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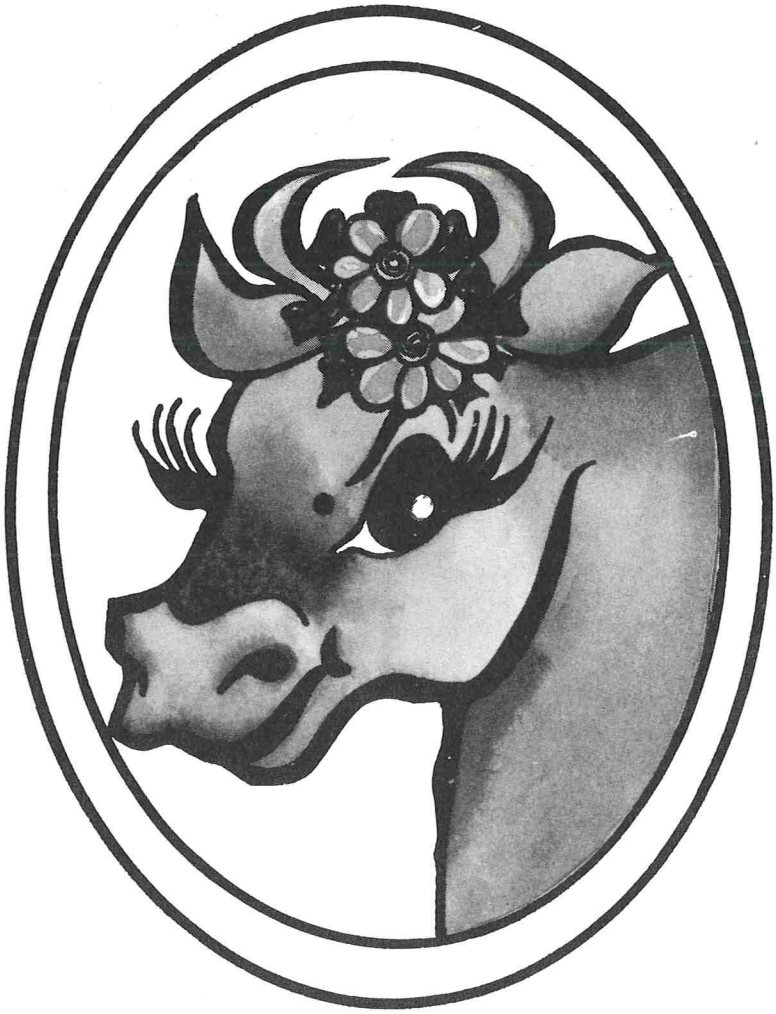

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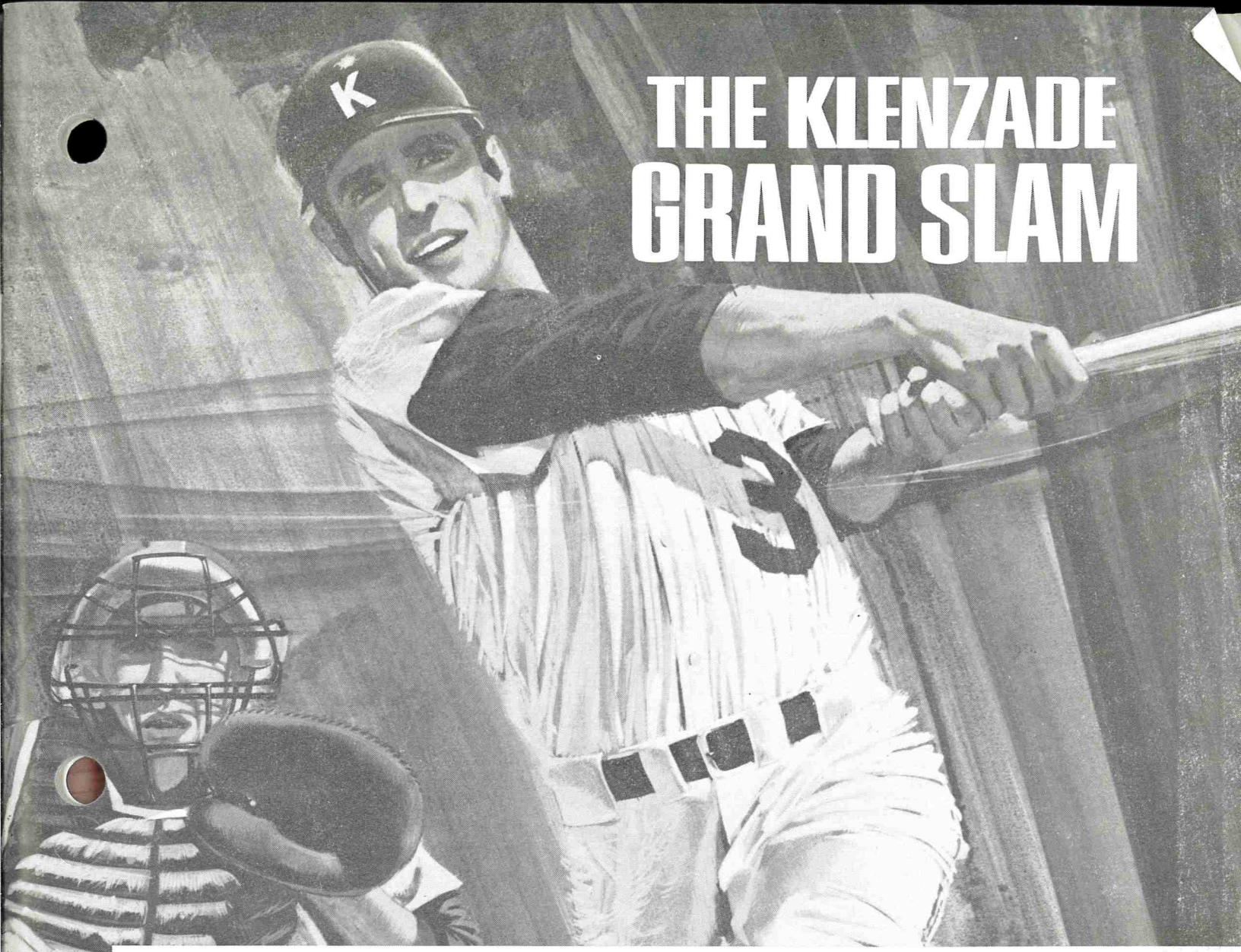

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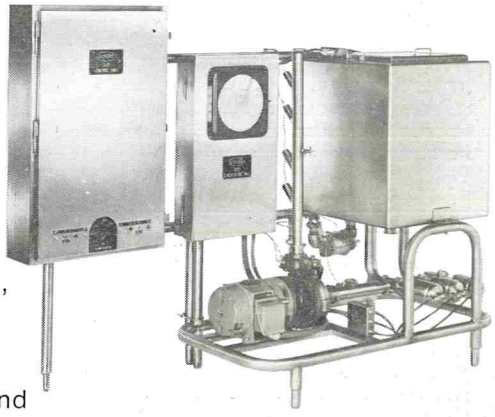

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INCLUDING MILK AND FOOD SANITATION

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Reg. U. S. Pat. Off.

