

AUGUST, 1964

Vol. 27

P. 227-258

No. 8

Journal of

MILK and FOOD TECHNOLOGY

Official Publication

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



products for examination of water and wastewater

Media and Materials for Routine Use, various / Media for Additional Procedures, various / KILIT Ampules (Autoclave Controls) / KILIT Sporestrips #1 (Autoclave Controls) / FALCON Petri Dishes and other plastic products / FALCON Plastic Pipets

check your water

B-D LABORATORIES provides all materials required for examination of **WATER AND WASTEWATER** by standard methods. Thirteen special media are also available for additional procedures. And **FALCON** sterile, disposable plastic labware is convenient and efficient for use in all microbiological procedures. Lot Control Number on every case assures sterility.

Products of **B-D LABORATORIES** are available through your local distributor. Further information on request.



B-D LABORATORIES, INC., RUTHERFORD, NEW JERSEY



Baltimore
Biological
Laboratory



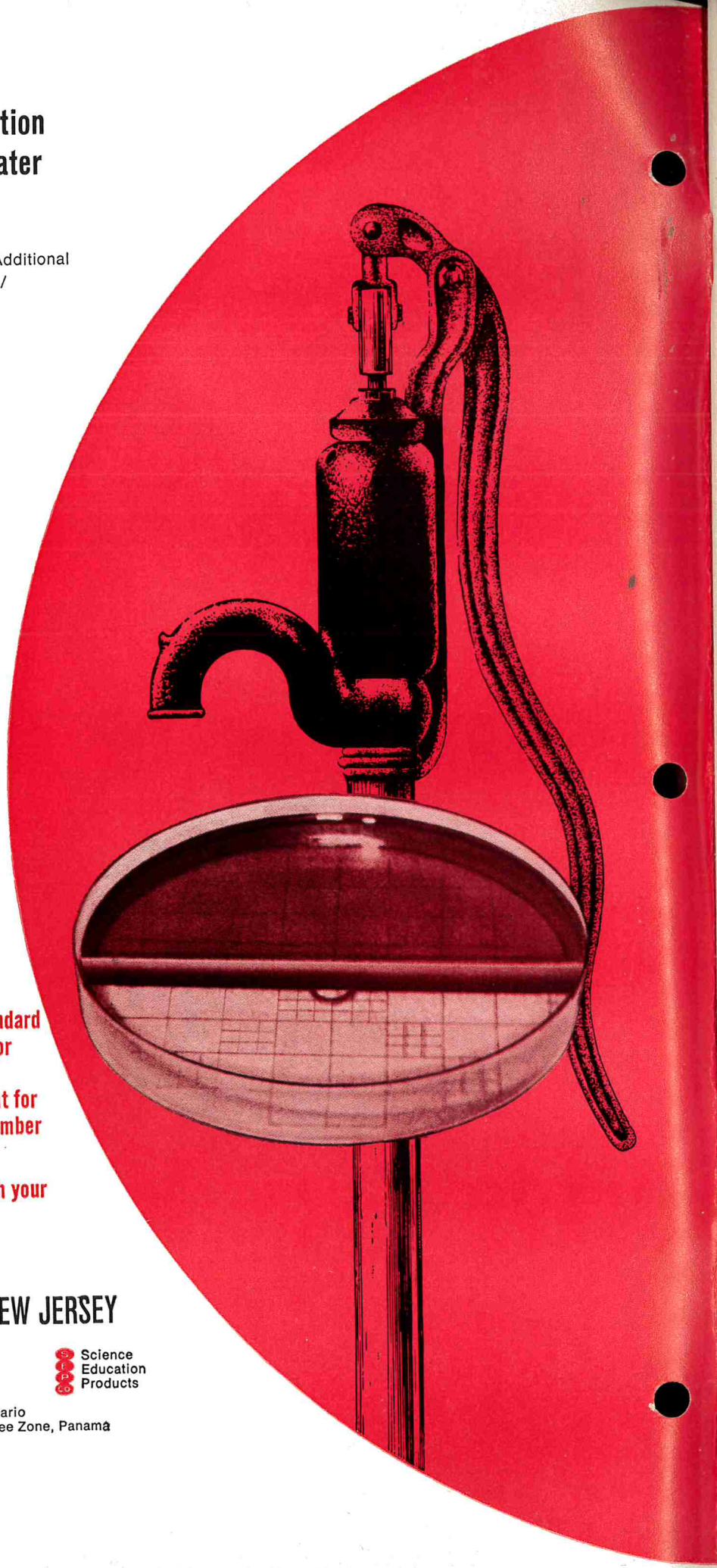
Falcon
Plastics



Science
Education
Products

In Canada: Becton, Dickinson, & Co., Canada, Ltd., Clarkson, Ontario
Overseas: Becton, Dickinson & Co., S.A., P.O. Box 1173, Colon, Free Zone, Panama

B-D, B-B-L, FALCON, KILIT AND SEPICO ARE TRADEMARKS. 06864





from American Can...

The strongest, most sanitary ½-gallon closures on the market

American Can's exclusive **TUFFY Form-N-Fill® Machine** for TUFFY plastic-coated, ½-gallon milk cartons was not designed to just meet sanitary standards — but engineered to surpass them.

It forms, sterilizes, fills and seals containers automatically. Throughout the entire operation, the cartons are touched only by sterile equipment and filtered air.

An instant before filling, ultraviolet light sterilizes the inside of the containers. The unique bottom filling design minimizes the possibility of contamination during fill by eliminating foaming. Filtered, heated air is directed only to areas to be sealed

at the top and bottom of the carton. The result is a ½-gallon carton that sets new sanitary standards . . . a ½-gallon milk container with the most sanitary closure on the market.

The TUFFY carton is 5-ply, plastic laminated for extra strength and protection. Both bottom and platform top (with supplementary adhesive) are stronger than any other comparable carton closures. TUFFY's unique "Flexi-Spout" guards against contamination when the milk is poured.

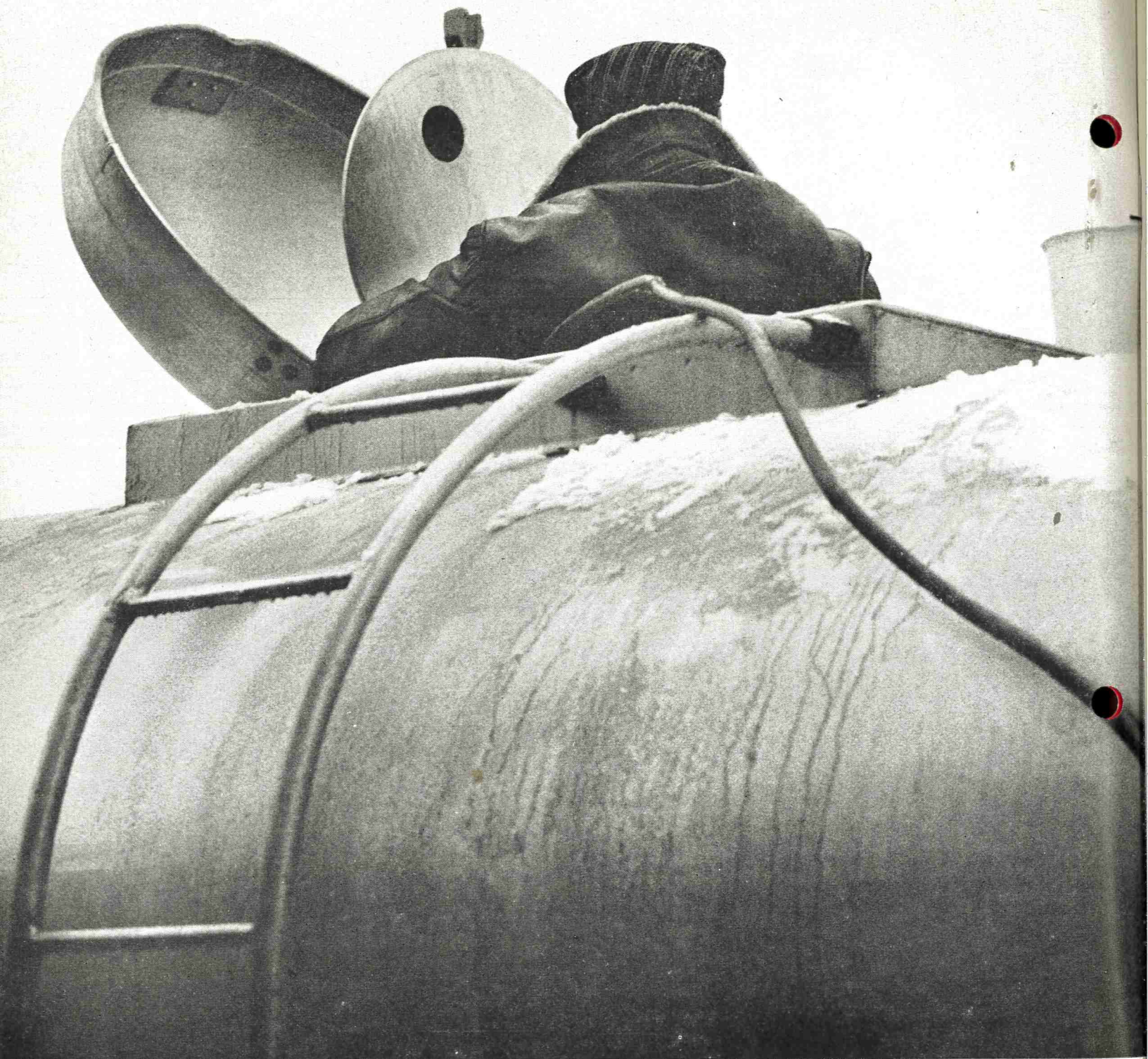
A TUFFY carton, always formed and filled by a TUFFY Form-N-Fill, is a SUPER-sanitary package. Another advance from American Can.

MILK CONTAINER *Division*

AMERICAN CAN COMPANY

100 Park Avenue, New York 17, New York





LO-BAX® is an OMCC trademark.

You can get in there and scrub it, scour it, scald it. But by the time you're ready to use it, those invisible little buggers are crawling all over it again.

Is there anything you can do about that?

There are two possibilities.

Scrub it, scour it and scald it again.

Or, immediately before you use any equipment, apply a Lo-Bax® Special solution to every part that comes in contact with the milk. (A good idea for your farmers, too.) Lo-Bax will kill bacteria before they get a chance to do any contaminating. And it won't add taste, color or odor of its own or change the milk in any other way.

Consider this, too: Lo-Bax is easy to use. And it's cheap: a single 1¼-lb. bottle of Lo-Bax makes over 1000 gallons of sanitizing solution. Now decide.

CHEMICALS DIVISION **olin**
HYPOCHLORITE PRODUCTS, 745 FIFTH AVE., NEW YORK, N.Y. 10022

OFFICERS

President, JOHN H. FRITZ, Washington, D. C.

President-Elect, WALLACE C. LAWTON, Minneapolis, Minn.

First Vice-President, FRED E. UETZ, West Englewood, N. J.

Second Vice-President, PAUL R. ELLIKER, Corvallis, Oregon

Secretary-Treasurer, KARL K. JONES, Indianapolis, Ind.

Executive Board

CHARLES E. WALTON

RAY A. BELKNAP

JOHN H. FRITZ

WALLACE C. LAWTON

FRED E. UETZ

PAUL R. ELLIKER

KARL K. JONES

Publication Board

DR. J. C. OLSON, JR. H. L. THOMASSON
KARL K. JONES

Editors

DR. J. C. OLSON, JR., *Associate Editor*,
Dept. Dairy Industries, University
of Minn., St. Paul 1, Minn.

H. L. THOMASSON, *Executive Secretary
and Managing Editor*, Box 437,
Shelbyville, Indiana.

Associate Editors

C. A. ABELE.....Chicago, Illinois

H. S. ADAMS.....Indianapolis, Indiana

M. P. BAKER.....Ames, Iowa

F. W. BARBER.....New York, New York

F. C. BASELT.....Monte Sereno, Calif.

L. A. BLACK.....Cincinnati, Ohio

J. C. FLAKE.....Chicago, Illinois

L. G. HARMON.....East Lansing, Mich.

E. K. HARRIS.....Cincinnati, Ohio

R. P. HAYWARD.....Bowie, Md.

C. A. HUNTER.....Topeka, Kansas

C. K. JOHNS.....Ottawa, Ontario, Canada

O. W. KAUFMANN.....East Lansing, Mich.

W. C. LAWTON.....St. Paul, Minnesota

W. S. MUELLER.....Amherst, Mass.

K. G. WECKEL.....Madison, Wisconsin

J. C. WHITE.....Ithaca, New York

The Journal of Milk and Food Technology is issued monthly beginning with the January number. Each volume comprises 12 numbers. Published by the International Association of Milk, Food and Environmental Sanitarians, Inc. with executive offices of the Association, Blue Ridge Rd., P. O. Box 437, Shelbyville, Ind.

Entered as second class matter at the Post Office at Shelbyville, Ind., March 1952, under the Act on March 3, 1879.

EDITORIAL OFFICES: J. C. Olson, Jr., Associate Editor, Dept. Dairy Industries, University of Minn., St. Paul, Minn.; H. L. Thomasson, Managing Editor, P. O. Box 437, Shelbyville, Ind.

Manuscripts: Correspondence regarding manuscripts and other reading material should be addressed to J. C. Olson, Jr., Associate Editor, Dept. Dairy Industries, University of Minn., St. Paul, Minn.

"Instruction to Contributors" can be obtained from the editor for the use of contributors of papers.

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.

Volume 27

August, 1964

Number 8

Contents

Editorial:

Internationalism 227

Relationship of Feulgen-DNA in Milk and of Milk Quality

Test (MQT) to the Number of Milk Somatic Cells

M. J. Paape, H. D. Hafs, and H. A. Tucker 228

Analysis of Animal Food Products for Chlorinated Insecticide

Residues. II. Some Factors Involved in Using

Electron Capture Gas Chromatography

A. R. Stemp, B. E. Langlois and B. J. Liska 231

The Quantification of Salmonellae in Foods Using

The Lactose Pre-enrichment Method of North

Herbert E. Hall, Daird F. Brown and Robert Angelotti 235

Effect of Time and Temperature of Incubation

on the Plate Counts of Dry Milk

Rafael Pedraja and Ainis Mengelis 241

Air-Borne Microorganism Populations

in Food Packaging Areas

D. R. Heldman, T. I. Hedrick, and C. W. Hall 245

News and Events 252

Index to Advertisers V

Classified Ads 259

Business Matters: Correspondence regarding business matters, advertising, subscriptions, orders for single copies, etc., should be addressed to H. L. Thomasson (address above).

Subscription Rates: One volume per year, individual non-members, Governmental and Commercial Organization subscription.

1 yr. \$8.00

Public and Educational Institution

Libraries, 1 yr. \$6.00

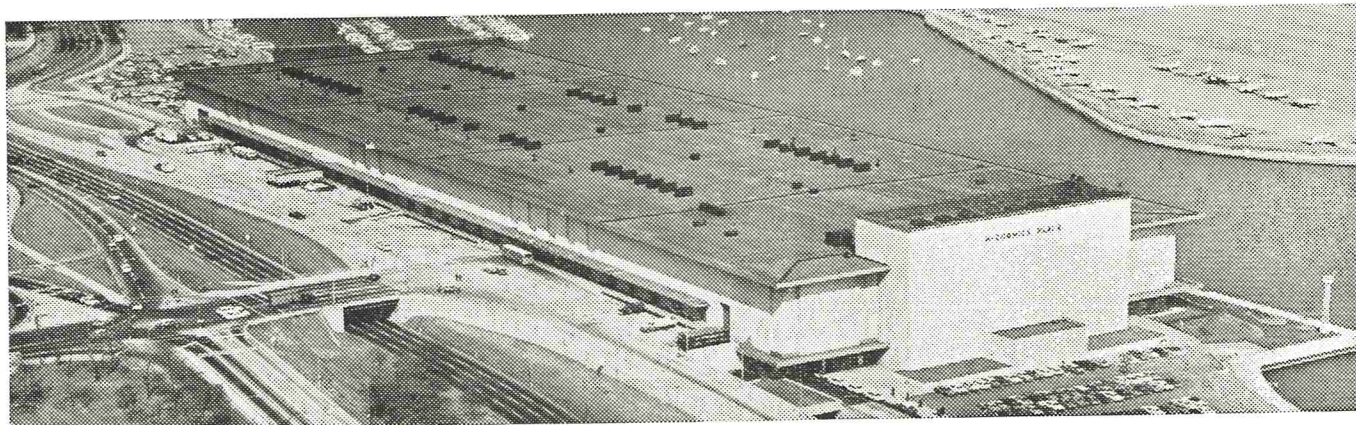
Single Copies \$1.00

Orders for Reprints: All orders for reprints

should be sent to the executive office of the Association, P. O. Box 437, Shelbyville, Ind.

Membership Dues: Membership in the International Association of Milk, Food and Environmental Sanitarians, Inc., is \$7.00 per year, which includes annual subscription to the Journal of Milk and Food Technology. All Correspondence regarding membership, remittances for dues, failure to receive copies of the Journal, changes in address, and other such matters should be addressed to the Executive Secretary of the Association, H. L. Thomasson, Box 437, Shelbyville, Indiana.

In the direct tradition of 23 great Dairy Industries Expositions IT'S NOW THE "ALL PROCESSES GO" SHOW!



THE DAIRY & FOOD INDUSTRIAL EXPOSITION OCTOBER 4-9, 1964 • MCCORMICK PLACE • CHICAGO, ILL.

ADMISSION FREE FOR

Dairy processors, food processors, distributors, marketers, transporters, scientific and technologic personnel, engineers, government officials, regulatory officers and sanitarians, educators and students.

ADMISSION FEES CHARGED TO

Representatives of non-exhibiting suppliers-equipppers.

The general public is not admitted.

BUS SERVICE

Just for dairy and food industry guests, private bus service will operate from major hotels to McCormick Place. Exposition may also be reached by public bus, subway, taxi, private car (aeres of parking lots).

FOR HOTEL ROOMS

If a member of a concurrently-convening association, use reservation forms provided by your association.

If not affiliated (and the majority of those attending are not members of any currently-meeting group), write Dairy and Food Housing, Chicago Convention Bureau, 332 S. Michigan Blvd., Chicago.

SHOW HOURS

Sunday, October 4 1 p.m.-5 p.m.
Monday, October 5 Noon-6 p.m.
Tuesday, October 6 10 a.m.-6 p.m.
Wednesday, October 7 10 a.m.-6 p.m.
Thursday, October 8 Noon-6 p.m.
Friday, October 9 10 a.m.-6 p.m.

ON DISPLAY

More than 300 exhibits featuring the latest and best in

- dairy and food processing equipment, including pasteurizers, homogenizers, freezers, evaporators, driers, cheese making equipment, tanks, vats, bottle washers, fillers and stackers, conveyors, and hundreds more
- cleaning and sanitizing equipment and systems
- delivery equipment, trucks, bodies, mobile refrigeration
- flavors, ingredients, stabilizers, additives
- containers of every variety and packaging equipment
- cabinets, soda fountains, venders, other point-of-sale equipment and aids
- specialized services and products, including architecture, financial, record keeping, advertising programs, and many more.

"FOOD IN THE FUTURE: Concepts for Planning"

A two-day seminar, set for October 8-9 at McCormick Place, will be open to all qualified to attend the Exposition. Meet with, discuss with the nation's top experts on technologic advance, economics, synthetics, microbiology, environmental adaptation, and more! Major meetings also planned throughout the Exposition week by DSI, IAICM, MIF, NICRA, EMA, DSF, NICMA, others!

List of 300-Plus Exhibitors, plus Detailed Floor Plan

Soon available from Exposition's sponsor.
For yours, drop postcard to

DAIRY AND FOOD INDUSTRIES SUPPLY ASSOCIATION

1145 19th STREET, NW, WASHINGTON, D.C. 20036



THE ONLY Approved
SANITARY METHOD OF APPLYING
A U. S. P. LUBRICANT
TO DAIRY & FOOD
PROCESSING EQUIPMENT

*Haynes
Spray*

U.S.P. LIQUID PETROLATUM SPRAY

U.S.P. UNITED STATES PHARMACEUTICAL STANDARDS

CONTAINS NO ANIMAL OR VEGETABLE FATS. ABSOLUTELY NEUTRAL. WILL NOT TURN RANCID—CONTAMINATE OR TAINT WHEN IN CONTACT WITH FOOD PRODUCTS.

SANITARY—PURE

ODORLESS—TASTELESS

NON-TOXIC

The Modern HAYNES-SPRAY Method of Lubrication
Conforms with the Milk Ordinance and Code
Recommended by the U. S. Public Health Service

The Haynes-Spray eliminates the danger of contamination which is possible by old fashioned lubricating methods. Spreading lubricants by the use of the finger method may entirely destroy previous bactericidal treatment of equipment.

PACKED 6-12 oz. CANS PER CARTON
SHIPPING WEIGHT—7 LBS.

THE HAYNES MANUFACTURING CO.
4180 Lorain Avenue • Cleveland 13, Ohio

HAYNES-SPRAY INGREDIENTS CONFORM WITH FDA REGULATIONS AND CAN BE SAFELY USED AS A SANITARY LUBRICANT FOR FOOD PROCESSING EQUIPMENT WHEN USED IN COMPLIANCE WITH A EXISTING FOOD ADDITIVES REGULATION.



