root crops and leafy vegetables was probably related to the adverse effect of sunlight and the desiccation of organisms on leaf surfaces as contrasted to more favorable environmental factors in the soil.

SOURCES OF FECAL CONTAMINATION

Prior to development of any bacteriological standards for farm produce, there must be an understanding of the possible sources of fecal contamination and their impact on raw food quality. Contamination may occur either through natural means or by agricultural practices.

Insects

Of the possible sources of natural contamination, insects contribute the least numbers of fecal coliforms. Insect pests, particularly species of beetles and grasshoppers, may contaminate leafy vegetables (Table 5), but the fecal coliforms are not part of the permanent flora of insects. Rather, these bacteria are transients that attach to the hairy exoskeltons during contact with fecal wastes or enter the digestive tract when the insects feed upon fecally contaminated plant debris and animal wastes. Individual flower blos-

TABLE 4. PERCENTILE DISTRIBUTION OF FECAL COLIFORM MPN VALUES PER 100 GRAMS OF FARM PRODUCE GROWN UNDER IRRIGATED CULTIVATION

Farm produce	No. of samples	Fecal coliform density per 100 g	
		50th Percentile	90th Percentile
Irrigation water quality—630 fecal coliforms per 100 ml[1]			
Root crops	18	18	1,400
Leafy vegetables	35	13	10,000
Irrigation water quality—58,000 fecal coliforms per 100 ml[1]			
Root crops	57	260	70,000
Leafy vegetables	80	16	2,900

[1]Mean values

TABLE 5. PERCENTILE DISTRIBUTION OF FECAL COLIFORM MPN VALUES PER GRAM OF GARDEN INSECT PESTS, POLLINATORS, FLOWER BLOSSOMS AND BUDS

Insect or plant specimens	No. of samples	Fecal coliform per g	
		50th Percentile	90th Percentile
Insect class			
Coleoptera Beetles	16	130	3,300,000
Orthoptera Grasshoppers	11	< 20	2,530
Hymenoptera Bees, wasps, ants	7	< 20	140
Plant organs			
Flower blossoms	79	< 2.0	130
Unopened buds	8	< 2.0	<2.0

soms occasionally have measurable densities of fecal coliforms, whereas unopened flower buds and leaves of the same plant do not. The occurrence of fecal coliforms in flowers is related to the activity of pollinating insects which may mechanically transfer fecal material trapped in their hairy body areas to the flowers. Available data indicate that the fecal contamination of plants by insects is generally low but measurable *(22)*.

Wild animals

The random contamination of garden and orchard produce by direct defecation of wildlife and farm animals is probably the most significant natural means of disease transmission. Leafy vegetables and root crops are attractive food sources for many rodents including meadow mice, house mice, and Norway rats. These crops can support several hundred or more rodents per acre *(5, 41)*. Jack rabbit or cottontail rabbit populations are also abundant wherever the food supply is plentiful. *Salmonella* isolations from 253 wild mammals located on seven Illinois farms indicate the residual level of these pathogens may be about 7.5% of the total population *(49)*.

Birds are the most frequent sources of fecal contamination of maturing fruits and berries. The potential health hazard from bird fecal contamination is also indicated by the report of 6.3% incidence of *Salmonella* in the intestinal flora of 127 wild birds captured in Colorado *(31)*. In another report *(71)*, *Salmonella typhimurium* strains were isolated from the intestinal tract of 45 moribund house-sparrows collected by bird lovers in southern Ontario during a period from January 1966 to April 1968. Samples of bird feed submitted from many of the feeding stations where outbreaks occurred were negative for *Salmonella*. The authors suggest that healthy carriers among the sparrow population may perpetuate infection between outbreaks. Thus, birds and rodents harbor a residual level of *Salmonella* which may be transmitted to man via fecal droppings that contaminate maturing fruits and vegetables.

Farm animals

Farm animals usually are fenced out of cultivated fields so that contamination of farm produce by direct defecation is a remote possibility. In this country the use of farm animal wastes as a fertilizer has declined because the high cost of handling manure removes it from competition with chemical fertilizer *(69)*. Farm animal and human manures, however, are used in other regions of the world and are a particularly hazardous source of many bacterial and viral pathogens and parasites of enteric origin *(45)*. Salmonellae are found in approximately 13% of farm animals in the United States and 14% of the farm animals in the Netherlands *(46)*. Between 3.7 and 15% of sheep are carriers. The percentage of symptomless pigs that are *Salmonella* carriers ranges from 15 to 20% in the Netherlands, 7% in France, 12% in England, 13.4% in Norway, and 22% in Belgium *(44)*. Many *Salmonella* species known to cause gastrointestinal disturbances in man have frequently been isolated from animal fecal wastes. Salmonellosis in farm animals is perpetuated by contaminated live stock feeds and water.

Soil

Soil in regions remote from habitation rarely contain fecal contamination, and the few occurrences reported are probably related to random contact with the wildlife population *(23)*. Land under cultivation may receive increased exposure to fecal contamination because of visitations of wild animals in search of food, use of poor quality irrigation water, and applications of manure. As an illustration, *Salmonella* were detected in an irrigated green onion field one day after irrigation with poor quality water (Table 6). The fecal coliform density of this soil was 460 per gram. Eight days later the fecal coliform count had decreased to 79 organisms per gram and no *Salmonella* strains could be detected. Salmonellae were not found on the green onion crop growing in this soil on either occasion. Frequent recontamination of the soil by repeated applications of polluted water or animal manure may counteract environmental factors unfavorable to fecal organisms and maintain bacterial indicator groups and pathogens for 2 months or longer *(3, 32, 33, 36, 52, 70, 74)*.

The water-soil interface of the irrigation ditch can be a reservoir for fecal pollution transported in the channel. A study on stream and lake bottom deposits indicates that when the overlying water contains less than 200 fecal coliforms per 100 ml, *Sal-*

TABLE 6. FECAL COLIFORMS AND SALMONELLA OCCURRENCE IN FARM SOIL, IRRIGATED CROP AND IRRIGATED DITCH

Source	Fecal coliforms (per g)	*Salmonella* Occurrence
Irrigated green onion field		
1 day after irrigation	460	+
8 days after irrigation	79	–
Irrigated green onion crop		
1 day after irrigation	21	–
8 days after irrigation	11	–
Irrigated ditch		
Wet mud zone	230,000	+
Dry mud zone	13,000	–
Dry land farming soil		
Wheat field	< 0.2	–
Pastureland "A"	< 0.2	–
Pastureland "B"	< 0.2	–
Open range	< 0.2	–

monella are recovered in 23.5%, or only four of the related mud samples *(21)*. There is a much greater frequency of *Salmonella* in the mud when fecal coliform density in the overlying waters is above the 200 organism level per 100 ml. In these waters, 68.2%, or 15 of 22 mud samples, contained *Salmonella*. The wet mud zone in one irrigation ditch near Denver was found to contain *Salmonella* organisms and 230,000 fecal coliforms per gram. In the dry-mud zone of the ditch, no salmonellae were found, and the fecal coliform density had decreased to 13,000 organisms per gram.

Dry-land farming practices depend on available soil moisture accumulated from meager rainfall. Soil cultivation techniques attempt to reduce evaporation losses to the atmosphere. Soil samples from these fields rarely contain fecal coliforms. Those that occur probably represent random contamination from wildlife, unconfined farm livestock, or poultry.

Unlike those organisms discharged into a water environment, bacteria deposited on soil via fecal excretions are immobolized and are subject to the ecology of a specific site. This point was verified from studies on the detection of both a fecal coliform and a fecal streptococcus strain periodically dosed into soil adjacent to a stormwater catchment basin *(67)*. Under optimum conditions of soil moisture and limited sunlight exposure, fecal coliform and fecal streptococcus tracer organisms were isolated from hillside runoff most frequently during periods of heavy rainfall and represented a very small proportion of the total population entrapped in the soil sites. Thus diffusion of fecal pollution is limited in soil depth and lateral migration from a point of contamination. This fact is further verified by several studies which concluded that soil is not a major factor in the fecal contamination of plants *(17, 51)*.

Polluted water

Irrigation agriculture requires approximately 2 acre-feet of water per acre of growing crops. The frequency and volume of application must be carefully programmed to compensate for deficiencies in rainfall distribution and soil moisture content occurring during the growing season. The quantity of irrigation water used annually in the 17 western states is about twice the average flow of the Columbia River *(15)*. The surface streams are tapped through irrigation canals, ground water is pumped from deep wells, and catchment basins are constructed to trap stormwater runoffs.

Because water availability is critical, little attention is given to the bacteriological quality of the water supply. In water-short areas, available streams are subjected to sewage discharges from small communities, cattle feedlot drainage, infrequent stormwater runoff, and return irrigation water. Since the streams are frequently small, these pollution discharges quickly exceed the normal self-purification capacity of the stream and extend the zone of potential health hazard downstream to other water users, generally farmers dependent upon irrigation water.

Bacteriological data for three selected western regions where irrigation agriculture is essential, North Platte Basin, Cache Valley, and South Platte Basin, illustrate (Table 7) the magnitude of the irrigation water quality problem. The North Platte River Basin contains a gridwork of supply and return canals. Above Torrington, Wyoming, during September 1961 the North Platte River supplied irrigation water of excellent bacteriological quality with a median value of 70 fecal coliforms per 100 ml *(59)*. The Belmont Diversion Lake and the Red Willow drain downstream from Torrington, however, demonstrated substantial degradation of water quality in terms of 8,000 and 13,000 fecal coliforms per 100 ml, respectively. The fecal coliform to fecal streptococcus ratios (5.0 and 3.9) indicated this irrigation water was contaminated with municipal sewage discharges.

In Utah the fertile Cache Valley Basin received irrigation water from an extensive canal system built in the early 1900's to divert mountain stream water to valley farms. The high fecal coliform densities and low fecal coliform to fecal streptococcus ratios found in August 1962 in Cub Canal and Worm Creek water (Table 7) reflect pollution from irrigation water returns and small herds of milk cows that use the irrigation ditches and canal as a water supply *(58)*. In addition to these sources Worm Creek received storm water discharges and domestic wastes from a small town of 3,600 inhabitants. Effluent from the treated sewage of this population entered Worm Creek downstream from this sampling station, and the combined flow in Worm Creek entered the West Canal just a few hundred feet downstream from the waste discharge point. Although the Worm Creek stream flow was less than one-third of the flow in West Canal, the proportionally large volume of treated sewage present greatly degraded the water quality in that canal.

A series of studies in the South Platte River Basin *(10, 12, 13, 39, 66)* report the danger of irrigating with polluted water vegetables which are eaten raw. In a more recent investigation *(64)* irrigation water of good quality was found in ditches diverting water from two creeks in an area west of Longmont, Colorado (Table 7.) The source of water flowing in these two creeks originates in the mountains and

TABLE 7. BACTERIOLOGICAL QUALITY OF IRRIGATION WATERS[1]

Region and water source	Date	Number of samples	Densities per 100 ml[2]		Ratio (FC/FS)
			Fecal coliforms	Fecal streptococci	
North Platte Basin, Wyo.-Nebr.	Sept. 1961				
North Plate River		7	70	22	3.2
Belmont Diversion Lake		7	8,000	1,600	5.0
Red Willow Drain		7	13,000	3,300	3.9
Cache Valley, Utah	Aug. 1962				
Cub Canal		26	700	1,700	0.4
Worm Creek		26	5,400	37,000	0.2
West Canal		26	12,000	6,300	1.9
South Platte Basin, Colorado	Sept. 1964				
Lyons - Longmont Area					
Lefthand Ditch		15	230	780	0.3
Niwot Ditch		16	790	950	0.8
Rough and Ready Ditch		12	230	590	0.4
Swede Ditch		13	790	660	1.2
Denver - Brighton Area					
Burlington Ditch		33	13,000	3,300	3.9
Duggan Ditch		16	28,000	2,600	10.8
Gardeners Ditch		18	49,000	3,000	16.3
Fulton Ditch		19	450,000	48,000	9.4

[1]Data summarized from references *58, 59, and 64.*
[2]Median values.

there is little intervening pollution. In contrast, water for agricultural use in the Denver, Colorado area is diverted from the South Platte River via major irrigation canals downstream from a sewage treatment plant. The quality of irrigation water in these canals, evidenced in the Burlington, Duggan, Gardeners, and Fulton Ditches, was found during the 1964 study period to be grossly polluted as a result of inadequate municipal sewage treatment. Since fecal coliform to fecal streptococcus ratios greater than 4 to 1 usually indicate the pollution is derived from domestic wastes *(20)*, the high ratios in these irrigation ditches verified the source of this fecal pollution to be domestic wastes.

FECAL COLIFORM CORRELATIONS WITH SALMONELLAE OCCURRENCE

The full impact of fecal contamination is best measured by the occurrence of pathogens in irrigation water. Field methods for pathogen detection are currently limited to salmonellae and are not quantitative. We do not know the minimal numbers of ingested organisms necessary to cause infection. Despite these gaps in our knowledge, we can obtain some estimate of the possible health hazard by determining the probability of *Salmonella* occurrence at specified fecal coliform levels.

The data presented in Table 8 were collected from nation-wide field investigations in which numerous streams were examined for *Salmonella* occurrence and fecal coliform concentrations. These surveys were conducted in the South Platte River Basin *(64)*, the Upper Colorado River Basin *(66)*, and many other locations *(8, 55, 56, 60, 62, 63)* in most of which irrigation agriculture is a common practice. Results of these studies were grouped into two fecal coliform density ranges which bracketed the limits recommended for irrigation water by the National Technical Advisory Committee on Water Quality Critera *(65)*. This committee proposed that the upper fecal coliform limits should not exceed a monthly arithmetic average of 1,000 organisms per 100 ml, with no single value above 4,000 fecal coliforms. Arrangement of the fecal coliform counts within these ranges showed the occurrence of *Salmonella* to be 53.5% for streams with fecal coliform levels from 1 to 1,000. In contrast, salmonellae occurred in 96.4% of the samples containing more than 1,000 fecal coliforms per 100 ml.

TABLE 8. FECAL COLIFORM CORRELATIONS WITH *Salmonella* OCCURRENCE IN STREAMS

Fecal coliform density (per 100 ml)	*Salmonella* detection		
	Total examinations	Number positive	Percentage occurrence
1 - 1,000	71	38	53.5
Over 1,000	140	135	96.4

Although this relationship between the occurrence of *Salmonella* and fecal coliform counts does not indicate the density of these pathogenic strains in water, or the occurrence of other pathogenic bacteria, vi-

ruses, parasitic cysts or ova, it underscores the health hazards of water degraded by fecal contamination.

PRODUCE HANDLING

Fecal contamination from farm laborers and from workers employed in produce packaging operations, is a latent source of public health hazard. Field hands are often migrant farm laborers who move from place to place during the growing and harvesting seasons. In some locations the living conditions provided farm hands, particularly migrants, are notoriously primitive and housing and sanitary facilities are frequently inadequate *(72)*. These agricultural workers often must live in close proximity to farm animals and their wastes which may be reservoirs of disease. Disposal of human and animal waste receives little attention in some camps provided for seasonal or migrant workers.

The cultivation, harvesting, and market preparation of fruits and vegetables offer many opportunities for contact by field and produce-house workers. Hand labor operations in the field reach a peak during harvesting when the crops are picked, sorted, trimmed, tied, bunched, and precleaned of soil particles in a rinse water. Rinse water used for field cleaning of produce is often of questionable bacteriological quality. Washing procedures may consist of simply dipping the produce in nearby irrigation ditches, rinsing in flow-through vats, or spraying with water of uncertain quality. Table 9 illustrates the quality of water routinely used to wash the large quantities of vegetables harvested from a group of farms located in the South Platte River Basin. The mean density of fecal coliforms in wash water was three times higher than that of the source water pumped from shallow wells into the tanks. Hand washing and sanitary facilities for the farm workers are often not available for field workers. The constant contact with sewage polluted irrigation water and contaminated material in the fields, in addition to the general lack of sanitation in living quarters and in personal habits, can make such agricultural food handlers a poor sanitation risk.

During transportation of farm produce there is ample opportunity for additional contamination. In the United States, increasing attention is being given to careful handling and sanitary shipping and storage conditions. In many areas, however, crops are often shipped in dirty freight cars, cargo ships or boats, trucks, and other vehicles in which a variety of commodities are constantly hauled without intervening cleansing. Open trucks, carts, and other means of transport offer easy access to dust, road dirt, insects, and rodents. Handlers frequently freshen produce enroute by spraying with water of questionable quality. Transport in unrefrigerated vehicles, or exposure to warm temperatures for other than brief periods, is undesirable.