Vol. 35 January, 1972 No. 1

SODIUM IN FOODS AND BEVERAGES[1]

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(Received for publication July 20, 1971)

ABSTRACT

Sodium in the human dietary is derived from many sources, some of which are obvious, while others might be termed "hidden." Table salt and other compounds used in food processing and preparation contribute large quantities of sodium to the day's total intake. Numerous additives commonly used by the food industry are contributing a larger and larger proportion of the sodium content in the usual dietary.

Chronic ingestion of increasing intakes of sodium can have serious consequences in susceptible persons. A second look at all sources of dietary sodium may be in order, particularly in view of the introduction of newer convenience-type foods into the retail and institutional markets.

Salt, and other sources of sodium, have played a vital role in man's physiological environment. Life began in the salty environment of the sea and it is not surprising that salt should be a necessary component of all protoplasmic systems.

Salt has been used in cooking and preservation of foods since prehistoric times. Although sodium and chloride are necessary constituents of the extracellular fluids in the body of man, the appetite for salt and many other sodium-containing additives seems to be an acquired habit rather than a reflection of need.

PHYSIOLOGIC ROLE OF SODIUM

Salt is dissociated in aqueous solution to form sodium and chloride. Therefore the constituent ions are considered separately in respect to their effects on the total organism.

Because sodium and chloride constitute the principal ions in the extracellular fluid of the body, their major role in the body is to maintain osmotic pressure of body fluid compartments. Concentration of these ions directly affects control of the volume of interstitial fluid, control of the water content of the tissues, and control of the volume and pressure of circulating blood.

Sodium also affects irritability of cells but in this role it is directly related to other ions which must be present in fixed ratios in order to promote normal physiologic activity. Relative concentrations of potassium, magnesium, calcium, and hydrogen affect the ability of sodium to maintain normal cell irritability. This relationship is sometimes observed clinically in the review of electrocardiograms. The well known effects of low levels of serum potassium on the electrocardiogram may not be seen in the presence of elevated serum sodium levels, or reduced levels of calcium and magnesium *(4)*.

Sodium in the body has the additional role of helping to maintain normal acid-base balance by the stabilizing action of the sodium buffer systems which are present in the extracellular fluid. Sodium exchanges for potassium and for hydrogen in the distal tubule of the kidney. The quantity of sodium in the tubular fluid determines the rate of excretion of both potassium and hydrogen ions. This relationship is responsible for the potassium losing effect following ingestion of large amounts of sodium salts and the accentuation of an existing potassium deficiency when a diet high in sodium content is eaten.

Conversely, when sodium-induced hypertension occurs in animals, potassium has a protective effect *(6)*. Regulation of the sodium content of body fluids and tissues is maintained normally by the balance between intake and excretion. Under usual circumstances the excretion of sodium is controlled by the kidney. If sodium intake is reduced, the kidney has the capacity to reduce sodium excretion to very low levels, a capacity which does not exist for potassium. Therefore, with normal kidney function and in the absence of unusual losses of sodium through the skin or bowel, a severe sodium restriction in the diet does not result in serious sodium deficiency.

USUAL INTAKE OF SALT AND SODIUM

Salt and sodium intakes vary considerably among individuals in the United States and throughout the world. French chefs, for example, are said to add 3% of salt in their baking while in many areas of England, very little salt is used for this purpose *(4)*.

It is common in medical and dietetic practice to express total sodium consumption in terms of grams, milligrams, or milliequivalents. Frequently the person who is advised to adhere to some level of sodium restriction because of a disease condition must learn what this terminology means and how to interpret it in respect to his daily consumption of an advised

[1]Contribution from the College of Agricultural and Life Sciences.

amount of sodium.

This usually means the patient will need to find sodium contents of food products readily available on the food label. For ease in converting milligrams of sodium to milliequivalents, the milligram weight is divided by the atomic weight of the monovalent sodium ion. For example a diet (or a single food) which contains 2300 mg of sodium would contain 100 milliequivalents (meq) of sodium. Label statements about the milligram and milliequivalent weights of sodium in a given portion would be exceedingly useful for the many people who must adhere to a sodium-restricted diet. The daily sodium intake of Americans has, for some years, been estimated as ranging between 2.3 and 7.0 g (2300 - 7000 mg or 100 - 304 meq of sodium). A part of this is derived from salt per se, a part from the sodium naturally present in foods and waters, and a part from the sodium containing substances added in food processing and preparation. It is probable that the sodium intake of many Americans now lies high above this upper range of estimate because of increased use of sodium-containing additives in food processing.

Sodium ingested via the salt route is then only one of many sources of sodium intake. Naturally, the use of baking soda, baking powder, monosodium glutamate, and other commonly used additives in food preparation also add to the sodium content of the usual dietary.

Many common foods in their natural state contain sodium in amounts that can be significant when dietary restriction is indicated because of disease. Unprocessed foods of animal origin, which contain protein, have significant natural contents of sodium. They include milk and other dairy products, meats, fish, eggs and variations thereof (Table 1). Non-fat milk solids contribute sodium as well as protein and other nutrients. Increasing use of milk-base convenience foods by the public probably is an important source of sodium for many individuals. Added to this, the salt and/or other additions used in processing or preparation of foods already fairly high in sodium, can make for very significant quantities of sodium in the day's total intake *(9)*. Fruits and vegetables, in their natural form, are generally low in sodium.

Analysis of foods used in hospital menu planning at the University of Wisconsin clearly showed the effects of sodium containing additives on final composition of foods which, in their usual state, contribute sodium *(2)*. American cheese has been thought to contain roughly 1,100 mg of sodium per 100 g of cheese. In the study mentioned, the content of sodium in 100 g of one brand of American cheese was close to 1,800 mg. The increase in sodium content of this one newer food item apparently resulted from addition of sodium aluminum phosphate which was added as an emulsifier. Commercially prepared stew may contain up to 9,000 mg of sodium per 10 oz can *(5)*.

TABLE 1. SOME EXAMPLES OF SODIUM CONTENTS OF FOODS AND BEVERAGES

Food	Amount	Approximate mg sodium
Foods from animal sources		
Butter, salted	1 tsp	50
Cheese, cottage	1 oz	130
Egg	1	60
Fish, fresh, unsalted (as walleye pike)	1 oz	80
Meat, fresh, unsalted	1 oz	20
Milk, buttermilk	1 cup	315
Milk, whole fresh	1 cup	120
Other common foods		
Bread, regular	1 slice	135
Fruit	1 serving	2
Low sodium vegetables as lettuce, sliced tomato	1 serving	5-10
Vegetables	1 serving	varies widely
Taste teasers		
Bacon	3 slices	300
Baking powder (tartrate type)	1 tsp	350
Baking soda	1 tsp	1000
Bologna	1 slice (1 oz)	390
Bouillon cube	1 (for 1 cup liquid)	1050
Canadian bacon	1 serving (2 oz)	1530
Cheddar cheese	1 slice (1 oz)	210
Corned beef	1 serving (2 oz)	1040
Cornflakes	3/4 cup	220
Dill pickle	1/4 medium	270
Monosodium glutamate	1 tsp	765
Prepared yellow mustard	1 tsp	60
Soda crackers	4	130
Soy sauce	1 tsp	365
Table salt	1/4 tsp	580
Wines		
Dessert (U.S. origin)	1 qt	75- 510
Red (U.S. origin)	1 qt	48-1210
Rosé (U.S. origin)	1 qt	115-1100
White (U.S. origin)	1 qt	32-1175

To list the contribution of various sodium containing additives to individual foods in the course of food preservation and processing would be an almost endless task. Brine solutions used in vegetable processing; mold retardants used in breads; and salted, corned, and pickled meat products are several examples of methods of introducing sodium into commonly consumed foods. In the latter connection, some highly spiced, highly salted sausage and other meat products have been known to produce "taste fatigue," the result of an over abundance of flavor in the product. New uses for sodium containing additives are constantly being discovered *(8)*. A new use will probably be found for precooked and reheat-

ed meats, now commonly used in institutional food service *(7)*. Undesirable organoleptic changes caused by oxygen and moisture can apparently be prevented by certain additives, some of which contain sodium. What these additives will do to sodium contents of foods, particularly for hospital food services, remains to be seen.