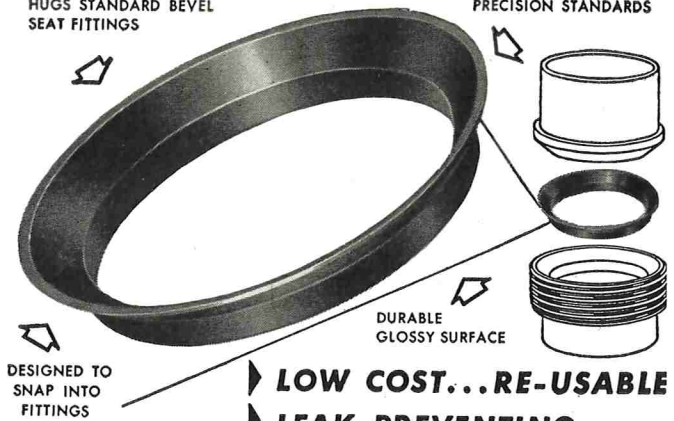
This Fine
Mist-like
HAYNES-SPRAY
should be used to lubricate:

SANITARY VALVES
HOMOGENIZER PISTONS — RING
SANITARY SEALS & PARTS
CAPPER SLIDES & PARTS
POSITIVE PUMP PARTS
GLASS & PAPER FILLING
MACHINE PARTS
and for ALL OTHER SANITARY
MACHINE PARTS which are
cleaned daily.

HAYNES SNAP-TITE GASKETS

"FORM-FIT" WIDE FLANGE
HUGS STANDARD BEVEL
SEAT FITTINGS

MOLDED TO
PRECISION STANDARDS



NEOPRENE GASKET for Sanitary Fittings

Check these **SNAP-TITE** Advantages

fight joints, no leaks, no shrinkage	Time-saving, easy to assemble
Sanitary, unaffected by heat or fats	Self-centering
Non-porous, no seams or crevices	No sticking to fittings
Odorless, polished surfaces, easily cleaned	Eliminate line blocks
Withstand sterilization	Help overcome line vibrations
	Long life, use over and over

Available for 1", 1½", 2", 2½" and 3" fittings.

Packed 100 to the box. Order through your dairy supply house.

THE HAYNES MANUFACTURING CO.
4180 Lorain Avenue • Cleveland 13, Ohio

INDEX TO ADVERTISERS

American Can Co.	I
Babson Bros. Co.	Back Cover
Baltimore Biological Laboratories, Inc.	Inside Front Cover
Cowles Chemical Co.	VII
Dairy and Food Industry Supply Ass'n.	IV
Difco Laboratories	257
Garver Mfg. Co.	257
Johnson & Johnson	VIII
Klenzade Products, Inc.	VI
Olin Mathieson Chemicals	II
Perfection Division, Sta-Rite Products, Inc.	258
Sep-Ko Chemicals	260
The Haynes Mfg. Co.	V
U. S. Stoneware	Inside Back Cover

A HEAVY DUTY SANITARY LUBRICANT



Available in both

SPRAY AND TUBE

All Lubri-Film ingredients are approved by F.D.A. and can be safely utilized as a lubricant for food processing equipment when used in compliance with an existing food additive regulation.

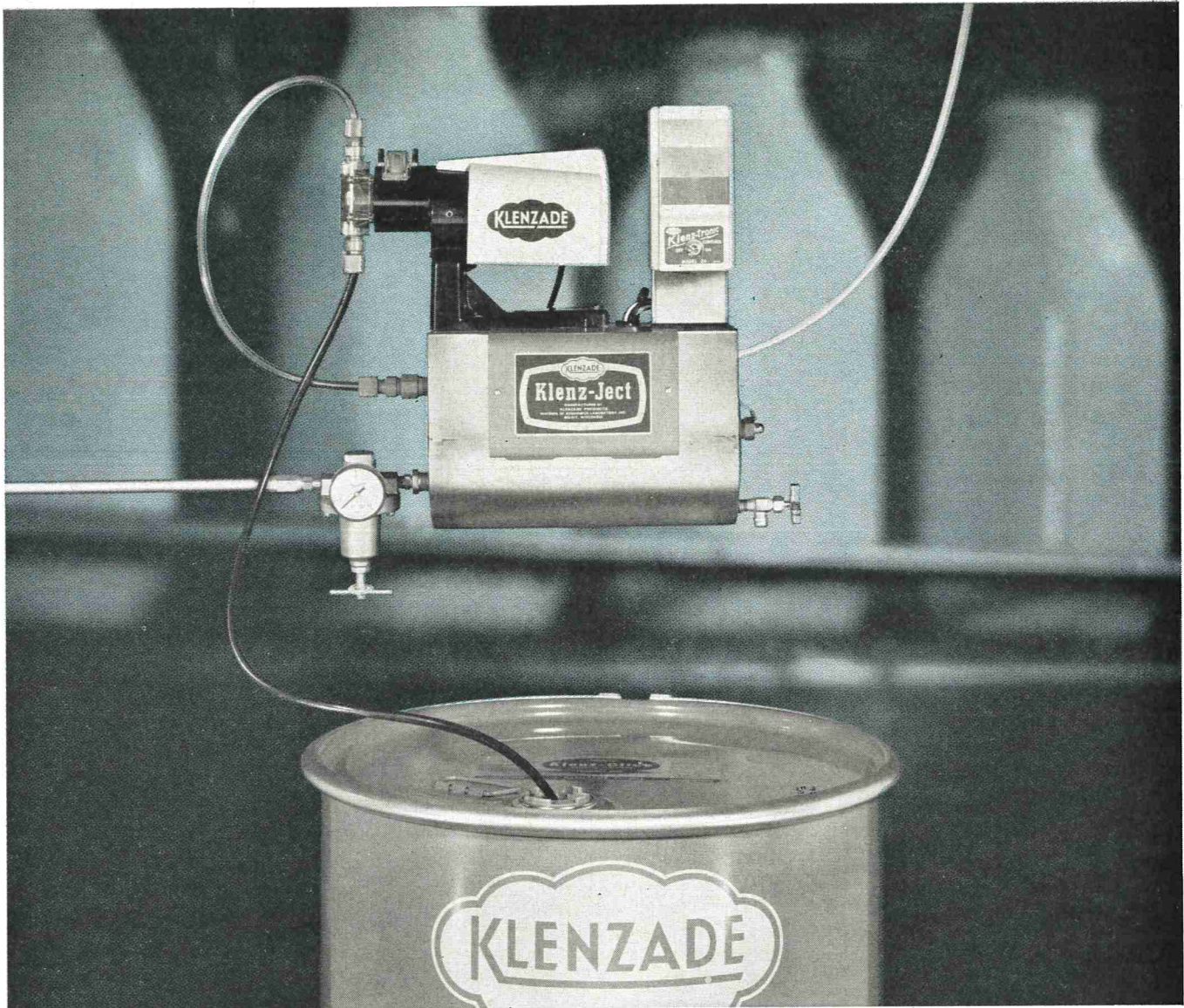
ESPECIALLY DEVELOPED FOR LUBRICATION OF FOOD
PROCESSING AND PACKAGING EQUIPMENT

For Use in Dairies — Ice Cream Plants — Breweries —
Beverage Plants — Bakeries — Canneries — Packing Plants

SANITARY • NON TOXIC • ODORLESS • TASTELESS

SPRAY — PACKED 6 — 16 OZ. CANS PER CARTON
TUBES — PACKED 12 — 4 OZ. TUBES PER CARTON

THE HAYNES MANUFACTURING CO.
CLEVELAND 13, OHIO



NEW ECONOMY...NEW EFFICIENCY NEW QUALITY PROTECTION FOR YOUR DAIRY PRODUCTS

Completely control chain conveyor lubrication with the automatic Klenz-Ject Central System. Klenz-Ject economically delivers a continuous spray of Klenz-Glide lubricant at optimum monitored rate. Single unit services up to fifty dispensing points. Does away with costly, laborious premixing and handling. Eliminates gumming, odor build-up and container contamination due to sporadic maintenance or the use of lubricants which do not have the detergent, controlled

foaming, rust inhibiting properties of Klenz-Glide. Lengthen conveyor life and sharply reduce conveyor maintenance cost by eliminating excessive friction due to inadequate or infrequent lubrication. Field-tested and field-proven under extremely varied conditions, the Klenz-Ject Central System is already achieving substantial savings in milk processing plants of all types and sizes. Installation is simple, Klenzade provides technical assistance.

Why milk processors spell clean with a "K"

When you add the "K" from Klenzade, you solve all your sanitation problems with the Klenzade Complete Plant Quality Program which includes all the necessary detergents, sanitizers and equipment from one dependable source. For optimum efficiency and economy, a Klenzade technical representative will help you solve your cleaning and sanitizing problems and best protect the consistent quality of your dairy products.



Division of Economics Laboratory, Inc.

For Completeness, Quality and Cost-Cutting, it's KLENZADE PRODUCTS Dept. 328, Beloit, Wisconsin

INTERNATIONALISM

To people who are associated through international agreement, action, or pact, there is implicit some type of co-operation or mutual assistance, liaison and coordination of effort by the persons concerned in the two or more countries involved. Under such conditions, this may be termed "Internationalism" in action. Can there be a difference in what is implied when we attach "International" to the title of an association?

One of Webster's definitions of the word "International" reads, ". . . common to or affecting two or more nations." When the adjective is part of the title of an association or organization, this definition, obviously, must be expanded to "common to or affecting the association members of two or more countries".

If this concept of meaning be accepted, we must also accept as an obligation entailed by the use of the adjective, the functions of such internationalism, as stated in the first paragraph.

With some associations and organizations, the use of the word "International" in the title lends a certain amount of prestige. This, they realize and pay little more than lip service to obligations entailed. A different situation is apparent when the International Association of Milk, Food and Environmental Sanitarians Inc., is considered.

The activities in which the I.A.M.F.&E.S. membership is engaged lend themselves to the broadest practice of the functions of Internationalism. This has not been forgotten by the association. This writer can speak only of the condition of internationalism that exists between the association, its membership and the membership in Canada.

In recognition of the obligation of internationalism during its history, this association has elected one Canadian as its president; it has held two annual conventions in Canada. Canadians have been appointed to some of its standing committees. Presently one Vancouver member and three Ontario members are members of current committees. Saskatchewan's William Kempa was the winner of the association's highest annual award for outstanding service. Canada's Dr. C. K. Johns, a past president, was honoured with a life membership.

Every member has the opportunity, at annual conventions, of gaining knowledge in his particular field, from the continent's outstanding authorities, and from the reports of the committees. The membership is kept informed of technological and scientific advances, and of the work being done in these spheres of endeavour in each issue of this splendid journal.

Without discounting the tremendous importance of what is being done, is it inappropriate to ask whether all the functions entailed by the use of the term "International" is being fulfilled.

It is realized that any association must cut its cloth according to its wool. Finances are the bane of many organizations and much effort and action is limited through lack of funds. However, anyone who listened to Ray Belknap, 1963 president, speaking at the annual meeting in Toronto, will realize there is much work to be done, all of which has a bearing on Internationalism. Mr. Belknap rightly deplored the lack of interest, a rather tardy approach and a display of little interest by some of the membership in association affairs. This is discouraging to the leaders of an organization particularly when the apathetic condition seems apparent among committee members. When there is enthusiastic interest in the association's work, there is also interest in its international aspects.

Of the four Canadian Committee members, one is on a committee whose work is definitely outside of his normal field. It is unavoidable that, in naming up to 150 or more members of committees, some round pegs will be found in square holes. However, through careful selectivity, an ever reducible minimum will be found which will be conducive to greater interest, and greater effort and hence, an even greater international association.

On the other hand it is a happy circumstance, to find a Canadian member on the Membership Committee. There is need for much work in Canada in this field. To meet the obligations of internationalism a strong membership is not only desirable but necessary.

We, in Canada, feel that our country can make a contribution to the work and the advancement of the association. Contribution has been made in the past, but it can be greater. However, the channels of liaison, cooperation and mutual assistance must be clearly defined, and regularly employed. If this condition is established, the professional status of sanitarians in both countries can be further enhanced, and all our peoples will benefit.

Lest there be any smug complacency among Canadian members, let it be understood that every word in all of the foregoing is as applicable to them as to anyone else; that their obligation is equal to that of other sections of the membership.

COLIN L. BRETHER, *Editor*
Ontario Milk Producer
Toronto, Ontario

RELATIONSHIP OF FEULGEN-DNA IN MILK AND OF MILK QUALITY TEST (MQT) TO THE NUMBER OF MILK SOMATIC CELLS¹

M. J. PAAPE, H. D. HAFS, AND H. A. TUCKER

Department of Dairy, Michigan State University, East Lansing

(Received for publication December 29, 1963)

SUMMARY

The relationships of Feulgen-DNA determinations and Milk Quality Test (MQT) score with the concentration of milk cells were determined from 75 milk samples. The correlation of the number of each cell type with Feulgen-DNA score was consistently higher than with MQT score, but the highest correlation of 0.876 between Feulgen-DNA scores and total cell numbers accounted for 77% of the total variation. Plotting the observed values revealed a linear relationship when both variables were transformed to logarithms. The correlation of the logarithms of the coded Feulgen-DNA scores with the logarithms of total cell numbers was 0.909. The coefficient of variation for Feulgen-DNA scores was 7%, much less than the 32% for the microscopic estimations of cell concentration. These data indicated that Feulgen-DNA scores may be at least as accurate in estimating milk cell numbers as microscopic estimations.

Although direct microscopic leucocyte counts have been generally regarded as being superior to other laboratory tests for diagnosing inflammation of the mammary gland (1, 8), they are relatively expensive in terms of the necessary manpower for widespread practical mastitis testing. Furthermore, Paape et al. (5) determined that the error variation associated with milk cell counts was large and suggested that ways of reducing the error variance should be investigated.

Since the deoxyribonucleic acid (DNA) of milk somatic cells was responsible for the gel formation in the California Mastitis Test (CMT) (10) reaction (2, 6), Paape et al. (6) adapted the DNA-specific Feulgen reaction (3) for detecting udder inflammation. This modified Feulgen-DNA test involved color development, the intensity of which was presumably proportional to the concentration of somatic cells in milk.

The purpose of the present research was to determine the reliability of the Feulgen-DNA test and of the milk quality test (MQT) in estimation of milk somatic cell numbers.

EXPERIMENTAL PROCEDURES

Seventy-five quarter milk samples were obtained from Holstein cows in various stages of lactation. Duplicate MQT (4, 7) and duplicate Feulgen-DNA (6) determinations were performed as indicators of

the milk cell concentration within 30 min after obtaining each milk sample. The MQT was used according to the manufacturer's recommendation with the exception that inflammation scores of 0, 1, 2, 3, and 4 were substituted for 0, T, 1, 2, and 3, respectively, to facilitate statistical analysis. Milk samples were selected at random until 15 were obtained for each MQT score. Feulgen-DNA determinations were performed according to the following procedure:

1. Each milk sample (2 ml) was hydrolyzed with an equal amount (2 ml) of 1 N HCl at 60 C for 24 min.

2. Schiff's reagent (4 ml) was then added in an amount equal to twice the volume of milk. Schiff's reagent was prepared by dissolving 1.0 g of basic Fuchsin² in 200 ml boiling distilled water, cooling to 50 C, adding 20 ml of 1 N HCl, cooling to 25 C, and dissolving 2.0 g of sodium metabisulfite. This solution was stored in the dark at room temperature for 10-15 hr and then 0.5 g activated charcoal was added, the mixture stirred for 1 min, and rapidly filtered through Whatman No. 1 paper. The filtrate was stored in a full, well stoppered, dark bottle at 5 C. The solution was discarded if a pink color or a white precipitate developed during storage.

3. Thirty min was allowed for color development. The color was stable for as long as 5 hr.

4. The degree of inflammation was scored by comparing the color intensity of the treated milk sample with a standard color chart³ which consisted of seven color blocks, ranging from white through pink to purple. The color was a mixture of process blue and white inks. Variation in color density was accomplished by varying the dot intensity from 0 to 80%.

Within 30 min after obtaining the samples, two milk smears were prepared from each milk sample by the method of Prescott and Breed (9), which consisted of transferring 0.01 ml of milk in a 10-lambda pipette to a microscope slide and distributing the milk over one square centimeter. The smears were air-dried on a level surface for 24 hr and then stained with pyronin Y — methyl green according to the procedure outlined by Paape et al. (5).

The concentration of cells per milliliter of each

¹Journal Article No. 3191 from the Michigan Agricultural Experiment Station.

²National Aniline, C.I. No. 42500.

³A standard color chart is available upon request.

milk sample was estimated from the number in 25 microscopic fields randomly chosen from other than the extreme periphery of the smear. The numbers of agranulocytes, granulocytes, leucocytes (the sum of agranulocytes and granulocytes), epithelial cells, and total cells (the sum of leucocytes and epithelial cells) were independently determined on two smears from each milk sample by each of two persons.

RESULTS AND DISCUSSION

The correlation of Feulgen-DNA score with MQT score was 0.907 ($P < .01$). The standard deviation of duplicate Feulgen-DNA scores was 0.114, considerably less than the 0.230 for duplicate MQT scores. The coefficients of variation were 7 and 12% for Feulgen-DNA and MQT scores, respectively.

The average number of cells was 24, 254, 278, 72, and 350×10^4 , for agranulocytes, granulocytes, leucocytes, epithelial cells, and total cells, respectively. The comparable coefficients of variation were 83, 34, 33, 40, and 32%, respectively. The latter values were determined by taking the square root of the sum of the smear and count variances, dividing by the mean, and multiplying by 100. The milk sample variance was excluded from these estimates because the samples included in the present experiment would not necessarily be representative of those chosen for a future experiment where, for example, one may begin with either infected cows or cows with no history of mastitis. Consequently, these coefficients of variation were minimal estimates for these data. Nevertheless, these values were much higher than the values of 7 and 12% for duplicate Feulgen-DNA scores and duplicate MQT scores, respectively.

The correlations of the estimated number of each cell type with Feulgen-DNA score and with MQT score are tabulated in Table 1. Each of these correlations was significant ($P < .01$) and those for the relationship of Feulgen-DNA with cell numbers were consistently higher than those for MQT with cell numbers. The data in Table 1 suggested that each cell type contributed to the Feulgen-DNA and MQT scores. The highest correlation of 0.876 between Feulgen-DNA score and total cells accounted for only 77% of the total variation, indicating that these relationships were somewhat low for prediction purposes.

Feulgen-DNA and MQT scores were plotted against the estimated number of total cells per milliliter of milk (Figure 1). Since these relationships were curvilinear, an attempt was made to create a linear relationship by logarithmic transformations of Feulgen-DNA scores and total cell numbers. The Feulgen-DNA scores were coded, for this purpose, by adding the number one to each score to eliminate

TABLE 1. CORRELATION COEFFICIENTS BETWEEN MILK SOMATIC CELL NUMBERS AND FEULGEN-DNA OR MQT SCORES

Cell classification	Feulgen-DNA score	MQT score
Agranulocytes	.805	.769
Granulocytes	.814	.730
Leucocytes	.834	.751
Epithelial cells	.816	.790
Total cells	.876	.799

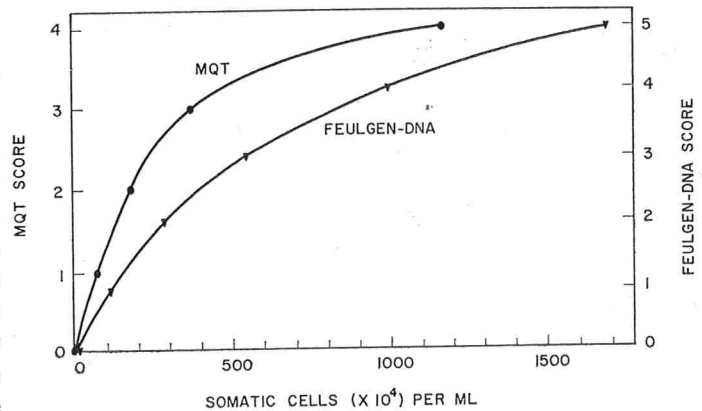


Figure 1. Relationship of concentration of total somatic cells to Feulgen-DNA and MQT scores.

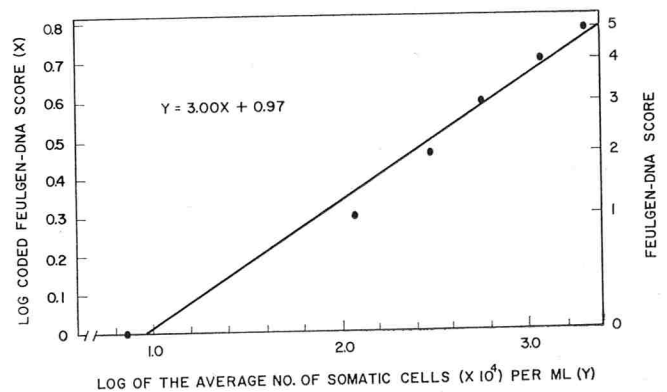


Figure 2. Regression of logarithm of average number of somatic cells (Y) on the logarithm of the coded Feulgen-DNA score (X).

the score of zero. The curve of the double logarithmic transformation of the coded data is illustrated in Figure 3.

The correlation between logarithms of coded Feulgen-DNA scores and logarithms of estimated numbers of total cells was 0.909 ($P < 0.01$), a value somewhat higher than that for the non-transformed data. The regression equation shown in Figure 2 should be useful for estimating total cell number from Feulgen-DNA scores. In view of the high variation ($C.V. = 32\%$) associated with microscopic estimations

of total cell numbers, this regression equation may be at least as accurate in estimating milk cell numbers as the routine methods of counting cells directly.

It should be pointed out that the colors in the standard color chart were selected from a large number of possibilities which ranged from white to dense purple. The selection of the colors was arbitrary, since it was based upon our ability to distinguish small differences in the intensity of this color. A more desirable procedure, which would eliminate subjectivity, would be to measure the intensity of the color of the Feulgen-treated milk samples by reflectance spectrophotometry. Although such a procedure would be expected to be more reliable, it would be more costly than the Feulgen scoring procedure used to obtain the Feulgen-DNA data in this paper.

ACKNOWLEDGMENTS

The authors thank Dr. M. J. Gordon for his helpful suggestions during the formative phases of this work. The interest and assistance of Prof. W. W. Snyder are also appreciated.