TABLE 9. MEAN FECAL COLIFORM DENSITIES OF PRODUCE WASH WATER

Sample	No. of samples	Mean fecal coliform density
Source water	7	2,000
Wash tank water	6	6,200

Warehouses and other storage facilities for produce do not always provide clean, temperature-controlled space free of insects and rodents. Flush toilets, handwashing facilities, and locker rooms in which produce handlers may change their clothing are often non-existent. Shipping and packing containers are frequently reused. Currently warehousemen are placing more emphasis upon prompt handling, with care being taken to prevent injury to the farm produce and contamination from handlers or other sources.

Additional handling occurs when the produce reaches the wholesale and retail markets where it is prepared for distribution and sale to the consumer. Market practice often includes unpacking, trimming, sorting, and repacking, sometimes accompanied by additional spraying with water to maintain freshness immediately prior to final dispersal. In many regions, fruits and vegetables are displayed in the open, without attention to sanitary conditions.

Various investigations have observed and substantiated contamination of produce between harvesting and marketing. The increase in fecal contamination occurring on vegetables ready for market, as compared to field crops from the same growing area, was observed in the South Platte study *(64)*. There was more than a six-fold increase of fecal coliforms at the 50th percentile, and a nine-fold gain at the 90th percentile level, on crops destined for market over those sampled in the field (Table 10). A recent study in Greece also found higher levels of coliform bacteria on market products than on field produce *(42)*. Four of 41 samples (9.7%) of fruits and 17 of 76 vegetable samples (22.3%) sold in the central market of Athens were contaminated with *E. coli*. *Escherichia coli* was found on only 5.8% of 204 plant foliage and flower specimens collected from a wide variety of habitats in the region of Attica over a period of 13 months. Contributing to farm produce contamination were: heavily polluted irrigation water, domestic animals frequenting fields and gardens, and the close proximity of fruit and vegetable plots to human or animal habitation. Evidence for the pres-

TABLE 10. MAGNITUDE OF FECAL CONTAMINATION OBSERVED ON PRODUCE GROWING IN THE FIELD AS COMPARED WITH MARKET PRODUCE

Produce	No. of samples	Fecal coliform MPN values per 100 g	
		50th Percentile	90th Percentile
Root crop			
Field	20	200	10,000
Market	13	1,300	90,000
Leafy vegetables			
Field	34	20	5,000
Market	31	13	6,300

ence of *Salmonella, Shigella,* and enteropathogenic *E. coli* was found on a wide variety of market fruits and vegetables sampled in Ceylon *(68)*. These pathogens were isolated from 1.1% of 1,806 samples consisting of 54 varieties of fruits and vegetables. Sources of pollution mentioned by the authors were irrigation water, animal manure, human carriers, and the handling and hand-sprinkling of produce.

Recovery of these pathogens from only 1.1% of the fruits and vegetables in Ceylon and the sporadic reports of pathogen detection on farm produce indicate the low incidence of disease-causing organisms in spite of recognized poor sanitary practices. These reports emphasize the adverse effects of environmental factors during the storage and handling of produce. As epidemiological evidence confirms, however, the low incidence does not obviate the disease-causing potential of farm produce.

RECOMMENDED QUALITY GOALS

Selection of agricultural irrigation water sources should not be based principally on availability of the water supply, but should also take into full consideration the bacteriological and chemical quality of the water. The scarcity of water supplies of acceptable quality in irrigation areas is fully recognized. Unfortunately, surface waters in arid and semi-arid regions are almost always small streams with disproportionately large pollutional additions from domestic wastes, sugar-beet lagoon discharges, feedlot drainage, and irrigation returns. These additions of low quality water bring varying numbers of pathogenic organisms to irrigation waters and ultimately in contact with field crops. Practical reduction of the public health hazard can be accomplished only through a concept of multiple safeguards designed to prevent raw plant food from contact with, and retention of pathogens.

Enforcement of the bacteriological quality guidelines for irrigation water, 1,000 fecal coliforms per 100 ml, recommended by the National Technical Advisory Committee on Water Quality Criteria *(65)* should result in reduced exposure of raw plant foods to pathogens. Data correlating fecal coliform levels to *Salmonella* occurrence indicate that the proposed standard is realistic, providing the safeguard measures and sanitary practices described are observed along with the use of water of this quality. The standard represents the best scientific information presently available. Further refinement of the fecal coliform limits for irrigation water awaits additional microbiological and epidemiological studies. Standards have as their primary objective the protection of public health, but may also recognize the importance of multiple use in water-short areas, and take into account the specific uses for which the water is needed. The fecal coliform level suggested is attainable only at a cost of adequate waste treatment by all stream users.

Because receiving streams may be small, and of lesser volume than the sewage effluent, secondary treatment and disinfection of domestic sewages are necessary to ensure substantial reductions of pathogens in irrigation waters. Wastes from food processing plants, meat packing plants and sugar beet mills, and runoff from cattle feedlots should be diverted to lagoons and held for 20 to 30 days to reduce the number of pathogens prior to discharge.

The method of water application influences the amount of fecal contamination to which farm crops are exposed. Flooding, spraying, sub-irrigation, and furrow irrigation are used in various agricultural communities. Waters which are not of potable quality should be applied to crops which may be consumed raw by furrow or sub-irrigation to limit contacts of the disease-causing microorganisms with plant surfaces *(11)*. Use of primary effluent for spray irrigation resulted in isolation of salmonellae from soil and potatoes after 40 days, from carrots after 10 days, and from cabbage and gooseberries after 5 days *(38)*.

As a further safeguard against exposure to pathogens and their survival on raw plant foods, farm management of irrigation water should include a program of selective application based on the bacteriological quality of available water. Irrigation water from nearby sources could be applied during the various stages of cultivation but should be discontinued four weeks prior to harvest, to further diminish the risk from waterborne pathogens. Water applied after this period should be derived from ground water supplies or farm holding ponds. Survival studies of salmonellae in farm drainage collected in a tank, diluted with well water and then used for irrigation, indicate that the water must be retained for 20 days to prevent possible salmonellae

transfer to field crops *(4)*. In another report, an outbreak of *S. typhimurium* infection occurred in a dairy herd that had been grazing on pasture lands previously sprayed three weeks before with a slurry of farm waste waters *(27)*.

Preparation of fruits and vegetables at harvest time includes fresh water rinses to remove soil particles and to maintain the quality of leafy vegetables. From harvest to consumption, all water applied to clean and to freshen raw produce must be of drinking water quality *(57)*. This water should be applied in a continuously flowing stream or spray with no recirculation of spent water through the system.

Equally important as the water quality are the sanitation practices of the farm laborers who cultivate and harvest the crops and of the produce workers who repackage these perishables for market. Adequate toilet and washing facilities should be provided for both. Every effort should be made to instruct the personnel in principles of hygiene for their own protection and for improvement of the sanitary quality of the produce. Farm workers must be made aware of the potential dangers of acquiring or spreading disease when working near animals and animal wastes as well as polluted water and soil. Recurring leptospirosis outbreaks in agricultural areas of Israel emphasize some of the hazards associated with irrigation agriculture *(53)*. In many areas, agricultural workers are in contact with water far exceeding approved bacteriological limits for recreational waters.

Medical services, clinics, and disease reporting regulations should be established so that good health protection can be developed among migrant farm laborers. Persons with skin lesions, open sores, wounds, or obvious sickness should not be permitted to harvest or package produce. Clean clothing should be worn. Field laborers should wear protective clothing such as boots, long-sleeved garments, and head coverings when insect vectors are present.

Vehicles used for transporting fresh fruits and vegetables should be designed and constructed to protect the produce from damage and contamination. Truck beds should be kept clean and produce should be covered. The produce should be packed only in clean containers; reused containers should be thoroughly cleaned, kept in good repair, and used only for the transport of uncontaminated food items. An example of contamination from containers is the wooden crates in which dressed poultry is iced and packed which were found to be a source of *Salmonella* and other pathogens *(61)*. The Food and Drug Administration states that shipments of vegetables or other food stuffs in unclean crates or containers will be regarded as adulterated because of the possibility of such contamination.

Obviously any program for increased sanitation is meaningless unless it is enforced. The quality of irrigation and processing waters should be monitored periodically during the growing and harvesting season by the responsible sanitation authority. Pollution abatement measures must be taken immediately when the bacteriological water quality limits are exceeded. A sanitary inspection procedure, including initial and follow-up visits, should be established; it would include the growing areas, packing sheds, transport equipment, and market processing facilities; the responsible authority would prepare a report and certify approval. Such inspections would reveal unsanitary conditions and practices that must be corrected.

SUMMARY

Microbiological data from a wide variety of field studies, including fruits, vegetables, related crops, the soils in which they were grown, and the water with which they were irrigated or processed, have been correlated to demonstrate the magnitude of fecal contamination on raw food products. Additional studies point to the many different sources of this contamination in the field and during handling and marketing.

Fecal coliform measurements proved to be the most practical and useful method for determining the degree of disease hazard caused by pathogen occurrence on fruits and vegetables that may be eaten raw. The correlation of fecal coliform densities with the occurrence of *Salmonella* in various stream waters was demonstrated. For values under 1,000 fecal coliforms per 100 ml, *Salmonella* occurrence in these streams was 53.5%; above this fecal coliform value, the occurrence was 96.4%. This high percentage of *Salmonella* occurrence in water in which there are more than 1,000 fecal coliforms per 100 ml serves to substantiate the validity of the water quality standard recommended by the National Technical Advisory Committee. No quantitative procedure is currently available to measure the pathogen densities that could be present.

In addition to the fecal coliform standard, a series of multiple safeguards against disease infection to be applied during cultivation, irrigation, handling, and processing of salad-type vegetables and fruits are proposed for the protection of public health.

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A Research Note

SIMPLE TEST TO PREDICT COMMERCIAL STERILITY OF HEATED FOOD PRODUCTS

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ABSTRACT

A simple test is described for predicting commercial sterility of batches of fluid products that have been heat sterilized.

When batches of food products are produced with a final heat sterilization step in the process, it is often impractical to do a statistically significant number of sterility tests on each batch. The test described in this paper could be used to minimize the number of routine sterility tests done on each batch of product produced. It also points out batches where additional tests should be made since the initial bacterial load was unusually resistant to heat treatment.

Food processors who heat sterilize products should know the degree of heat treatment needed for each combination of product and container in terms of F_s units. According to Stumbo, F_s is "the equivalent, in minutes, at 250 F, (of the) . . . integrated lethal value of heat received by all points in a container during process" *(1)*. There is usually a considerable difference between the heat treatment that is sufficient most of the time and the one that is actually used for continuous assurance of sterility. By an approximate measurement based on this difference it is possible to predict whether the safety margin is being utilized to adequately sterilize the product or whether it's effectiveness has been exceeded to the point that nonsterile units are being produced.

Ulrich *(2)* described an indicator system which reacts with most bacteria that can survive in an understerilized product. It will respond to changes in pH and Eh. Addition of dextrose, methylene blue, and chlor phenol red to the product tested, at levels recommended by Ulrich, provides an effective and representative medium for determining if any bacteria which survive heat processing will grow in the product subsequent to sterilization.

MATERIALS AND METHODS

Glucose indicator solution

Dissolve 75 g glucose, 0.075 g methylene blue, and 0.225 g chlor phenol red in distilled water and adjust to 1000 ml. Autoclave at 250 F for 15 min.

Test method

Obtain one unsterilized, filled container from the beginning and end of the filling of each batch. For each unit to be tested add 1 ml of glucose indicator solution to each of two test tubes. Pipette 15 ml of the product under test into each of the two tubes and plug or cap them.

Autoclave the tubes for one-half the time in minutes of the calculated F_s processing value at 250 F. The time and temperature must be carefully controlled. Vent the autoclave and cool the tubes in water as rapidly as possible without boilover. Incubate the tubes at 100 F for 7 days with daily visual inspection of the tubes for any change.

RESULTS AND DISCUSSION

With milk the sample tubes, when first taken from the autoclave, are nearly white in color. They soon develop a bluish-gray top layer with a pink underportion. As air oxidizes the methylene blue, the blue color extends downward until the entire tube is uniformly blue. With condensed milk, pink with a blue top color is the final appearance of the sample. A positive reaction is any deviation from the final, stabilized color and indicates that the batch should have additional sterility tests performed on it. The nature of the product tested will determine what color or reaction should be considered sterile and what constitutes a nonsterile tube. A positive test here indicates only that additional sterility tests should be performed in the traditional manner on the batch in question. It should not be interpreted as definitely showing a batch having spoiled units.

The autoclaving time of one-half the F_s value was chosen to provide substandard sterilization to the product. It is realized that autoclaves vary in time required to attain the desired temperature and to return to ambient temperature so it may be necessary to adjust this time to give meaningful results. If possible, the time at 250 F in the autoclave that is necessary to give the product a process equal to one-half of it's normal process time at 250 F should be determined.

Because of this inherent weakness in the test's performance, it is not meaningful to give data on the accuracy and precision of the procedure. The error introduced in conducting the test may produce a tenfold variation in results. The value of this tech-

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TABLE 1. BACTERIAL SPOILAGE RATES

F_s (min)	D_{250} (min)	Units spoiled (%)	Initial count (Spores/ml)
4	0.5	0.01	10.0
4	1.0	0.01	0.001
4	0.5	0.1	100.0
4	1.0	0.1	0.01
6	1.0	0.01	0.1
6	1.0	0.1	1.0
6	1.0	1.0	10.0
8	1.0	0.01	10.0
8	1.0	0.1	100.0
10	1.0	0.01	1000.0

nique remains because: *(a)* it is only a predictive test and *(b)* commercial sterilization is usually on the order of 12 log cycles of bacterial reduction *(1)*. Most of this is the safety factor which is rarely needed and varies with each batch.

Some representative spoilage rates (the per cent of the batch which spoils upon incubation) that might be encountered in normal production are correlated with F_s values, D_{250} values (the time in minutes at 250 F required to kill 90% of the bacteria), and initial counts for a fluid product in Table 1. These values are calculated for a 32-oz can and are only close approximations.

As can be seen from data in Table 1, some fairly low initial bacterial levels that might be acceptable in a processing sample, can produce significant levels of spoilage that could be missed in the normal sterility testing of a product. The proposed predictive test can be of value in locating a batch which has a potential problem with a higher than acceptable rate of spoilage.

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SUPPLEMENT NO. 2 TO THE 3-A SANITARY STANDARDS FOR INSTRUMENT FITTINGS AND CONNECTIONS USED ON MILK AND MILK PRODUCTS EQUIPMENT

Serial #0904

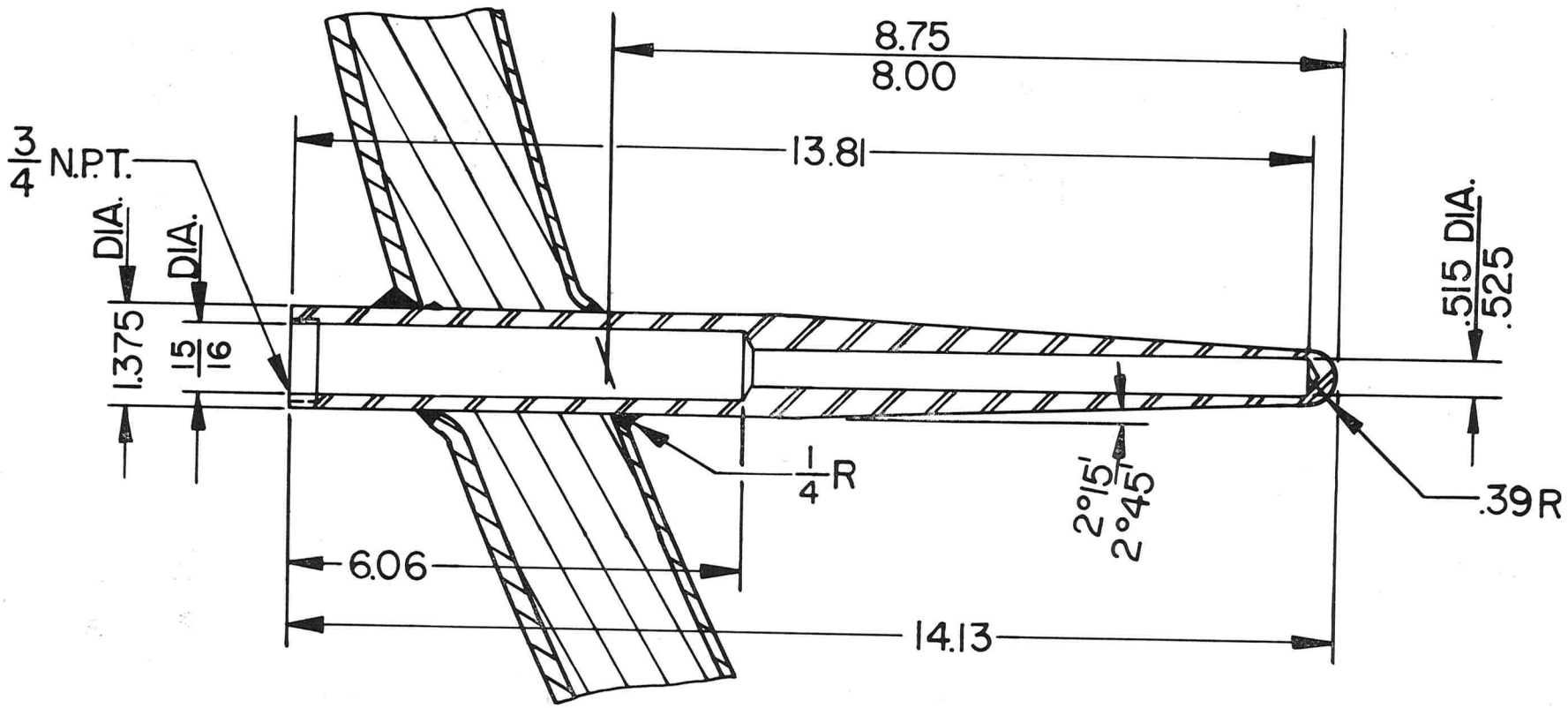
Formulated by
International Association of Milk, Food and Environmental Sanitarians
United States Public Health Service
The Dairy Industry Committee

This supplement incorporates the following fittings into this standard:

	Page No.	3-A Drawing No.
3A9 Temperature sensor well (short) for milk storage tanks	13	3A-101-11
3A10 Temperature sensor well (long) for milk storage tanks	14	3A-101-12

Note: The temperature sensor portions of the above wells are longer than those of the 3A7 and 3A9 thermometer wells. Other dimensions are different.