TABLE 2. SOME EXAMPLES OF SODIUM CONTAINING CHEMICALS USED IN FOOD PROCESSING

Preservatives
Sodium acetate, diacetate, benzoate, hypochlorite
Sodium propionate, chloride, sorbate
Antioxidants
Sodium ascorbate, thiosulfate, sulfite, bisulfite, metasulfite
Sequestrants
Sodium diacetate, citrate, gluconate metaphosphate, aluminum phosphate, tartrate, thiosulfate, calcium disodium salt of EDTA.
Surface active agents
Sodium sulfoacetate glycerides, monosodium glycerides, stearate, taurocholate
Stabilizers, thickeners
Sodium alginate, methyl sulfate, pectinate
Bleaching and maturing agents
Sodium chloramine, chlorite, hypochlorite, tri-metaphosphate
Buffers, acids, alkalis
Sodium acetate, carbonate, bicarbonate, citrate, aluminum phosphate, acid pyrophosphat, hydroxide, potassium tartrate
Non-nutritive sweeteners
Sodium saccharin
Flavoring agents
Synthetic flavors
Spices, herbs
Miscellaneous—Firming agents, texturizers, binders, anti-caking agents
Sodium sulfate, sulfide

Frequency of use of individual food items must be considered in evaluating the sodium contribution of a given food in the dietary. When an individual eats large quantities of bread, for example, the use of salted bread as a single type of food in the diet will soon increase sodium intakes. Breads prepared without added salt contain some sodium depending on the kind and proportion of ingredients and on the local water supply. One product developed in cooperation with a bakery in the Madison area averages 5 mg sodium for a 1-oz slice, whereas in the same area, a 23-g slice of salted bread contains 200 mg or more of sodium.

Drinking waters vary in sodium content but often are a significant additional source of sodium intake. A study conducted by the U. S. Public Health Service indicated that in samples derived from 2,100 municipalities in the United States, the sodium ion varied considerably, with highest sodium concentrations found consistently in the waters of the Far West and Midwest areas of the United States. All areas of the country had some municipal water supplies showing a high sodium content *(10)*. Here again, the significance of sodium contents of drinking waters becomes most important when dietary sodium must be restricted. A sodium content of more than 20 mg per liter could be of much importance when sodium restriction on the order of 500 mg a day is indicated in disease conditions. A 500 mg sodium intake is less frequently advised now than it has been formerly, in part because of the development of more effective medications which promote body sodium loss. However, a dietary prescription of 1,000 mg of sodium is not at all uncommon in the food services of most hospitals and in the home care of the patient for whom sodium intake must be restricted and controlled. The water and syrup in which foods are packed may in themselves contain large amounts of sodium. Like drinking waters, wines in the diet may also be an additional unexpected source of sodium (Table 1).

Ingestion of sodium from foods and liquids can be further increased by use of sodium containing toothpastes and medications such as cough remedies, laxatives, antibiotics, and sedatives.

The introduction of new food items, especially the convenience type food item, into the retail and institutional markets has posed problems for the physician, patient, and dietitian. Although various tables of sodium composition of foods commonly used in American households have been available in past years, the lack of information regarding sodium composition of newer food products has made dietary planning for the patient difficult.

IMPLICATIONS OF HIGH SODIUM INTAKE IN HEALTH AND DISEASE

Because of the healthy kidney's capacity to excrete excess sodium, the assumption has been made by many that a liberal intake of sodium is not injurious to the healthy adult. This assumption has been refuted by some. There appears to be growing support for the thesis that excessive sodium intake over a period of time might precipitate hypertension and atherosclerosis in susceptible individuals *(1)*. The food industry is alerted to the withdrawal of monosodium glutamate from foods processed for babies. It is known, of course, that the infant's renal excretory mechanisms are not mature and do not function with the same efficiency as the healthy adult kidney.

The implications of high sodium intake in certain disease conditions are profound and serious. Sodium retention occurs in a number of clinical conditions. Congestive heart failure, hypertension, kidney diseases, cirrhosis of the liver with fluid retention, severe malnutrition, and toxemia of pregnancy are among the more common. Sometimes medications,

in themselves, cause sodium retention and therefore must be used in conjunction with a sodium-restricted diet. Certain steroids, for example, can cause this situation to occur.

In many hospitals a temporary loss of normal kidney function is sometimes noted post-operatively in patients of all ages, and excessive sodium (and solute) intake at this time can be injurious. Elderly persons often have a change in kidney efficiency which may or may not be reflected in the way the kidney can handle sodium.

When sodium restriction is obviously indicated as often happens in congestive heart failure and various forms of kidney failure, strict adherence to a regimen of diet and medications is often a life or death proposition. As mentioned previously, sodium restriction of the order of 1,000 mg a day is not uncommon nor are prescriptions for less or more than this amount of sodium.

In recent months and years medical attention has focused also upon the total osmolality of a diet, since it is known that diets with high osmolalities (derived from ionic constituents and dissolved substances, notably sugars) can provoke diarrheas in some sick persons and increase serum osmolality levels in others.

Depending upon the total composition of a special diet, a diet high in sodium may have a high osmolality. The osmolality effects of processed convenience type liquid diets and mixtures consisting of liquified foods have received attention. Reports of deaths in persons fed liquid diets via nasogastric tube have been attributed to the effects of the osmolality of the feeding unaccompanied by adequate fluid intake.

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GE INTRODUCES NEW HIGH-TEMPERATURE VORTEX I VORCINERATOR SYSTEM TO CUT POLLUTION-IN SOLID WASTE DISPOSAL

The General Electric Industrial Heating Department announces Vortex I, a new high-temperature modular Vorcinerator system affording high-speed elimination of industrial solid waste materials without violating air and water pollution regulations.

The Vortex I system, which meets Federal air pollution control standards, was especially designed for industrial manufacturing plants, warehouses, institutions and food processors who have solid waste disposal requirements in the range of 5- to 20-tons daily.

Vortex I provides 10- to 20-times the hourly combustion rate of conventional incinerators. The new Vorcinerator system uses a horizontal vortex combustion chamber measuring 7-feet wide by 11-feet long, and has an operating temperature range of 1800 to 2000 F.