REFERENCES

1. Babel, F. J. Problems of the Dairy Bacteriologist Resulting from Mastitis. *J. Am. Vet. Med. Assoc.*, 133:161. 1958.
2. Carroll, E. J., and Schalm, O. W. Effect of Deoxyribonuclease on the California Test for Mastitis. *J. Dairy Sci.* 45:1094. 1962.
3. Feulgen, R., and Rossenbeck, H. Mikroskopisch-chemischer Nachweis einer Nucleinsäure vom Typus der Thymonucleinsäure und die darauf beruhende elektive Färbung von Zellkernen in mikroskopischen Präparaten. *Hoppe-Seyler's Ztschr. f. Physiol. Chemie.* 135:203. 1924.
4. Noorlander, D. O. Milking Machines and Mastitis. Compton Press, Inc. Chino, California. 1960.
5. Paape, M. J., Hafs, H. D., and Snyder, W. W. Variation of Estimated Numbers of Milk Somatic Cells Stained with Wright's Stain or Pyronin Y — Methyl Green Stain. *J. Dairy Sci.* 46:1211. 1963.
6. Paape, M. J., Snyder, W. W., and Hafs, H. D. Feulgen DNA in Milk as a Measure of Udder Irritation. *J. Animal Sci.* 21:1028. 1962.
7. Paape, M. J., Snyder, W. W., and Hafs, H. D. The Michigan Mastitis Test for Udder Irritation. *Mich. Agr. Expt. Sta. Quart. Bull.* 45:245. 1962.
8. Plastridge, W. N. Bovine Mastitis: A Review. *J. Dairy Sci.* 41:1141. 1958.
9. Prescott, S. C., and Breed, R. S. The Determination of the Number of Body Cells in Milk by a Direct Method. *J. Infect. Diseases.* 7:632. 1910.
10. Schalm, O. W., and Noorlander, D. O. Experiments and Observations Leading to Development of the California Mastitis Test. *J. Am. Vet. Med. Assoc.*, 130:199. 1957.

ANALYSIS OF ANIMAL FOOD PRODUCTS FOR CHLORINATED INSECTICIDE RESIDUES.

II. SOME FACTORS INVOLVED IN USING ELECTRON CAPTURE GAS CHROMATOGRAPHY^{1, 3}

A. R. STEMP, B. E. LANGLOIS², AND B. J. LISKA

*Department of Animal Sciences,
Purdue University, Lafayette, Indiana*

(Received for publication December 23, 1963)

SUMMARY

The use of ECGC along with proper sample clean-up provides an accurate analytical method for the detection and measurement of trace amounts of chlorinated insecticide residues. Levels of 0.01 ppm of several insecticides may be determined, providing a degree of accuracy comparable to other analytical procedures, if the following precautions are observed: (a) a representative sample is available; (b) the linearity of the instrument is determined for each compound; (c) all reagents are redistilled; (d) only high purity nitrogen is used as the carrier gas; (e) the chromatographic clean-up column is not overloaded with fat; (f) proper elution techniques are followed; (g) the best column packing is used for the insecticides being analyzed; (h) an all glass system is used including a borosilicate glass injector tube and a borosilicate glass column; (i) careful control of temperature and rate of gas flow is performed; and (j) analytical instrument is carefully calibrated with standard insecticide solutions at specified times.

This paper contains more details and problems encountered in developing the technique reported in the first paper of the series (8). Some of these problems can only be avoided by the analyst if he is aware of certain details about reagents and procedures.

The use of microcoulometric (4) and electron capture (9) gas chromatography for the trace analysis of chlorinated insecticides in animal products has certain advantages over chemical methods including the Mills procedures (10) and Schechter, et al. (13). Instrumental methods are more sensitive and in most cases require less sample clean-up.

There are a number of factors which must be considered in using gas chromatography for insecticide residue analysis. Cassil, et al. (3) attempted to inject solvent extracts of vegetable samples directly into a microcoulometric gas chromatograph for analysis. However, failure to separate some residues from impurities resulted in instrument contamination,

reduced sensitivity and inaccurate analysis. Coulson, et al. (4) and Morley (11) reported that absorbents used for sample clean-up occasionally contained impurities which made it difficult to achieve the desired sensitivity and accurate results. Beckman and Bevenne (1) and Phillips, et al. (12) indicated that certain insecticides were decomposed when metal analytical columns were used for gas chromatographic analysis.

Langlois, et al. (8) in the first paper in this series outlined a rapid one-step sample clean-up procedure for animal products prior to chlorinated insecticide residue analysis with electron capture gas chromatography (ECGC). However factors other than sample clean-up also must be considered in order to obtain accurate analyses. This paper includes more information on several factors which are involved in the analysis of samples for chlorinated insecticide residues using the one-step sample clean-up method in combination with ECGC.

METHODS AND PROCEDURES

Clean-up of samples.

The sample clean-up procedure previously described (8) was used with some modifications. All solvents and chemicals used in the procedure were checked for possible contaminants by gas chromatography. Florisil and Florex absorbents were used with and without partial deactivation with water. Two methods of solvent evaporation from samples were examined. The effect of type of sample on accuracy achieved was investigated.

Analytical conditions.

Analytical columns for the Wilkens Aerograph Hi-Fi Model 600 gas chromatograph were prepared from 1/8-inch OD stainless steel, monel metal and borosilicate glass tubing. The columns were packed with acid washed Chromosorb "W" or HMDS treated Chromosorb "W" coated with different amounts of Silicone Dow 11, QF-1 and Epon 1001 as the liquid phase. The columns were conditioned by baking out at 225 C for at least 24 hr with nitrogen gas flow before being placed in a gas chromatograph equipped with an electron capture detector. Standard in-

¹Supported in part by Research Grant No. EF-00049-02 from the Division of Environmental Engineering and Food Protection, Public Health Service.

²Present address is Department of Dairy Science, University of Kentucky, Lexington, Kentucky.

³Published with the approval of the Director of the Purdue Agricultural Experiment Station as Journal Series Paper Number 2269.

secticide solutions in hexane were injected into the gas chromatograph. Peak areas were measured with a Leeds and Northrup Model H 1 mv recorder equipped with a disc integrator unit.

Factors which were studied included column length, column material, rate of gas flow, type of packing material and column temperature. Retention times and breakdown of insecticides on columns made from various column tubing materials were compared. Studies were made on the effects of a completely borosilicate glass system including a glass injector port liner on insecticide analysis.

The linear range of the analytical instrument for each insecticide was determined. Factors which might affect the linear range such as impurities in the samples, were considered. A calibration curve for each insecticide was constructed by plotting quantity of insecticide versus area response on log-log paper. Factors affecting the necessity for frequent injection of standard solutions to insure quantitative results were studied. This included the effect of impurities in reagents and samples.

RESULTS AND DISCUSSION

During development of the one-step column clean-up technique it was found that acetonitrile and sodium sulfate could not be used due to contaminants which each contained. These contaminants interfered in the analysis for nanogram quantities of insecticides. This may have contributed to the artifact as reported by Klein, et al. (7). No artifact problem was encountered in this work using the confirmation test for DDT described by Klein, et al. (7). Florex absorbent was used in preliminary work but considerable fatty material was eluted from florex columns and work with it was discontinued.

Two methods of solvent evaporation were considered, the use of a steam bath and a 50-60 C water bath. Early in the study, recovery experiments indicated that the steam bath method caused too much bumping of samples with occasional loss of solvent and reduction in recovery of insecticide residues.

Variations may be obtained between duplicate samples analyzed by this method depending on the type of sample being analyzed. Fluid milk samples and other dairy products are no problem to sample and appear to have an even distribution of the insecticide residues on a fat basis. Coulson, et al. (4) reported large variations in results on a series of samples from a human liver. The same problem was encountered with poultry and other meat tissue samples in this study. It is desirable to grind a chicken thigh or breast and sample the homogenous mixture when possible. Sampling procedures from non-homogenous materials are of great importance as only 1 or 2 g of tissue are used for an analysis.

Attempts to reduce the volume of eluant needed for sample clean-up were not successful. If florasil was partially deactivated with over 5% water or the concentration of methylene chloride in the eluant exceeded 20%, impurities which interfered with the analysis were eluted from the clean-up column. All samples were carefully checked by visual observation for fatty residue on the bottom of the beakers following the evaporation of the eluant. Any fatty residues indicated a need for repetition of the particular sample to prevent contamination of the analytical column.

Gunther, et al. (6) reported that DDT breakdown on analytical columns could be prevented by addition of tris (2-biphenyl) phosphate to the sample.

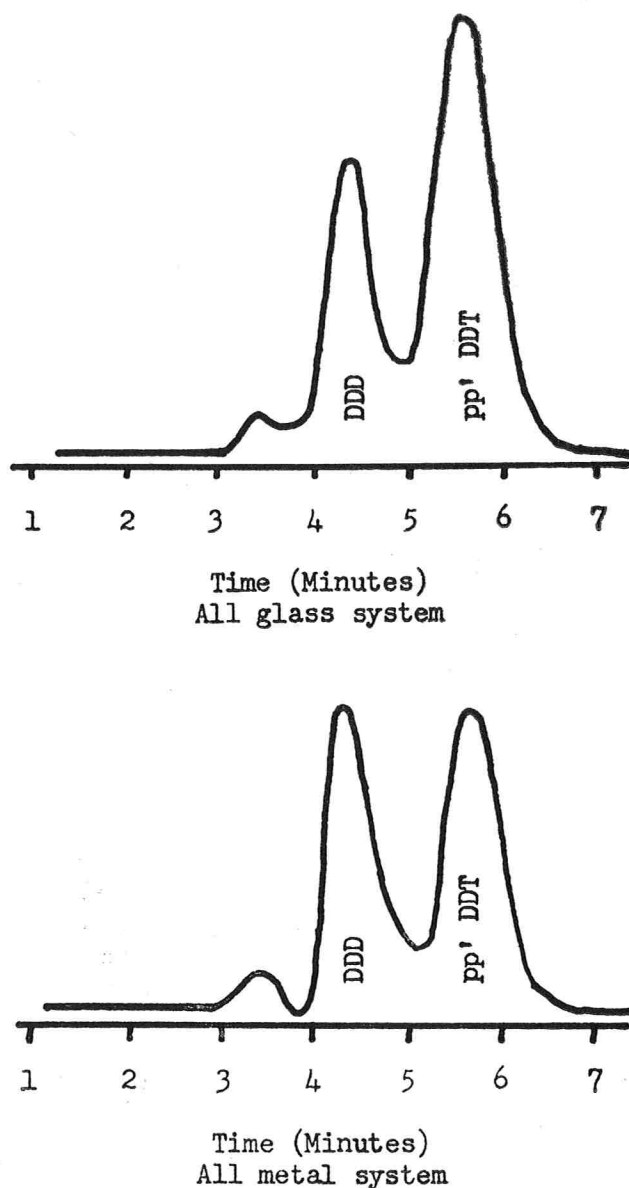


Figure 1. Comparison of DDT analysis on all glass analytical column system versus all metal analytical column system.

TABLE I. RETENTION TIMES OF INSECTICIDES ON VARIOUS ANALYTICAL COLUMN SYSTEMS AT VARIOUS COLUMN CONDITIONS

Column packing	Column material	Column length in feet	Temp.	Nitrogen flow rate (c) (ml/min)	Retention time in min				
					DDT	Lindane	Heptachlor	Dieldrin	Endrin
5% Dow 11 on HMDS Chromosorb W (60/80 mesh)	Stainless steel	5	200	80	13.0	4.0	7.0	9.0	17.0
			193	60	19.0	4.5	10.0	14.0	25.0
			193	40	22.0	5.0	12.0	17.0	—
5% Dow 11 on HMDS Chromosorb W (60/80 mesh)	Stainless steel + pyrex insert	5	195	60	13.0	2.0	7.0	9.0	15.0
			195	80	10.0	1.0	5.0	7.0	11.0
			185	60	14.0	2.0	9.0	11.0	18.0
2.5% Dow 11 on HMDS Chromosorb W (60/80 mesh)	Pyrex	4	195	60	6.0	1.5	3.0	4.0	6.5
			190	60	7.0	2.0	4.0	5.0	8.0
			185	60	8.5	2.5	5.0	6.5	10.0
2.5% Dow 11 on HMDS Chromosorb W (60/80 mesh)	Pyrex + pyrex insert	4	195	60	5.0	1.0	2.5	3.0	5.0
			190	60	5.5	1.5	3.0	3.5	7.0
			183	60	7.0	1.5	4.0	5.0	12.0

However, Phillips, et al. (12) found no noticeable improvement in elimination of DDT breakdown on metal columns upon use of this compound. Beckman and Bevenne (1) noted that the greatest amount of breakdown occurred on copper columns, less on stainless steel or aluminum columns, and the least on quartz columns. A comparison of results with DDT on borosilicate glass and stainless steel columns are presented in Figure 1. The results presented here agree with results reported by Beckman and Bevenne (1). The same type of breakdown occurs when a glass injector insert is not used with a glass column.

Breakdown of endrin into two products on metal and borosilicate glass columns as reported by Phillips, et al. (12) was also noted. Temperature and gas flow rate studies were not performed on these breakdown products.

A series of column packings including QF-1, SE 30, Epon 1001, and Dow 11 were used with metal and borosilicate glass columns. Most of these were discarded for a variety of reasons: tailing of peaks, inability to separate combinations of insecticides, long retention times, instability at high column temperatures, and breakdown of insecticides.

Increasing the amount of liquid resulted in longer retention times and more diffuse peak areas. Attempts to use a smaller mesh chromosorb to improve separation led to greatly increased retention times.

The effects of a borosilicate glass column and liner in the injector port of the instrument on the retention time of the five insecticides studied are presented in Table 1. The use of the injector liner resulted in decreased retention times and more sharply defined peaks. An increase in temperature or rate of gas

flow also resulted in decreased retention times. Poor peak resolution was obtained at temperatures below 180 C and a nitrogen flow rate of 40 ml per minute.

The optimum resolution of the five insecticides studied was obtained using a 1/8-inch by 5 ft coiled borosilicate glass column packed with 5.0% Dow 11 on 60/80 mesh hexamethyldisilazane (HMDS) treated Chromosorb "W". Separation of the insecticides was achieved within 10 min at the temperature of 191 C and a nitrogen flow rate of 60 ml per minute. DDE and dieldrin were separated as one peak under these conditions.

Work performed by Bonelli, et al. (2) has shown that a polar QF-1 analytical column can separate insecticides which are not separated by a non-polar Dow 11 Column. By this means, compounds such as dieldrin and pp' DDE, which are not separated by a Dow 11 column can be readily identified. Goodwin, et al. (5) have also studied ways by which identification of peaks can be achieved. The use of various detection systems, changing of column parameters, and use of different stationary phases were studied. By noting peak retention times on various analytical columns, the identity of individual peaks may be more positively determined.

To obtain quantitative results, it is important to determine the linear range of the analytical instrument with electron capture detector. The linear ranges for selected insecticides are presented in Table 2.

During the study, results indicated that the injection of standard solutions at the start of analysis, after each series of ten samples and at the end of the analysis, was sufficient providing no contaminated samples were encountered. When a contaminated

TABLE 2. LINEARITY OF GAS CHROMATOGRAPH FOR SELECTED CHLORINATED INSECTICIDES

Insecticide	Linear range (nanograms)
DDT	0.5 to 100
Lindane	0.1 to 10
Heptachlor	0.1 to 10
Dieldrin	0.1 to 50
Endrin	0.1 to 50

sample was injected, it was necessary to check the accuracy of the instrument with an injection of a standard solution before proceeding further. Contamination shows up as large peaks on the chromatograph with retention times of 16 to 25 min under the column conditions used in this study.

ACKNOWLEDGMENT

The authors wish to thank Dr. J. W. Amy, Purdue University and Wilkens Instrument Company, Walnut Creek, California, for technical assistance.

REFERENCES

- H. Beckman and A. Bevenne. The Effect of the Column Tubing Composition on the Recovery of Chlorinated Hydrocarbons by Gas Chromatography. *J. Chromatography*. 10: 231-233. 1963.
- E. J. Bonelli, H. Hartmann, and K. P. Dimick. Gas Chromatographic Retention Times and Sensitivity Data for Insecticides and Herbicides. Presented at the American Chemical Society Fall Meeting, New York, New York, September 8-13, 1963.
- C. Cassil. Pesticide Residue Analysis by Microcoulometric Gas Chromatography. *Residue Reviews*. 1:37-65. 1962.
- D. M. Coulson, L. A. Cavanaugh, and J. Stuart. Gas Chromatography of Pesticides. *J. Agr. Food Chem.* 7:250. 1959.
- E. S. Goodwin, R. Goulden, and J. G. Reynolds. Rapid Identification and Determination of Residues of Chlorinated Pesticides in Crops by Gas-Liquid Chromatography. *Analyst*. 86:697. 1961.
- F. A. Gunther, R. C. Blinn, and G. K. Kahn. Labile Organo-Halogen Compounds and Their Gas Chromatographic Detection and Determination in Biological Media. *Nature*. 193:573. 1962.
- A. K. Klein, J. O. Watts, and J. N. Damico. Electron Capture Gas Chromatography for Determination of DDT in Butter and Some Vegetable Oils. *J. Assoc. Off. Agr. Chem.* 46:165. 1963.
- B. E. Langlois, A. R. Stemp, and B. J. Liska. Analysis of Animal Food Products for Chlorinated Insecticide Residues I. Column Cleanup of Samples for Electron Capture Gas Chromatographic Analysis. *J. of Milk and Food Technol.* 27:..... 1964.
- J. E. Lovelock and S. R. Lipsky. Electron Affinity Spectroscopy A New Method for Identification of Functional Groups in Chemical Compounds Separated by Gas Chromatography. *J. Am. Chem. Soc.* 82:431. 1960.
- P. A. Mills. Detection and Semi-Quantitative Estimation of Chlorinated Organic Pesticide Residues in Foods by Paper Chromatography. *J. Assoc. Off. Agr. Chem.* 42:734. 1959.
- H. V. Morley. Clean-up for Pesticide Residue Analysis in Plant Material with Particular Reference To Dieldrin. Presented at the Fifth International Pesticides Congress, London, England, July 17, 1963.
- D. D. Phillips, G. E. Bollard, and S. B. Soloway. Thermal Isomerization of Endrin and Its Behavior in Gas Chromatography. *J. Agr. Food Chem.* 10:217. 1962.
- M. S. Schechter, M. A. Pogorelskin, and H. L. Haller. Colorimetric Determination of DDT in Milk and Fatty Materials. *Ind. Eng. Chem. Anal. Edit.* 19:51. 1947.

THE QUANTIFICATION OF SALMONELLAE IN FOODS BY USING THE LACTOSE PRE-ENRICHMENT METHOD OF NORTH

HERBERT E. HALL, DAVID F. BROWN, AND ROBERT ANGELOTTI

*Milk and Food Research,
Robert A. Taft Sanitary Engineering Center
Public Health Service, Cincinnati, Ohio*

(Received for publication March 25, 1964)

SUMMARY

The lactose pre-enrichment technique of North (8) modified only in the amount of original specimen (60 g as compared with 34 g) and of diluent (540 ml as compared with 406 ml) has been successfully employed to isolate and enumerate salmonellae in foods. Quantitative studies using laboratory-prepared chicken pie filling indicate that excellent recovery in the range of four to hundreds of thousands of salmonellae per 100 g of food can be obtained. The technique is excessively cumbersome for the quantification of large numbers (hundreds per g) of salmonellae, but excellently adapted to the detection and quantification of smaller concentrations. The specificity of the method was maintained in the presence of natural food flora and of additions of relatively large numbers of organisms frequently associated with food. None of the natural food constituents used in the study affected the sensitivity or specificity of the method.

The value of enrichment methods for the recovery of salmonellae from natural sources has been well established. Selective enrichment with selenite and tetrathionate broths has proved valuable with feces (5, 6), and modifications of these broths have enhanced their usefulness in the isolation of *Salmonella* from foods. Galton, Lowery, and Hardy (3) found that the addition of Tergitol No. 7 to tetrathionate increased its efficiency for the detection of salmonellae in pork sausage, and the results obtained with selenite broth have been improved by the addition of cystine (9), sulfapyridine (10), and brilliant green (12). Pretreatment or pre-enrichment of specimens in which it is suspected that the salmonellae have been injured during processing has also improved detection. Thomson (13) employed nutrient broth with fecal specimens. Byrne et al. (2) suspended dried eggs in distilled water before inoculating selenite-cystine broth, and North (8) used lactose broth pre-enrichment with dried egg albumin. North (8) indicated that recoveries of less than 3.6 organisms per 100 g of albumin were possible with this method and attributed the advantages of the lactose broth pre-enrichment technique over direct inoculation into selenite-cystine or tetrathionate broths and over the centrifugation method of Silliker and Taylor (11) to the fact that lactose broth offers more favorable conditions for growth than does a selective medium. Furthermore, when there is a mixed flora present, a lowering of the pH occurs, which is detrimental to

the development of many concomitant organisms, but has no adverse effects upon the salmonellae. The results reported by North suggested that the lactose broth pre-enrichment method might be equally advantageous for the enumeration of salmonellae in other foods.

This report deals with the results from experiments made to determine the limits of recovery, accuracy, and adaptability of this method when applied to foods experimentally contaminated with salmonellae.

MATERIALS AND METHODS

The salmonellae strains were from stock cultures originally obtained from the Communicable Disease Center, Public Health Service, Atlanta, Georgia. Other species were stock cultures from the collection of the Food Microbiology Unit, Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio. Cultures were maintained at room temperature on trypticase soy agar slants after incubation at 35 C for 24 hr. These cultures were transferred frequently, and fresh 24-hr cultures were used to prepare the inocula.

The foods were laboratory-prepared chicken pie filling containing chicken, carrots, peas, celery, potatoes, and onions in natural gravy, and dehydrated, raw, or canned food from retail markets.

The media employed were the commercially prepared, dehydrated products reconstituted and sterilized as directed by the manufacturer (lactose broth, selenite broth, brilliant green agar, etc.), or were laboratory prepared according to the directions in the publications from which the formulas were obtained, for example selenite-cystine broth (8) and brilliant green sulfadiazine agar (3).