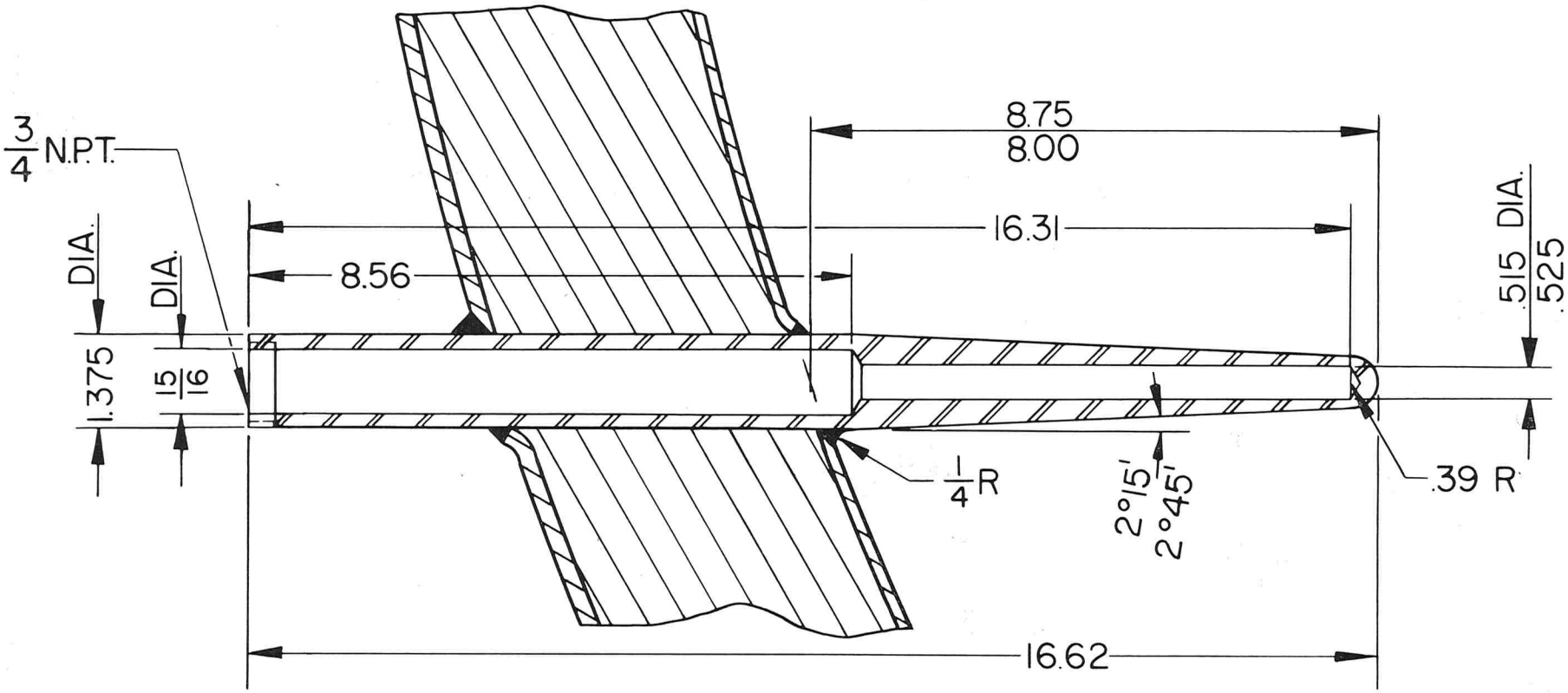
This amendment is effective June 1, 1971.



3A9 TEMPERATURE SENSOR WELL (SHORT) FOR LIQUID EGG AND LIQUID EGG PRODUCTS STORAGE TANKS

Note: The temperature sensor portion of this well is longer than that of 3A7 Thermometer Well. Other dimensions are different.

3A STANDARD
INSTRUMENT
FITTINGS & CONNECTIONS
3A-101-11



3A10 TEMPERATURE SENSOR WELL (LONG) FOR LIQUID EGG AND LIQUID EGG PRODUCTS STORAGE TANKS

Note: The temperature sensor portion of this well is longer than that of 3A8 Termometer Well. Other dimensions are different.

3A STANDARD
INSTRUMENT
FITTINGS & CONNECTIONS
3A-101-12

EVALUATION OF PRODUCTION CONDITIONS OF MANUFACTURING-GRADE RAW MILK BY FIELDMEN RATINGS AND BY BACTERIAL TESTS

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ABSTRACT

Standard microbiological tests, Standard Plate Count, direct microscopic count, methylene blue reduction test, and several variations of the resazurin reduction test were correlated with fieldmen's ratings of sanitary condition of milking area, milk house, and milking utensils. Correlation coefficients were low, in general, approximately 0.2. The effect of different production facilities and practices on these correlations was variable. Results suggest that bacterial tests and fieldmen's inspection be used concurrently since they appear to measure different sanitary factors on the producing farms.

The sanitary conditions under which manufacturing-grade milk is produced are generally assessed by several standard bacterial tests and by periodic farm inspections. Correlations between bacterial tests and farm's scores have been shown to be low in farms producing Grade A milk *(3, 5)*. For manufacturing-grade milk, Hartley et al. *(2, 4)* in a comprehensive review of literature, indicate that barn conditions can account for only a small part of the total bacterial load of raw milk.

This paper presents data obtained with manufacturing-grade milk with regard to correlations between fieldmen's ratings of sanitary conditions of milking area, milk house, and milking utensils, with six commonly used bacterial tests and indicates the effect of various production facilities and practices on these correlations.

EXPERIMENTAL METHODS

Sources of milk samples and methods of bacteriological analysis were the same as previously reported *(1)*. In addition to Standard Plate Count (SPC), direct microscopic count (DMC), methylene blue reduction test (MBRT), and "triple reading" resazurin reduction test, samples were analysed by 1-hr resazurin reduction test *(6)* and by a modification of the resazurin reduction test with a 10 P 7/8 end point on the Munsell color chart *(7)*.

Plant fieldmen were asked to rate the sanitary condition of milking area, milk house (if present), and milking utensils as "excellent," "good," "fair," or "poor." Data on type of cooling used, size of dairy herd, type of milking, and other also were entered in the rating sheets by fieldmen. All data were pre-coded and entered directly on IBM cards for computer analysis of correlations and of their statistical significance.

TABLE 1. STATISTICS ON 970 RANDOMLY SELECTED MANUFACTURING GRADE MILK PRODUCERS IN THREE GEOGRAPHICAL AREAS IN 1963-4.

	All areas %	Area A %	Area B %	Area C %
Type of refrigeration				
None	3.0	0.4	5.6	2.5
Farm bulk tank	29.3	42.1	19.1	29.0
Can cooling	67.7	57.5	75.3	68.5
Size of dairy herd				
≤ 5 cows	16.6	6.5	22.4	18.7
6 to 25 cows	69.8	80.2	71.9	59.7
> 25 cows	13.6	13.3	5.7	21.6
Weight of milk delivered				
≤ 150 lb.	26.5	12.8	35.7	28.2
151 to 750 lb.	48.5	58.1	47.7	41.8
> 750 lb.	25.0	29.1	16.6	30.0
Temperature of milk at sampling				
≤ 40 F	33.2	41.0	27.0	33.1
41 to 50 F	24.4	21.0	28.7	23.0
51 to 60 F	23.9	22.2	25.5	23.7
> 60 F	18.5	15.8	18.8	20.2

RESULTS AND DISCUSSION

Nine hundred and seventy randomly selected producers from three widely separated geographical locations covering 7 states were sampled in winter, spring, summer, and fall. A total of 3880 samples were collected and analyzed. Since the sampled producing farms were selected at random, and the geographical areas sampled covered the major manufacturing-grade milk areas of the United States, several generalizations could be made briefly on manufacturing-grade raw milk production in 1963-

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TABLE 2. STATISTICS ON 690 RANDOMLY SELECTED MANUFACTURING GRADE MILK PRODUCERS HANDLING MILK IN CAN IN THREE GEOGRAPHICAL AREAS 1963-4.

Production facility and/or practice	All areas %	Area A %	Area B %	Area C %
Milk house	37.9	60.4	27.7	34.9
No milk house	62.1	39.6	72.3	65.1
Machine milking	81.6	93.1	65.4	91.1
Hand milking	18.4	6.9	34.6	8.9
Electrical cooling	54.3	50.9	64.2	45.5
Non-electrical cooling	45.7	49.1	35.8	54.5
Temperature at time of sampling ≤50 F	41.9	34.3	43.3	45.0
Temperature at time of sampling >50 F	58.1	65.6	56.7	55.0
Producers with a milk house				
Machine milking	96.2	97.4	89.2	99.4
Hand milking	3.8	2.6	10.8	0.6
Producers with no milk house				
Machine milking	72.6	84.1	56.3	88.3
Hand milking	27.4	15.9	43.7	11.7

1964.

As shown in Table 1, about 68% of all producers cooled and shipped milk in cans. Three per cent of all producers did not use any type of cooling, whereas 29% used farm bulk tanks. About 70% of the dairy herds numbered 6 to 25 cows and nearly one-half of the milk deliveries were in the range of 151 to 750 lb per farm. The temperature of milk at time of delivery ranged from less than 40 F to more than 60 F.

Producers handling milk in farm bulk tanks had generally uniform types of facilities and practices. Most had a milk house and practiced machine milking and the temperature of milk at time of pickup was rarely above 40 F and never above 50 F. On the other hand, milk production practices and facilities varied considerably among producers shipping milk in cans (Table 2).

As shown in Table 3, the bacterial estimates by SPC for raw milk ranged from less than 100,000/ml to more than 20,000,000/ml. Although 41.5% of all farm bulk tank samples had SPC's of 100,000/ml or less, 4.4% had SPC's over 20,000,000/ml.

Correlation tests between the six commonly used bacterial tests and ratings of sanitary conditions by fieldmen was done for 3622 samples from about 900 farms. As shown in Table 4, every bacterial test used is significantly correlated to the fieldmen ratings of milking area, milk house, and milking utensils, although the correlation coefficients are very low. Hartley et al. *(4)* using elaborate farm scoring systems in Grade A milk producing farms reported a correlation coefficient of 0.27 between RRT and farm scores. This compares quite closely with our 0.23 to 0.24 correlation coefficients between rating of fieldmen and RRT-Tr. In all instances, correlation coefficients were lower for DMC than for any other test.

The magnitude of correlation between fieldmen ratings and bacterial tests was variably affected by type of handling practiced on farms, i.e. farm bulk tank or can (Table 5), by geographical location of farms (Table 6), by temperature of milk at time of sampling (Table 7), by type of can cooling practiced, i.e., electrical or non-electrical (Table 8), and finally by type of milking practiced, i.e., machine or hand milking (Table 9). Correlation coefficients varied from 0.03 to 0.39

While most bacterial tests were significantly correlated with the fieldmen's rating of milking area, milk house, and milking utensils, the correlation coefficients were low, generally under 0.30. Johns et al. *(5)* have attempted to use preliminary incubation of samples prior to bacterial tests but have concluded that detection of improper sanitary conditions at the producing level cannot be assessed by bacterial tests alone with or without preliminary incubation.

Our results and those of other workers show that commonly used bacterial tests are not greatly affected by factors evident on field inspection. There is, at the present time, no basis for choosing one approach

TABLE 3. QUALITY OF MANUFACTURING-GRADE MILK ACCORDING TO BACTERIAL ESTIMATES BY STANDARD PLATE COUNTS.

Standard plate count (Range/ml)	All samples (%)	Can samples (%)	Farmbulk Tank samples (%)
≤100,000	28.0	22.3	41.5
>100,000 - ≤200,000	7.6	6.8	9.3
>200,000 - ≤500,000	9.2	9.6	8.4
>500,000 - ≤1,000,000	13.9	14.5	12.4
>1,000,000 - ≤2,000,000	8.7	9.7	6.3
>2,000,000 - ≤3,000,000	2.2	1.4	4.0
>3,000,000 - ≤5,000,000	5.8	6.7	3.5
>5,000,000 - ≤10,000,000	10.5	11.9	7.0
>10,000,000 - ≤15,000,000	3.8	4.7	1.7
>15,000,000 - ≤20,000,000	2.5	2.9	1.5
>20,000,000	7.8	9.5	4.4

TABLE 4. CORRELATIONS BETWEEN SANITARY CONDITION OF MILKING AREA, MILK HOUSE, AND MILKING UTENSILS WITH SIX BACTERIOLOGICAL QUALITY TESTS FOR ALL PRODUCERS SAMPLED[1].

Quality tests	Milking area	Milk house	Milking utensils
SPC[3]	0.19**[2]	0.19**	0.22**
DMC	0.13**	0.09**	0.14**
MBRT	−0.21**	−0.17**	−0.20**
RRT-Minn.	−0.24**	−0.21**	−0.23**
RRT-Tr.	−0.24**	−0.23**	−0.23**
RRT-1 hr	0.18**	0.15**	0.16**

[1]3622 samples (2085 with milk house).
[2]**Significant at the 0.01 level.
[3]See text for explanation.

TABLE 5. CORRELATIONS BETWEEN SANITARY CONDITION OF MILKING AREA, MILK HOUSE, AND MILKING UTENSILS WITH SIX BACTERIOLOGICAL QUALITY TESTS FOR ALL PRODUCERS SAMPLED ACCORDING TO THE TYPE OF MILK HANDLING (BULK TANK OR CAN).

Quality tests	Milking area		Milk house		Milking utensils	
	Can[1]	Bulk[2]	Can	Bulk	Can	Bulk
SPC	0.12**[3]	0.17**	0.17**	0.17**	0.16**	0.24**
DMC	0.16**	0.05ns	0.13**	0.05ns	0.11**	0.11**
MBRT	−0.06**	−0.13**	−0.15**	−0.12**	−0.12**	−0.21**
RRT-Minn.	−0.14**	−0.16**	−0.19**	−0.16**	−0.13**	−0.24**
RRT-Tr.	−0.15**	−0.18**	−0.18**	−0.18**	−0.12**	−0.25**
RRT-1 hr.	0.14**	0.08*	0.15**	0.06ns	0.12**	0.12**

[1]2510 can samples (1014 with milk house).
[2]1112 farm bulk tank samples (1034 with milk house).
[3]**Significant at the 0.01 level.
*Significant at the 0.05 level.
nsNot significant at the 0.05 level.

TABLE 6. CORRELATIONS BETWEEN FIELDMEN RATING OF SANITARY CONDITIONS OF MILKING AREA AND MILKING UTENSILS WITH SIX BACTERIOLOGICAL QUALITY TESTS FOR PRODUCERS HANDLING MILK IN CANS ACCORDING TO THE GEOGRAPHICAL LOCATION OF THE SUPPLY.

Quality tests	Milking area			Milking utensils		
	Locations					
	1[1]	2[2]	3[3]	1	2	3
SPC	0.19**[4]	0.17**	0.19**	0.16**	0.14**	0.19**
DMC	0.19**	0.15**	0.19**	0.12**	0.11**	0.19**
MBRT	−0.20**	−0.17**	−0.22**	−0.14**	0.13**	−0.18**
RRT-Minn.	−0.28**	−0.15**	−0.19**	−0.21**	−0.12**	−0.15**
RRT-Tr.	−0.24**	−0.15**	−0.17**	−0.18**	−0.12**	−0.15**
RRT-1 hr.	0.23**	0.14**	0.18**	0.13**	0.11**	0.17**

[1]Location 1: 564 samples.
[2]Location 2: 1031 samples.
[3]Location 3: 915 samples.
[4]**Significant at the 0.01 level.