The cylindrical combustion chamber can handle up to 1-1/2-tons-per-hour of industrial waste materials, such as highly combustible paper, cardboard, wood boxes and plastics. The waste can contain up to 10 percent non-combustible materials, and small rubber items from commercial and industrial sources.

The new modular Vorcinerator system is comprised of the Vortex combustion chamber with automatically regulated secondary air system, primary air/feed blower and material feed rate control system, exhaust stack assembly, control, panel, and ash separation system.

The automatic control system contains two separate overtemperature control systems which monitor the exhaust gas temperatures through thermocouples and meter-relays to provide double protection by shutting down the electrical power system and secondary air supply in case the pre-selected maximum temperature limits are exceeded. The entire unit will shut down to the preheat condition automatically after its last load if left unattended.

The cylindrical combustion chamber receives waste material blown in from a high-capacity shredder. The

(Continued on Page 14)

IMPROVED HEALTH FOR YOUR HEALTH DEPARTMENT'S FILING SYSTEM

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(Received for publication July 2, 1971)

ABSTRACT

Poor management of filing systems has become a costly inconvenience to many operating offices. The Johnson County, Iowa, Health Department has adopted a new filing system which eliminates the problem of lost records. Consideration is given to drawer crowding, size and weight of folders, labeling of folders, and how much is filed in each. The scheme of arrangement provides index headings common to the user, and easily identifiable color coded index tabs for all primary and secondary guides and folders. In addition, a cross reference index provides easy location of materials. The system now functions satisfactorily, but only time can indicate the trouble spots.

"Where is that letter? I know I filed it in this drawer very carefully so that I could find it quickly!" Tempers often flare, embarrassing moments occur, and valuable time is wasted searching through files for a misfiled record. A survey by Records Control, Inc. shows that from 1 to 5% of records are being misfiled and one-half of the misfiled records are never found *(4)*. After a 7-year study of our nation's companies, savings and loan associations, banks, etc. Industrial Psychology, Inc. has calculated the cost of a misfiled letter or record to be $61.23 *(4)*. The point of the problem is often management indifference *(2)* which probably is why managers sometimes fail to take the responsibility to secure and properly supervise skilled clerical employees *(3)*. Managers and executive must be made aware of the high cost of clerical errors to do something about the problem.

Officers of Health Departments are not magically immune to misfiled letters and poorly organized filing systems or non-systems. The Johnson County, Iowa, Health Department in 1970 was a victim of such a non-system. The purpose of this paper is to show what was done to correct the problem.

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[2]Present address: Comprehensive Health Planning Unit, P. O. Box 7053, Grand Rapids, Michigan 49510.
[3]Page 83.

THE REORGANIZATION PROCESS

Several mornings were spent studying the organization of the files. "In revising a system you must know how the present system works and what its weaknesses are." (4)[3] At the same time, a review of the different types of filing was made. Principal references included, *Progressive Filing and Records Management* and *How to Appraise Files in 60 Seconds (2)*. Following is a list of questions considered.

(a) Are the drawers crowded?
(b) Are all the folders uniform in size and weight?
(c) Is there a scheme of arrangement?
(d) How are the file folders labeled (typed or handwritten)?
(e) How much material is filed in each folder? *(2)*

While examining the files, minor changes were made—scrap pieces of paper were removed, papers that were clipped together were stapled, file folders were turned into proper position, papers had to be straightened out or turned so all would be facing in the same direction, and misfiled records were properly filed.

The Health Department is engaged in numerous activities and programs as can be seen by the list given in the project section. Because of the large amount of records required for just one program or activity, there are separate file drawers for milk and milk products, restaurants and food service establishments, trailer parks and mobile homes, schools, administrative activities, etc. These alphabetic files seemed to be working well and thus were left intact. The filing of nuisance investigations and project activities seemed to be the big problems.

Although there was some semblance of subject filing in the old system, it was ineffective. The trouble appeared to be one of categorizing subjects in some kind of order. Categorizing was done by using the list of sanitation activities and programs.

At this point, a filing truism was put into efficient use. "With 'subject' indexing, select words for the thesaurus which already are common to the users of the files" *(1)*. An example of this is "weeds" was used instead of "noxious weeds."

The new system was adopted and revisions were made while files were being transferred. At this time the basics of proper filing procedures were performed. In many instances records were coded which had never been coded before. The final list of main divisions (primary guides) and subheadings (secondary guides and folders) that was developed is found in the project section, Table 1. To differentiate between the guides, red tabs were used for the primary guides and green tabs for the secondary guides.

From the list of main divisions and subheadings, a relative index was prepared on 3 inch × 5 inch index cards which served as a cross reference. Also a list of the filing procedures was prepared to serve as a reminder to the secretary of the steps that must be followed for subject filing to be functional.

The filing system which was employed is adopted to the situation in the Johnson County, Iowa Health Department. But "no one system is universal" *(1)*. The system may have to be modified to suit different Health Departments with special problems.

"Bugs" or trouble in a system may not appear immediately after adoption of the new system. "Bugs" may not appear for months or years. The test of the effectiveness of the system is when a record has to be retrieved from the files. How quickly can this be performed? The secretary who worked with the new system, for a period of time, indicated that the system seems to be functioning satisfactorily. Time will tell the true story.

TABLE 1. LIST OF SANITATION ACTIVITIES AND PROGRAMS

ADMINSTRATIVE ACTIVITIES
- Control of labeling and advertising
- Correspondence
- Enforcement; laws, ordinances, regulations
- Formulate rules and regulations
- Inter-agency programs
- Inter-departmental coordination
- Inter-state programs
- Licensing
- Record keeping and reports
- Responsibility for sanitation programs
- Sanitation program evaluation
- Supervision of personnel

AIR POLLUTION
- Commercial
- Governmental
- Industrial
- Public
- Sampling
- Solid waste
- Transportation

DRUGS
- Cosmetics
- Drug mfg. firms
- Drug storage
- Retail drugs-compounding
- Retail drug outlets (bulk)
- Therapeutic devices

FOOD PREPARATION AND SERVICE
- Catering points—airports, etc.
- Eating and drinking establishments
- Factory cafeterias
- Fairs and carnivals
- Food handler training courses
- Itinerant service stands
- School lunch programs
- Swab sample exams
- Vending machine sanitation

FOOD STORES AND PROCESSING
- Bakeries
- Bottling plants: milk, soda pop, etc.
- Candy factories
- Canning factories
- Coffee packaging
- Confectionaries
- Dried fruits
- Flour mills
- Food warehouses
- Fruit stands
- Grocery stores
- Honey processing plants
- Ice and ice plant sanitation
- Rice and other cereals
- Sugar factories
- Tobacco warehousing

GARBAGE DISPOSAL and VECTOR CONTROL
- Community sanitary surveys
- Disposal of dead animals
- Garbage storage, collection, disposal
- Livestock sanitation
- Nuisance control
- Poultry sanitation
- Radioactive waste disposal
- Vector control:
  - Bed-bug, cockroach, flea, fly,
  - Mosquitoe, rat, etc.
    - Insecticides
    - Rodenticides
    - Supplies
    - Controls