The method of enumerating salmonellae in food was adapted from North (8) and performed as follows: 60 g of food to which the desired number of salmonellae and other organisms had been added were blended with 540 ml of lactose broth (1) for 2 min at slow speed (8,000 rpm) in a Waring Blendor to obtain an initial 1:10 dilution of food homogenate. Five 100-ml portions of the homogenate (containing 10 g of food per 100 ml) were placed in separate sterile, screw-capped, 6-ounce jars. Five 10-ml portions (1 g of food per 10 ml) were placed in sterile,

20 x 125-ml, aluminum-capped tubes; five 1-ml portions (0.1 g of food per milliliter were placed in similar tubes containing 10 ml of lactose broth; and finally five 0.1-ml portions (0.01 g of food per 0.1 ml) also were placed in tubes containing 10 ml of lactose broth. One-milliliter volumes of further tenfold dilutions of the 1:10 dilution, as needed to give a negative end point, were placed in 10 ml of lactose broth in some experiments.

The jars and tubes were incubated for 24 hr at 35 C in a water-jacketed incubator. They were then subcultured, by using a 5-ml wire loop, to tubes containing 1 ml of selenite-cystine broth (8) that were then incubated for 6 to 7 hours at 35 C and again subcultured by wire loop to plates of brilliant green sulfadiazine (BGS) agar (3) or other differential media. Each plate of BGS was divided into five sections by marking the back of the plate with a glass-marking pencil, and one plate accommodated the inoculum from the tubes of one set of dilutions. The BGS plates were incubated for 24 hr at 35 C and then held for an additional 24 hr at room temperature for final observation.

The sections showing colonies typical of salmonellae were recorded for each dilution, and the number of organisms present per 100 g of the original sample was determined by means of the most probable number (MPN) tables of Hoskins (4).

RESULTS

Repeated experiments with single strains of 9 *Salmonella* species (*S. anatum*, *S. choleraesuis*, *S. derby*, *S. lexington*, *S. manhattan*, *S. montevideo*, *S. senftenberg* 775W, *S. reading*, and *S. typhimurium*) and with laboratory-prepared chicken pie filling were performed to determine the degree of accuracy and limits of recovery possible with the lactose broth pre-enrichment technique. Inoculum concentrations were determined by plate count with duplicate plates of brain heart infusion agar incubated at 35 C for 24 hr. The inocula ranged from 2×10^5 to less than 10 organisms per 100 g of food. Of a total of 85 experiments with these nine species, 69 (81%) gave MPN values whose 95% confidence limits included the inoculum concentration as determined by plate count (14). Twenty gave results below the limit and one above. With two species (*S. anatum* and *S. reading*), such agreement was obtained with all tests, whereas with one (*S. senftenberg* 775W), all results were too low for such agreement. With the remaining six species, the results of only a few experiments failed to agree. It was noted that the out-of-range results, with the exception of those obtained with *S. senftenberg* 775W, were usually associated with small concentrations (40 or less per 100 g), or with large concentrations (10^3 to 10^5 per 100 g) in

which no negative end point (one set of five tubes all negative) was obtained.

Representative results of these tests are shown in Table 1. The first two results at each inoculum concentration are typical of the usual findings, while the third represents one of the instances in which recovery was not in the acceptable range. For accurate results at concentrations of 10^5 organisms, it was necessary to make tenfold dilutions so that the last set of 5 tubes contained 0.001 ml of the blend equivalent to 0.0001 g of the original specimen. At lesser concentrations, however (10^3 to less than 10 per 100 g of food), the series of volumes (100, 10, 1, and 0.1 ml) were found to be adequate. In preliminary studies with this method, the brilliant green sulfadiazine agar (BGS) plates were incubated at 35 C for 24 hr. On several occasions it was noted that no color change in the medium had occurred around colonies that in most instances were very small. When these plates were incubated an additional 24 hr, the color change appeared, and, in addition, some sections that had not shown growth after 24 hr became positive. Larger colonies and more pronounced color reactions were obtained if the second incubation period was at room temperature. Consequently, in the test reported here, the BGS agar was incubated for 48 hr, the final 24

TABLE 1. QUANTIFICATION OF SALMONELLAE FROM CHICKEN PIE FILLING

Salmonella species	Inoculum level (plate count)	Recovery level lactose pre-enrichment (MPN)	95% confidence limits for MPN ^a
<i>S. lexington</i>	240,000	540,000	130,000 - 2,000,000
<i>S. typhimurium</i>	210,000	160,000	3,500 - 5,300,000
<i>S. montevideo</i>	350,000	3,500,000	89,000 - 1,400,000
<i>S. manhattan</i>	30,167	33,000	8,900 - 120,000
<i>S. anatum</i>	29,333	33,000	8,900 - 120,000
<i>S. typhimurium</i>	29,500	540	130 - 2,000
<i>S. choleraesuis</i>	7,250	4,900	1,300 - 18,000
<i>S. lexington</i>	2,875	1,700	500 - 4,400
<i>S. derby</i>	3,183	16,000	3,400 - 53,000
<i>S. derby</i>	318	330	89 - 1,200
<i>S. anatum</i>	352	350	89 - 1,400
<i>S. choleraesuis</i>	256	79	21 - 240
<i>S. reading</i>	39	79	21 - 240
<i>S. montevideo</i>	35	49	13 - 180
<i>S. manhattan</i>	33	7.8	1.6 - 24
<i>S. manhattan</i>	6.8	7.8	1.6 - 24
<i>S. choleraesuis</i>	7.3	14	4.8 - 35
<i>S. lexington</i>	2.8	0	-

^aTaken from Woodward, R. L., 1957.

TABLE 2. LIMITS OF RECOVERY OF SALMONELLAE FROM VARIOUS TYPES OF FOOD

Salmonella species	Food substrate	Inoculum level (plate count)	Recovery level lactose pre-enrichment (MPN)	95% confidence limits for (MPN) ^a
<i>S. lexington</i>	Vanilla pudding	7.5	6.8	1.4 - 19
<i>S. tennessee</i>	Raw beef kidney	7.4	4.5	0.55 - 17
<i>S. choleraesuis</i>	Chicken pie filling	7.3	14.0	4.8 - 35
<i>S. manhattan</i>	Chicken pie filling	6.8	7.8	1.6 - 24
<i>S. newport</i>	Banana cream pie	6.4	7.8	1.6 - 24
<i>S. senftenberg</i>	Chicken pie filling	4.1	0	0 - 5.4
<i>S. reading</i>	Chicken pie filling	4.0	2.0	0.052 - 12
<i>S. manhattan</i>	Chicken pie filling	3.3	0	0 - 5.4
<i>S. lexington</i>	Chicken pie filling	2.8	0	0 - 5.4
<i>S. choleraesuis</i>	Chicken pie filling	2.5	0	0 - 5.4

^aTaken from: Woodward, R. L., 1957.

hr at room temperature (24-26 C).

The results from this study indicate that the least number of organisms consistently recovered by this method was about 6 per 100 g of food (Table 2). It is indicated, therefore, that the initial 60-g sample of food must contain a minimum of 3 or 4 organisms if recovery is to be achieved. In one instance, however, an inoculum concentration of four organisms per 100 g was positive. An estimate based on the 95% confidence limit would indicate that a concentration of more than 5.4 organisms per 100 g must be present.

Although most of our work was done with laboratory-prepared chicken pie filling, some experiments were carried out with other foods. By using an inoculum concentration of 6.4 organisms per 100 g of food, we recovered *S. newport* quantitatively from shrimp and banana cream pie; *S. lexington* from angel food cake mix, egg white mix, and vanilla pudding mix at 7.5 per 100 g; *S. typhimurium* from commercial beef pot pie and lemon cream pie at 365 per 100 g; *S. anatum* from vegetables with lamb, and from chicken with vegetables and egg yolk (moist baby foods) at 960 per 100 g; *S. tennessee* from raw chicken meat and raw beef kidney at 7.4 per 100 g; and *S. derby* from several dried foods (high protein baby cereal, white cake mix, and vanilla pudding mix) at 54.5 per 100 g. In none of the above experiments did food constituents appear to affect recovery adversely.

Selenite-cystine broth has frequently been used as an enrichment for the isolation of salmonellae from foods without previous treatment. North (8) reported that the lactose broth pre-enrichment method gave better recovery from naturally infected egg white powder than did the selenite-cystine broth. A comparison was made between the two methods in this

study by using inoculum concentrations ranging from 10^4 to less than 10 organisms per 100 g of food. When selenite-cystine was used as the enrichment, the 60-g specimen was homogenized in 540 ml of selenite-cystine broth, and this 1:10 dilution was portioned as indicated for the lactose broth pre-enrichment method and then incubated for 24 hr. The BGS plates were then streaked from the jars and tubes and incubated as previously described.

Table 3 shows some comparative results obtained by using the two methods in parallel experiments. In almost every instance the recovery obtained with direct enrichment in selenite-cystine broth was less than that obtained with the lactose broth pre-enrichment method, although in many instances this lesser recovery was still within the 95% confidence limit range. At the small concentration, however (15 to 35 organisms per 100 g), frequently no recovery was obtained with the selenite-cystine enrichment; and, in repeated tests with the *S. montevideo* strain, no recovery was obtained at any concentration. Hence, it appears that better recovery and lower levels of detection are possible after lactose broth pre-enrichment.

To determine whether the lactose broth pre-enrichment method would recover experimentally injured or debilitated salmonellae as well as it had recovered inocula prepared from fresh cells, several experiments were performed in which recovery from inoculated chicken pie filling was conducted immediately after inoculation and again after storage at -15 C for 24 hr. Representative results are shown in Table 4.

Inoculum concentrations ranging from 10^2 to 10^4 organisms per 100 g of food were used. Recovery was obtained in every test with the lactose pre-enrichment technique. Acceptable agreement between

TABLE 3. COMPARISON OF RECOVERIES OF VARIOUS *Salmonella* STRAINS FROM CHICKEN PIE FILLING BY MEANS OF LACTOSE BROTH PRE-ENRICHMENT AND SELENITE-CYSTINE ENRICHMENT

Salmonella species	Inoculum level (plate count)	Recovery lactose pre-enrichment (MPN)	95% confidence limits of MPN ^a		Recovery selenite-cystine pre-enrichment (MPN)	95% confidence limits of MPN ^a	
<i>S. manhattan</i>	2,441	5,400	1,300	- 20,000	5,400	1,300	- 20,000
"	244	490	130	- 1,800	240	65	- 740
"	24	17	5.5	- 43	7.8	1.6	- 24
<i>S. choleraesuis</i>	2,558	790	210	- 2,400	33	8.9	- 120
"	256	79	21	- 240	2	0.052	- 12
"	26	2	0.052	- 12	0	0	- 5.4
<i>S. senftenberg</i>	2,058	1,300	352	- 3,600	490	130	- 1,800
"	206	11	2.8	- 28	7.8	1.6	- 24
"	21	2	0.052	- 12	0	0	- 5.4
<i>S. lexington</i>	2,875	1,700	500	- 4,400	1,300	350	- 3,600
"	288	330	89	- 1,200	170	55	- 430
"	29	23	6.5	- 68	7.8	1.6	- 24
<i>S. typhimurium</i>	1,516	3,500	890	- 14,000	2,400	650	- 7,400
"	152	790	210	- 2,400	490	130	- 1,800
"	15	27	8.6	- 69	0	0	- 5.4
<i>S. anatum</i>	3,525	3,500	890	- 14,000	23	6.5	- 68
"	353	350	89	- 1,400	78	16	- 240
"	35	79	21	- 240	2	0.052	- 12
<i>S. derby</i>	3,183	16,000	3,500	- 53,000	16,000	3,500	- 53,000
"	318	330	89	- 1,200	330	89	- 1,200
"	32	23	6.5	- 68	23	6.5	- 68
<i>S. montevideo</i>	3,300	5,400	1,800	- 20,000	0	0	- 5.4
"	330	490	130	- 1,800	0	0	- 5.4
"	22	49	13	- 180	0	0	- 5.4

^aTaken from: Woodward, R. L., 1957.

plate count values and MPN determinations was attained with concentrations above 100 per 100 g of food, and recovery was possible when the numbers were too low to yield positive plate count values. Although a reduction in the number of viable *Salmonella* occurred in almost every instance when the food was stored for 24 hr at -15 C, it was not marked in most instances.

Although some of the foods used in all the preceding experiments contained sufficient natural flora to give good competitive growth in relation to the *Salmonella* inoculum, most were sterile. To check further the effects of competition on the recovery of salmonellae, a number of experiments were done in which other organisms (*Staphylococcus aureus*, *Proteus vulgaris*, *Streptococcus faecium*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Streptococcus agalactiae*) were added to chicken pie filling in various combinations. These organisms were added at con-

centrations ranging from 1×10^5 to 1×10^6 per 100 g of food. Representative results of these studies are shown in Table 5. The additions of these organisms, even at great concentrations, did not appear to affect the recovery and quantification of the salmonellae. In most instances the recovery was as good as or better than that obtained with the same strains of *Salmonella* added to sterile chicken pie filling in pure culture.

DISCUSSION

Our work with the lactose broth pre-enrichment technique has confirmed the findings of North (8) that quantitative enumeration of salmonellae in foods is possible when they are present in concentrations that range from as few as six per 100 g of food to several thousand per 100 g. Our observations during this study indicate when the salmonellae are present in large numbers that the pre-enrichment method is

too cumbersome to use as a quantitative method. The number of jars and tubes of lactose broth, selenite-cystine broth, and plates of brilliant green sulfadiazine agar needed to enumerate hundreds of salmonellae per g of food is prohibitive. On the other hand, for the examination of foods in which the number of salmonellae is very small, the technique has great merit. The limits of recovery obtained in this study correspond closely to those reported by North (8) in that the smallest recoverable number, as determined by plate count, was four per 100 g, while he reported <3.6 from specimens of unknown content. Although the results obtained by plate count procedures cannot be directly compared with those obtained by MPN determinations, these determinations do indicate an expected range of actual numbers of organisms. At the 95% confidence limit, these ranges are relatively broad, and the MPN procedure described in this report consistently yielded values of salmonellae recovery within the range.

Admittedly, eleven salmonellae species is a small

number compared with the total number of serological types recognized today. They do, however, include members of five serological groups (B, C₁, C₂, E, and E₄), and all have at one time or another been associated with food-borne disease. Undoubtedly some strains among the numerous sero-types will not be recovered quantitatively by this method, but no inhibition or consistent reduction in recovery levels was noted for *S. manhattan* or *S. anatum* as described by Leistner, et al. (7) when these strains were subcultured to selenite-cystine broth. On the other hand, the strain of *S. anatum* and *S. montevideo* used was markedly inhibited when the selenite-cystine broth was used as the enrichment medium (Table 3).

A number of recovery media of various degrees of inhibitory activity were examined in the course of preliminary studies. The brilliant green sulfadiazine agar (3) was found to give the best results. None of the strains of salmonellae tested were inhibited by it, and outgrowth of either natural flora or added organisms was completely suppressed. Colonies of

TABLE 4. EFFECT OF FREEZING ON THE RECOVERY OF SALMONELLAE FROM CHICKEN PIE FILLING

Salmonella species	Inoculum level (plate count)	Recovery level lactose pre-enrichment (MPN)	95% confidence limits for MPN ^a	Salmonella level after 24 hr at 15° C (plate count)	Recovery level lactose pre-enrichment (MPN)	95% confidence limits for MPN ^a
<i>S. montevideo</i>	44,000	24,000	6,500 - 74,000	13,100	24,000	6,500 - 74,000
<i>S. lexington</i>	8,900	7,900	3,100 - 24,000	6,600	3,300	890 - 12,000
<i>S. manhattan</i>	650	790	210 - 2,400	690	330	89 - 1,200
<i>S. reading</i>	100 ^b	33	8.9 - 120	No growth.	13	6.3 - 49
<i>S. anatum</i>	200 ^b	33	8.9 - 120	No growth.	7	1.9 - 19

^aTaken from: Woodward, R. L., 1957.

^bCount based on one and two colonies in three plates containing a total volume of 10 ml. of 1:10 blend.

TABLE 5. EFFECT OF ADDED ORGANISMS ON THE RECOVERY OF SALMONELLAE FROM CHICKEN PIE FILLING

Salmonellae species	Inoculum level (plate count)	Other organisms added — total No. (10 ⁵ - 10 ⁶ /100 g.)	Recovery level lactose pre-enrichment (MPN)	95% confidence limits for MPN ^a
<i>S. montevideo</i>	3,300	<i>S. aureus</i>	5,400	1,300 - 20,000
	330	<i>P. vulgaris</i>	490	130 - 1,800
	33	"	49	13 - 180
<i>S. reading</i>	3,733	<i>S. faecium</i>	3,500	890 - 14,000
	373	<i>P. aeruginosa</i>	790	210 - 2,400
	37	"	79	21 - 240
<i>S. choleraesuis</i>	254	<i>P. vulgaris</i>	540	130 - 2,000
	25	<i>S. aureus</i>	92	21 - 300
	2.5	"	0	0 - 5.4

^aTaken from: Woodward, R. L., 1957.

salmonellae on brilliant green agar without sulfadiazine were slightly larger at 24 hr than they were on the medium containing sulfadiazine, but the numbers were of the same order. It is most interesting that the observations of Leistner, et al. (7) with brilliant green agar and our own with the medium containing sulfadiazine indicated that a secondary 24-hr incubation period at room temperature was desirable with both media. The number of different foods examined was relatively small, but included most of the types subject to examination for salmonellae. With none of these foods was any adverse effect noted.

In his original article, North (8) noted that the pH of the selenite-cystine medium did not require adjustment, but would be approximately 7.1 as made. It was our experience, however, that some of the lots of the medium were pH 7.4 to 7.5. It also appeared that recovery was not quite as good with these lots. We adjusted, therefore, our selenite-cystine to pH 7.1 before autoclaving. In their discussion of enrichment media, Leistner, et al. (7), indicated that a pH of 7.3 for selenite broth was desirable when pig feces was being examined for salmonellae because of the acid nature of the inoculum. They consider a pH range of 5.8 to 6.3 as optimal for the selective increase of salmonellae. It is, therefore, possible that some adjustment of pH of the selenite-cystine broth would make it more effective, depending upon the nature of the food inoculated into the lactose broth pre-enrichment tubes. The carry-over from these tubes is rather small (one 5-mm loopful), so it appears doubtful that this aspect is as critical in this method as it is in the selenite enrichment technique.

It is hoped that the findings of North (8) with egg albumin and our findings with frozen specimens are indications that the lactose broth pre-enrichment technique recovers salmonellae that have been injured by adverse conditions. The test of its effectiveness in this respect will, however, have to await field trial with many types of foods. The indications ob-

tained in this study are favorable for the use of this method both to quantitate and isolate salmonellae from foods.

REFERENCES

1. American Public Health Association. Standard Methods for the Examination of Dairy Products. 10th ed. Am. Public Health Assoc. New York, N. Y. 1960.
2. Byrne, A. F., Rayman, M. M. and Schneider, M. D. Methods for the detection and estimation of numbers of *Salmonella* in dried egg and other food products. *Appl. Microbiol.* 3:368-372. 1955.
3. Galton, M. M., Lowery, W. D. and Hardy, A. V. *Salmonella* in fresh and smoked pork sausage. *J. of Infectious Diseases.* 94:232-235. 1954.
4. Hoskins, J. K. Most Probable Numbers for evaluation of Coli-Aerogenes test by fermentation tube method. *Public Health Reports.* 49:393-405. 1934.
5. Kauffman, F. Weitue erfahwngen mit dem kombinierten Anreicherungsnerfahren fur Salmonellabacillen. *Ztschr. f. Hyg.* 117:26-32. 1935.
6. Leifsen, E. New selenite enrichment media for the isolation of typhoid and paratyphoid (*Salmonella*) bacilli. *Am. J. Hyg.* 117:26-32.
7. Leistner, L., Deibel, R. H., Johantges, J. and Niven, C. F., Jr. Contribution to the methodology of *Salmonella* detection. *Am. Meat Inst. Foundation Bull.* 56. 1963.
8. North, W. R., Jr. Lactose pre-enrichment method for isolation of *Salmonella* from dried egg albumin. *Appl. Microbiol.* 9:188-195. 1961.
9. North, W. R., Jr. and Bartram, M. T. The efficiency of selenite broth of different compositions in the isolation of *Salmonella*. *Appl. Microbiol.* 1:130-134. 1953.
10. Osborne, W. W. and Stokes, J. L. A modified selenite brilliant green medium for the isolation of *Salmonella* from egg products. *Appl. Microbiol.* 3:295-299. 1955.
11. Sillicker, J. H. and Taylor, W. I. Isolation of Salmonellae from food samples. II. The effect of added food samples upon the performance of enrichment broths. *Appl. Microbiol.* 6:228-232. 1958.
12. Stokes, J. L. and Osborne, W. W. A selenite brilliant green medium for the isolation of *Salmonella*. *Appl. Microbiol.* 3:217-220. 1955.
13. Thomson, S. The numbers of pathogenic bacilli in faeces in intestinal diseases. *J. Hyg.* 53:217-224. 1955.
14. Woodward, R. L. How probable is the most probable number? *J. Am. Water Works Assoc.* 49:1060-1068. 1957.

EFFECT OF TIME AND TEMPERATURE OF INCUBATION ON THE PLATE COUNT OF DRY MILKS

RAFAEL PEDRAJA AND AINIS MENGELIS

American Dry Milk Institute, Inc., Chicago, Illinois

(Received for publication January 3, 1964)

ABSTRACT

Bacterial counts of nonfat dry milk of various heat types and of dry buttermilk were determined by the agar plate method using three incubation conditions, 35 C for 48 hours, 32 C for 48 hours, and 32 C for 72 hours. Generally higher plate counts were obtained when incubating samples at 32 C for 72 hours. No outstanding differences were found when samples were incubated at 35 C for 48 hours, as compared to incubation at 32 C for 48 hours. There was no relation between the direct microscopic clump count and differences in plate counts due to varying incubation conditions. Results clearly demonstrate that only a very small percentage of samples were affected in their grade classification as a result of extending the incubation time to 72 hours.