TABLE 7. CORRELATIONS BETWEEN SANITARY CONDITION OF MILKING AREA, MILK HOUSE, AND MILK UTENSILS WITH BACTERIOLOGICAL QUALITY TESTS FOR PRODUCERS SHIPPING MILK IN CAN AND ACCORDING TO TEMPERATURE AT TIME OF SAMPLING.

Quality Tests	40 F[1]			41-50 F[2]			51-60 F[3]			60 F[4]		
	Area	House	Utensils	Area	House	Utensils	Area	House	Utensils	Area	House	Utensils
SPC	0.18**[5]	0.29**	0.21**	0.16**	0.28**	0.22**	0.07*	0.09ns	0.14**	0.10**	0.09ns	0.10**
DMC	0.11ns	0.17ns	0.09ns	0.17**	0.26**	0.22**	0.05ns	0.04ns	0.05ns	0.08*	0.06ns	0.08*
MBRT	−0.18**	−0.34**	−0.17**	−0.17**	−0.21**	−0.20**	−0.11**	−0.08ns	−0.11**	−0.07ns	−0.07ns	−0.03ns
RRT-Minn.	−0.19**	−0.34**	−0.15*	−0.17**	−0.24**	−0.17**	−0.11**	−0.13**	−0.13**	−0.11**	−0.10ns	−0.06ns
RRT-Tr.	−0.11ns	−0.26*	−0.08ns	−0.15**	−0.22**	−0.15**	−0.09**	−0.14**	−0.13**	−0.12**	−0.11ns	−0.08*
RRT-1 hr.	0.09ns	0.30*	0.15*	0.18**	0.24**	0.18**	0.09**	0.07ns	0.09**	0.08*	0.11ns	0.08*

[1]224 samples (62 with milk house).
[2]756 samples (331 with milk house).
[3]855 samples (382 with milk house).
[4]676 samples (239 with milk house).
[5]**Significant at the 0.01 level. *Significant at the 0.05 level. nsNot significant at the 0.05 level.

over the other and it would appear that the concurrent use of field inspection and bacterial tests will give a better indication of the sanitary quality of manufacturing milk than either inspection or bacterial tests alone.

ACKNOWLEDGMENT

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TABLE 8. CORRELATIONS BETWEEN SANITARY CONDITIONS OF MILKING AREA, MILK HOUSE, AND MILKING UTENSILS WITH BACTERIOLOGICAL QUALITY TESTS FOR PRODUCERS SHIPPING MILK IN CAN AND ACCORDING TO THE TYPE OF COOLING USED.

Quality Tests	Milking area		Milk house		Milking utensils	
	Elect.[1]	Non-Elect.[2]	Elect.	Non-Elect.	Elect.	Non-Elect.
SPC	0.17**[3]	0.09**	0.23**	0.05ns	0.18**	0.14**
DMC	0.13**	0.06**	0.20**	0.03ns	0.14**	0.08*
MBRT	−0.16**	−0.10**	−0.19**	−0.03ns	−0.15**	−0.09**
RRT-Minn.	−0.18**	−0.11**	−0.23**	−0.08ns	−0.16**	−0.09**
RRT-Tr.	−0.18**	−0.08**	−0.23**	−0.06ns	−0.16**	−0.08*
RRT-1 hr	0.15**	0.09**	0.22**	0.03ns	0.15**	0.08*

[1]1356 samples.
[2]1155 samples.
[3]**Significant at the 0.01 level.
*Significant at the 0.05 level.
nsNot significant at the 0.05 level.

TABLE 9. CORRELATIONS BETWEEN SANITARY CONDITION OF MILKING AREA, MILK HOUSE, AND MILKING UTENSILS WITH BACTERIOLOGICAL QUALITY TESTS FOR PRODUCERS SHIPPING MILK IN CAN AND ACCORDING TO THE TYPE OF MILKING USED.

Quality Tests	Milking area		Milk house		Milking utensils	
	Hand[1]	Machine[2]	Hand	Machine	Hand	Machine
SPC	0.13**[3]	0.14**	0.45**	0.16**	0.17**	0.15**
DMC	0.11*	0.12**	0.39**	0.12**	0.11*	0.11**
MBRT	−0.11*	−0.16**	−0.39**	−0.14**	−0.07ns	−0.13**
RRT-Minn.	−0.10ns	−0.18**	−0.29ns	−0.19**	−0.07ns	−0.15**
RRT-Tr.	−0.11*	−0.16**	−0.27ns	−0.18**	−0.08ns	−0.13**
RRT-1 hr	0.08ns	0.15**	0.23ns	0.15**	0.07ns	0.13**

[1]454 samples.
[2]2057 samples.
[3]**Significant at the 0.01 level.
*Significant at the 0.05 level.
nsNot significant at the 0.05 level.

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INFLUENCE OF HEAT TREATMENT OF SKIMMILK UPON GROWTH OF ENTEROPATHOGENIC AND LACTIC BACTERIA[1]

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Abstract

By use of agar plate count techniques, growth patterns of *Streptococcus cremoris* R-1, *Streptococcus lactis* C-2, *Streptococcus agalactiae* OARDC, enteropathogenic *Escherichia coli* OSU 66, *Salmonella typhimurium* OSU 471, and *Staphylococcus aureus* OSU 256 were determined in nonheated skimmilk and in skimmilks heated at 62.8 C – 32 min, 80 C – 12 min, and 121 C – 10 min. Calculations were made of the generation time, number of generations, and rate of multiplication expressed as the velocity coefficient, k, = 0.692/generation time. The k values of the organisms in nonheated skimmilk following 2 hr incubation ranged from 0.38 for *S. cremoris* to 1.15 for *E. coli*. Heat treatment of skimmilk stimulated the rate of multiplication of the organisms and in most instances better growth occured in skimmilk heated at 80 or 121 C than in the skimmilk heated at 62.8 C. The average per cent stimulation of the growth rate at 2 hr upon heat treatment of the skimmilk ranged from 9% for *S. agalactiae* to 45.5% for *S. cremoris*. *Streptococcus agalactiae* and *E. coli* were least stimulated by heat treatment of skimmilk and *S. aureus* and *S. cremoris* the most. Growth inhibitory properties of nonheated skimmilk were dissipated upon incubation for 4-8 hr.

The bactericidal and bacteriostatic properties of raw milk have been known for some time *(1)*. Within the last 3 years, two succinct reviews have appeared *(6, 9)*. These have been primarily concerned with the lactic streptococci with only limited information on the antimicrobial properties of raw skimmilk against other microorganisms. Unfortunately where such information is available, comparisons are difficult because *(a)* investigators have usually worked with a single bacterial species, *(b)* antimicrobial properties of milk may vary with the milk supply, *(c)* nature of heat treatments rendered the milk, and/or *(d)* various means used to achieve a sterile nonheated product. In some instances, pH or titratable acidity was used to measure efficacy of the inhibitory agents.

This research was designed to overcome some of these deficiencies and to report growth rates of selected lactic and enteropathogenic microorganisms in nonheated sterile skimmilk and in the same product following different heat treatments.

[1]Article 29:70. Department of Dairy Technology. This investigation was supported by Public Health Service Grant FD-00161 from the Office of Research and Training Grants, Food and Drug Administration.

Materials and Methods

Microorganisms

The organisms studied formed part of our collection and include: the lactic cultures *Streptococcus cremoris* R-1 and *Streptococcus lactis* C-2; *Streptococcus agalactiae* OARDC, isolated from a case of bovine mastitis; *Escherichia coli* OSU 66, a pathogenic strain associated with infant diarrhea; *Salmonella typhimurium* OSU 471, etiological agent of salmonellosis; and *Staphylococcus aureus* OSU 256, a food poisoning strain producing enterotoxin.

Skimmilk

Raw milk obtained from the University Dairy was centrifuged at 13,300 × *g* for 15 min at 2 C. The skimmilk was passed through 1 μ and then 0.02 μ Seitz filters under aseptic conditions. Sterility was checked by agar plate counts of the filtered milk following incubation at 30 or 37 C for 48 hr.

Seven milliliter portions of the filtered skimmilk were distributed into a series of sterile screw-cap test tubes and then subjected to the following heat treatments: nonheated (control); 62.8 C – 32 min (pasteurized); 80 C – 12 min; and 121 C – 10 min (autoclaved). After heat treatment, tubes were cooled immediately in ice water and stored at 2-4 C.

Stock cultures

The microorganisms were activated from a lyophilized state and carried in reconstituted Matrix Mother Culture medium (11% total solids). Incubation was at 30 C for the lactic cultures and 37 C for *S. agalactiae* and the enteropathogens. For stock cultures Matrix milk was inoculated with a loopful of the activated culture and depending upon the organism, incubation was at either 30 or 37 C for 12-14 hr.

Growth rate determinations

One tube (7 ml) each of the nonheated and heated skimmilk samples was inoculated by use of a micropipette with 0.07 ml of the 12-14 hr stock culture, mixed well, and then distributed in 1 ml lots into individual sterile screw-cap tubes. Where the stock culture was coagulated, it was diluted 1 to 9 with sterile distilled water to facilitate measurement of the 0.07 ml inoculum. Tubes were incubated in a water bath at either 30 or 37 C. At selected time intervals, a single tube was withdrawn and the bacterial population was determined by the agar plate count method. Agars used were: Elliker (Difco) for the streptococci; Standard Methods (BBL) for *E. coli;* and Trypticase Soy (BBL) for *S. typhimurium* and *S. aureus*. Plates were incubated for 24 to 48 hr at either 30 or 37 C.

Calculations

The number of generations, n; generation time, g; and velocity coefficient, k; were calculated as suggested by Porter *(7)*.

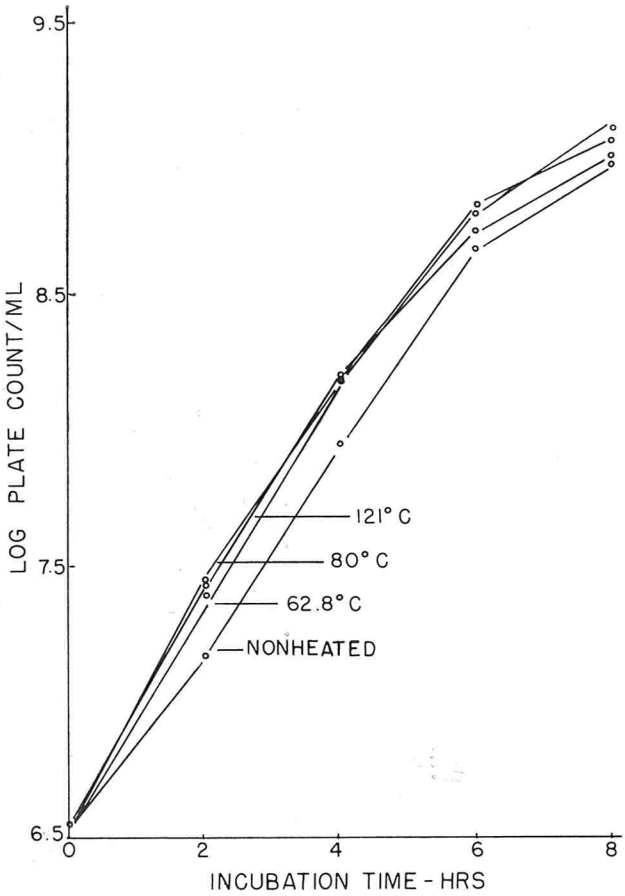

Figure 1. Growth curves for *Streptococcus lactis* C-2 in nonheated and variously heated skimmilks.

RESULTS

To illustrate the influence of heat treatment of skimmilk on the growth of selected bacteria, agar plate count data were used to prepare standard growth curves for the organisms in nonheated skimmilk and in skimmilk following heat treatment at 62.8 C — 32 min, 80 C — 12 min, and 121 C — 10 min. Only the growth curves for S. *lactis* C-2, *E. coli* OSU 66, S. *typhimurium* OSU 471, and S. *aureus* OSU 256 are presented. The growth curves for S. *cremoris* R-1 and S. *agalactiae* OARDC are not shown since they resembled the curves for S. *lactis* C-2 and *E. coli* OSU 66, respectively.

The growth curves for S. *lactis* in nonheated and heated skimmilks are presented in Fig. 1. These reveal that the growth rate in all systems was essentially exponential during the first 6 hr of incubation. However, the growth rate was slower in the nonheated system than in the heated systems during the first 4 hr of incubation. For example, the number of generations, n, at 4 hr was 4.70 in the nonheated skimmilk and 5.50 in the autoclaved skimmilk. Only slight differences were noted in the number of generations at 4 hr in the heated skimmilks, with n being in the range of 5.43 - 5.50.

For *E. coli*, the growth rate (Fig. 2.) was rapid and exponential with no evident lag during the first 6 hr of incubation. Only slight differences were noted between the nonheated and heated systems. The number of generations at 2 hr ranged from 3.33 in the nonheated system to 3.83 in the system heated at 80 C. The n values at 4 hr ranged from 7.43 in nonheated skimmilk to 7.77 in pasteurized skimmilk. This strain of *E. coli* proliferated more rapidly in nonheated skimmilk than any of the other organisms studied. *Escherichia coli* OSU 66 varied from most species of the genus *Escherichia* in that it produced little or no gas in skimmilk.

The growth curves of S. *typhimurium* are presented in Fig. 3. For this enteropathogen, a lag period of 1 hr preceded the initiation of exponential growth in all skimmilk systems. Rate differences between the various skimmilks were evident: the number of generations at 2 hr was 2.07, 2.90, 2.87, and 3.17 in nonheated, pasteurized, 80 C heated, and autoclaved skimmilk, respectively. The growth depressing effect of nonheated skimmilk presisted throughout the 4 hr incubation period.

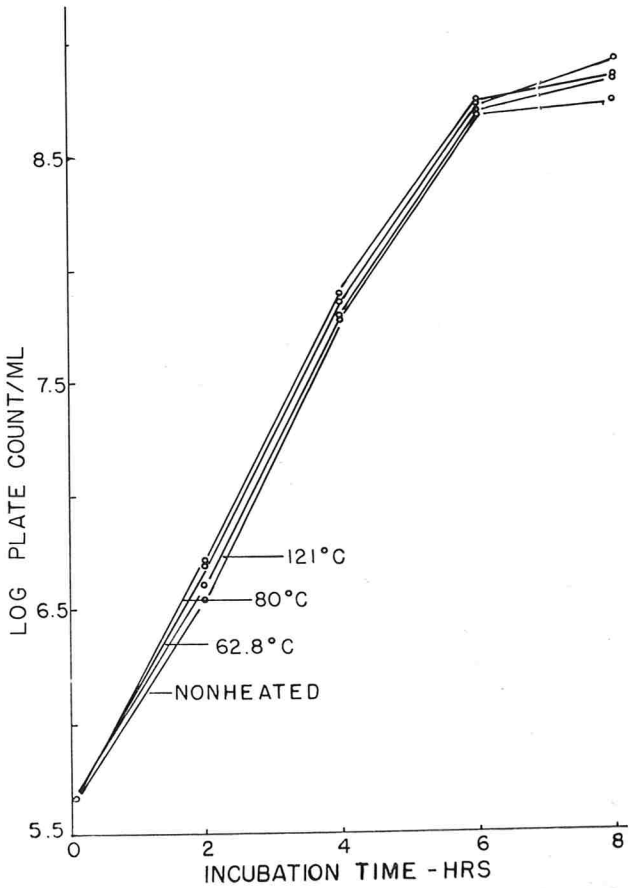

Figure 2. Growth curves for enteropathogenic *Escherichia coli* OSU 66 in nonheated and variously heated skimmilks.

In Fig. 4, growth curves are presented for the food poisoning strain of *S. aureus*. From the curves, it is evident that nonheated skimmilk depressed the growth of the organisms. At 4 hr, the number of generations was 6.97 in nonheated skimmilk as compared with 8.57 in the pasteurized, 9.13 in 80 C heated, and 8.77 in autoclaved skimmilks. This strain of *S. aureus* proliferated more rapidly in heated skimmilks than any of the other organisms studied.

For all of the bacteria during the first 4 hr of incubation, the rate of growth in nonheated skimmilk was slower and the total bacterial population was less than in the heated systems. The degree to which growth was depressed in the nonheated system when compared with the heated systems varied with the particular organism being negligible for *E. coli* and *S. agalactiae*, intermediate for *S. lactis* and pronounced for *S. cremoris*, *S. typhimurium*, and *S. aureus*. No constant relationship was noted between the degree of heat treatment of the skimmilk and the growth of the organism.

In Table 1 are shown rates of multiplication of the organisms in the nonheated and heated skimmilks.