HOUSING AND BUILDING
- Airport sanitation—stations and planes
- Boarding homes and institutions
- Bus and depot sanitation
- Camps for migratory workers
- Comfort stations—mobile and stationary
- Commercial establishments, service stations, etc.
- Day care centers,
- Fraternity and sorority houses
- Hospital sanitation
- Hotels and motels
- Housing sanitation and slum clearance
- Industrial hygiene sanitation (Occupational hygiene)
- Labor and construction camps
- Mobile home sanitation
- Nursing homes

Professional offices
Public building, jails, etc.
Railway sanitation—stations and trains
School buildings and grounds
Scout camps
Ship and vessel sanitation—including docks and warehouses
Trailer courts and tourist parks

MEAT AND MEAT PRODUCTS
Eggs including egg breaking plants
Fish processing plants (seafood)
Meat processing plants
Meat storage and curing
Poultry plant inspection
Refrigerated locker plants
Rendering plant sanitation
Slaughter houses and abbattoirs

MILK AND MILK PRODUCTS
Grade A fluid—pasteurized
Grade A fluid—raw
Manufacturing—raw
Retail inspected—raw
Testing milk or milk products
Uninspected milk

OCCUPATIONAL HEALTH AND SAFETY
Commercial
Industrial
Accident
Biological hazards
Energy stresses
Toxic chemicals

RADIATION
Education and training
Hospitals
Industry
Inventory of sources
Medical and dental operations
Schools
Surveillance
University

RECREATION SANITATION
Athletic areas
Camp grounds
Municipal parks
National parks
State parks
Marinas
Reservoirs

SANITATION
Disaster
Emergency
Fringe area
Highway
Radioactive fallout
Research
Rural
Virology
Weeds

SANITATION EDUCATION
Development and use of audio-visual aids
Community health committees
Industrial relations
Professional development
In-service training
Public relations
Use of mass communications media

SEWAGE DISPOSAL
Community sewer systems
Disposal of wastes from septic tanks and cesspools after cleaning
Inspection and/or abatement of outdoor privies
Oxidation ponds
Private disposal systems
Urban and rural

SWIMMING POOLS AND BEACHES
Private beaches
Private swimming pools
Public beaches
Public swimming pools
Wading pools

WATER PROGRAMS
Drinking fountain standards
Emergency water treatment
Private water supplies
Public water supplies
Sampling or testing of water
Water for industrial use
Water fluoridation

WATER POLLUTION PROBLEMS
Domestic wastes
Industrial wastes
Natural contaminants

MISCELLANEOUS
Accidents
Bacteriological standards
Carbon monoxide
Garages
Police cars
School bus
Public transportation
Disinfectant, insecticide, pesticide testing
Epidemiological survey
Hazardous substances
Laundromats and self-service dry cleaning establishments
Lead poisoning
Noise
Planning and zoning
Product safety
Radiological health
Schools
Ventilation, air conditioning, lighting, plumbing, etc.

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PROCEEDINGS OF THE THIRTEENTH NATIONAL CONFERENCE ON INTERSTATE MILK SHIPMENTS

CHASE-PARK PLAZA HOTEL, ST. LOUIS, MISSOURI MAY 16-20, 1971

J. C. McCaffrey
National Conference on Interstate Milk Shipments
1800 West Fillmore Street
Chicago, Illinois 60612

(Received for publication September 6, 1971)

Executive Board Meeting, May 17, 1971

The Executive Board meeting was convened by Secretary-Treasurer J. C. McCaffrey at 10 a.m. All Board members except C. E. Henderson, New Mexico; Dr. Howard K. Johnston, Pennsylvania; and Shelby Johnson, Kentucky; were in attendance. The Secretary explained to the Board that Chairman Johnson had suffered a blackout on the previous Saturday which, according to his doctor, was brought on by complete physical exhaustion.

The Treasurer's report indicating a balance of $4,575.98 as of May 15, 1971 was accepted. John C. Schilling, Chairman of the Local Arrangements Committee, and Earl Wright, Chairman of the Program Committee, reported on the current status of their activities. Both reports were unanimously accepted as presented.

Secretary McCaffrey informed the Board that it was its duty to appoint a Nominating Committee, a Resolutions Committee, and a Credentials Committee. The Nominating Committee consisted of: Richard A. Parry, D.V.M., Connecticut; Sam Noles, Florida; R. L. Van Buren, California; N. E. Kirschbaum, Wisconsin; John Baghott, Colorado; and E. G. Huffer, Illinois, Chairman. The Resolutions Committee consisted of Clinton VanDevender, Mississippi; Carlus Blevins, Wyoming; H. H. Vaux, Indiana, Chairman. The Credentials Committee consisted of E. P. Gadd, Missouri; Paul Carpenter, Ohio; and Brace Rowley, Kansas, Chairman.

There being no further business, the meeting adjourned at 11:40 a.m.

First General Session

The first general session of the Conference was called to order at 1:30 p.m. on Monday, May 17, by Secretary J. C. McCaffrey. The Secretary explained to the Conference why Chairman Shelby Johnson was not in attendance and then explained further that he and Earl Wright, Program Chairman, would handle the various operations of the Conference. After these opening remarks, management of the session was turned over to Earl Wright who introduced Sam Noles, Florida State Health Department, to give the invocation.

The address of welcome was delivered by William C. Banton II, M.D., Health Commissioner, St. Louis, Missouri. The keynote address entitled "In the Inter-Space Age Era with IMS" was given by D. Paul Alagia, Jr., Executive Director, Dairymen, Inc., Louisville, Kentucky. The keynote address was followed by a two-part presentation of a subject entitled "Some Concerns About Present and Future of NCIMS". Mr. Robert H. North, Executive Vice-President, Milk Industry Foundation, Washington, D. C. and Patrick B. Healy, Secretary, National Milk Producers Federation presented this discussion.

"The FDA Role in NCIMS" was very ably presented by Virgil O. Wodicka, Director, Bureau of Foods, FDA, Washington, D. C. The biennial report of the U. S. Public Health Service to the Conference was presented by H. E. Thompson, Jr., Chief Milk Sanitation Officer, Washington, D. C.

The first general session closed with the charges by Acting-Chairman Wright to the Nominating Committee, the Resolutions Committee and the Credentials Committee.

Second General Session

The second general session was convened by acting-Chairman Wright at 8:35 a.m. on Tuesday, May 18. The first roll-call of delegates authorized to vote on Conference Agreements was made by Secretary McCaffrey.

The following committee reports were presented at this session: (1) "To Study the Scope of Survey Ratings" by D. J. Conner; (2) "Reciprocity" by K. G. Weckel; J. F. Speer, and B. Heinemann were co-chairmen; (3) "Single-Service Containers" by R. Parry, D.V.M., (4) "Laboratory Committee" by Dr. W. W. Ullman; (5) "Over The Road Tankers" by E. G. Huffer; (6) "Structure and Organization of the Conference", by C. K. Luchterhand; M. W. Jefferson and R. R. Perkins were co-chairmen; and (7) "Abnormal Milk Control" by Dr. J. C. Flake.

Program Chairman Earl Wright concluded this general session by announcing additional problems which would be assigned to the task forces.