In the 11th edition of "Standard Methods for the Examination of Dairy Products" (1), the incubation conditions for colony counts as determined by the agar plate method for dry milks were changed from 48 ± 3 hr at either 32 C or 35 C to 3 days at 32 C. The incubation temperature of 35 C, according to "Standard Methods" is no longer optional. These changes apply only to dry milks. The agar plate method for other dairy products, including raw and pasteurized fluid milk, still allows incubation of the plates for 48 ± 3 hr at either 32 C or 35 C.

The present American Dry Milk Institute procedure, as described in Bulletin 916 "Standards for Grades for the Dry Milk Industry, including Methods of Analysis", (2) specifies incubation at 35 C for 48 ± 3 hr.

It is the purpose of this work to determine the effect of these specific changes of incubation conditions in the bacterial estimate of dry milks and to evaluate the results of such changes.

EXPERIMENTAL PROCEDURE

Samples of nonfat dry milk (NDM) from a number of manufacturers and processed under various heat treatments, as well as of dry buttermilk, representing production from different manufacturers, were analyzed in duplicate for bacterial estimate using three different sets of incubation conditions, namely, 35 C, 48 hr; 32 C, 48 hr; and 32 C, 72 hr.

Buffered distilled water tempered to 45 C was used for reconstituting the samples. Plates for the three sets of incubation conditions were prepared from the same dilution bottles. The media used in the preparation of the plates was standard plate count

TABLE 1. EFFECT OF TIME AND TEMPERATURE OF INCUBATION ON PLATE COUNTS PER GRAM OF PRODUCT

Product	No. of samples	Incubation conditions		
		35 C/48 hrs	32 C/48 hrs	32 C/72 hrs
Low heat NDM ^a	57	32,900	37,400	52,100
Medium heat NDM ^a	19	11,100	10,100	20,100
High heat NDM ^a	18	11,600	10,400	14,400
NDM - heat treatment unknown	73	3,340	3,530	5,660
Buttermilk	54	9,150	7,250	9,240
All Data	221	13,700	14,300	20,500

^aHeat treatment classification:

Class	Whey protein nitrogen
High heat	Not more than 1.5 mg/g.
Low heat	Not less than 6.0 mg/g.
Medium heat	1.51 - 5.99 mg/g.

media, as specified in "Standard Methods" (1). Only the incubation conditions were varied in the experiments.

Undenatured whey protein nitrogen (WPN) according to the Harland-Ashworth (2) method was determined in a number of samples of nonfat dry milk in order to classify them into low, medium, and high heat and thus observe the influence of the heat treatment as evaluated by the WPN values on the bacterial counts obtained. WPN was also run on some of the dry buttermilk samples investigated.

The Direct Microscopic Clump Count (DMCC) was determined in some of the nonfat dry milk samples analyzed to illustrate any possible relationship of this value and the bacterial counts obtained at different incubation conditions.

RESULTS

Table 1 presents a summary of all the plate counts obtained using the three sets of incubation conditions under study. The data are presented graphically in Figure 1. When 57 samples of low heat NDM were incubated at 32 C for 48 hr, there was a 13.6% in-

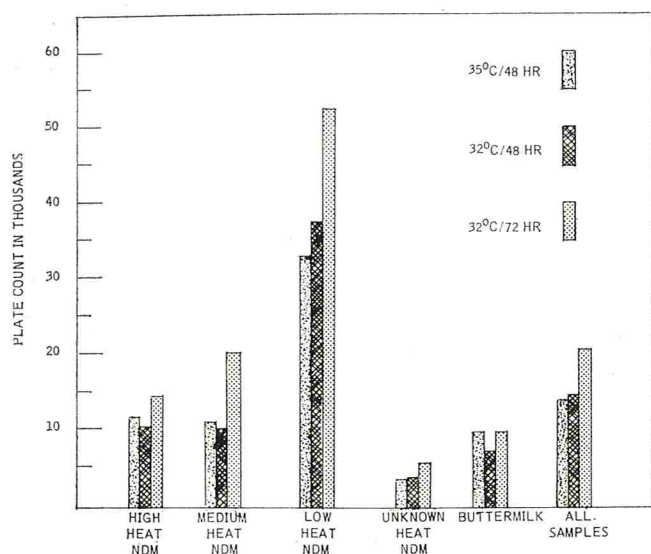


Figure 1. Effect of varying conditions of incubation on the plate count of dry buttermilk and nonfat dry milk.

crease in counts over results obtained at 35 C for 48 hr. When samples were incubated at 32 C for 72 hr, there was a 39.3% increase in counts over those incubated at 32 C for 48 hr and a 58.3% increase over those incubated at 35 C for 48 hr. The WPN in these samples ranged from 6.0 to 7.9 mg per g. The DMCC on 32 of the samples tested ranged from 6 million to 72 million per g. There was no relation between the DMCC and differences in plate counts due to varying incubation conditions. The plate counts at 35 C for 48 hr ranged from 200 to 930,000; those obtained at 32 C for 48 hr ranged from 100 to 1,100,000; and those obtained at 32 C for 72 hr ranged from 410 to 930,000 per g of dry milk.

When the 19 medium heat samples of NDM were incubated at 32 C for 48 hr, there was a 9.0% decrease when compared to the count obtained at 35 C for 48 hr. Samples incubated at 32 C for 72 hr showed a 99% and 81% increase in counts over those obtained at 32 C for 48 hr, and 35 C for 48 hr, respectively. The DMCC of 14 of the samples ranged from 12 to 440 million per g and again there was no relation between the DMCC and the differences in plate counts due to different incubation conditions. The plate counts obtained ranged from 210 to 95,000, 300 to 82,000 and 300 to 120,000 per g when incubation was carried at 35 C for 48 hr, 32 C for 48 hr and 32 C for 72 hr, respectively. The WPN ranged from 1.7 to 5.9 mg per g on the 19 samples of medium heat NDM.

The plate count obtained on 18 samples of high heat NDM at 32 C for 48 hr was 10.3% less than the count obtained at 35 C for 48 hr. The count obtained at 32 C for 72 hr was 38.5% higher than the count obtained at 32 C for 48 hr, and 24.1% higher than the resulting count at 35 C for 48 hr. The WPN

of these 18 samples ranged from 0.3 to 1.5 mg per g. The DMCC of 11 of these samples ranged from 4 to 167 million per g and again there was no relation between the DMCC and the differences in plate counts due to different incubation conditions. The plate counts obtained ranged from 300 to 100,000, 370 to 80,000 and 300 to 98,000 per g when samples were incubated at 35 C for 48 hr, 32 C for 48 hr and 32 C for 72 hr, respectively.

It was also observed on the samples of NDM studied that there was no relation between the DMCC and the plate counts obtained using any of the three sets of incubation conditions above mentioned. The relation between the DMCC and the differences in plate counts obtained at 32 C for 48 hr and 32 C for 72 hr is shown in Figure 2 for data described in the preceding paragraphs.

When 73 samples of NDM of unknown heat treatment were plated at 32 C for 48 hr, the plate count was 5.7% higher than the count obtained by incubating at 35 C for 48 hr. Samples incubated at 32 C for 72 hr were 60.3% and 69.5% higher than those obtained by incubating at 32 C for 48 hr and 35 C for 48 hr, respectively. The plate counts obtained at 35 C for 48 hr ranged from 190 to 44,000; those obtained at 32 C for 48 hr ranged from 210 to 44,000; and those obtained at 32 C for 72 hr ranged from 300 to 75,000 per g. The DMCC and WPN tests were not run on these samples.

The results obtained by incubating 54 samples of dried buttermilk at 32 C for 48 hr were 20.8% lower

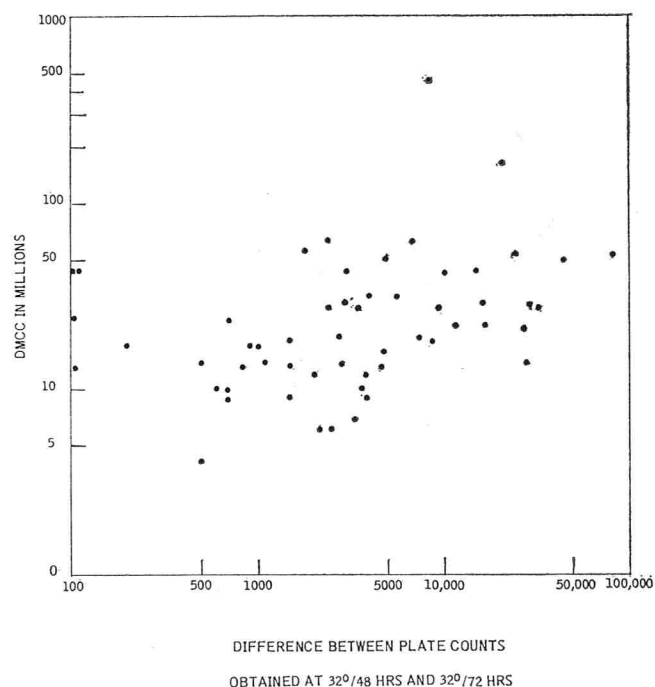


Figure 2. Relation between the direct microscopic clump count and the difference between plate counts obtained at 32 C for 48 hours and 32 C for 72 hours.

TABLE 2. GRADE CHANGES AS A RESULT OF VARYING INCUBATION CONDITIONS FROM 35 C FOR 48 HR TO 32 C FOR 72 HR

Product	Total	Extra to standard	Extra to below grade	Standard to below grade
Low heat NDM	57	5	1	4
Medium heat NDM	19	0	0	1
High heat NDM	18	0	0	0
NDM - heat treatment unknown	73	2	0	0
Buttermilk	54	0	0	0
Total	221	7 (3.1%)	1 (0.45%)	5 (2.2%)

than the counts obtained by incubating at 35 C for 48 hr. There was a 27.4% increase in the counts obtained at 32 C for 72 hr compared with those obtained at 32 C for 48 hr. The average counts for the 32 C 72 hr, and the 35 C 48 hr incubation, were practically similar. The plate count yielded results ranging from 320 to 54,000, 280 to 29,000, and 280 to 45,000 per g for samples incubated at 35 C 48 hr; 32 C 48 hr; and 32 C 72 hr, respectively. The WPN analyses performed in some of these samples yielded results ranging from 0.4 to 2.1 mg per g.

The mean plate count of all of the above samples, a total of 221, obtained at 32 C for 48 hr was 4.3% higher than that obtained at 35 C for 48 hr. The mean plate count of all samples incubated at 32 C for 72 hr was 43.4% higher than that obtained at 32 C for 48 hr. The mean plate count obtained at 32 C for 72 hr was 49.6% higher than that obtained at 35 C for 48 hr.

Since the plate count obtained on dry milks is part of a grading specification, it is of value to determine the effect of the various incubation conditions on the grade assigned. "Extra" grade of nonfat dry milk and dry buttermilk respectively, should have a bacterial estimate no greater than 50,000 per g. The plate count of "Standard" grade must not be greater than 100,000 and 200,000 per g for nonfat dry milk and dry buttermilk, respectively, and if this is exceeded, no grade may be assigned.

Table 2 presents the grade changes in the various types of dry milk products as a result of varying the incubation conditions. It will be noted that most changes occurred in the low heat type of NDM. For the 221 samples tested when the incubation conditions were changed from 35 C for 48 hr to 32 C for 72 hr, 3.1% of the samples were changed from "Extra" grade to "Standard" grade, 0.45% were changed from "Extra" grade to below grade and 2.2% were changed from "Standard" to below grade. Out of a

total of 221 samples, 13 or 5.9% were affected by the change in incubation conditions.

DISCUSSION AND CONCLUSIONS

The data presented in this paper indicates that higher plate counts were obtained at 32 C when the incubation time was increased from 48 hr to 72 hr.

Very slight differences in counts were obtained when plates were incubated at 32 C for 48 hr, as compared to plates incubated at 35 C for 48 hr, although the colonies were commonly larger and consequently easier to count at the higher temperature.

While higher plate counts were obtained by using the 72-hr incubation period, still higher counts may result by incubating for 5 or 7 days at lower temperatures as shown by Thomas, et al. (3). A method to be included in "Standard Methods" (1) must be practical. Therefore, considerations other than the one criterion of obtaining the highest possible count must be evaluated. As generally understood, there are four major criteria for judging the practicability of a method: accuracy, precision, economy and safety. With the exception of safety, which is not relevant in the evaluation of the plate count, the other factors will be mentioned in this discussion.

Of the above criteria, the only one usually considered in evaluating standard plate count procedures is that of accuracy. The term accuracy, may be considered the degree to which the results of the method approach the true or absolute value of the quantity present of the substance or character measured in the sample. Accuracy also depends upon the fundamental knowledge of the basis for the method, so that the more empirical a method is, the less accurate it is likely to be. The standard plate count is an empirical method, since the results obtained by its use depend upon certain arbitrary conditions.

An assumption has been made that those techniques which result in the highest total count are those which achieve the greatest accuracy, yet it seems that this simple assumption has not been carefully explored. It is generally recognized that dairy products contain a variety of organisms, some of which necessitate specialized techniques for their detection. It would appear difficult to devise any single method capable of detecting at one time all viable organisms, due to the numerous specific conditions which the different bacteria present in the product require for growth. Consequently, it may be seen that the absolute accuracy of the standard plate count, sought by some investigators, will not be readily attained.

Another factor which should be considered in evaluating the standard plate count method is that of precision. The term precision, according to the

technical usage of the word, means the degree to which the results obtained by a given method are reproducible. A method may be precise, yet lack accuracy. Precision assumes a great deal of importance when a method is used to determine the compliance of a product with a standard. Under these circumstances a laboratory must be capable of duplicating its own results, as well as those obtained by other laboratories. The complexity of a method or the ease of its performance, influences its precision.

Precision of the standard plate count also leaves much to be desired, since the method is highly empirical. Close adherence to the prescribed method is essential until all the variables have been investigated from the standpoint of their effect upon precision. The consideration of precision, however, does assume importance from the standpoint of the use to which the results will be put. Nonfat dry milk, for example, is purchased on the basis of grade, one of the requirements of which is the standard plate count. The standard plate count requirement for "Extra" grade instant nonfat dry milk is a maximum of 35,000 per g and for "Extra" grade nonfat dry milk, a maximum of 50,000 per g. These values are 3,500 and 5,000 per ml, respectively, on a reconstituted basis. Compared with existing standard for fluid milk on the order of 30,000 per ml, these standards for nonfat dry milk are, in fact, exceptionally rigid, yet a majority of the samples tested complied with these specifications, most of them being well below the maximum. The precision of the method, then, is not of great importance, since in many cases even doubling the standard plate count would not change the grade.

The level of the standard, however, is low enough to usually ascertain and prevent improper processing methods or any possible health considerations. It does not seem reasonable, therefore, from either an accuracy or a precision standpoint that there is an advantage in using a 3-day incubation period over the 2-day.

It would seem that the criterion of economy should carry some weight in considering the relative merit of the 2-day and 3-day incubation period. Speed or time required, is a factor which affects economy. The speed of analysis may be of considerable importance, particularly in routine control tests. Where products are graded before use, the time involved in grading

influences the amount of product which must be held in inventory until the product is released. Furthermore, in testing for quality control, rapid methods may result in more adequate control since the knowledge obtained from the results may be used by the plant operator to alter the process favorably.

In most drying plants today, grade analyses are made continuously with the manufacture of the products. Since the present edition of "Standard Methods" (1) requires a 3-day incubation period instead of a 2-day, as previously specified, additional storage space for dry milks is required and a larger inventory maintained before the product can be released for shipment. In the case of Government purchase or the testing of preshipment sample by a customer, the 3-day incubation period again results in one more day's delay in the release of the product by the Government or customer's laboratory. In addition, a 3-day incubation period requires 50% more incubator space and petri dishes.

It would appear that certain thermophilic bacteria surviving the heat treatment as may be used during processing, are impaired to an extent and require a slightly longer time for their development during incubation. These organisms, however, are apparently not of wide importance since only approximately 6% of the samples tested in this survey were affected by the longer incubation period. It is possible, however, that such organisms could be of importance to some users of dry milk and in this case special specifications may be formulated for these purposes by the parties concerned thus allowing the large majority of the users to save one day's time in the grading of dry milk products.

ACKNOWLEDGMENT

The authors wish to extend their grateful acknowledgment to Mr. Burdet Heinemann, Technical Manager, Producers Creamery Company, Springfield, Missouri, for his valuable assistance in the preparation of this work.

REFERENCES

1. American Public Health Association. Standard Methods for the Examination of Dairy Products, 11th edition. 1960.
2. American Dry Milk Institute. Bulletin 916 - Standards for Grades for the Dry Milk Industry, including Methods of Analysis. 1963.
3. Thomas, W. R., Reinbold, G. W., and Nelson, F. E. Effect of Temperature and Time of Plate Incubation on the Enumeration of Pasteurization-resistant Bacteria in Milk. *J. Milk and Food Technol.* 26:357. 1963.

AIR-BORNE MICROORGANISM POPULATIONS IN FOOD PACKAGING AREAS^{1, 2}

D. R. HELDMAN, T. I. HEDRICK AND C. W. HALL

*Departments of Agricultural Engineering and Food Science,
Michigan State University, East Lansing*

(Received for publication January 22, 1964)

SUMMARY

A slit air-sampler was used to ascertain the numbers of air-borne bacteria, yeasts and molds in dairy product packaging areas. The mean air-borne bacteria count for all food packaging areas investigated was below 6 per ft³. The highest mean count for a daily sampling period was just over 10 per ft³. The mean count for air-borne yeasts was 2 per ft³ for all samples collected. About 17% of all counts were less than 1 yeast per 5 ft³. The mean air-borne mold count for all samples was over 12 per ft³ with nearly 10% of the counts over 20 per ft³. A mean count in cottage cheese packaging was over 22 mold per ft³ while mean counts for individual sampling days revealed populations as high as 67 mold per ft³. There were significant day to day variations in all areas and standard deviations of daily sampling periods indicated significant variations within 4-hr test periods. Some degree of correlation between worker activity and air-borne counts was indicated by the results. However, it is evident that other factors also contribute to the air-borne population.

The trend toward sterile processing of milk and other food products introduces new problems associated with the packaging of such products. One of the problems involved is that of preventing contamination of the product from air-borne microorganisms. However, before this type of contamination can be controlled, the microorganism populations associated with air in food packaging areas must be investigated.

Contamination of milk and food products from air has been given very little consideration in the past since the effect on shelf life was not appreciable. In products with high heat treatments and low microorganism populations, contamination from air-borne microorganisms can greatly reduce the shelf-life.

The objective of the investigation reported in this paper was (a) to determine microorganism populations of the air associated with various food packaging areas under normal operating conditions, and (b) to determine factors which contribute to air-borne contamination.

LITERATURE REVIEW

Quantitative evaluation of air-borne microbiologi-

¹Presented at Twenty-Third Annual Dairy Manufacturers Conference, Michigan State University, East Lansing, Michigan, October 17, 1963.

²Approved for publication as Journal Article No. 3283 of the Michigan Agricultural Experiment Station.

cal particles was first introduced by Wells (9), who used centrifugal force to collect the particles on a solid surface. Since this initial development, many other methods and improvements have been introduced. Among the more effective of these methods are the slit samplers (2), filtration samplers (8), electrostatic precipitation samplers (1) and thermal precipitation samplers (5).

Any of the above methods will provide information on air-borne microorganism populations. Information on these populations in dairy and food plants is very limited. Labots (6) and Cerna (3) have reported on air-borne bacteria counts in dairy plants in foreign countries. Labots (6) used a slit air sampler and reported counts of 18 colonies per liter. In addition, the same worker reported counts of 300 colonies per petri dish per minute when using the sedimentation technique. Using the same technique, Cerna (3) revealed that counts ranged from 1 to 550 colonies per petri dish per 10 min of exposure. It was found that counts were effected by: (a) the presence of workers in the given area, (b) the number of workers in the area, and (c) the activity of the workers.

PROCEDURE

All phases of the reported investigation were conducted in food packaging areas of the Michigan State University dairy plant. The study extended over a 7-month period from January to August, 1963. In all cases, experiments were conducted in a manner which would create the least interference with normal operating conditions.

Equipment.

A Casella slit sampler was used to quantitatively sample the air in various food packaging areas. This sampler operates on a solid impaction technique, using a solidified agar medium as a collection surface. A vacuum source is used to draw air through a narrow slit, which is calibrated to provide an air-flow rate of 1 ft³ per min. The amount of air sampled can be carried by changing duration of the sampling time to 0.5, 2 or 5 min. The number of microorganisms collected on the agar surface was determined by counting the colonies after suitable incubation periods.