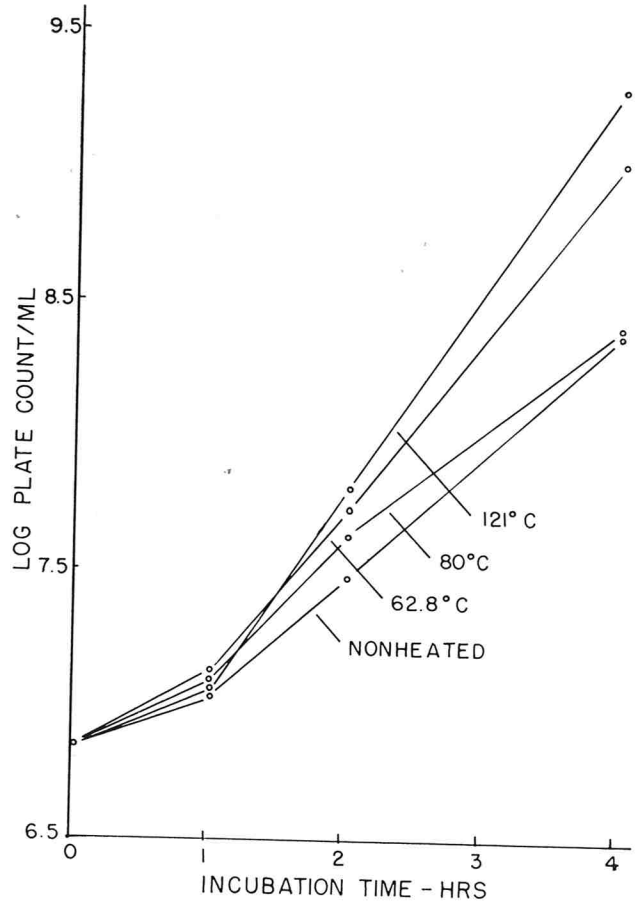

Figure 3. Growth curves for enteropathogenic *Salmonella typhimurium* OSU 471 in nonheated and variously heated skimmilks.

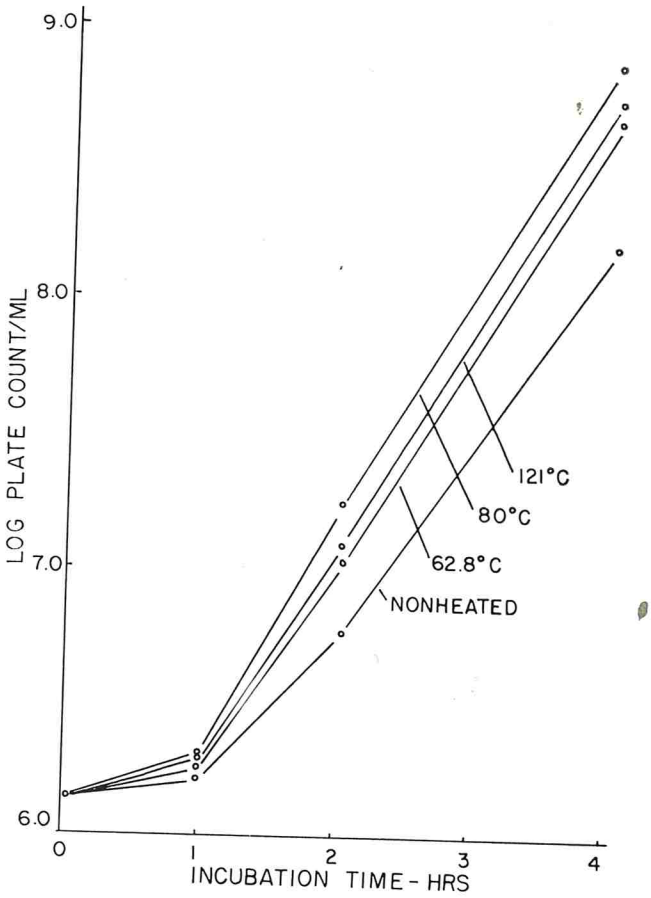

Figure 4. Growth curves for enterotoxin-producer *Staphylococcus aureus* OSU 256 in nonheated and variously heated skimmilks.

These are expressed as the velocity coefficient, k. It may be seen that with the exception of *S. agalactiae* the growth rates of all of the organisms were more rapid in the heated than in the nonheated systems. For *S. agalactiae*, the non-heated skimmilk was a more suitable growth medium than pasteurized skimmilk. However, heat treatment of the skimmilk at 80 or 121 C stimulated the growth rate to a higher level than in the nonheated system.

As a means of expressing the degree to which heat treatment of the skimmilk stimulated growth of the organisms, the per cent increases in k values for each of the heated systems over the nonheated system were calculated. Data are presented in Table 2. Average values are also shown.

With the exception of *S. agalactiae* and *E. coli*, average growth rates greater than 21% occurred upon heat treatment of the skimmilk. For *S. agalactiae* and *E. coli*, the values were less than 14%. Heat treatment of the skimmilk in some manner produces changes in the system making it a more suitable growth medium. There was a direct relationship between the degree of heat treatment of the skimmilk and its growth stimulatory properties for *S.*

TABLE 1. EFFECT OF HEAT TREATMENT OF SKIMMILK ON THE RATE OF MULTIPLICATION OF SELECTED LACTIC AND ENTEROPATHOGENIC MICRORGANISMS

Microorganism	Incubation period	Skimmilk treatment				
		Nonheated	62.8 C — 32 min	80 C — 12 min	121 C — 10 min	Heated[1]
	(hr)	(κ^2)				
S. cremoris R-1	2	0.38	0.52	0.75	0.96	0.74
	4	0.53	0.56	0.79	0.88	0.74
S. lactis C-2	2	0.73	0.97	1.03	1.02	1.01
	4	0.77	0.96	0.99	0.99	0.98
S. agalactiae OARDC	2	1.05	0.89	1.33	1.33	1.18
	4	0.98	0.93	1.23	1.31	1.15
E. coli 66	2	1.15	1.30	1.33	1.19	1.27
	4	1.22	1.33	1.33	1.24	1.30
S. typhimurium 471	2	0.58	0.84	0.77	0.82	0.81
	4	0.68	0.97	0.81	1.01	0.93
S. aureus 256	2	0.48	0.67	0.85	0.76	0.76
	4	0.72	0.93	1.09	1.01	1.01

[1]Average κ for the heated systems.
[2]The rate of multiplication was calculated as suggested by Porter *(7)* where κ, velocity coefficient, $= 0.692/$generation time, g, for each organism at the two incubation periods shown.

cremoris. For the other organisms, the results were variable and could not be associated with the degree of heat treatment of the skimmilk. In most instances, the per cent stimulation of growth of the organisms upon heat treatment of the skimmilk was less at 4 hr than at 2 hr incubation. This effect was particularly pronounced for *S. cremoris* where the average per cent decreased from 45.5 to 26.0. These results indicate that growth inhibitory properties of nonheated skimmilk are dissipated upon extended incubation with the organisms.

DISCUSSION

The nonheated skimmilk prepared by Seitz filtration exhibited antimicrobial activity against a wide range of microorganisms. However, the response was variable even between closely related organisms. This variability is probably related to several factors not the least of which is the susceptibility or resistance of the individual organisms. Since the degree of inhibition in the nonheated system was expressed relative to that of the same system upon heat treatment, consideration must be given not only to the heat lability of the inhibitory agents *per se* but also to the physical and chemical changes which occur in the heated milk, some of which are stimulatory whereas others are inhibitory.

Wright and Tramer *(10)* have shown that lactoperoxidase activity in milk will inhibit acid production of lactic starter cultures. However, lactoperoxidase is not the only inhibitory factor in milk *(1, 6, 9)*. Some earlier studies in our Department *(8)* and those of Emmons et al. *(3)* indicate that the lactic organisms are also susceptible to specific antibodies associated with the immune globulins of milk. Thus, the observed differences between *S. lactis* C-2 and *S. cremoris* R-1 are probably the combined effect of two or more inhibitory agents present in nonheated skimmilk. The progressive reduction in inhibition exibited upon increased heat treatment of the milk would be a reflection of the known thermolability of both lactoperoxidase and immune globulins.

The loss of inhibitory activity by the nonheated system during incubation with the organisms is harder to explain. This loss may be the result of a two-fold effect: chemical/physical inactivation of the agent(s) upon storage and/or changes in microbial susceptibility to the agent(s). For *S. cremoris* R-1, the data indicate that cells in the initial lag growth phase were more susceptible to the inhibitory agent(s) than those in exponential growth. For *S. lactis* C-2, this effect was not evident.

S. agalactiae responded somewhat differently from the lactic starter cultures in that nonheated and pasteurized skimmilks apparently exerted only slight inhibition of growth when compared with skimmilks heated at 80 or 121 C. These results are in agreement with those reported by Brown *(2)*. He also has shown that maximum inhibitory activity of the heat labile agents of milk against *S. agalactiae* became apparent only after 24 hr of incubation. During the first 8 hr of incubation, he noted only slight differences in culture activity as expressed by changes in pH between the variously heated systems. The anaerobic conditions of the udder plus increased catalase activity would deter the lactoperoxidase system of raw milk. In essence, raw milk would not be an unfriendly growth medium for *S. agalactiae*, an

TABLE 2. PER CENT INCREASE (DECREASE) IN K VALUES OF SELECTED LACTIC AND ENTEROPATHOGENIC MICROORGANISMS UPON HEAT TREATMENT OF SKIMMILK

Microorganism	Incubation period	Skimmilk heat-treated at: 62.8 C — 32 min	80 C — 12 min	121 C — 10 min	Avg[1]
	— hr —	(% increase)[2]			
S. *cremoris* R-1	2	26.9	49.3	60.4	45.5
	4	5.4	32.9	39.7	26.0
S. *lactis* C-2	2	24.7	29.1	28.4	27.4
	4	19.8	22.2	22.2	21.4
S. *agalactiae* OARDC	2	(15.2)[3]	21.1	21.1	9.0
	4	(5.1)[3]	20.3	25.2	13.5
E. coli 66	2	13.0	15.7	3.5	10.7
	4	9.0	9.0	1.6	6.5
S. *typhimurium* 471	2	31.0	24.7	29.3	28.3
	4	29.9	16.1	32.7	26.2
S. *aureus* 256	2	28.4	43.5	36.8	36.2
	4	22.6	33.9	28.7	28.4

[1]Average per cent increase for the three heated systems.

[2]Per cent increase $= \dfrac{(\kappa \text{ at 2 or 4 hr in heated system}) - (\kappa \text{ at same time in nonheated system})}{(\kappa \text{ at 2 or 4 hr in heated system})} \times 100.$

[3]Per cent decrease.

invasive mastitic organism.

For the enteropathogen *E. coli*, nonheated skimmilk also lacked growth deterrent properties. Whether factors responsible for lack of inhibition by nonheated skimmilk are the same as those for S. *agalactiae* are not known. It is noteworthy that both *E. coli* and S. *agalactiae* had the highest k values among the organisms studied and exhibited almost no lag growth phase.

Microbial growth was stimulated by heat treatment of the sterile Seitz-filtered skimmilk. However, the influence of the filtration process on bacterial growth was not determined. Although we have found in previous studies *(4, 5)* that Seitz filtration of skimmilk did not influence (*a*) the electrophoretic properties of the major milk proteins, (*b*) the reducing sugars, (*c*) the soluble calcium and magnesium, or (*d*) the inherent protease activity. Conceivably, the filtration procedure could create subtle changes in the skimmilk which could influence bacterial growth. This is now being investigated.

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EFFECT OF PASTEURIZATION ON THE DIRECT MICROSCOPIC COUNT OF EGGS

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Abstract

When pasteurized whole eggs from breakers were examined by the Direct Microscopic Count (DMC) procedure, the bacterial count frequently appeared to be too low to correlate with the observed state of decomposition. The DMC of whole egg was found to decrease during pasteurization. To determine why, DMC's were done using the North Aniline Oil - Methylene Blue Stain and the Levowitz-Weber modification of the Newman-Lampert stain. Total bacterial counts also were made using the Petroff-Hausser counting chamber. Results indicated that the reduction in count resulted from lysis of some of the bacterial cells in egg rather than to loss of stainability. Crystalline lysozyme at the concentration found in egg and whole egg preparations produced similar reductions in the DMC of bacteria isolated from egg.

The Direct Microscopic Count (DMC) on egg *(2)* has been a useful tool in determining the sanitary condition of eggs from commercial egg breaking plants. With the advent of egg pasteurization, it became apparent that the results of the DMC done on pasteurized egg did not consistently correlate with results from organoleptic examination in that the DMC would often be lower than that expected from organoleptic examination. Accordingly, a limited study was done to determine why results of organoleptic examination and those by DMC did not correlate for pasteurized egg.

Materials and Methods

Eggs

Two sources of eggs were used in this study. Breaker eggs were obtained from a commercial breaker who prepared eggs especially for us so as to include the contents of cracked and dirty eggs as well as normal breaker eggs. These were frozen by the breaker and were maintained frozen in the laboratory until used. Fresh, whole eggs were obtained from local supermarkets, were hand broken, and, where appropriate, yolks and whites were separated by hand.

Preparation of bacterial suspension from egg culture

Whole breaker eggs were incubated at room temperature for 24 hr and 0.1 ml was plated on the surface of Trypticase Soy Agar plates. These were incubated for 24 hr at 35 C and the growth was washed from the plates with sterile phosphate buffer. A homogenous suspension of microorganisms was prepared by shaking the suspension with glass beads and by passing the shaken suspension through cotton.

[1]Deceased.

Table 1. Effect of heating whole egg on the direct microscopic count

Heating time (min)	Temp (C)	Direct microscopic count	Reduction in count (%)
0	27	9,200,000	—
2	54	5,000,000	46
4[a]	60	2,500,000	73
5.5	60	1,700,000	82
6.5	60	1,500,000	84
7.5	60	1,200,000	87

[a]End of heating time and beginning of pasteurization process.

Table 2. Reduction in DMC by the pasteurization of whole egg, egg yolk, and egg white

Product	DMC		Reduction (%)
	Before pasteurization	After pasteurization	
Whole egg	7,300,000	900,000	88
Egg yolk	8,600,000	3,700,000	57
Egg white	6,900,000	850,000	88

Table 3. Comparison of DMC and Petroff-Hauser chamber counts on whole egg before pasteurization

Whole egg preparation	DMC	Petroff-Hausser	Difference (%)
1	3,400,000	4,100,000	+21
2	6,400,000	5,000,000	−22
3	6,500,000	6,800,000	+ 5
4	8,600,000	7,000,000	−19
5	13,000,000	10,000,000	−23
6	16,000,000	13,000,000	−19
		Average	−10

Table 4. Bacterial counts of egg before and after pasteurization as measured by the Petroff-Hausser technique

Whole egg preparation	Petroff-Hausser		Reduction in count (%)
	Before pasteurization	After pasteurization	
1	6,400,000	2,700,000	58
2	13,000,000	2,000,000	84
3	3,600,000	1,200,000	67

Pasteurization

Eggs were thawed and pipetted in 9-ml volumes into 15 x 100-mm test tubes. To pasteurize the eggs, tubes of egg were placed in a water bath so that all the egg was below the water line of the bath. Care was taken to prevent egg from adhering to the lip of the tubes or to any portion of the tube that was not immersed. Four minutes were required to bring the egg to 60 C, which is the temperature used commercially for egg pasteurization *(5)*. Tubes were held at 60 C for various times (3.5 min were used as the holding time for pasteurization) and cooled in immersion of the egg in an ice bath.

Enumeration

The North Aniline Oil-Methylene Blue stain was prepared and used as outline by A.O.A.C. *(2)*. The Levowitz-Weber modification of the Newman-Lampert stain was prepared and used, as described in *Standard Methods for the Examination of Dairy Products (1)*. Simple stains were prepared in water or acid solutions and applied for various staining times.

Petroff-Hausser counting chamber counts were done as described in *Clinical Diagnosis by Laboratory Methods (4)*. Several standard diluting fluids for direct counting were tried, but these were unsatisfactory because of their effect on the egg. Finally, a satisfactory diluting fluid was developed by adding 0.1 ml of saturated alcoholic solution of methyl violet to 10 ml of a 4% aqueous acetic acid solution.

Results and Discussion

Breaker eggs that had been intentionally abused by improper refrigeration were pasteurized in the laboratory and DMC done at various times during the pasteurization process (Table 1). Reduction in DMC occurred rapidly; the count was reduced almost one log cycle by the end of the pasteurization process.

A similar study was done with hand-separated egg yolk and egg white prepared from fresh eggs. The egg yolk and egg white portions were inoculated with an incubated whole egg preparation to give a 2% inoculum. The same reduction in DMC occurred in the egg white preparation as in the whole egg (Table 2). The reduction in DMC done on the egg yolk material was about 30% less than that for the egg white. The yolk and white preparations were not free of white and yolk, respectively, since hand separation was used, and a 2% inoculum of whole egg was added to both preparations.