THIRD GENERAL SESSION

Program Chairman Earl Wright convened the third general session at 2 p.m. on Tuesday, May 18. Chairman Wright explained the operational procedures of the various task forces. He specifically explained the manner in which the minority report could be presented. Chairman Wright then assigned the various task force meeting rooms and stated that the task forces would be in operation during the remainder of Tuesday afternoon and on Wednesday morning until 11 a.m.

FOURTH GENERAL SESSION

The fourth general session was convened by Program Chairman Earl Wright at 11:00 a.m. on Wednesday, May 19. The Chairman of each task force reported on the problems submitted, and the disposition thereof. Each of these preliminary reports was duly accepted by the membership by means of an official motion and second. Names of individuals making and seconding the motions are in the Secretary's files.

FINAL GENERAL SESSION

The final general session convened at 8:30 a.m., Thursday, May 20 with Program Chairman Earl Wright presiding. John Newlin served as parliamentarian. The final roll call of states and delegates authorized to vote on Conference Agreements was presented by Secretary McCaffrey. The roll call showed that 43 states were represented: 16 by both agriculture and health; 11 by agriculture only and 16 by health only. The health departments of the District of Columbia and Puerto Rico were also represented. Minutes of the previous Conference were accepted as mailed to those who had been in attendance at Denver, Colorado in 1969. The Treasurer's report was accepted as read.

E. G. Huffer, Illinois Department of Public Health, reported the selections of the Nominating Committee for Board members representing Region II. These selections were: H. H. Vaux, Indiana State Board of Health; Kenneth Van Patten, Michigan Department of Agriculture; Burdett Heinemann, Mid-America Dairymen, Inc.; J. C. Schilling, St. Louis, Missouri Health Department, Earl O. Wright, Iowa State University; and J. C. McCaffrey, Illinois Environmental Protection Agency.

RESOLUTIONS

The Resolutions Committee, under the Chairmanship of Herbert Vaux, presented the following resolutions, all of which were unanimously accepted by the Conference.

a. The Conference expresses its sincere appreciation to Mr. and Mrs. John Schilling and the members of the Local Arrangements Committee for the excellent manner in which they have fulfilled their responsibilities by making available the facilities, services, and activities to the participants and their ladies. The Conference Secretary is directed to express the thanks of the Conference to the Chase-Park Plaza for the cooperation, accommodations, and services extended by its management and staff.

b. The Conference expresses its sincere regrets that Mr. Shelby Johnson, because of his untimely illness, was unable to attend these proceedings and to offer the guidance and leadership which he so capably demonstrates.

c. The Conference expresses its deep and sincere appreciation to Secretary-Treasurer J. C. McCaffrey and Program Chairman Earl Wright on the splendid manner in which they accepted the responsibilities of leadership and guided this Conference to a successful conclusion.

d. The Conference emphatically requests the Department of Health, Education, and Welfare to make every effort to (1) up-date uniform standards, (2) continue the certification and re-certification of state survey officers, (3) continue the certification and publication of sanitation compliance and enforcement ratings for qualified milk shippers, (4) spotcheck listed shippers, (5) channel information to all parties concerned, (6) train state survey personnel, for example, survey officer seminars, etc., (7) issue uniform national interpretations from the central office to all parties concerned, (8) standardize procedures and interpretations by both federal and state personnel, (9) provide funds for research, (10) provide qualified consultants and spotcheck personnel, (11) establish milk sanitation programs on a division basis, all for the purpose of strengthening the Conference program will full recognization of the importance of the historical cooperative relationship between federal and state agencies.

TASK FORCE REPORTS

Program Chairman Earl Wright next called for reports of the task forces. Complete task force reports are in the Secretary's files. However, only the changes in or additions to procedures are included in this report.

TASK FORCE ON STANDARDS

This task force was given three problems. *Problem 1*: Should "sterilized" milk products be subjected to Grade A labeling and date requirements. The task force voted to reaffirm the position now provided in Section I of the 1965 PHS Grade A Pasteurized Milk Ordinance relating to sterilized milk and milk products in hermetically sealed containers. The delegates concurred in the action of the task force.

Problem 2: Should the Pasteurized Milk Ordinance be changed to include sediment standards? The task force voted that no action be taken. The delegates refused to accept the recommendation of the task force casting 29-1/2 "Nay" votes and 15-1/2 "Yea"

votes.

Problem 3: Should a section be added to both inspection and survey forms to include single service items? The task force reported that this matter was already adequately covered and that the problem be referred to the Committee on Single-Service Containers and Closures to be further resolved. The delegates approved the task force recommendation.

Task Force on Supervision

This task force was given four problems. *Problem 1*: Laboratory examinations for the presence of inhibitors in raw milk for pasteurization and processed products shall be made at the same frequency as specified for bacteriological tests in the Public Health Service's recommended "Milk Ordinance". The task force recommended that no action be taken on this problem and the delegates concurred.

Problem 2: The somatic cell count of 1,500,000 per milliliter is too high to produce a high quality, wholesome milk. I would recommend a 1,000,000 somatic cell count by July 1, 1972. The task force recommended that no action be taken and the delegates concurred.

Problem 3: Suggest amendment of Procedure "B" when confirmatory counts indicate the presence of greater than 1,500,000 somatic cells per milliliter, as contained in the "Guidelines For The Control of Abnormal Milk," by deleting the mandatory requirement that an inspection shall be made by the regulatory agency or certified personnel as specified in Section V, Grade A Pasteurized Milk Ordinance. Also delete the last sentence of this "B" procedure. The task force recommended that no action be taken on this problem and the delegates concurred.

Problem 4: In the second paragraph of Procedure "C" July 1970 "Guidelines For The Control of Abnormal Milk," the second sentence reads as follows: "If the results of the herd milk sample indicate a somatic cell count of less than 1,500,000 cells per milliliter, a temporary permit should be issued." In the 1969 proceedings of the 12th National Conference on Interstate Milk Shipments as adopted by the Conference, this second paragraph, appearing about midpage of Column 1 of page 3 reads: "If the results of the farm inspection indicate that there is no longer a violation of lr, a temporary permit shall be issued. The Conference should amend the 1969 Task Force Committee report to read as in the July 1970 "Guidelines For The Control of Abnormal Milk" published by the USPHS in July of 1970. In this publication, Section IV, Subsection B, states in part "In addition to written notice, the inspection shall be made by the regulatory agency or by certified personnel as specified, Section V". It is requested that the task force on supervision give consideration to recommend that the PHS amend its guidelines to delete the requirement that the inspection shall be made by certified personnel. It is recommended that the statement should read: "In addition to the written notice, an inspection shall be made by the regulatory agency or approved personnel." The task force recommends that Section IV, Subsection B, of the "Guidelines For The Control of Abnormal Milk" revised July 1970 be amended to read as follows: "Whenever two of the last four consecutive somatic cell counts exceed 1,500,000 cells per milliliter, the health authority shall send a written notice thereof to the producer concerned. This notice shall be in effect so long as two of the last four consecutive somatic cell counts exceed 1,500,000 per milliliter. In addition to the written notice, an inspection shall be made by the regulatory agency or by certified personnel as specified in Section V of the Grade "A" Pasteurized Milk Ordinance or by approval personnel as appointed by the official regulatory agency." The delegates concurred.