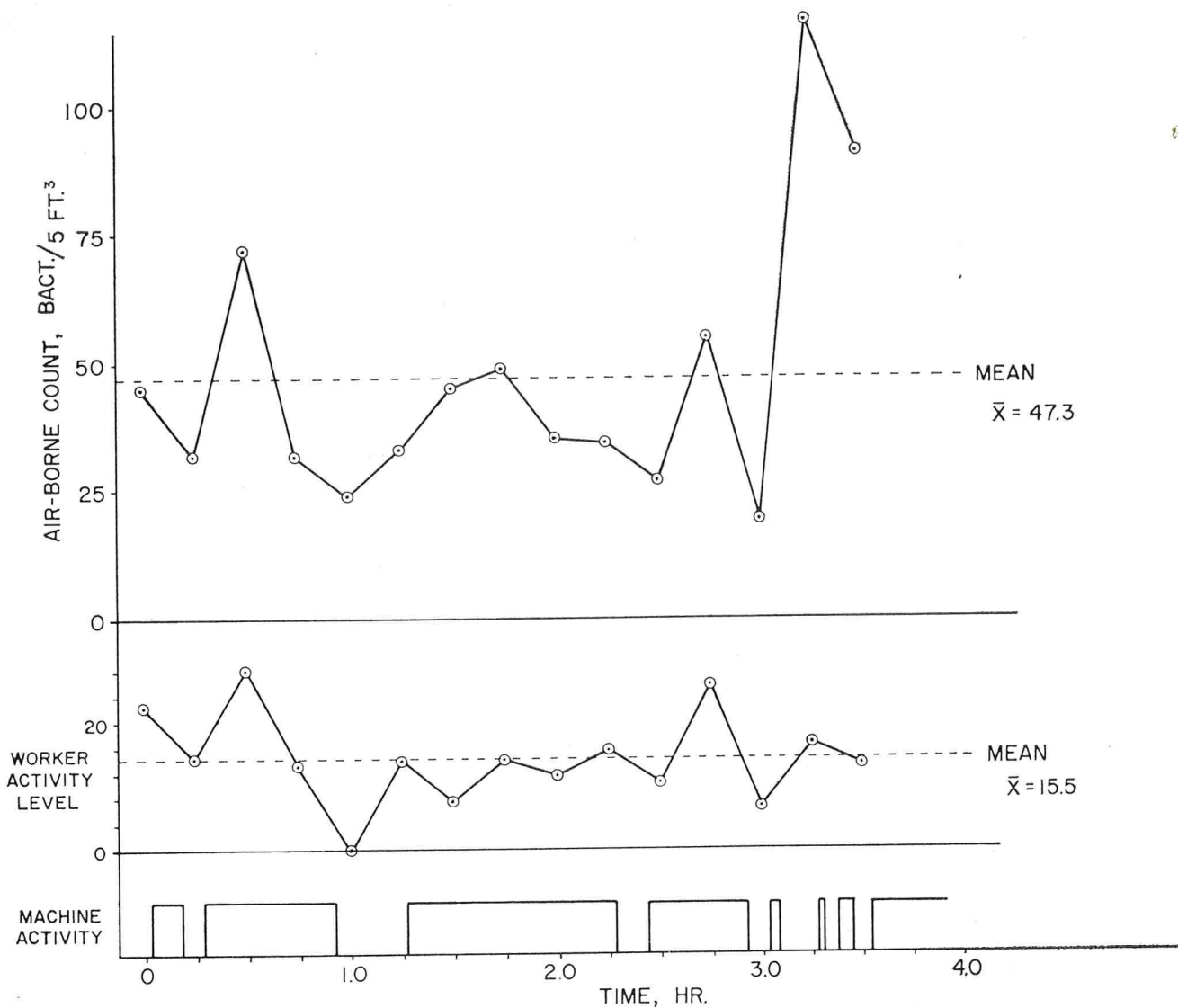


FIG. 1 VARIATION IN AIR-BORNE BACTERIA COUNT IN MILK PACKAGING AREA

Sampling locations.

Air samples were collected adjacent to milk, cottage cheese, and butter packaging locations. Milk packaging is located in a large room which also contains the milk and ice cream processing areas. The room has 16 doors or openings leading to other areas of the plant. These openings provide access for air mixing from area to area. Additional air movement is created by a central ventilation system. The sampling point at the milk packaging location was next to the paper filling operation and as near as possible to the point where air and milk contact occurs.

The cottage cheese and butter packaging locations are in a room separate from the rest of the plant, with only two doors leading into it. Two air conditioning units and the plant ventilation system provide a nearly consistent air movement except for that created by opening and closing of the doors.

The sampling point in the cottage cheese area was next to the manufacturing and packaging operations. Samples in the butter area were collected at a point next to the butter printing operation.

Sampling methods.

At each sampling location, samples were collected at 15 min intervals throughout a test period of 4 hr or more according to the procedure of Greene, et al. (4). These tests were conducted on 6 or more days at each location, and samples were collected at a level approximately 4 ft above the floor. In every case, precautions were taken to prevent contamination of the area by the operator of the sampler. Sampler parts were sterilized with 95% ethyl alcohol before each 4-hr test to prevent contamination from this source.

A tryptone glucose yeast extract agar medium was used in the standard plate count determination. In

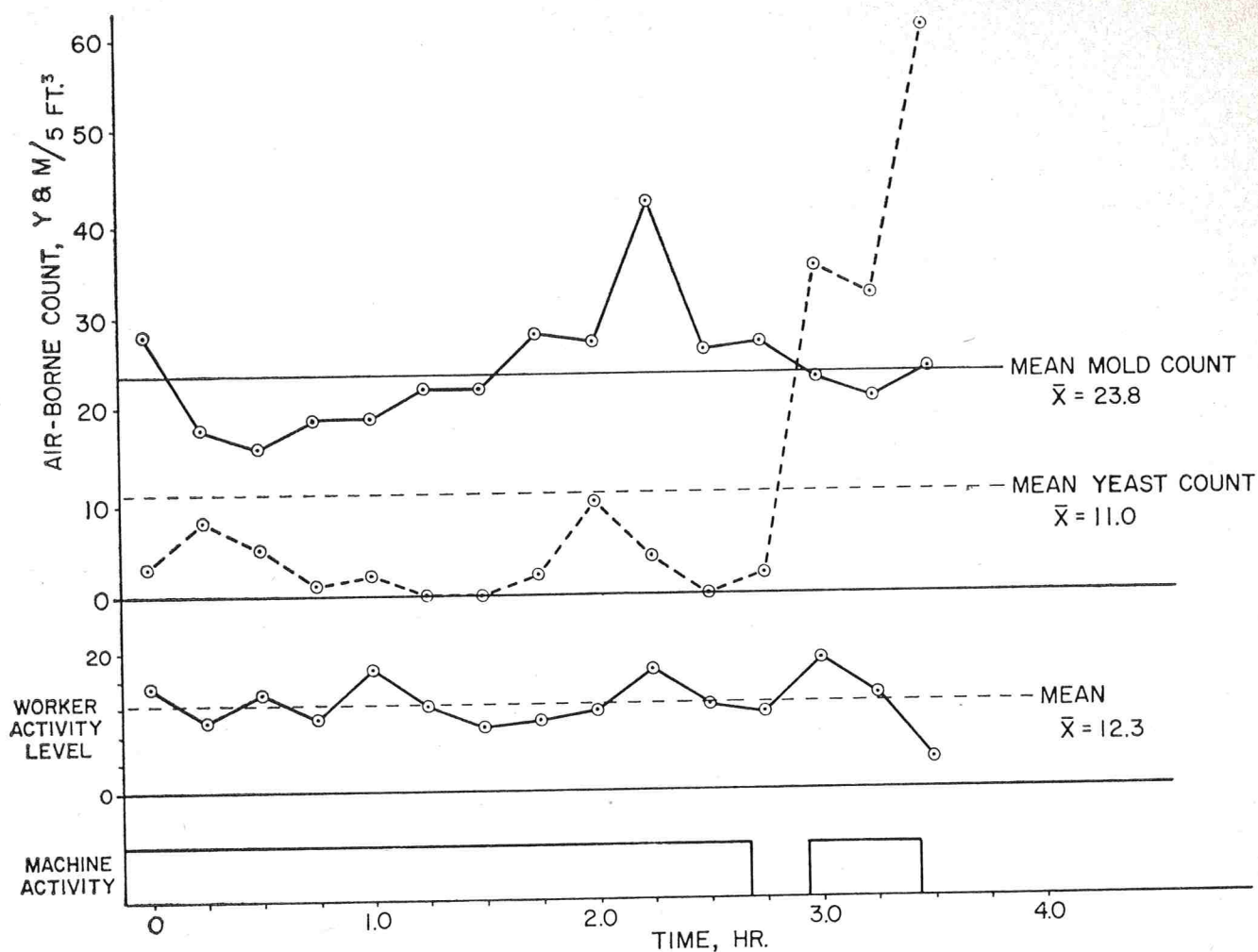


FIG. 2 VARIATIONS IN AIR-BORNE YEAST AND MOLD COUNTS IN THE MILK PACKAGING AREA.

addition, potato dextrose agar adjusted to pH 3.5, by addition of a tartaric acid solution, was used to evaluate the number of air-borne yeast and mold.

Exposed plates for the standard plate count were incubated and counted after 48 hr at 35 C. However, to obtain maximum growth and a better indication of the total number of microorganisms, the plates were given additional incubation at 37 C for 48 hr and 40 F for 7 days. Exposed plates for the yeast and mold count were incubated for 7 days at 70 F.

Worker activity evaluation.

During each 4-hr test, an evaluation of worker activity, within the test area, was made. This evaluation was based on factors of the following type: (a) activities which cause air movement within the test area, (b) activities which may cause the worker to contribute microorganisms to the air, and (c) activities which bring contaminated air directly into the air sampler. In order to set some quantitative value

on the factors mentioned, the following worker activity factors were considered: a = the number of workers in the test area during sampling, b = the number of times workers passed directly by the air sampler, and c = the number of times that workers were directly next to the sampler for extended periods of time. These factors were then used to establish a "worker activity level" (WAL) which was calculated by the equation:

$$WAL = a + b + 2c$$

This relationship was established in the following manner. Although the first factor (a) probably results in a continuous contribution of microorganisms to air in the test area, the second factor (b) results in movement of these air-borne microorganisms to the sampling point. Using this assumption, these factors appear to be relatively equal in degree of contribution to the activity level. However, the third factor (c) not only causes movement of air to the sampling point, but in addition, the worker may contribute additional microorganisms at the

TABLE 1. NORMALIZED DISTRIBUTIONS OF DAILY AIR-BORNE BACTERIA COUNTS AND CORRESPONDING WORKER ACTIVITIES IN THE MILK PACKAGING AREAS

Sampling day	No. of samples	Normalized mean - \bar{x}	SD	Mean WAL ^a	WAL SD
		(per 5 ft ³)			
1	16	8.3	4.1	7.2	3.5
2	16	30.6	8.4	8.3	7.4
3	15	41.3	19.8	13.1	6.1
4	15	25.3	7.4	16.3	7.3
5	14	12.8	6.9	11.7	5.1
6	16	23.4	4.9	13.6	3.8
7	15	21.0	13.5	12.6	2.2
8	15	45.2	29.9	13.7	5.9
TOTAL	122	26.0	—	12.1	—

^aWorker activity level.

sampling point while there for the extended period of time. For this reason, the third factor (c) was given double influence.

RESULTS

An initial survey of air-borne bacteria counts, conducted by collecting air samples at random points throughout the plant, reveal a mean count of 1 colony per 5 ft³ of air for 53 samples collected under non-operating conditions (no workers present). This level compared to a mean count of 22 colonies per 5 ft³ for 100 samples collected at random points under normal operating conditions, with large variations among counts. However, the random sampling approach was not designed to provide an indication of the factors causing: (a) differences between oper-

ating and non-operating conditions or (b) variations during operating conditions. Therefore, the air-sampling approach described in the procedure was adopted.

Figure 1 illustrates typical variations in air-borne bacteria counts within a 4-hr test period during normal milk packaging operations. These variations are also typical of results obtained in the butter and cottage cheese packaging areas. Of significance in Figure 1 are the changes in air-borne count which can occur in a 15 min interval. In the illustration, the count changes from a low of 19 colonies per 5 ft³ to a high of 117 per 5 ft³ within 15 min. These changes result in a standard deviation of 26 from a mean of 47 colonies per 5 ft³.

The changes in worker activity level (WAL) and machinery (Pure-Pak filler) activity are presented in Figure 1. In this case, the variations in air-borne bacteria count appear to be related to variations in worker activity level. However, this relationship did not appear in all test periods, indicating that other factors were contributing to the variations. The effects of machine activity on air-borne count are not evident; however, other factors such as air movement or worker activity could overshadow these effects. Process activities such as operation of the butter printer in the butter area or draining or whey in cottage cheese area resulted in increased air-borne counts.

Figure 2 illustrates the variations in air-borne yeast and mold counts within a 4-hr test period in the milk packaging area. These data are typical of results obtained in the butter and cottage cheese packaging areas, also. Again significant variations are evident with yeast counts varying from 0 to 61 per 5 ft³ and mold counts from 16 to 42 per 5 ft³ within this test period.

Possible correlation between the WAL and air-borne yeast and mold counts (Figure 2) is less evident than with air-borne bacteria. In general,

TABLE 2. AIR-BORNE BACTERIA COUNTS IN FOOD PACKAGING AREAS^a

Area	Samples	Mean count ^b	SD	No. of sampling days	Range of daily mean counts ^b	Range of daily SD	Percentage distribution of counts/5 ft ³			
							<15	<30	>30	>50
							(%)	(%)	(%)	(%)
Milk	122	28	15.7	8	9 - 47	3.4 - 30.2	15.6	40.2	59.8	9.8
Butter	98	31	32.7	6	14 - 53	16.4 - 54.3	38.8	59.2	40.8	25.5
Cottage Cheese	95	22	17.0	6	15 - 34	9.4 - 24.5	44.2	73.7	26.3	8.4
Total	315	27	22.6		9 - 53	3.4 - 54.3	31.4	56.1	43.9	14.3

^aCounts based on 35 C-48 hr incubation.

^bPer 5 ft³.

TABLE 3. AIR-BORNE YEAST COUNTS IN FOOD PACKAGING AREAS

Area	Samples	Mean count ^a	SD	No. of sampling days	Range of daily mean counts ^a	Range of daily SD	Percentage distribution of counts/5 ft ³			
							<1	<5	>5	>10
							(%)	(%)	(%)	(%)
Milk	89	10	12.9	6	2 - 26	3.3 - 24.8	25.8	61.8	38.2	30.3
Butter	98	12	17.6	6	1 - 26	1.3 - 31.6	7.1	43.9	56.1	36.7
Cottage Cheese	98	9	23.8	6	2 - 30	1.2 - 58.6	20.0	66.3	33.7	12.6
Total	282	10	18.7		1 - 30	1.2 - 58.6	17.4	57.1	42.9	26.6

^aPer 5 ft³.

results indicated somewhat closer relationships between air-borne mold counts and worker activity than between air-borne yeast counts and worker activity. There is little evidence of a direct effect of machine activity on these air-borne counts.

Results in Table 1 represent air-borne counts and worker activity levels from 8 different sampling days at the same point in the milk packaging area. In each case, the mean and standard deviation have been obtained for a normalized distribution of the values from each sampling day. Normalization was performed by plotting the experimental values on probability paper. This transformation allows a somewhat more detailed statistical analysis of day to day variations in the air-borne counts.

In general, the results (Table 1) indicate considerable variations in the means of daily air-borne counts, with a range from 8.3 colonies per 5 ft³ on sampling day no. 1 to 45.2 per 5 ft³ on sampling day no. 8.

Since the standard deviations within sampling periods are as high as $\pm 66\%$, it is evident that the variations of air-borne counts within periods are considerable, also. In addition, the results reveal that standard deviations of daily air-borne counts vary on a day to day basis, indicating that the amount of variation within sampling periods changes from day to day.

In order to establish some quantitative magnitude for the day to day variations, tests were conducted comparing the mean air-borne counts of 2 different sampling days. For the case of homogeneous variances (sampling days no. 1 and no. 6), the means were different at 0.1 level of significance. When the variances are non-homogeneous as is the case for sampling days no. 1 and no. 8, the means are significantly different at a significance level less than 0.01.

The results indicate that significant variations between air-borne count means do not exist for all sampling days. For example, a test comparing the

means from sampling days no. 4 and no. 6 reveals that the difference is not significant even at a significance level of 0.4. However, the tests which have been conducted indicate that significant day-to-day variations do exist in most cases.

Table 2 provides a summary of air-borne bacteria counts for 315 air samples collected in three food packaging areas. The mean count for all samples was 27 colonies per 5 ft³ with mean counts for individual sampling days ranging from 9 to 53 per 5 ft³. Over one-half (56.1%) of the counts were below 30 per 5 ft³, but 14.3% were over 50 per 5 ft³.

A summary of air-borne yeast counts for 282 air samples collected in the milk, butter, and cottage cheese packaging areas is presented in Table 3. The mean count for all samples was 10 yeasts per 5 ft³ of air, with daily means ranging from 1 to 30 per 5 ft³. Only 26.6% of the counts were greater than 10 per 5 ft³ and 17.4% were less than 1 per 5 ft³.

Air-borne mold counts for 282 air samples collected in three dairy food packaging areas are summarized in Table 4. The mean count for all samples was 68 molds per 5 ft³ with a range from 7 to 334 per 5 ft³ for means of daily sampling periods. Nearly 10% (9.2%) of the counts were over 100 per 5 ft³ with 57.1% being less than 30 per 5 ft³.

The air-borne counts for the three packaging areas were all relatively close to the overall mean, except for the air-borne mold counts. Although the mean air-borne mold count for the milk packaging area was relatively low, the mean counts for the butter and cottage cheese packaging areas were much higher in comparison.

The results in Table 1 also reveal rather good agreement between mean daily air-borne bacteria counts and mean daily WAL for the milk packaging area. A calculated correlation coefficient for these values is 0.417, indicating some degree of agreement between worker activity and air-borne counts. Although this does not represent a high degree of correlation, the coefficient does indicate the influence

TABLE 4. AIR-BORNE MOLD COUNTS IN FOOD PACKAGING AREAS

Area	Samples	Mean count ^a	SD	No. of sampling days	Range of daily mean counts ^a	Range of daily SD	Percentage distribution of counts/5 ft ³			
							<10	<30	>30	>100
							(%)	(%)	(%)	(%)
Milk	89	21	9.0	6	7 - 42	2.6 - 13.7	29.2	78.6	21.4	0
Butter	98	69	272.0	6	17 - 276	6.5 - 690.0	2.1	57.1	42.9	9.2
Cottage Cheese	98	111	332.0	6	19 - 334	6.7 - 831.0	4.2	36.8	63.2	17.9
Total	282	68	249.5		7 - 334	2.6 - 831.0	11.4	57.1	42.9	9.2

^aPer 5 ft³.

of worker activity on the air-borne counts. However, this correlation also reveals that factors other than worker activity must contribute to air-borne counts.

A comparison of standard deviations of air-borne bacteria counts and standard deviations of WAL obtained in the cottage cheese packaging area is presented in Table 5. The correlation coefficient for the values obtained on six different sampling days is 0.825. This coefficient reveals very good agreement between variations in air-borne bacteria counts and variations in worker activity, and indicates that changes in worker activity result in corresponding changes in air-borne bacteria counts. However, this degree of correlation was not evident in other areas indicating that factors other than worker activity are involved in air-borne count variations, also.

DISCUSSION

The data provide an indication of air-borne microorganism populations in food packaging areas of a dairy plant. These data are not expected to apply to food packaging area of all plants; however, this information provides a general view of the air conditions in such areas. Furthermore, the information presented should provide an indication of the factors

TABLE 5. COMPARISON OF VARIATIONS IN AIR-BORNE BACTERIA COUNT AND VARIATIONS IN WORKER ACTIVITY

Sampling day	Samples	SD of air-borne counts	SD of worker activity
1	16	12.9	3.33
2	16	19.5	3.69
3	16	18.1	3.44
4	16	9.4	3.06
5	16	24.5	3.67
6	15	16.5	3.12
Total	95	17.0	

which contribute to air-borne microorganism populations and must be controlled in order to maintain desired low counts.

All air-borne bacteria counts reported (28 per 5 ft³ in milk packaging; 31 per 5 ft³ in butter packaging; 22 per 5 ft³ in cottage cheese packaging) are 6 bacteria per ft³ or less. These are low counts when compared to the only other reported value for a dairy plant by Labots (6) of 18 bacteria per liter or approximately 510 bacteria per ft³. Also, they are very low when compared to mean counts of 22.3 and 13.2 colonies per ft³ for 3079 and 934 samples, respectively, collected in many areas of two different hospitals as reported by Greene, et al. (4). The air-borne bacteria counts in these areas compares favorably with recent reports for hospital operating rooms and industrial white rooms by Michaelsen and Vesley (7). Populations in hospital rooms ranged from 1 to 81 bacteria per ft³ with a mean of 10.5 per ft³ for 795 samples, while counts in the industrial white rooms ranged from 1 to 10 colonies per ft³ with a mean of 3.32 per ft³ for 76 samples. However, counts in food packaging areas may depend on outside air counts which may be as low as 2 colonies per ft³ or as high as 28 per ft³ (10) for a particular geographical location. Approximately 10% of the counts reported in the milk and cottage cheese packaging areas and 25% of the counts in the butter packaging area were greater than 50 colonies per 5 ft³ or greater than 10 per ft³ indicating the potential of higher counts.

The air-borne yeast counts encountered (highest was 12.1 yeast per 5 ft³ in butter packaging area) were all relatively low. Air-borne mold counts, such as 111.3 per 5 ft³ in the cottage cheese packaging area, do seem significantly high and could be detrimental to the shelf-life of a product such as cottage cheese.

Theoretically, it would be desirable to maintain the air-borne population in food packaging areas at zero to prevent any degree of contamination. However, under operating conditions it is impossible to

avoid all contamination due to the presence of workers and the many other factors which contribute to the over-all count. On the other hand, methods available for removing microorganisms from air provide a means of limiting the population at least in an isolated area in which the factors contributing to the count can be controlled.

Significant day to day variations in all types of air-borne counts were found. The day to day variation in air-borne bacteria count could be related to a corresponding day to day variation in worker activity. In many cases, it was evident that worker activity was not the only contributing factor. Day to day variations in outside air-borne bacteria counts and variations in operation activities also could contribute to the day to day variations.

Significant variations in air-borne microorganism counts within 4-hr test periods are also evident in data reported. In many cases these variations were related to variations in worker activity. In other cases, variations in air-borne counts occurred without corresponding variations in worker activity. These results indicate that although worker activity is a contributing factor to air-borne microorganism population; other factors must be involved.

Additional exceptions to the relationship between worker activity and air-borne microorganism count are air-borne yeast and mold counts. All results obtained indicate a lack of agreement between worker activity and air-borne yeast counts. There was a closer relationship between worker activity and air-borne mold counts; but the degree of correlation was less than that for air-borne bacteria.