Since reduction in count could be from loss of stainability by the North Aniline Oil-Methylene Blue stain, simple stains as well as the Levowitz-Weber modification of the Newman-Lambert were evaluated. Results from the North and Levowitz-Weber stains were identical, and further studies were based on the North stain.

Because of the inability of several stains to give a count higher than that obtained with the North stain, loss of count by the DMC procedure appeared not to be from loss of stainability. To investigate this point in greater depth, Petroff-Hausser counts were made in parallel with DMC on egg before pasteurization to establish the relationship between these counts (Table 3). The Petroff-Hausser count results ranged from +21 to −23% of the DMC, with an average of −10%. We believe that the differences in count were well within the limit of error of the two techniques and that the results obtained demonstrated that the Petroff-Hausser technique could be used to count unstained bacteria in egg.

Since the Petroff-Hausser procedure did not depend on stainability of the cells being counted, it was used to count whole egg before and after pasteurization (Table 4). The reduction in count by pasteurization ranged from 58 to 84%, which is typical of the results as measured by DMC. These data suggest that the loss of count by DMC in whole egg during pasteurization resulted from lysis of bacterial cells rather than from a decrease in stainability since unstained cells are counted in the Petroff-Hausser technique.

The enzyme lysozyme, which is lytic to many bacteria, is found in relatively high concentration in egg white, and the activity of this enzyme is known to

Table 5. Effect of lysozyme on isolates of bacteria from whole egg

Morphology	Gram stain	Reduction in count after pasteurization (%)
Cocci in clumps	Positive	0
Bacillus	Negative	4
Small bacillus	Positive	66
Large bacillus	Positive	83
Small pigmented bacillus	Positive	87
Diptheroid	Positive	91

Table 6. Effect of pasteurization and lysozyme on mixed bacterial flora isolated from egg

System				
Mixed culture	Lysozyme	Pasteurization	DMC ($\times 10^9$)	Reduction in DMC (%)
+	−	−	9.9	−
+	−	+	9.3	6
+	+	−	6.5	34
+	+	+	3.5	65

Table 7. Effect of substitution of crystalline lysozyme for egg on the DMC of a mixed culture isolated from egg

System	Pasteurized	Reduction in DMC count (%)
Culture + lysozyme	−	24[a]
Culture + egg	−	25[a]
Culture + lysozyme	+	50
Culture + egg	+	55

[a]Left standing at 25 C for 3 hr.

increase with temperatures up to 60 C, which is the temperature of egg pasteurization *(3)*.

To establish whether egg lysozyme could be the reason for the reduction in bacterial count during the pasteurization of egg, bacterial isolates from egg were subject to the egg pasteurization process in buffer containing the normal lysozyme content for egg (2600 μg/ml). Reduction in count varied from 0 to 91% with the various morphological types isolated (Table 5). Similarly, the effect of lysozyme and pasteurization on a mixed bacterial culture isolated from egg was determined (Table 6). Here, again, lysozyme in the concentration found in egg reduced the bacterial count of a mixed bacterial suspension from egg in an amount typical of that found during egg pasteurization. Finally, in another study of the effect of pasteurization on the DMC of mixed bacterial flora from egg, crystalline lysozyme in the concentration found in egg was substituted for egg with similar results (Table 7).

All data from this study were consistent with the thesis that egg lysozyme is effective in lysing bacterial cells of some species during egg pasteurization. As a result, the bacteria disappear, and the DMC or the other techniques tested here for enumeration of total bacterial numbers are no longer useful in establishing the sanitary condition of pasteurized egg products using the criteria established for raw egg products.

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AMENDMENT TO 3-A SANITARY STANDARDS FOR PUMPS FOR MILK AND MILK PRODUCTS, REVISED

Serial #0205

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

The "3-A Sanitary Standards for Pumps for Milk and Milk Products, Revised, Serial #0203" are further amended by adding a new subsection 7 to A. *MATERIAL.*

7. Pump impellors or rotors, and cases or stators, which operate in conjunction with a metallic counterpart and the sealing faces of rotary seals may be covered with a ceramic material. Ceramic materials shall be inert, non-porous, non-toxic, non-absorbent, insoluble, resistant to scratching, scoring and distortion when exposed to the conditions encountered in the environment of intended use and in cleaning and bactericidal treatment.

This amendment is effective June 1, 1971.

THE FREEZING POINT OF HERD MILK PRODUCED IN KENTUCKY[1]

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Abstract

Freezing point measurements were made on 24 hr composite herd samples of milk obtained monthly for one year. Samples were obtained from 45 herds located in five soil areas of Kentucky, representing 975 individual cows. The mean freezing point was −0.540 C, the range −0.567 to −0.511 C, and the standard deviation 0.0073 C. It is recommended that, for regulatory purposes, −0.530 C be adopted as the official freezing point standard for herd milk, compliance to be ascertained on the basis of a three-sample moving average with the sampling interval being not less than 30 days.

The freezing point of milk has long been a reference for controlling adulteration by watering. Those using this method recognize that it is not ideal. It lacks in accuracy because normal, known pure milk does vary. This has been documented by several investigators (2-9). Most of these reports are based on limited data. The official freezing point standard for unadulterated milk used in Kentucky is −0.530 C. Although this standard is used rather extensively in the United States, many persons in Kentucky believe that it has not been substantiated statistically for milk produced in this state. This paper reports an extensive survey to provide data to find sources of variation and to verify the currently used standard or recommend a different one.

Methods

Sampling

The sampling program was designed to obtain data that would be representative of milk produced throughout the state. With the assistance of a soils specialist, five soil areas were selected for milk sampling. These five areas represent a wide diversity in Kentucky soils, are well distributed geographically, and are representative of the slight variations in climate within the state. The five are Areas 2, 5, 7, 9, and 10 in Fig. 1. They encompass from 6 to 14 counties each.

Forty-five herds were sampled, the number in each area varying from 7 to 10. The average herd size was 21.7 cows, with a range of 4 to 81. The total number of cows varied from one sampling period to another as fresh cows were brought into the sampling program and dry cows taken out. The average total number was 975. Herds were selected without regard to grade of milk produced or to breed. However, an effort was made to select herds so a similar number would be subjected to above average and below average feeding and management practices. Classification of herds into these two categories was done by dairy plant fieldmen, the persons most familiar with the quality of management employed with each herd. Twenty-four-hour composite samples were obtained from each herd once a month for 12 months, for a total of 507 herd samples.

To be assured of unadulterated samples, all sampling was supervised by a person from the experiment station staff or the health department, or by a dairy plant fieldman. This person arrived at the farm before each milking, night and morning, to inspect all milking machines, milk lines, pails, cans, and other milk-handling equipment for incomplete drainage of rinse water. After the morning and evening milking the herd composite sample, consisting of a mixture of individual cow samples, was placed in crushed ice and remained in possession of the sampler. The evening and morning samples were combined, placed in ice, and transported immediately to Lexington, where freezing point measurements were made within 30 hr of the morning milking. Although mastitis tests were not made on individual cows at the time of sampling, milk from cows known to be or suspected of being mastitic or otherwise abnormal was excluded.

Analyses

All freezing point determinations were made with an Advanced Instruments Model 30 L Milk Cryoscope, employing the technique recommended by the manufacturer (1). Milk samples, upon receipt at the laboratory, were placed in a refrigerator at 1.7 C, where they remained quiescent overnight. The following morning 2-ml test portions of "skim milk" were withdrawn from each sample jar by inserting the tip of a pipet through the cream layer. (Preliminary trials indicated that better repeatability was obtained on skim milk than on whole milk.) The average of three measurements was recorded as the freezing point of the sample.

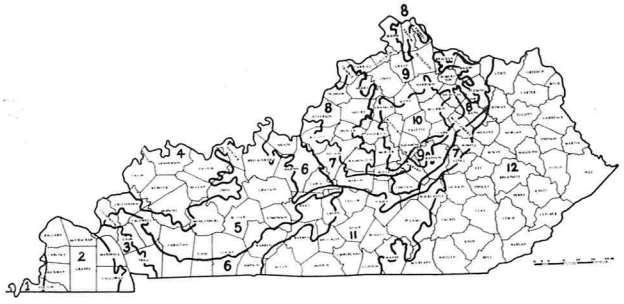

Figure 1. Location of the soil areas where milk samples were obtained.

Results and Discussion

The mean freezing point of the 507 samples was

[1]The investigation reported in this paper (No. 69-5-146) is in connection with a project of the Kentucky Agricultural Experiment Station and the paper is published with approval of the Director.

TABLE 1. DISTRIBUTION OF FREEZING POINTS OF HERD MILK SAMPLES FROM FIVE SOIL AREAS OF KENTUCKY

Soil area	No. of herds	No. of samples	Freezing point[a] 0.551 or below (No.)	(%)	0.550-0.541 (No.)	(%)	0.540-0.531 (No.)	(%)	0.530 or above (No.)	(%)	Range	Mean	Std. dev.
No. 2	9	106	7	7	40	37	52	49	7	7	0.526 to 0.558	0.540	± .0041
No. 5	10	117	8	7	64	55	44	37	1	1	0.524 to 0.559	0.540	± .0068
No. 7	10	111	9	8	54	49	41	37	7	6	0.512 to 0.554	0.541	± .0074
No. 9	9	95	8	8	31	33	42	44	14	15	0.518 to 0.567	0.540	± .0073
No. 10	7	80	6	8	37	46	26	32	11	14	0.511 to 0.567	0.540	± .0099
Summary	45	509	38	8	226	44	205	40	40	8	0.511 to 0.567	0.5406	± .0073

[a]All freezing point values are degrees centigrade below zero.

–0.5406 C and the overall standard deviation was ± 0.0073. Distribution of freezing points by soil areas is shown in Table 1. They were not affected by soil areas nor by geographical location of the herd.

Sources of variance in freezing point were analyzed with a nested classification model which included a component of variation for month-to-month fluctuation within farms, a component for farm-to-farm fluctuation within areas, and a component for between areas *(10)*. Analysis (Table 2) showed that farm-to-farm fluctuations accounted for 16% of the variance within an area and month-to-month fluctuations within farms accounted for 84%. Variance between areas was practically zero, as one would expect from viewing the means of the freezing points from the soil areas.

Since all milk produced by a farm cannot be analyzed for freezing point, a sampling procedure must be employed to estimate the true freezing point of the herd milk.

To operate with efficiency, regulatory administrators should know the sources and magnitudes of causes of sample variation in the freezing point from milk samples obtained at a particular farm. The analysis shown in Table 2 revealed that 84% of the variation in freezing points was caused by factors associated with a month-to-month fluctuation within farms. Such factors might be variations in weather, changes in feeding regime, changes in milk production, and stage of lactation.

Table 3 is presented to serve as an aid to regulatory agencies who wish to determine the number of monthly samples and the regulatory standard required to control adulteration by watering. To construct Table 3 we assumed that monthly fluctuations of milk freezing point within a farm are normally distributed and have a variance equal to 44.5×10^{-6}, as estimated from these data and shown in Table 2.

Utilizing the well-known sampling properties of averages *(10)*, values presented in Table 3 show the probability that a single estimate would be judged to be "suspect" when in fact the true freezing point was –0.540 C (average freezing point of Kentucky milk). Moreover the number of judgment errors (false accusations) made on normal milk would be expected to decrease as more monthly samples are averaged and as the freezing point of the regulatory standard is raised. Stated differently, judgment errors vary inversely with number of months in the average, and with the freezing point level of the regulatory standard. For example, assuming a regulatory standard of –0.530 C, a single monthly sample of normal unadulterated herd milk in Kentucky would have a 6.8% chance of being erroneously judged "suspect," but an average based on two monthly samples would reduce the chance to 1.7%; furthermore, an average of 3 monthly samples would result in less than a 1% chance of error.

The regulatory official must, without implicating the innocent, attempt to apprehend all violators. To establish regulatory standards that achieve these goals is a practical impossibility. For example, aver-

TABLE 2. ANALYSIS OF VARIANCE IN MILK FREEZING POINTS[a]

Source	Degrees of freedom	Mean squares	Per cent contribution to total variance
Areas	4	120.6	0.0
Farms/Area	40	140.5	16.1
Months/Farm	462	44.5	83.9
Total	506	52.7	

[a]Freezing points were multiplied by 1,000.

TABLE 3. PROBABILITY OF MAKING A JUDGMENT ERROR IN THE FREEZING POINT OF NORMAL MILK[a]

Number of months in the average	Regulatory standard C		
	—0.535	—0.530	—0.525
	(Per cent)		
1	22.7	6.8	1.2
2	14.5	1.7	0.1
3	9.7	0.5	0.0
4	6.8	0.1	0.0
5	4.6	0.0	0.0
6	3.3	0.0	0.0
7	2.4	0.0	0.0
8	1.7	0.0	0.0
10	1.0	0.0	0.0
12	0.5	0.0	0.0
14	0.3	0.0	0.0

[a]Based on average freezing point of —0.540 C

age Kentucky milk (freezing point —0.540 C) could be adulaterated to the extent of 2.7% added water and still have a freezing point below —0.525 C. Therefore, adoption of the highest freezing point (—0.525 C) in Table 3 as a regulatory standard could allow many violators to be unchallenged. But adoption of the most stringent standard (—0.535 C) would result in numerous judgment errors; the labor cost of the subsequent supervised samples would be prohibitive. Adoption and enforcement of a freezing point standard for controlling adulteration of milk with water thus becomes an administrative decision which must be guided by an adequate understanding of the distribution of freezing points of normal, unadulterated milk.

Because of these problems, we suggest that a "moving average" based on three samples is the most feasible routine method for the regulatory detection and elimination of watered milk. This also would be a practical approach for quality control laboratories. The method would involve systematic random checking of all individual producers. Any two consecutive samplings should be at least 30 days apart to allow month-to-month fluctuations to average out. The most reliable estimate of the true freezing point for milk from a single herd would, at any time, be the average of the freezing points of the three most recent samples.

Referring again to data in Table 3, it would seem that, for milk produced in Kentucky, the freezing point —0.530 C based on a 3-sample moving average should be adopted as a legal standard. It would be possible to enforce this standard with a minimum of supervised farm sampling because less than 1% of normal milk would erroneously be judged "suspect." At the same time no serious amount of adulteration would escape detection.

If regulatory officials feel that watering of milk is a serious problem in a specific milk shed, they might need to employ a different strategy until conditions are brought back to "normal." In such a situation a more stringent standard, e.g., —0.535 C, might be adopted for the interim, with the realization that sampling more frequently than at monthly intervals or supervised sampling, or both, would be required pending elimination of the difficulty.

CONCLUSIONS

Based on analysis of the results obtained from an extensive survey of milk freezing points in Kentucky, an "official" freezing point standard for regulatory officials in the state has been suggested. This standard may or may not be suitable, without modification, for other states.

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A Research Note

GROWTH OF *CLOSTRIDIUM PERFRINGENS* IN MEAT LOAF WITH AND WITHOUT ADDED SOYBEAN PROTEIN

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Abstract

The effects of various soybean proteins used in meat loaf formulations on growth of *Clostridium perfringens* were studied under actual meat loaf conditions. Beef products offer an excellent medium for the rapid growth of this organism and addition of soybean additives did not show any significant effects.

Food-borne disease caused by *Clostridium perfringens* has become a major concern in cooked meat products that have been improperly handled *(2)*. Soybean protein is presently being incorporated into many meat products such as meat loaves and patties as a meat extender. The soybean serves as a source of protein enrichment and improves textural properties. Recent research *(3)* has shown that some soybean protein stimulates growth of *C. perfringens*. This study was designed to determine effects of various soybean proteins used in meat loaf formulations on growth of *C. perfringens*.

Materials and Methods

Clostridium perfringens strains S40 and S45 were obtained from H. E. Hall (National Center for Urban and Industrial Health, Cincinnati, Ohio). Stock cultures were maintained in cooked meat medium (BBL) at room temperature. Inoculation cultures were grown at 45 C for 18 hr in thioglycollate medium without added dextrose (BBL). The culture inoculum was centrifuged at 4080 × g for 10 min. The supernatant liquid was decanted. The pellet was dispersed in 200 ml of sterile 6.25×10^{-4} M phosphate buffer pH 7.0 and centrifuged again. The pellet was dispersed in buffer and the procedure repeated twice. The final pellet was resuspended in 20 ml of buffer and serially diluted to obtain the proper inoculum. All procedures were done aseptically.