Task Force on Ratings and Certification

This task force was given seven problems. *Problem 1*: Recommend Section III, paragraph E1, F1, be amended to delete the words "but not more often than semi-annually" and wording added to allow resurveys to be made at any time. The task force accepted the recommendation to delete, "but not more often than semi-annually". After considerable discussion, Delegate Pais, Maryland, proposed an amendment: "Instead of deleting the frequency of 'not more than semi-annually' to insert 'not more often than 90 days.' Delegates refused to accept the proposed amendment by a vote of 24-1/2 "nay" to 20-1/2 "yea". Delegates then voted on the original recommendation of the task force. The recommendation was not approved by the delegates.

Problem 2: Should Section III, paragraph E, be changed to allow temporary continuation of listed rating if a survey was made under extenuating circumstances? The problem was withdrawn.

Problem 3: Change Section III, D to agree with Section 1, C., Section 11A, paragraph 3 and Section V, paragraph Cl and 4. The action taken by the task force was to insert a new D as follows: D. Sampling surveillance personnel, (1) Evaluation of sampling practices shall be made by a qualified state milk sanitation officer and/or state designated survey officer, (a) who has been standardized and approved by the Public Health Service as a state sampling survey officer and holds a currently valid certification

of qualification for milk sanitation officer, or laboratory survey officer. Continuing on, this will change the present D to E, E to F, F to G, G to H, and H to J in Section III. Delegate Noles, Florida, proposed an amendment to the recommendation as follows: To the present Section III, C, 1 on page 3 add the words "and the evaluation of sampling techniques of sample collectors" between the words reading "and shall". This would then read: Milk sanitation clinics, compliance and enforcement ratings, and evaluation of sampling techniques of sample collectors shall be made by qualified state milk sanitation officers" and continue rating as it is. The amendment was put to a vote of the delegates and lost. There were 35 "nay" votes and 9 "yea" votes. The delegates voted on the original recommendation of the task force. The recommendation was accepted.

Problem 4: Should Section III, paragraph H1 be changed regarding the publication of enforcement ratings? The task force recommendation was that no action be taken and this was accepted by the delegates.

Problem 5: Should the same state agency have responsibility for enforcement and survey? The task force recommended no action on this problem. The recommendation was accepted by the delegates.

Problem 6: Clarification of the grounds for industry asking for a survey before the inspection date. The task force recommended no action. The recommendation was accepted by the delegates.

Problem 7: Section III, E1, delete words "but not more often than semi-annually", and add "but new ratings shall not be established until 6 months has elapsed from the previous rating date." The task force recommended no action and the delegates concurred.

TASK FORCE ON UNIFORM BILL OF LADING AND SEALS

This task force was given one problem which was a recommendation to change Section IV, A1, to require indication that milk be free of inhibitors. The task force recommended no action and the delegates agreed.

TASK FORCE ON RESPONSIBILITIES OF PARTICIPATING STATES

This task force was given two problems. *Problem 1*: Recommend that the National Conference delist the states that do not practice reciprocity. The task force endorsed the committee report on reciprocity as a possible answer to the problem of multiple inspection and trade barriers and recommended that the Executive Board undertake studies to determine the magnitude of the problem. The delegates concurred.

Problem 2: Change the wording of Section V, paragraph C to be consistent with Section V, paragraph A regarding the most recent rating of milk supplies. The task force recommended Section V, paragraph C4 be amended to read as follows: "if results of the most recent official laboratory and sampling surveys are not received by the appropriate PHS regional office within 2 years and 6 months of the last survey date, the regional office shall notify the state milk sanitation rating agency to withdraw certification of the shipper". The delegates accepted the recommendation.

TASK FORCE ON RESPONSIBILITIES OF THE PUBLIC HEALTH SERVICE

This task force was given seven problems. *Problem 1*: Recommend the Public Health Service spell out the confirmation test to be used for abnormal milk. The task force recommended no action on the problem but did recommend that the Laboratory Committee review the proposed standard of tests and procedures before action by the PHS through the Food and Drug Administration. The recommendation was accepted by the delegates.

Problem 2: Recommend the Conference urge USPHS continued participation and contribution to the Conference. The task force moved the adoption of this recommendation. The delegates concurred.

Problem 3: Recommend the PHS re-evaluate its farm water supply standards. The task force agreed with the existence of the problem and urged the National Conference on Interstate Milk Shipments to contact the Public Health Service and the Food and Drug Administration to take another look at their interpretation of the farm water supply standards. The recommendation was accepted by the delegates.

Problem 4: Recommend PHS review 19r and 17p with the idea of lowering temperature standards. The task force recommended no action on this problem and motion was carried.

Problem 5: Recommend that the USPHS reconsider their interpretation of the PMO requirements on thermometer for plant milk storage tank. The task force recommended that the Public Health Service and the Food and Drug Administration clarify the requirements for thermometers on milk plant storage tanks. The recommendation was accepted by the delegates

Problem 6: The voluntary Interstate Milk Shippers certification program, with ratings based on compliance with basic sanitation standards in the Grade "A" Pasteurized Milk Ordinance, is the most effective

procedure now available for developing reciprocal inspection agreements between states. However, there is substantial indication that equivalent uniformity does not exist between regions. Therefore, it is proposed that the Public Health Service promote more cooperation and standardization of regional personnel as an additional service to the various states. The task force recommended the adoption of this proposal. The recommendation was accepted by the delegates.

Problem 7: Non-survey enforcement agencies are not invited to attend or participate in regional survey seminars. The task force recommended that Section VI, C, 2, be continued to read "and by invitation, personnel charged by law with the enforcement of Grade A milk regulations." Delegate Rowley, Kansas, suggested a change in the wording of the recommendation to insert between the word "invitation" and the word "personnel" the phrase "from regional personnel". The task force chairman, with the approval of members who were present accepted this change in wording. The official task force recommendation read "and by invitation from regional personnel, personnel charged by law with the enforcement of Grade A milk regulations". The recommendation was accepted by the delegates.

Task Force on Procedures for Handling Complaints and Challenges of Validity of Ratings

This task force was given six problems. *Problem 1*: Can participating agencies be required to accept milk products properly labeled Grade A? The problem as submitted dealt with cultured products. The task force recommended that (1) PHS/FDA provide a uniform definition for cultured products, and (2) states and communities be encouraged to develop Grade A programs of uniform labeling for such cultured products. The delegates accepted the recommendation.

Problem 2: This is a combination of Problems 2, 3 and 4. They deal with (1) duplication of inspections, (2) restraint of trade, and (3) application of routine inspections by out of state markets. The task force felt that these problems can be resolved by complete reciprocity and therefore, the task force recommended that the Committee on Reciprocity be continued and progress reports be submitted. It recommended also that the Conference Executive Board takes steps to implement recomendations of the reciprocity committee. The delegates approved the recommendation.

Problem 3: This is a combination of Problems 5 and 6 dealing with the fact that (1) Section VII, C1, C2 does not provide for the resurvey of shippers whose certification has been withdrawn, and (2) that PHS change in Conference Agreements Section VII, C2 to be consistent with III, E1 and III, F1. The task force recommended that VII, C1 and C2 remain as previously agreed upon and published. The delegates accepted the recommendation.