From observations during test periods and results obtained, many specific factors which contribute to air-borne microorganism counts are apparent. These contributing factors can be divided into two areas: (a) the factors which cause air movement and (b) the sources of the air-borne microorganisms.

Within a food packaging area, several factors may contribute or cause air movement: (a) workers or people in the area, (b) moving parts of the packaging machine or related operations, (c) ventilation systems, (d) movement of materials, and (e) opening and closing of doors. Workers and material will cause air movement to different extents depending on activities, while movement created by machines and ventilation systems are relatively consistent. How-

ever, all factors may be contributing simultaneously.

Without some major sources of air-borne microorganisms, air movement would not be an important factor. In a food packaging area, there are sufficient number of contributing sources such as workers, dust particles, outside air, and drains. Workers may act as a source of air-borne microorganisms by talking, coughing, or even natural breathing. In addition, workers may carry dust particles and associated microorganisms from area to area within a food or dairy plant. Materials being moved into the packaging area may also carry dust particles, thereby acting as a source of dust. Outside air acts as a source of air-borne microorganisms in the food packaging area by movement from outside into the plant. This movement occurs by opening and closing of doors between areas. Drains also appear to be a definite source of air-borne microorganisms. Because of the availability of nutrients in food packaging areas, drains provide excellent locations for multiplication of microorganisms. When drains become flooded with liquids, it appears that a certain portion of the microorganisms become air-borne and are picked-up by passing air currents.

REFERENCES

1. Berry, C. M. An electrostatic method for collecting bacteria from air. *Public Health Rep.* 56:2044-2051. 1941.
2. Bourdillon, R. B., Lidwell, O. M., and Thomas, J. C. A slit sampler for the collecting and counting of air-borne bacteria. *J. Hyg.* 41:197-224. 1941.
3. Cerna, M. Study of microbiological purity of air in dairies. *Promysl. Protravin.* 12:374-379. 1961.
4. Greene, V. W., Vesley, D., Bond, R. G. and Michaelsen, G. S. Microbiological contamination of hospital air. I. Quantitative Studies. *Applied Microbiol.* 10:561-566. 1962.
5. Kethley, T. W., Gordon, M. T., and Orr, C. A thermal precipitator for aerobacteriology. *Science.* 116:368-369. 1952.
6. Labots, H. The estimation of the bacteria count of the atmosphere in dairy factories. *Off. Org. K. Ned Zuivelb.* 53:772-774. 1961.
7. Michaelsen, G. S. and Vesley, D. Industrial clean rooms vs. hospital operating rooms. *Air Eng.* 5:No. 9, 24-29. 1963.
8. Silverman, L. and Viles, F. J. A high volume air sampling and filter weighing method for certain aerosols. *J. Indust. Hyg. & Toxicol.* 30:124-128. 1948.
9. Wells, W. F. Apparatus for study of the bacterial behavior of air. *Am. J. Public Health.* 23:58-59. 1933.
10. Wolf, H. W., Skaliy, P., Hall, L. B., Harris, M. M., Decker, H. M., Buchanan, L. M. and Dahlgren, C. M. Sampling microbiological aerosols. *Public Health Rept.* 74:12. 1959.

NEWS AND EVENTS

SCIENTISTS BELIEVE INSECT ATTRACTANTS MAY LESSEN NEED FOR PESTICIDES

Writing in the August issue of *SCIENTIFIC AMERICAN*, Martin Jacobson and Morton Beroza, research chemists in the Pesticide Chemicals Branch of USDA's Entomology Research Division in Beltsville, Md., suggest that the success achieved in eliminating the Mediterranean fruit fly in Florida through sex lures offers new hope for using such lures to fight undesirable insects, rather than the pesticides which today are under heavy fire from groups which believe they are harmful to man and desirable species as well as to undesirable insects.

"...On the strength of this (Florida) demonstration," the authors write, "and a wealth of information acquired by intensive research, it can be said that the outlook for eradicating insect pests by means of attractants is now much more encouraging."

"The principal difficulties in such a program are the problem of identifying the specific odorous substances to which various insect pests respond and, in the case of the natural sex lures, the task of obtaining large enough amounts of these substances to carry out the program. . ."

The program is based on the fact that "Many insects find their food, their partners for mating and favorable sites in which to deposit their eggs by means of automatic responses to various scent cues. Male moths, for example, can smell potential sex partners at a considerable distance. Not surprisingly, each species tends to have its own distinctive odor, which facilitates the meeting of partners capable of mating with each other.

"This behavior makes the odor-baited lure a most promising means of getting rid of malignant species of insects. Instead of spraying the whole countryside with insecticide, one could attract the unwanted insects to traps where they would make contact with an appropriate insecticide, with a sterilizing chemical, or with some other exterminating device. Females could be induced to lay their eggs not on nourishing plants but in places where the emerging larvae would starve for lack of food. The odorous lures and the lethal chemicals could be applied only at the most effective time and place. Any new infestation of insects could be detected early and eliminated before it had a chance to spread. In sum, the battle against harmful insects would be much less costly and more efficient, and the problem of the contamination of the environment by toxic materials would be vastly reduced."

The authors conclude their article with this optimistic note: "With the sensitive devices that chemists

now have for analyzing extremely tiny amounts of material, the prospects for fighting the insects with their own lures and for investigating their behavior have suddenly become much brighter than they have been in the past. We can now mark insects and recall them at some later time by means of attractants. Thus armed with the ability to follow the lives of individual insects, we are in a position to learn more about the ecology, flight habits, longevity and other attributes of the insects, which in number of species constitute four-fifths of the animals that inhabit the earth."

Research, by USDA and other scientists, into the so-called "biological" means of controlling undesirable insects has been greatly increased as a result of the furor which followed the publication of Rachel Carson's "Silent Spring." There are advantages in the biological, or attractant, controls inasmuch as the insects do not develop immunity to the lures as they do to many of the insecticides now in use. The elimination of the need for applying chemical insecticides would be a boon to agriculture which has been under fire because of uncertainties about the dangers of the insecticides to other species.

USE OF PETROLEUM WAXES

Petroleum waxes which meet certain specifications may be safely used in or on food and in food packaging materials, the Food and Drug Administration, Department of Health, Education, and Welfare, has concluded on the basis of extensive studies.

Final Food Additive regulations published in the Federal Register of July 8, 1964, establish specifications for conventional uses of petroleum waxes as coatings on cheese, fruits and vegetables, in chewing gum, as a defoamer component and as a component of wrapping or other containers designed for contact with food. The action was taken on a petition filed by the American Petroleum Institute in October, 1962, under the Food Additives Amendment to the Food, Drug, and Cosmetic Act.

The regulations prescribe testing methods which eliminate from food use waxes that contain polynuclear compounds. Only waxes clearly shown to be safe can pass the FDA tests.

Results of extensive research on animals were submitted by the Petroleum Institute. Dr. Edward O. Haenni of FDA's Division of Food Chemistry developed the analytical methods used by the Institute. Application of these methods now make it possible for industry and FDA to identify and keep off the market any waxes that fail to meet the specifications.

MILK INDUSTRY FOUNDATION URGES U. S. PUBLIC HEALTH SERVICE TO CHANGE PROPOSED NEW MILK ORDINANCE AND CODE

The Milk Industry Foundation urges the U. S. Public Health Service to include in its proposed new Milk Ordinance and Code provisions which would assure safe wholesome milk and permit the milk industry to be vigorous, inventive, creative, and competitive in providing milk and milk products to the public.

The USPHS proposal for revision of the Milk Ordinance and Code was released last March for study purposes and MIF recommendations are based on studies by its Product Regulations Committee, approved by its Board of Directors.

MIF comments to the Public Health Service urge full opportunity for the milk industry to develop new or changed products, to apply new technology, to develop new methods and procedures, to meet the competition of substitute products and other foods and beverages, and to utilize milk processing plants to produce other than milk and milk products if doing so does not, or is unlikely to, contaminate the milk or milk products regulated by the proposed ordinance.

Proposals submitted by MIF include:

1. Definitions for products made by use of food grade acids, for multiple vitamin-mineral fortified milks, for skim milk of not more than 0.5 percent milk fat, and for low-fat milk of more than 0.5 percent and less than 3.25 percent milk fat (actual fat content to be slated on the label).
2. Exclusion from the ordinance of sterile milk.
3. Provisions for the addition of skim milk solids to whipped cream, culture type products, skim milk, low-fat milk, half and half, and the wide use of food additives which may be useful in development of new products.
4. A comprehensive procedure to govern the application for, and the suspension, revocation, and reinstatement of permits, (licenses).
5. Procedure for acceptance of farm inspections made by industry.
6. Recommendation for cooling raw milk in bulk tanks to 45°, can milk to 50°, and maintenance (by plants) at 45° or below at all times prior to pasteurization.
7. Bacterial standards not to exceed 100,000 per ml. at farms, 200,000 per ml. as received at receiving station or processing plant and at no time to exceed 400,000 per ml.
8. Standards for pasteurized milk and milk products as follows:
 - a. Cooled to 45° or below and maintained below 50° (this to accommodate retail delivery during warm weather).

b. Coliform count not exceeding 5 per ml. within 24 hours of processing or while at plant where processed.

c. Bacterial count not to exceed 15,000 per ml. at plant where processed and not to exceed 30,000 per ml. at any time while in possession of processor.

Many additional changes are included in the MIF proposals submitted to the U. S. Public Health Service. All are intended to assure safe and wholesome milk and milk products while at the same time modifying or eliminating provisions believed unnecessary to this purpose or unnecessarily restrictive and costly.

A third draft of the USPHS recommendations is now being prepared, as MIF has requested. It is scheduled for release by USPHS by late August. Sixty days will be granted for study of this draft and filing of comments by industry and regulatory authorities. MIF will continue to insist that the provisions of the new code do not force industry into a rigid regulatory strait jacket, but be limited to requirements necessary to assure for the nation a safe, clean, and wholesome milk supply.

DR. EDGAR ALLAN DAY RECEIVES AWARD FOR RESEARCH

Dr. Edgar Allan Day, associate professor in the Department of Food Science and Technology at Oregon State University, has been named the first recipient of the Institute of Food Technologists' Award for Research.

Dr. Day was nominated on the basis of his fundamental contributions to knowledge and methods in the detection and identification of trace components in foods. His work in this field was supported by four grants from the Public Health Service Division of Environmental Engineering and Food Protection.

The Award recognizes scientists 35 years of age or younger who have demonstrated outstanding ability in some area of food science and technology research.

The specific area for which Dr. Day was cited involved knowledge of components affecting flavor of milk and milk products. Research in this field aims to define chemically the flavor attributes in dairy products so that manufacturing processes can produce acceptable foods consistent with the highest public health standards.

Working under Public Health Service grants, Dr. Day discovered and identified many flavor compounds in dairy products and contributed significant knowledge in the area of deteriorative changes in dairy products.

His achievements were cited as being of both fundamental and applied nature.

Dr. Day received his B.S. from the University of

Maryland in 1953. His M.S. was received from Pennsylvania State University in 1955, where he also received his Ph.D. in 1957.

NEW FOOD SERVICE MANUAL FOR NURSING HOMES

A 32-page manual, "Feeding With Paper Service—The Newest Trend For Nursing Homes" which shows how paper service can be used to improve sanitation, cut down noise, lighten trays and dress up dining in nursing homes of any size, has been published by the Paper Plate Association. The illustrated booklet is designed to assist dietitians, nursing home administrators and kitchen planners in establishing a more efficient food service program.

Drawing its information from actual case histories, the booklet provides a comparative breakdown of cost and time savings; indicates consumer reactions; reports on controlled portioning and freezing procedures; and describes operations with complete and partial use of paper.

Of major importance is paper's role in providing utmost sanitation standards and preventing cross-infection. The booklet devotes an entire chapter to this subject, explaining why sanitarians not only recommend but often insist on paper service whenever there is a question about the sanitary efficiency of dishwashing operations.

Using paper to save time, labor and food is stressed through the use of portion control, pre-portioning and freezing. Of help to the efficiency-minded operator is a table of paper portion cup sizes and standard containers. Foods that are particularly well suited for freezing are also cited.

Partial use of paper service is discussed in a number of instances including: one meal a day, Sundays and holidays, snacks, special occasions, medical uses and water service.

Finally, a general description of the most commonly used paper items can be helpful to the operator in selecting the right styles and sizes for his particular serving needs. Items covered are: paper plates, dishes, hot drink cups, cold drink cups, portion cups, hot and cold food containers, soup bowls, medicine cups, place mats, tray covers and doilies.

Material for the brochure was taken not only from the files of the Paper Plate Association but also from the Linen and Lace Paper Institute and the Paper Cup and Container Institute, both of which are also making the book available to their members.

Copies are available at no charge from the Paper Plate Association, 441 Lexington Avenue, New York, New York 10017.

ARTHROPOD-BORNE ENCEPHALITIS—ITS EPIDEMIOLOGY AND CONTROL FILM

Throughout the United States, there have been periodic outbreaks of a devastating disease of man and horses . . . encephalitis, inflammation of the brain. Transmitted by the bites of infected mosquitoes, the disease is one of the major insect-borne diseases of this country.

This film shows clinical signs of encephalitis in humans and horses, discusses diagnosis, treatment and prevention.

Three distinct viruses may cause encephalitis in the U. S. One, the etiological agent of western encephalitis has been isolated from birds and mosquitoes throughout much of the United States.

Eastern encephalitis appears restricted to the Eastern seaboard, Gulf of Mexico and localized areas in the midwest. A third type, St. Louis encephalitis, and the associated virus was later recognized in California and Florida epidemics.

The film explains the transmission cycle of encephalitis from birds, to mosquitoes, to other animals and man. It describes research which determined the cause of seasonal fluctuations in incidence of the disease and laboratory methods for determining virus-carrying mosquitoes. It also discusses control methods.

This film was produced for vector control personnel, epidemiologists, students of the health professions, and civic groups under monitored conditions.

ANNOUNCEMENT OF NEW PUBLICATION

Examination of Farm Milk Tanks—A Manual for Weights and Measures Officials, by Malcolm W. Jensen, National Bureau of Standards Handbook 98; May 22, 1964; 36 pages; 35 cents. (Order from the Superintendent of Documents, U. S. Government Printing Office, Washington, D. C. 20402, or from local U. S. Department of Commerce Field Offices.)

This publication, one of a number of Handbooks of the National Bureau of Standards designed to present in compact form comprehensive technical guides for State and local weights and measures officials, is a manual describing the devices, testing equipment, gaging, inspecting and testing procedures, and reporting systems used in examining farm milk tanks.

Handbook 98 is the first of a series that will treat the examination of individual types of measuring devices. The entire series will succeed National Bureau of Standards Handbook 45, *Testing of Measuring Equipment*, published in 1951 and now out of print.

This Handbook has been published in "pocket" size to further its usefulness to the official in his field operations.

Although this Handbook is prepared primarily for use by weights and measures officials of the States, counties, and cities, it is believed that the information presented will be useful to manufacturers, calibrators, and others interested in farm milk tanks as measuring devices.

NOTE: Foreign remittances must be in U. S. exchange and should include an additional one-fourth of the publication price to cover mailing costs.

INTERNATIONAL GUESTS TO BE WELCOMED AT OCTOBER DAIRY AND FOOD EXPOSITION

Dairy and food processors from all over the globe are completing their plans to attend the 1964 Dairy and Food Industrial Exposition to be held October 4-9 at McCormick Place in Chicago, Illinois.

The Exposition is the world's largest display of dairy and food industrial processing supplies and equipment, with more than 300 exhibits. It regularly attracts between 500 and 700 guests from beyond the borders of the United States, among a total attendance of nearly 25,000.

A book-sized Directory, given to every person qualified for admission to the Exposition, will clearly show which items on display are exportable beyond the borders of the United States and indicate export managers and other personnel.

Many visitors from abroad, however, will be in the non-buying category — e.g. scientists and educators and students, similar to their counterparts among American visitors. These visitors find the Exposition a major gathering center for ideas, since all exhibitors traditionally show their finest and latest lines and products.

Admission for all processors, public officials, educators, scientists, and students is free. The general public is not admitted, however, and some fees are charged to persons who represent manufacturers of dairy and food industrial supplies and equipment not on display.

Once inside, guests from abroad will find special welcomes for them in numerous booths which feature linguists (the Exposition Directory clearly identifies these booths and the languages spoken there) and additionally, overseas guests will find an extra warm welcome awaiting them at the International Lounge. Staffed by Dairy Society International, a non-profit organization whose aims are education and the building of trade, the lounge will serve guests as a social center or message center, and volunteer personnel will help visitors arrange tours, visits to plants, farms and factories.

OTHER CONVENTIONS

Convening concurrently with the Exposition are a number of organizations. Dairy Society International will hold its annual meeting on Sunday, October 4, the opening day of the Exposition. Other groups meeting include International Association of Ice Cream Manufacturers, October 5-7; Evaporated Milk Association, October 6; National Ice Cream Mix Association, October 7; Milk Industry Foundation, October 7-9; and National Ice Cream Retailers Association, October 8-10.

Occupying seven acres of space, the more than 300 exhibits will feature dairy and food industrial equipment of nearly every variety; the latest advances in cleaners and sanitizers, clean-in-place systems, and automated systems; delivery equipment; flavors and ingredients, stabilizers and emulsifiers; containers; point-of-sale equipment and supplies; and a host of miscellaneous services and supplies, including architectural services, financing advice, accounting systems, advertising programs, and technical publications and periodicals.

Of world-wide concern is the subject of a forum for management to be held the final two days of the Exposition, October 8-9, "Food and the Future: Concepts for Planning." The seminar, arranged by the Show's sponsor, Dairy and Food Industries Supply Association, will emphasize the problems of providing food for the world's growing millions, as well as opportunities for top management to anticipate and take advantage of the far-reaching technological changes occurring almost hourly in dairy and food processing.

FORUM SPEAKERS

Dr. K. G. Weckel, Professor of Dairy and Food Technology, University of Wisconsin, will serve as forum moderator. Speakers will be: Dr. James R. Bright, Professor of Business Administration, Harvard Graduate School of Business, who will discuss the impact of technological change on the food industry; Dr. E. M. Foster, Professor of Bacteriology of the University of Wisconsin, who will discuss microbiology of the food supply and environmental control in the food industries; Dr. A. T. McPherson, Physical Sciences Administrator, U. S. Department of Commerce, and a recognized authority on synthetically produced nutrients, who will discuss possible chemical bases for the food supply of the next century; and Dr. R. G. H. Siu, Chairman of the Army Research Council, U. S. Department of Defense, who will discuss new principles in preservation of a wide range of food products.

To be held at McCormick Place, the forum will be open to all those who are qualified to attend the

Exposition. Due to space requirements, however, advance reservations will be necessary for forum attendance.

Another highlight of the Exposition week will be the Collegiate Students' International Contest in Judging Dairy Products, jointly by the American Dairy Science Association and Dairy and Food Industries Supply Association. Teams from 30 or more colleges and universities in the United States and Canada are expected to enter. Contestants are generally college seniors and top prizes are DFISA cash fellowships for graduate study.

A full calendar of evening social events will round off the week's activities, and a special program of daytime events for the ladies is also planned.

A booklet describing the Dairy and Food Industrial Exposition is available on request from the Show's sponsor, Dairy and Food Industries Supply Association, 1145 - 19th Street, N.W., Washington, D. C. 20036.

A MACHINE CALLED IRMA

A new machine called IRMA may come to the aid of the dairy industry and indirectly help provide high protein, low butterfat milk for the American consumer.

The machine, called the Infrared Milk Analyzer (IRMA), was demonstrated July 22, by its inventor, J.D.S. Goulden, at meetings of the American Dairy Science Association eastern division being held at the N. Y. State College of Agriculture, Cornell University, July 20-22.

Goulden, who works for the National Institute for Research in Dairying, Reading, England, put a sample of homogenized milk in the machine and in less than one minute the amounts of fat, protein, and lactose (milk sugar) were flashed on the read-out meter. This is far less time than with present chemical methods and tests indicate it is nearly as accurate.

Goulden says a beam of infrared light passes through the milk sample. The various components of milk absorb different wave length of the light and the machine reads the absorption at these wave length—converting the actual percentage of each component.

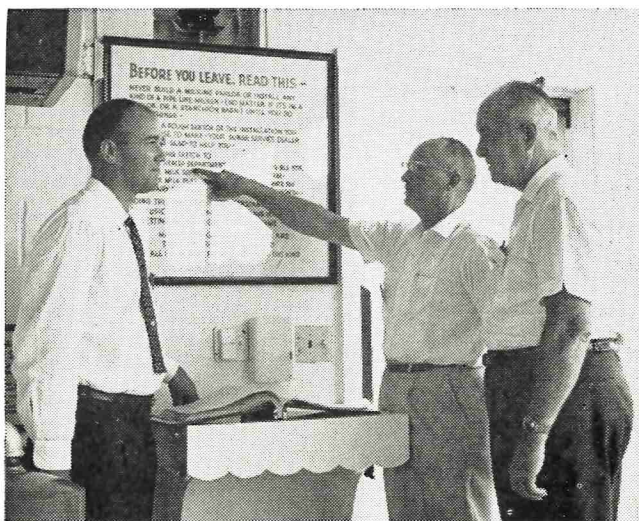
At the present time, in most of the U. S., dairymen are paid for milk on the basis of the amount of butterfat in it. With the calorie-conscious consumer demanding less butterfat, the dairy industry

has been working on other methods and test procedures on which to base payments. In England and Canada, payments will soon be made on the basis of both milk solids and fat content of milk.

When tests are available for milk solids, farmers will be able to cull and breed dairy cattle for these factors and produce the kind of milk wanted by the consumer. The problem has been in finding accurate tests that could be done quickly at small cost.

Development of the machine has been sponsored by the National Research Development Corporation in England where it is now being tested for routine use. The model demonstrated at the meeting by Goulden is on its way to Canada—the first machine to be used outside England.

Goulden pointed out while the cost of running each sample is only a fraction of a dollar, the cost of the machine is great and it would probably be used in a centralized place where it could be kept working all the time. He says the individual dairy would not have enough samples to test to make it feasible.