Ground boneless round of beef adjusted to 18% fat (w/w) was mixed with soy protein additives in amounts recommended for commercial use. These additives included a textured soy flour, soy grits, isolated soy protein, and a soy protein concentrate. Cooked meat medium (BBL) was used as an additive to demonstrate the influence of a laboratory medium. Beef alone constituted the control. Each sample consisted of seven individual meat loaves each made up of 150 g of beef or 125 g beef plus 6.25 g of the protein additive mixed in 18.75 ml of water. The meatloaves, 7 cm × 7 cm × 4.5 cm in dimension, were made up in blender jars (Mini Blend, 0.5 pint, John Oster Service Company, Milwaukee, Wisconsin) and heated in an oven at 190 C for approximately 30 min. The center temperature of the loaf reached at least 66 C. Prior to inoculation, the meat loaves were steamed 30 min and tempered at 45 C. A 10 ml quantity of cell suspension was then inoculated into the meat loaf using multiple injections with a syringe giving the meat loaf an initial population of between 10^1 to 10^3 per gram. The meat loaves were incubated at 45 C. At timed intervals one of the meat loaves was taken from the series of samples and sacrificed to determine the extent of the lag, exponential, and stationary phases of growth. The whole loaf was blended for 3 min and the cell population was estimated by plate count on freshly prepared sulfite polymyxin sulfadiazine agar *(1)*. The plates were overlayed and incubated anaerobically at 35 C for 18 hr in an atmosphere of 90% N_2 and 10% CO_2. The pH and E_h values during the growth period were monitored using a combination pH electrode and an E_h platinum inlay electrode (Corning Glass Works, Medford, Mass.). These electrodes were kept in the meat loaves throughout the incubation period.

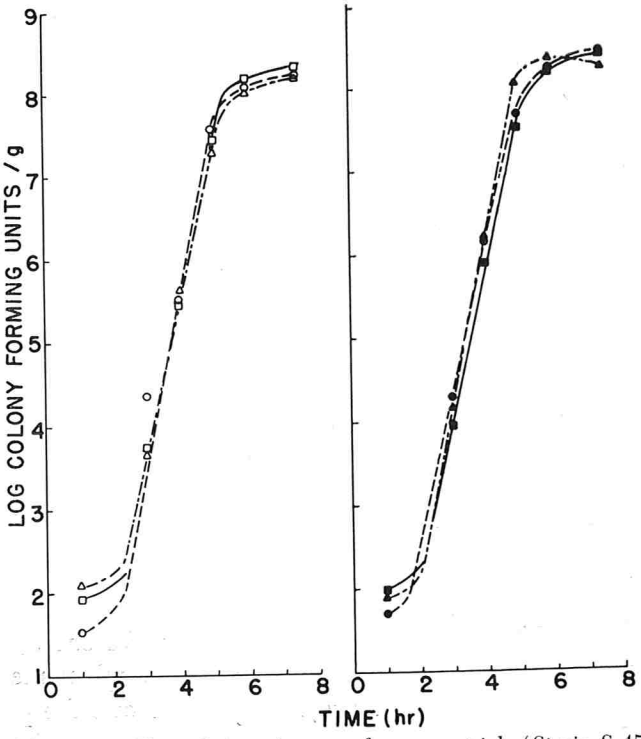

Figure 1. Typical growth curve from one trial. (Strain S-45 in beef alone vs. beef supplemented with five protein additives). Meat loaf formulations are: beef control, △; beef plus soy protein concentrate, □; beef plus soy protein isolate, ○; beef plus textured soy flour, ■; beef plus Cook Meat Medium, ▲; and beef plus soy grits, ●.

[1]Paper No. 7416, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul.

TABLE 1. GENERATION TIME OF TWO STRAINS OF *C. perfringens* IN MEAT LOAVES SUPPLEMENTED WITH FIVE PROTEIN ADDITIVES VS. BEEF ALONE. TWO REPLICATES.

Strain	Replicate	Beef alone	Soy protein concentrate	Soy protein isolate	Textured soy flour	Cooked meat medium (BBL)	Soy grits
		(min)					
S40	1	12.3	10.6	10.3	12.9	9.9	11.0
	2	12.6	12.3	12.7	10.5	9.1	11.0
	Average	12.45	11.45	11.5	11.7	9.5	11.0
S45	1	10.0	9.8	10.0	10.1	11.2	10.7
	2	11.5	9.9	8.7	9.1	10.7	9.1
	Average	10.75	9.85	9.35	9.6	10.95	9.9

The generation time was calculated *(4)* by the formula $G_t = \frac{t}{n} = \frac{t}{3.3 \log_{10} b/B}$ where the generation time G_t is equal to t (the time elapsed between b, the final population after time t and B, the initial population) divided by the number of generations, n, the number of generations being equal to 3.3 $\log_{10}$ b/B. This study consisted of two replicates of each sample tested with two strains.

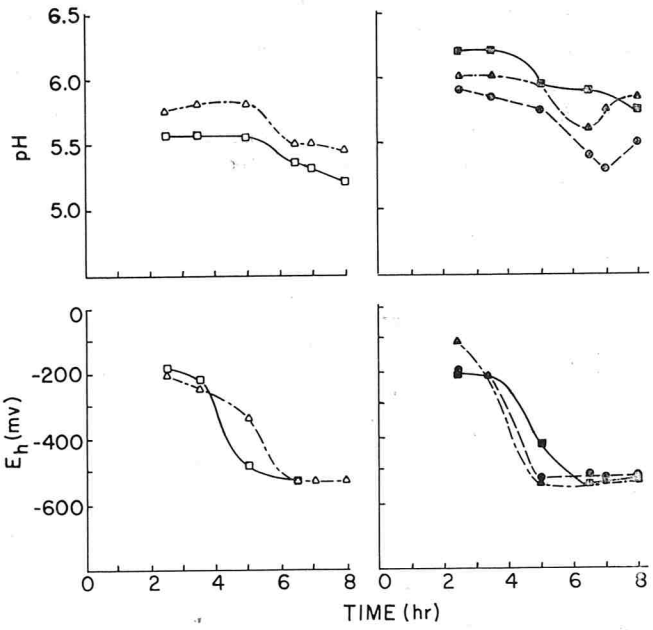

Figure 2. pH and E_h curves representing the growth curves in Figure 1. Meat loaf formulations are: beef control, △; beef plus soy protein concentrate, □; beef plus textured soy flour, ■; beef plus Cooked Meat Medium, ▲; and beef plus soy grits, ●.

RESULTS AND DISCUSSION

Figure 1 shows a typical growth curve for one trial (strain S-45 in beef alone versus beef supplemented with five protein additives). Figure 2 shows the pH and E_h curves from the same trial. Strain S-40 in beef alone gave an average generation time of 12.5 min (Table 1), a slightly lower average value for the protein additives, and a 9.5 min average for the beef plus cooked meat medium. Strain S-45 in the beef alone had an average generation time of 10.75 min, whereas the value was 10.95 min in cooked meat medium and slightly less in beef with soybean additives. Table 2 presents the analysis of variance of data in Table 1 *(5)*. The analysis showed no apparent significant differences, at the 5% level, in generation times of the bacteria as a result of commercial soybean products or cooked meat medium added to beef meat loaves.

These results indicate that (*a*) beef products such as a meat loaf provide an excellent medium for the rapid growth of these microorganisms so the potential for food-borne disease exists and (*b*) soybean additives that are presently used commercially to prepare meat products had no noticeable effect on growth of *C. perfringens* when added to a beef medium.

Further testing with the other strains of *C. perfringens* may be warranted as these results represent the effects on only two strains.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service Research Grant FD 00178-01 from the Food and Drug Administration. The authors thank Central Soya Company and Swift Chemical Company for the supply of soybean proteins. The authors also acknowledge the able technical assistance of Lorraine B. Smith and Kathleen A. Stewart.

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TABLE 2. ANALYSIS OF VARIANCE OF GENERATION TIME OF TWO STRAINS OF *C. perfringens* IN MEAT LOAVES SUPPLEMENTED WITH FIVE PROTEIN ADDITIVES VS. BEEF ALONE. TWO REPLICATES.

Source of variation	Degrees of freedom	Sum of squares	Mean square	*F Value
Total	23	76.8		
Within subclasses (error)	12	54.3	4.53	
Strains	1	8.7	8.7	1.92
Protein sources	5	4.75	.95	.21
Treatment interaction (subclass discrepance)	5	9.1	1.82	.40

*No significant differences at 5% level

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PUBLICATIONS RECOMMENDED BY THE DAIRY FARM METHODS COMMITTEE

Circular, book, etc.	Name and cost	Write to:
Circular 532	Sampling, Collecting, and Testing Milk Cost - one free - more than one, 25 cents	Pennsylvania State University College of Agriculture, Extension Service University Park, Pennsylvania
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Publication AXT-94	Milking Management and Its Relationship to Milk Quality by: University of Cal. Agriculture Extension Service Cost - 50 cents	University of California Agriculture Extension Service Davis, California
Publication No. 1306	Screening Tests for the Detection of Abnormal Milk by: Dept. of Health, Education and Welfare Public Health Service Cost - 20 cents	Superintendent of Documents U. S. Government Printing Office Washington, D. C. 20402
DeLaval Handbook S.A. 1175 Revised 8-67	The DeLaval Handbook of Milking by: DeLaval Separator Company Cost - free	DeLaval Separator Company 5724-48 North Pulaski Road Chicago, Illinois 60646
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Bulletin A-37	A Milker's Manual by: University of Arizona Extension Service W. R. Van Sant Extension Dairymen Cost - free	Extension Service University of Arizona College of Agriculture Tucson, Arizona
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KENTUCKY ASSOCIATION OF MILK, FOOD AND ENVIRONMENTAL SANITARIANS 1971 EDUCATIONAL CONFERENCE

The 1971 Educational Conference for Fieldmen and Sanitarians was held February 23-24, 1971, at the Executive Inn Motor Hotel, Louisville, Kentucky.

A total of 266 (county and state health department sanitarians, milk and food industry fieldmen, plant managers and related service companies) were registered. States other than Kentucky represented w re as follows: Georgia (2), Illinois (1), Indiana (7), Minnesota (2), Missouri (2), Ohio (7), and Tennessee (2).

The program was broken into general sessions, food and environmental sanitarians sections and milk sanitarians section.

The following awards were presented to all past presidents of the Kentucky Sanitarians Associations. (all presently combined into KAMFES).

Past presidents plaque (1970) presented to outgoing president Jim McDowell, Dairymen Inc., Kyana Division, Louisville, Ky.

Outstanding Sanitarians Award – A. P. Bell, Louisville-Jefferson Co. Health Dept., Louisville, Ky.

Outstanding Fieldman Award – J. D. Gadberry, Dairymen Inc., Kyana Division, Glasgow, Ky.

Outstanding Service Award – Forest Borders, Border's Pure Milk Company, Bowling Green, Ky.

The Educational Conference is sponsored by the following: Kentucky Association of Milk, Food and Environmental Sanitarians; Kentucky State Health Department, Division of Environmental Health; University of Kentucky, Departments of Dairy Science and Extension Service.

EGG PROCESSORS ADOPT E-3-A STANDARDS, AMENDMENT

Three new E-3-A Standards and one amendment have been adopted by the E-3-A Sanitary Standards Committees since their meeting in November. Standards for Leak Protector Valves, Batch Pasteurizers and Sanitary Fittings, and a brief amendment to the Pump Standard, were adopted at the Houston meeting and signed as official since then.

The E-3-A Sanitary Standards will be published in the Journal of Milk and Food Technology and will be available for distribution at different times during the ensuing year. These timely standards should serve the industry well as new requirements are inaugurated under the federal mandatory egg inspection law.

E-3-A is a cooperative effort by the Sanitary Standards Committees of five groups to establish voluntary criteria for cleanability of processing equipment and product protection. Dairy & Food Industries Supply Assn., Institute of American Poultry Industries, International Assn. of Milk, Food & Environmental Sanitarians, U. S. Dept. of Agriculture and U. S. Public Health Service are involved in the effort.

Secretariat for the organization is housed at Dairy & Food Industries Supply Assn., 5530 Wisconsin Avenue, Washington, D. C. 20015, telephone (301) 652-4420.

NATIONAL CONFERENCE ON FOOD PROTECTION SPONSORED BY AMERICAN PUBLIC HEALTH ASSOCIATION

Action plans to combat the growing problem of microbial contamination of foods, and discussion of the role played by government and industry in consumer protection was developed at a National Conference on Food Protection, April 4-8 in Denver, sponsored by the American Public Health Association.

The Conference was a working meeting to determine new methods of preventing contamination during the processing and handling of foods. It did *not* discuss contamination through mercury or artificial additives and papers were not presented by individuals.

Ten workshop groups studied and revised position papers drawn up by expert panels on contamination of raw and processed foods, consumer education, food hazards, manpower training and utilization, and evaluation of current programs. The 400 participants in the invitational Conference recommended programs to reduce the incidence of food contamination and outbreaks of food-related disease. They did not simply restate the problems, but provided detailed proposals for methodology, administration, costs, and benefits of new programs.

Featured speakers included Senator Peter H. Dominick (R-Colo.), and the Food and Drug Administration's Keith H. Lewis, Ph.D., Director, Office of Food Sanitation, and Dale R. Lindsay, Ph.D., Associate Commissioner for Science. Also appearing was P. Walton Purdom, Ph.D., Director, Center for Urban Research and Environmental Studies, Drexel

University, and President of APHA; George J. Kupchik, Dr. Eng., Director of APHA's Division of Environmental Programs; Virgil O. Wodicka, Ph.D., Director of the Food and Drug Administration's Bureau of Foods; and William O. Beers, President, Kraftco Corp.

3-A COMMITTEES SIGN NEW STANDARD, AMENDMENTS

Seven documents, including an unusually significant Accepted Practice, amendments, revisions and supplements, have been signed by the 3-A Sanitary Standards Committees since their November meeting.

The completely new Practice for Spray Dryers for Milk and Milk Products, which provides guidelines for the drying operation, is a landmark accomplishment of 3-A. When the Practice is coupled with an Instantizer Practice pending final action, and previously published standards for fillers and sifters, the dry milk industry will have 3-A santitation criteria for the complete powder handling system.

Amendments for sanitary pumps, farm tanks and thermometer fittings, and a thermometer fittings supplement, were adopted. Two complete revisions of major 3-A standards for storage and transportation tanks were also signed.

These completed projects have varying effective dates in the coming year, when they will subsequently be published in the Journal of Milk & Food Technology. Copies of all new 3-A publications will be available to the industry following publication in the Journal.

REPORT OF THE 3-A SANITARY STANDARDS SYMBOL ADMINISTRATIVE COUNCIL, 1969-1970

The action of the meeting of the Board of Trustees preceding the Annual Meeting of this Association at Louisville, in August, 1969, was included in the Report presented at that meeting. Only one other meeting of the Trustees was held during the interval between the 1969 and 1970 Annual Meetings of this Association. This meeting was held in Chicago, on January 29, 1970.

Reports of the Secretary and of the Treasurer were presented, as was an audit of the Treasurer's records for the years 1967, 1968, and 1969. A report of the action of a DFISA Task Committee on the application of the 3-A Sanitary Standards for Fillers and Sealers of Single Service Containers for Milk and Milk Products—Serial 1702 to a type of fillers and sealers of flexible plastic packets was reviewed. Because the said sanitary standards were declared to apply to the new devices the Secretary was instructed so to evaluate application for authorizations. Two authorizations have been issued; several other applications are pending, and one has been denied.

It is the consensus of the Trustees that the 3-A Sanitary Standards for Fillers anl Sealers should be reviewed with respect to their application to devices of the type now being marketed. These sanitary standards were developed to apply to fillers of a fundamentally different design and function.

Six initial authorizations were issued during the 12 months ending July 31, 1970, and three authorizations were relinquished ending July 31, 1970, and three authorizations were relinquished. Thus, the number of authorizations in effect on July 31, 1970 has increased by 3 to 146. Comparative numbers of authorizations, covering each type of equipment, on July 31, 1969 and 1970 appear in Table 1.

D. C. Cleveland, J. A. Meany, K. G. Weckel, and C. A. Abele.

TABLE 1. NUMBERS OF AUTHORIZATIONS IN EFFECT

	Sanitary standard	Serial number	7-31-69 7-31-70
Storage tanks	0102	16	15
Pumps	0204	13	15
Homogenizers	0402	3	3
Transportation tanks	0506	19	18
Piping fittings	0809	17	18
Thermometer fittings	0902	1	2
Filters	1002	1	1
Plate type heat exchangers	1102	7	7
Tubular heat exchangers	1202	3	3
Farm bulk milk tanks	1303	17	16
Leak-detector plug valves	1401	4	4
Evaporators	1604	6	6
Fillers and sealers	1702	5	7
Freezers	1901	2	2
Silo-type storage tanks	2201	8	8
Packaging equipment	2300	3	3
Batch pasteurizers	2400	7	7
Batch processors	2500	6	6
Dry milk sifters	2600	5	5
Dry milk packaging equipment	2700	0	0
Total		143	146

NEWS & EVENTS

DAIRY AND FOOD INDUSTRY SUPPLY ASSOCIATION 52nd ANNUAL MEETING

Three new members and three incumbents were elected to the board of directors of Dairy & Food Industries Supply Assn. at its March 24-26 annual meeting in San Diego.