Good advice for the dairy farmer is given on this notice pointed to by T. W. Caton, Sanitarian, City Health Department, Lexington, Kentucky. It stresses that each dairy farmer building a new milking barn or installing a pipeline milking system be sure to do this:

"Take a sketch to the health department involved before any project is started." In hearty accord are John Phillips, right, also a sanitarian with the Lexington Health Department, and James Wise, who represents the Surge dairy equipment dealer in the Lexington area. The scene is in the milkroom at the Surge Training Center near St. Charles, Illinois, where these men recently attended the Dairy School.



Media for MYCOLOGY

DIAGNOSTIC for the isolation, identification and cultivation of pathogenic fungi. These media are also widely used in phytopathological studies. Several are neutral in reaction, giving optimum conditions for growth of a variety of fungi. The following may be prepared as selective media by the adjustment of reaction, addition of antibiotics or other agents:

Bacto-Brain Heart Infusion Agar	Bacto-Mycological Agar
Bacto-Sabouraud Maltose Broth	Bacto-Mycological Broth
Bacto-Sabouraud Liquid Medium	Bacto-Corn Meal Agar
Bacto-Littman Oxgall Agar	Bacto-Corn Meal Agar with Dextrose
Bacto-Bean Pod Agar	Bacto-Prune Agar
	Bacto-Lima Bean Agar

CONTROL for sanitary and sterility procedures as well as for general use in mycological procedures:

Bacto-Sabouraud Dextrose Agar	Bacto-Neurospora Culture Agar
Bacto-Sabouraud Maltose Agar	Bacto-Potato Dextrose Agar
Bacto-Malt Extract	Bacto-Mildew Test Medium
Bacto-Malt Agar	Bacto-W.L. Nutrient Medium
	Bacto-W.L. Differential Medium

CLASSIFICATION and nutritional studies of fungi:

Bacto-Yeast Morphology Agar	Bacto-Czapek Dox Broth
Bacto-Yeast Carbon Base	Bacto-Czapek Solution Agar
Bacto-Yeast Nitrogen Base	Bacto-Vitamin Free Yeast Base

THE DIFCO MANUAL, NINTH EDITION,

including descriptions of these media and their use, is available on request.

DIFCO LABORATORIES
DETROIT 1, MICHIGAN

START DRIVE AMONG VETERINARIANS TO GIVE BLOOD FOR BETTER RABIES TREATMENT

A physician faced with the problem of administering rabies antiserum to a severely bitten child may soon have available a product that includes no risk of dangerous reaction to the treatment.

Currently under way is a drive among veterinarians throughout the country to donate blood to improve treatment for persons exposed to rabies.

The ultimate aim is to eliminate shock syndrome and "delayed serum sickness," occurring in some persons after rabies treatment.

Joining in the drive to collect blood from the veterinary profession are the U. S. Public Health Service, Communicable Disease Center, Atlanta, Ga.; state health departments; state and local veterinary associations, and the National Red Cross.

Explaining the program in the Aug. 1 issue of the *JOURNAL OF THE AMERICAN VETERINARY MEDICAL ASSOCIATION*, Dr. James H. Steele, Chief of CDC's Veterinary Public Health Section, reveals that approximately 32,000 individuals each year are given rabies vaccine because they are exposed to a rabid or suspected animal.

He says that in ordinary cases of rabies treatment,

new improved duck embryo vaccine has reduced vaccination accidents until they are almost unheard of today.

However, Dr. Steele points out, where hyperimmune horse serum is used to treat more severe types of exposure, such as bites about the face, neck, head, or hands, "the danger involved in its administration is well known to physicians who have used it to any



Consistently Accurate!

GARVER
Milk & Cream Testing Equipment

Faulty testing equipment can turn profit into loss—fast. Insure now against inaccuracy with this efficient Garver combination.

1. Garver "Super" Babcock Tester. Speed controlled and speed indicated for extreme accuracy.
2. Garver "ovate action" Test Bottle Shaker. Thoroughly integrates test ingredients—saves time—eliminates dangerous, haphazard hand twirling.

Write today for catalog.

THE GARVER MANUFACTURING CO.
Dept. JM, Union City, Ind., USA

"Babcock Tester Manufacturers for Four Decades"

extent."

"Reactions following administration of this horse-serum product have been reported in 20 to 50% of serum-treated individuals."

Dr. Steele says that the replacement of horse serum with human serum in tetanus treatment has eliminated the reaction problem in the administration of tetanus antitoxin, continuing

"The supplanting of horse serum with the high titering human serum globulin may be the answer to the present problem of passive immunization in rabies as well as tetanus exposures."

According to Dr. Steele, the highest antibody titers against rabies occur in those individuals who have received Pasteur rabies treatment, a treatment commonly used before the introduction of the improved duck embryo vaccine.

Veterinarians, because they are most frequently exposed to rabies, are a group with many individuals thus treated, and are therefore considered the most likely donors for the program.

A pilot program was recently completed among Georgia veterinarians. Similar programs are in the planning stage in several other states.

After the research and development phase of this project is completed, Dr. Steele says, it is hoped that a potent and safe biological product for use in treatment of exposures to rabid animals will then be made available for distribution throughout the country on an emergency basis.

"We feel that this is an excellent opportunity for the veterinary profession to make still another contribution to public health in a positive and personal way," he concludes.

CLASSIFIED ADS

FOR SALE

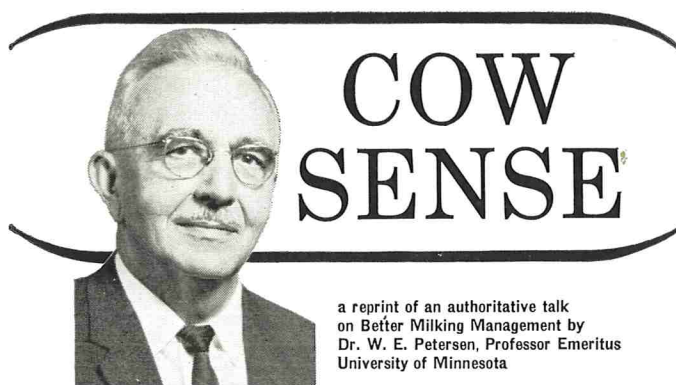
Single Service milk sample tubes. For further information and a catalogue please write, Dairy Technology, Inc., P. O. Box 101, Eugene, Oregon.

PLANT SANITARIAN

Multi-plant operation, including brewery, grain mill, malt house, and fermentation plant. Permanent assignment with responsibility for directing and improving present sanitation program. College degree, preferably with Entomology major and at least two years experience in food, grain or processing industry or with FDA. Starting salary commensurate with the background. Comprehensive benefit program. Interviewing and relocation expenses covered by employer. Send replies to Industrial Relations Manager, P. O. Box 3217, Peoria, Illinois.

NOTICE

Anyone having extra copies of Journal of Milk and Food Technology, March, June, 1960, January, February, August, 1962 and April, 1964, please send them to JMFT, Box 437, Shelbyville, Indiana.



COW SENSE

a reprint of an authoritative talk on Better Milking Management by Dr. W. E. Petersen, Professor Emeritus University of Minnesota

"There are many offenses committed against the dairy cow, but none is more serious nor more costly than that of *overmilking*. . . leaving the milking machine on the cow after the milk has ceased to flow out of the teats. It causes no harm as long as the milk flows out of the teat. No forces are exerted on the inside of the teat, but as soon as the milk ceases to flow, then the vacuum extends from the teat cup to the inside of the teat with damaging effects.

"The teat is lined with a very delicate lining and the application of the vacuum to this lining causes damage to it, frequently rupturing it. This paves the way for micro-organisms to enter into the tissue and mastitis may result. As a matter of fact, the biggest single cause of mastitis is that of injury to the teat and the lower part of the udder. Improper use of the milking machine in overmilking is more responsible for this injury than any other single cause.

"The milking machine emulates the calf's nursing almost perfectly. In each case, the milk is extracted by means of vacuum. There is this one big difference, however, between the calf's nursing and that of extracting it by the milking machine. The calf quits nursing when no more milk comes, but the average milking machine goes on with damaging effects as long as it's left on."



Now there is an automatic milker that shuts off vacuum, just like a calf, as each quarter is milked out. The Perfection automatic milker is the only milker in the world that times itself, with "Stop Watch" accuracy for each quarter. Perfection starts, strips, and stops itself, so your cows can't be injured by overmilking.

Ask for a free demonstration of Perfection's

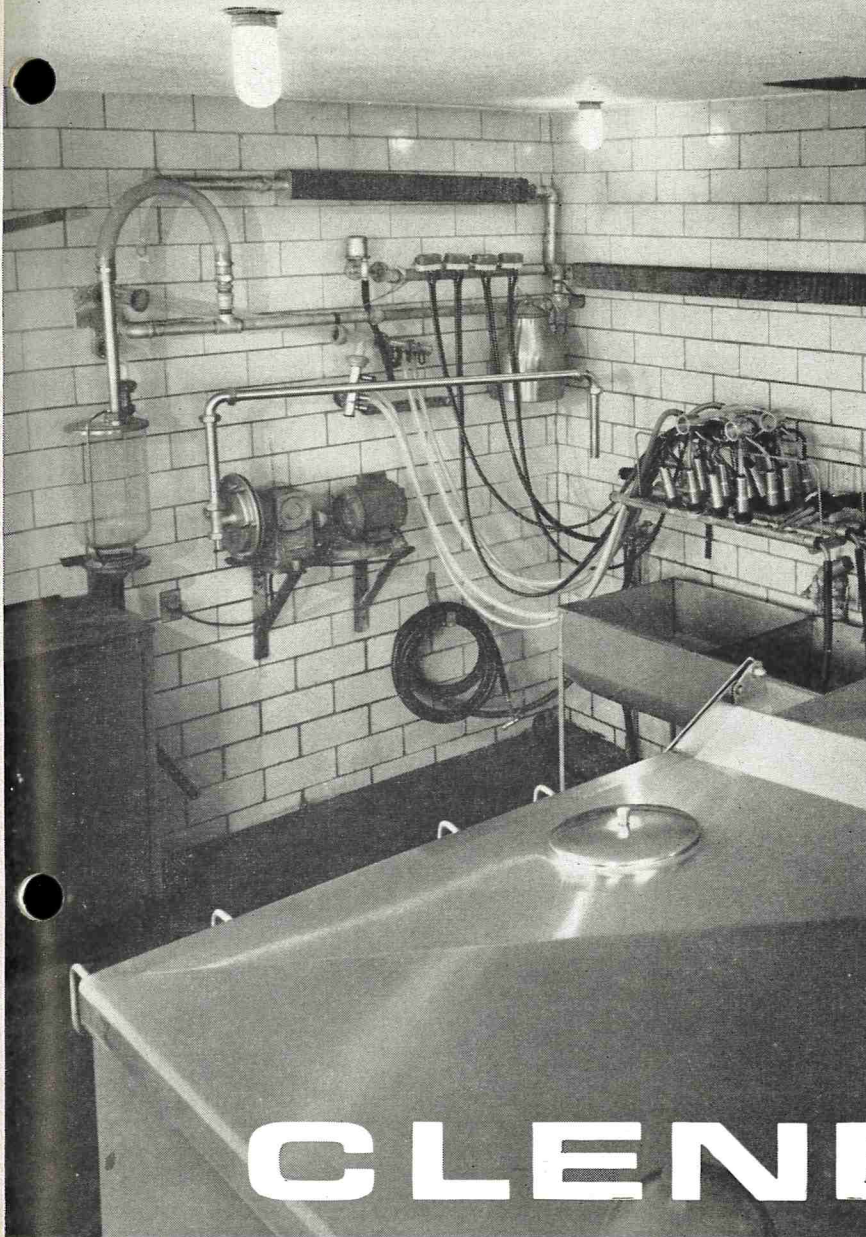
"STOP WATCH" milking



PERFECTION

A Great Name in Milkers . . . Now Better Than Ever
PERFECTION DIVISION, Sta-Rite Products, Inc.
 234 S. EIGHTH STREET • DELAVAN, WISCONSIN

...from milk house



...thru the dairy



CLENESCO

is a total cleaning and sanitizing concept

Today, CLENESCO offers a *complete* line of products for fast, efficient cleaning and sanitizing of all areas of potential contamination. Cowles research technicians have carefully formulated and tested CLENESCO products so you will get hygienically clean tanks, pipelines, and processing equipment with minimum effort and expense.

These products are supplemented by Cowles Technical Men, who can help the dairy industry meet new standards in sanitation control. For complete information about CLENESCO general cleaners, sanitizers, can-washing compounds and milkstone removers, write to —



CLEVELAND, OHIO 44120

DAIRY AND FOOD SHOW HOURS SET

The First Dairy and Food Industrial Exposition, which occurs October 4-9, 1964, in McCormick Place in Chicago, Ill., will be open for a total of 40 hours.

Dairy and Food Industries Supply Association, the Show's sponsor, has announced the following schedule of hours for the Show:

Exposition Hours

Sunday, Oct. 4 ----1 p.m.-5 p.m.

Monday, Oct. 5---12 noon-6 p.m.

Tuesday, Oct. 6----10 a.m.-6 p.m.

Wednesday, Oct. 7_10 a.m.-6 p.m.

Thursday, Oct. 8--12 noon-6 p.m.

Friday, Oct. 9-----10 a.m.-6 p.m.

You'll wonder
where the
ACID
went!

When you are on
**MONARCH
CLEANING
PROGRAMS**

Find out why:

**"THERE'S NOTHING LEFT ON
EQUIPMENT BUT SHINE!**

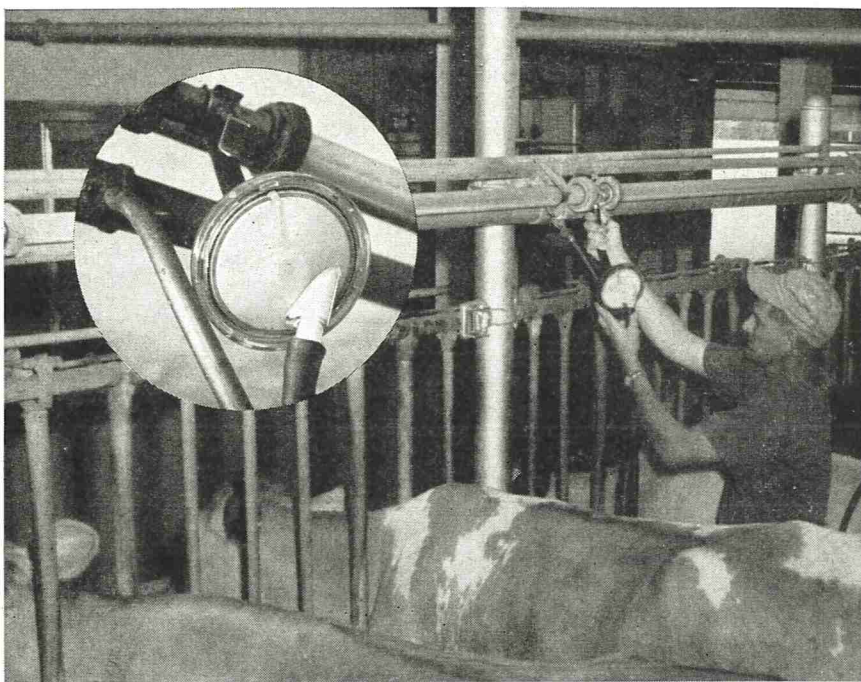
Check with your Dairy... Farm Supply Dealer or write for FREE "Planned Programs" folder.

SEP-KO CHEMICALS, INC.

3801 N.E. 5th St. — Minneapolis 21, Minn.

Sep-Ko Chemicals of Canada Ltd.
Toronto, Ontario, Canada

GUARANTEED UNBREAKABLE!



New **RAPID-FLO®** FILTER HOLDER by Johnson & Johnson

GIVES YOU A MANAGEMENT REPORT AT EACH MILKING

With the new guaranteed unbreakable **RAPID-FLO®** Filter Holder, and 4-9/16" **VACULINE®** Filter disks, you get a visual check on sediment. You spot mastitis flare-ups—detect abnormal milk problems at a glance, without guesswork. You help insure the quality of your milk.

RAPID-FLO® Filter Holder by Johnson & Johnson complies with applicable items of Milk Ordinance and Code of U. S. Public Health Service. Meets all safe vacuum requirements.

AND NOW IT'S GUARANTEED! Johnson & Johnson will replace any **RAPID-FLO®** Filter Holder plastic parts that break under normal milking practices within one year of date of purchase. Order today. Only \$10.45 complete.



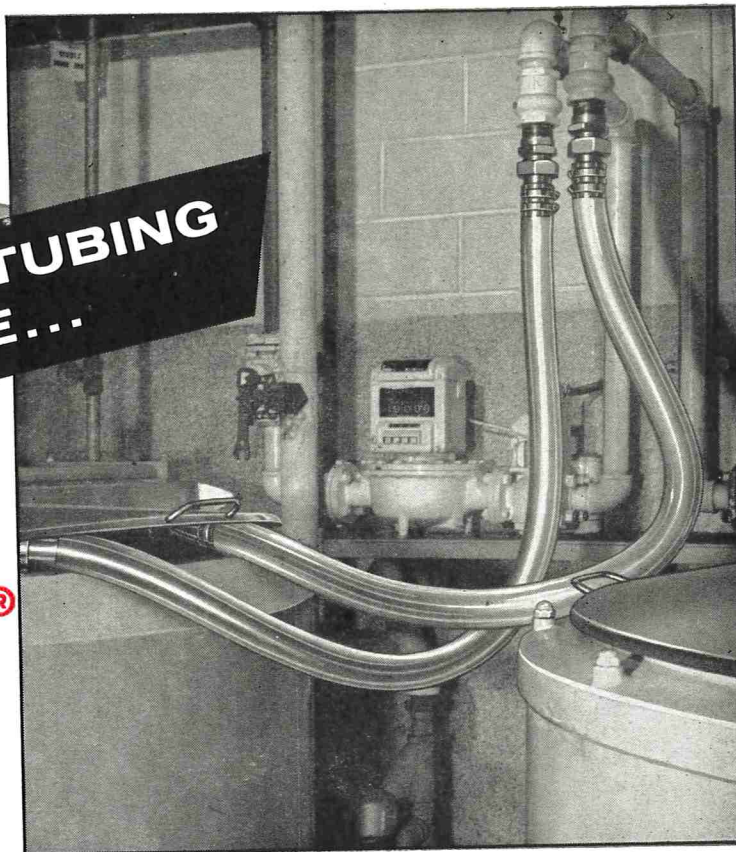
Dept. 22084

Johnson & Johnson
4949 W. 65th St. Chicago, Ill. 60638

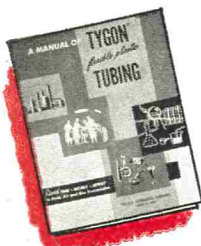
(DAIRY DEPARTMENT)

NO OTHER MILK TUBING
IS EXACTLY LIKE...

TYGON[®] B44-4X



TYGON B44-4X TUBING
MEETS ALL CRITERIA IN THE
NEW 3-A PLASTICS STANDARD.



Compare the unique advantages of Tygon Tubing B44-4X with any other piping medium and you'll see why more and more dairies and processors of milk products are using Tygon. Write today for free Bulletin T-101.

Not just another so-called "General purpose" hose, Tygon Tubing Formulation B44-4X was developed solely and specifically to meet the critical requirements of handling processed milk and milk products.* As such, it meets every physical, sanitary and chemical requirement of the job.

CLEAR — Tygon's sparkling clarity permits visual inspection and control of flow, facilitates maintaining high standards of cleanliness.
FLEXIBLE — Retained throughout the full range of normal operating temperatures, Tygon's rubber-like flexibility speeds set-ups, requires a minimum of couplings and fittings, simplifies revisions as needs change.

COMPLETELY SAFE — Tygon Tubing B44-4X is completely harmless and non-toxic, complies fully with the FDA's "Food Additive Amendment". Moreover, Tygon will not affect the taste or odor of milk or milk products.

EASILY CLEANED AND SANITIZED — Smooth, dense bore cannot trap tiny particles, is easily flushed clean. Any commercially available cleaner or sanitizer, including all common dairy cleaners, can be safely used.

RANGE OF SIZES — Running lengths of Tygon Tubing B44-4X are available in more than 66 sizes with bores from 1/16" to 4".

FOR YOUR PROTECTION every foot of Tygon Tubing is branded with the name TYGON and the formulation number.

* For piping liquid foods and beverages other than milk, use Tygon Tubing, Formulation B44-3.

Plastics and Synthetics Division


U. S. STONWARE
AKRON 9, OHIO

197H-3

Here's something you can do to help Dairymen fight MASTITIS

Authorities are pretty generally agreed that a good job of cow milking is an important factor in Mastitis control. And . . . a good job of milking is dependent on both the mechanical condition of the milking machine . . . and the men who operate that equipment.

Babson Bros. Co. and Your local SURGE Dealers are now equipped to help you DO something about both conditions.

Two precision instruments are available. The Babson Flow-Meter for determining vacuum system capacity . . . and, the Babson Levograph Vacuum System Analyzer that records operating vacuum levels as well as pulsator action. With them, the adequacy and efficiency of the milking machine's vacuum system can be analyzed so that corrective steps can be taken.

Proper operation can best be accomplished by education of the men who run the milkers. Here too, Babson Bros. Co. and SURGE Dealers have available special training sound-slide films to help you get the story of good cow milking explained . . . and aid you in YOUR Mastitis Control problems.

Write or contact your nearest Authorized SURGE Dealer or Babson Bros. Co. to arrange for use of the films, "The Story of Vacuum" or "Mastitis Is Your Problem", as well as availability of actual vacuum system tests.

SURGE is a Babson Bros. Co. trademark © Babson Bros. Co., 1964

BABSON BROS. CO.

2843 WEST 19TH STREET—CHICAGO, ILLINOIS 60623