Elected to the 25-man board as At-Large Directors were: W. Gordon Cousins, Jr., Dairy Division Manager, Southern Biscuit Co., Richmond, Va.; and Robert Walker, Walker Stainless Equipment Co. President, New Lisbon, Wisc. G. F. "Lefty" Barnum, marketing manager of Sybron Corp.'s Taylor Instrument Process Control Division, Rochester, N. Y., was chosen as director for the Processing & Handling

Equipment & Components Commodity Group.

Incumbents re-elected for additional three-year terms were: Ralph F. Anderson, chairman of the board of Anderson Bros. Mfg. Co., Rockford, Ill. — At-Large; Gordon A. Houran, vice-president, sales, Milk & Food Equipment Division, The DeLaval Separator Co., Poughkeepsie, N. Y. — At-Large; and James H. Brunt, Jr., sales manager, Hackney Bros. Body Co., Wilson, N. C. — Delivery Commodity Group.

G. L. HUFFMAN RECEIVES DFISA HONOR PLAQUE

George L. Huffman, former president of Dairy & Food Industries Supply Assn., received the Association's Honor Plaque March 24 at the annual meeting in San Diego. Mr. Huffman, vice-president of the Packaging Equipment Group of Ex-Cell-O Corp., Detroit, was the 18th member to receive the honor in the organization's history.

Universal and dedicated service are requisites for the award, which was presented by another president, Fred M. King, marketing director of BASF Wyandotte Corp., Wyandotte, Mich.

Mr. Huffman was cited for his 15 years on the board of directors (since 1956), one year as vice-president (1965), two years as president (1966-67) and activities on numerous Association committees.

A graduate of the University of Detroit, Mr. Huffman joined Ex-Cell-O in 1934 and has risen through the engineering and sales ranks to his present position. He was elected chairman of the board of Dairy Society International last year, having served since 1961, and is a former director of National Dairy Council and National Assn. of Food & Dairy Equipment Mfrs.

Other honor plaque recipients still active in the industry and DFISA are Paul Girton, Girton Mfg. Co.; Sid Crofts, Johnson Truck Bodies; Donald Colony, Gaulin Corp.; Roy Cairns, Waukesha Foundry; and Fred King, BASF Wyandotte Corp.

ROBERTS EVERETT HONORED AS DFISA LEADER

Roberts Everett, leader of the Dairy & Food Industries Supply Assn. for its first 41 years of existence, was commended for his dedicated service to the industry at the Association's March 24-26 annual meeting.

From 1919 until 1960, his guiding hand, first as secretary-manager and then as executive vice-president, was strongly felt. He has served as secretary in a consulting capacity since 1960, but will go into full retirement June 30.

"RE", as he was fondly known, received an antique silver muffin warmer and a certificate of appreciation on behalf of the members from former president Paul Girton, Girton Mfg. Co. president.

Praising the former newspaperman for "his sagacity, tempered judgment, foresight, perseverance, devotion and loyalty," the certificate noted that Mr. Everett "be remembered as one of the all-time important men in the dairy industry, and that the tradition of integrity and strength that he initiated continue to be the cornerstone for the future of the Association."

Mr. Everett's influence on the Association and the Dairy Industries Exposition, now Food & Dairy Processing Expo, which he helped shape, will linger for many years. He was also instrumental in forming the 3-A Sanitary Standards program in the 1930's and starting Dairy Society International in 1946.

An Oberlin College and Columbia University Journalism graduate, he celebrated his 77th birthday on March 17.

FLORIDA DAIRY FOOD PROCESSORS URGED TO CONFRONT QUALITY CONCERNS

DR. C. BRONSON LANE
Department of Dairy Science
University of Florida, Gainesville, Fla. 32601

Two hundred dairy industry representatives, meeting in Orlando for the First Annual Florida Dairy Food Industries Conference, were told that Florida's dairy food industry has launched a drive urging consumers to become 'partners in quality' to assure a continuing supply of fresh, palatable dairy products.

Speaking to an overflow audience at the Orlando Robert Meyer Motor Inn, Joe Antink, Executive Director of the Florida Dairy Products Association, outlined the initiation of a massive consumer education program. He said that attractive colored time-temperature charts on optimum storage conditions for milk and milk products have been distributed to food store managers, school lunch program employees, and consumers. "In addition," he continued, "the recommended storage temperature data will be printed on milk carton side panels, resulting in thousands of exposures." Results of a study by the University of Florida Dairy Science Department concluding that hot cars and cold milk don't mix were also given wide exposure in the mass media. The report stated that with Florida's almost perpetually warm weather, keeping quality of milk can be significantly decreased if stored in the shopper's car even for short periods of time.

"We must inform the consumers of the important role they play in the final stage of product handling to assure maximum satisfaction," Antink concluded.

Jim Smathers, Field and Quality Director for the

Participants on the First Annual Florida Dairy Food Industries Conference program included: Mr. Ed Sing, Moseley Laboratories, Indianapolis, Indiana; Joe Antink, Florida Dairy Products Association, Orlando, Florida; Dr. C. Bronson Lane, University of Florida, Gainesville, Florida; Dr. Bruce Langlois, University of Kentucky, Lexington, Kentucky.

Pete Sedler, Sealtest Food, Miami, Florida discusses his luncheon presentation at the First Annual Florida Dairy Food Industries Conference with Mel Neff, Upper Florida Milk Producers Association, Jacksonville, Florida.

Maryland and Virginia Milk Producers Association, conveyed the procedures his organization follows to assure the production of top quality raw milk. He said that standard plate counts, thermoduric counts, somatic cell counts, sediments, antibiotic tests, and direct microscopic analyses are performed on every producer's milk each month. "Continued surveillance of tank truck milk helps us troubleshoot quality problems," he stated. Smathers concluded by saying that it is the coop's responsibility to obtain odor-free and long shelf-life milk.

Dr. Bruce Langlois, an Associate Professor in the Animal Science Department at the University of Kentucky, urged the processors to prevent dairy product dropouts, those products that are spoiled by the time they reach the consumer's refrigerator. Langlois suggested that each plant establish a systematic program for testing the potential shelf-life of its products. "Too often," he said, "processors don't realize they have spoilage or off-flavor problems and the buyers who get stung don't complain, but merely switch brands." The dairy scientist discussed some of the available laboratory tests for determining shelf-life of the products, and highly recommended the Moseley testing procedure.

Edmond L. Sing, Executive Director of Moseley Laboratories, Indianapolis, Indiana, told the dairymen what to look for when troubleshooting quality problems in their plants. He said that psychrophile problems still persist and that these organisms might enter the product from leaky air valves, pipe joints, dead end lines, and filler condensate. Sing also cautioned the processors not to add fresh milk to that left over in silo tanks and to pay particular attention to the amount of air agitation in these tanks. "Too much air pressure can cause rancidity problems," he stated.

Ernest Glaser, Vice-President of Avoset Food Corporation, Oakland, California, related that the aseptic and sterile packaging of dairy food products has a tremendous potential. Glaser reviewed the development of these packaging systems, spelled out the regulatory problems confronting long shelf-life packaging procedures, and stated that the high cost of these processes will cause diversification into other product lines such as sauces and puddings. "Sterilized speciality dairy products has allowed our industry to get back on the grocery shelf and has caused the consumer to switch from non-dairy products back to the real thing," he said.

Earl Kimsey, Market Promotion Specialist for the American Dairy Association, Chicago, Illinois, stated, "The retail level is the most crucial point for our product, because this is when we in the dairy industry lose direct control of our milk and dairy products; however, we still have a responsibility to work with the retailer." Kimsey then discussed the results of an ADA conducted survey of grocer executives which showed the grocers wanted their personnel to be trained in product rotation and spoilage prevention, maintenance of a clean dairy case, allocation of products, and proper use of dairy equipment facilities. "ADA has met this challenge," Kimsey continued, "and established a seminar for dairy department management personnel in the supermarkets. In working with grocers through this program, we will not only encourage the importance of fresh quality products—but will show what effect the quality of products

have on grocery store sales and profits."

E. L. Szabo, Quality Control Specialist, Winn-Dixie Stores, Jacksonville, Florida, stated that shoppers desire neat, trim, and clean surroundings. "Her buying decisions are influenced by the store environment," he stated, "and we must strive to establish good housekeeping programs in all of the departments. Our employees are motivated to do just this, and are taught how to handle consumer complaints in a cool, tactful, and courteous manner."

Dr. C. Bronson Lane, Associate Professor of Dairy Science at the University of Florida summarized the conference by listing some solutions for survival. Lane stated that the dairy foods industry must guarantee long shelf-life dairy products, become more involved in consumer education programs, rectify plant sanitation problems, spend more money for new product development and promotional programs, deliver high quality milks to schools and institutions, and help topple some legislative barriers which are restricting new product development.

The conference was jointly sponsored by the Florida Dairy Products Association and the University of Florida Dairy Science Department. Program chairmen were Jim Beatty, Farmbest, Inc., Jacksonville, Florida and Dr. Leon Mull and H. H. Van Horn, University of Florida, Gainesville, Florida. Pete Sedler, Sealtest Foods, Miami, was the luncheon speaker. He told of his dramatic escape from East Germany by swimming the Baltic Sea. A standing ovation greeted the cultured products specialist at the conclusion of his presentation.

LITTER-PREVENTION TEACHING MANUAL BEING DISTRIBUTED

A new guide for elementary teachers, "Litter Prevention . . . A First Step To Improving The Environment," published by Keep America Beautiful, Inc., is now being used in schools in 46 states and the District of Columbia.

The new handbook is an updated and expanded version of an earlier teacher's aid developed by KAB, "Nobody Loves A Litterbug," which was released in 1967. It gives over 30 suggestions for classroom, school-wide and community-wide anti-litter projects which can be supplemented with other activities developed by teachers and pupils.

Keep America Beautiful, Inc., which is the national public service organization for the prevention of litter, furnished copies of the guide, in quantity, to state departments of education or state litter-prevention groups. These organizations then distributed them to all public and private schools in their state.

Distribution in (name of state) was made by (name of organization), and should be contacted if your school has not yet received copies.

"Teachers can do much to inculcate in students a sense of responsibility for improving the quality of the environment," said Allen H. Sneed, Jr., executive vice president of Keep America Beautiful. The handbook is part of KAB's continuing public-service effort to meet the growing need of educators for material which can stimulate student interest in helping solve pollution problems. And litter is one problem they can each do something about!

The manual was developed in cooperation with a special advisory committee made up of members of the National Education Association, the National Catholic Educational Association, the American Federation of Teachers, the National Congress of Parents and Teachers, and other educational environmental organizations.

According to a survey of elementary school principals conducted by KAB in 1967, the majority replying said litter was a problem at school. Over 71 per cent reported they had litter-prevention programs in operation, and 69 per cent said such programs were successful in reducing littering among children.

Since that time, KAB has expanded its youth education program to provide teachers and youth-group leaders with material to help teach litter control to young people.

KAB has also developed a new 16½-minute, sound and color anti-litter film, "The Litter Monster," for elementary-school-age children. It shows litter-prevention projects being conducted by youngsters in California, Colorado, Connecticut and Maryland, and encourages young people everywhere to "look around" to see what they can do about litter in their own communities. The movie is open-ended to promote discussion. Prints of the 16mm film are available from Alfred Higgins Productions, 9100 Sunset Boulevard, Los Angeles, California 90069, for $110 each.

GMPs FOR FOOD HANDLERS

A new educational audio-visual "GMPs For Food Handlers" available from the National Canners Association Research Foundation, 1950 Sixth Street, Berkeley, Calif. 94710.

The A-V kit price of $30.00 is to cover the cost of duplicating and handling sets and is made possible by the partial support of the production costs by the Henry L. Guenther Foundation. The Guenther Foundation has as its prime objectives the financing of research and education in the fields of food technology, processing and packaging.

IFT ANNOUNCES '71 ANNUAL MEETING

The 31st Annual Meeting and Exposition of the Institute of Food Technologists will be held on May 23-27, 1971, at the Americana Hotel in New York City. The program is being built around the theme, "New York—New Ideas."

Host for the meeting is the New York Section of IFT which has named E. E. Alt, Jr., CPC International, as the Chairman of the General Arrangements Committee. Program Chairman is Dr. William J. Hoover, Kansas State University, Manhattan, Kansas, and the Exhibitors Advisory Committee Chairman is H. B. Rogers, Nestle Co., White Plains, N. Y.

Advance programs and registration forms will be available on request during March. Exhibitors brochures are available on request right now. Over 85% of the Exposition space is already committed.

Requests for exhibitors brochures and advance programs should be addressed to D. E. Weber, Director of Convention Services.

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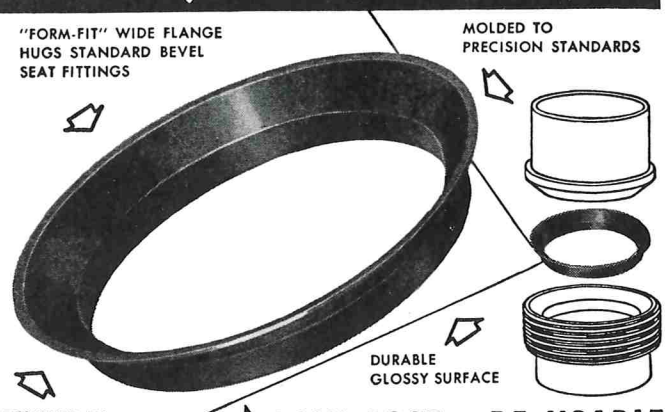

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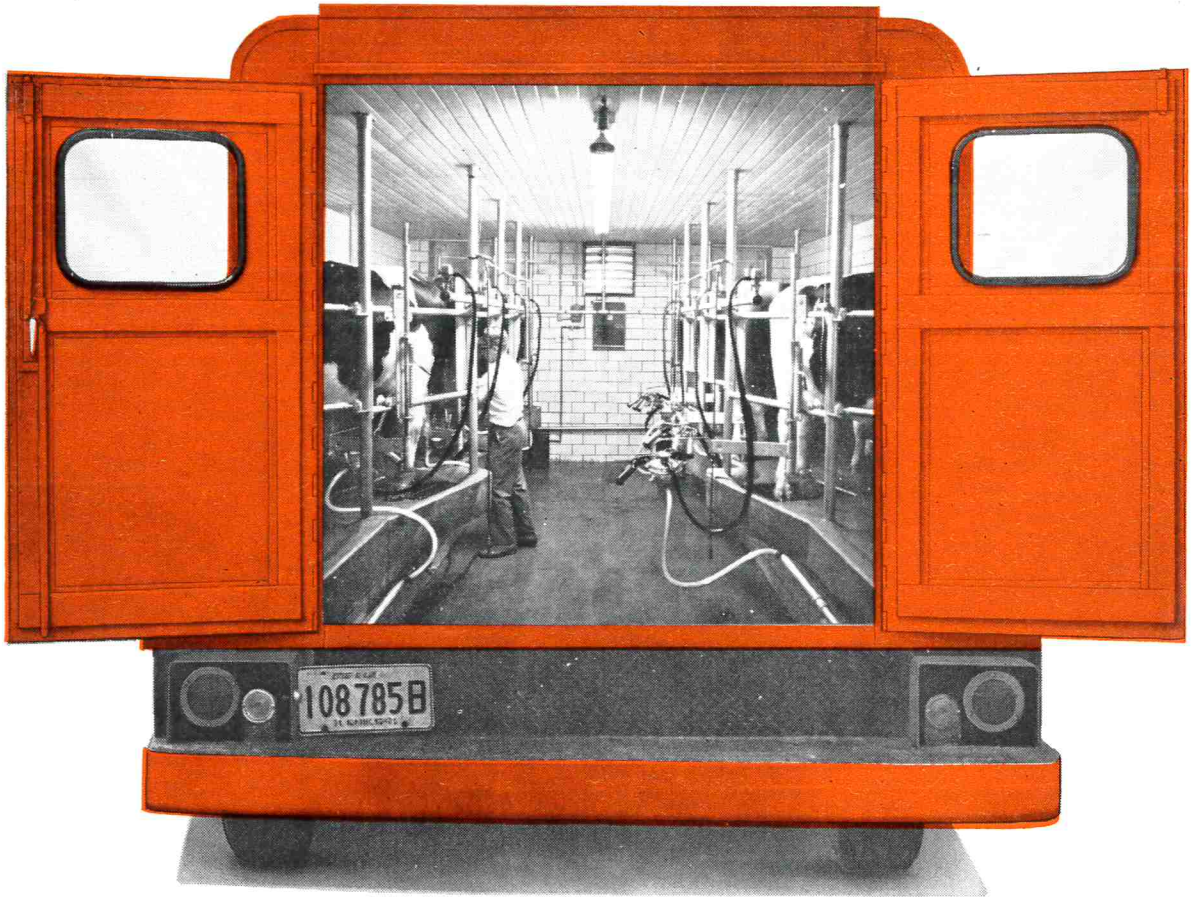

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