Task Force on the Application of Conference Agreements and Special Problems

This task force was given five problems. *Problem 1*: Should the movement of manufactured milk products come under the scope of the Conference? The task force recommended that the scope of the NCIMS not be expanded to include additional products. The recommendation was accepted by the delegates.

Problems 2, 3, and 5 were similar to Problem 1 and were therefore all considered together.

Problem 2: Should products such as frozen desserts, cottage cheese, etc. come under the scope of the Conference. *Problem 3*: Should all "aseptic sterile" or long life fluid milk and cream be included under the NCIMS Agreements?

Problem 5: To include frozen desserts in the interstate milk shippers program. The task force recommended for Problems 2, 3, and 5 that the scope of the NCIMS not be expanded to include additional products. The delegates accepted the recommendation.

Problem 4: Should the task force composition be changed? The task force recommended the adoption of the Committee on the Structure and Organization of the NCIMS relative to composition of groups previously called "task forces" and to be called "councils" in the future. The delegates accepted this recommendation.

Task Force on Laboratory Procedures

This task force was given 13 problems. *Problem 1*: Should goat's milk sold in interstate commerce be required to meet the somatic cell count of cow's milk as outlined in the "Guidelines For The Control of Abnormal Milk" published by the PHS July 1970? The task force recommended that this matter be referred to the Laboratory Committee for further study of the problem. The delegates accepted the recommendation.

Problem 2: Request the PHS to coordinate laboratory inspections to permit a single visit for an inspection for shellfish, milk, food, and, if possible, water laboratories. The task force recommended that the Conference forward this request to the PHS/FDA and Environmental Protection Agency for ac-

tion. The delegates accepted the recommendation.

Problem 3: Request the PHS to conduct additional research on the Wisconsin Mastitis Test in an effort to standardize the procedures, reagents, and methods of interpreting results. The task force felt that this problem was taken care of in the "Guidelines—Screening and Confirmatory Tests for the Detection of Abnormal Milk," 1970 revision of the PHS and FDA. Therefore, no action was recommended. The delegates concurred.

Problem 4: Recommend that the PHS work with the laboratory committee to develop a method for the bacteriological examination of aseptic milk products. The task force recommended no action. However, delegate Noles, Florida, moved to amend the action as follows; "That the Conference recommend that the FDA, USPHS work with the laboratory committee to develop an appropriate test to be used for the bacteriological examination of ultra high treatment for aseptically packaged milk and milk products." Chairman Ullman canvassed the members of his task force and it was agreed that Mr. Noles modification would be accepted. The amendment, therefore, was withdrawn and a modified recommendation was accepted by the delegates.

Problem 5: Recommend that the PHS publish specific instructions for conducting laboratory tests for the control of abnormal milk including established and definable limits of accuracy and accepted tolerance limits. These instructions should also contain adequate information to permit laboratories to determine the accuracy of their results. The task force recommended that the PHS/FDA work with the USDA research laboratory at Beltsville, Maryland, to obtain the requested information and to make this information available to the Conference. The delegates accepted the recommendation.

Problem 6: Recommend that when a chemical screening test for the detection of abnormal milk before confirmation is used, it shall be the Wisconsin Mastitis Test. The task force reworded the problem as follows: "Recommended that beginning July 1, 1971, that when a laboratory chemical screening test for the detection of abnormal milk is used before confirmation, it shall be the Wisconsin Mastitis Test." The task force recommended the adoption of the revised problem wording. At this time, delegate Lapinski, New York, presented a minority report as follows: "Since screening tests must be followed by a confirmatory test, the type of test is less significant. At the 1969 Conference, no recommendations as to the screening test of choice were given. Thus in the intervening time, no action has been taken by any states. At the present time all screening tests have their problems, but different states using different tests are carrying out effective abnormal milk control programs. It is a recommendation that this problem be considered after further data has been made available to the Conference, and be resolved at the 1973 Conference." Delegate Lapinski moved the adoption of the minority report, seconded by delegate Boosinger, Florida. The delegates voted to accept the minority report.

Problem 7: Recommend that new or changes in laboratory procedures shall become official for Conference purposes only after the following steps have been taken: The laboratory committee has reviewed the procedure and made recommendations to the Executive Board. The Executive Board has informed and obtained a concensus of the voting delegates of the Conference and based on the above, the Executive Board shall either approve or disapprove of the use of the procedure. The task force recommended the adoption of this problem. After considerable discussion, delegate Conner, Kentucky, moved that action on this problem be tabled until the next Conference. The delegates voted affirmatively to table the motion.

Problem 8: It is requested that this Conference consider delegating the PHS/FDA as sole enforcement agency in respect to the surveillance of laboratories that are engaged in Vitamin D Assays. The task force recommended that tests for vitamins and minerals be made a part of the regular laboratory surveillance program of the PHS/FDA and that the laboratories testing for vitamins and minerals be subject to this program. The recommendation was approved by the delegates.

Problem 9: (a) Define the adulterants such as pesticides which are of public health concern. (b) Determine at which point surveillance samples would be statistically significant to consuming public. (c) What sampling frequency would yield a statistically significant surveillance program? (d) Program of laboratory certification similar to that presently used in bacterial and inhibitory substances examination should be developed. (e) The study of the economic feasibility of such a program; what would be its cost as related to the value of the protection of the health of the consuming public. The task force recommended that FDA be asked to furnish the Conference with a summary of what is being done on the surveillance of pesticides and heavy metals and to answer the questions posed in this problem and to assist in the establishment of state conducted programs. The recommendation was defeated by the delegates.

Problems 10, 11, 12 and 13 all received the same

recommended action so will be listed together. *Problem 10*: Recommend a change in the bacteriological procedure for the determination of bacterial counts of both Grade A raw and pasteurized milk.

Problem 11: Evaluate the significance of current bacterial standards that are applied to raw and pasteurized milk.

Problem 12: Develop statistically significant sampling, laboratory procedures, and standards that more truly reflect the degree of the sanitary production and quality of raw and pasteurized milk.

Problem 13: To undertake a study with the PHS to determine acceptable maximum number of bacteria in milk, determine acceptable maximum number of bacteria in raw milk determined by the coliform, psychrophilic, and laboratory pasteurized tests. The task force recommended that the laboratory committee review existing bacterial standards and procedures and if necessary work toward development of new standards and procedures for the examination of Grade A raw and pasteurized milk. The laboratory committee shall report back to the 1973 Conference on its findings. The delegates approved the recommendation.

After conclusion of the task force reports, Program Chairman Wright called for unfinished business. Since there was no unfinished business, the Conference moved on the consideration of new business. There being no new business, the Chairman called for election of new members of the Executive Board. The Nominating Committee recommendations were read for the second time. Delegate Boosinger, Florida, moved that the nominations be closed, and the Secretary be directed to cast a unanimous ballot for candidates. The motion was carried. The new members of the Executive Board were duly announced and the final general session of the 13th National Conference adjourned at 12:10 P.M. on Thursday, May 20.

Executive Board Meeting, May 20, 1971

Secretary-Treasurer J. C. McCaffrey convened the meeting of the new Executive Board at 12:25 p.m. on Thursday, May 20, 1971. All members with the exception of Carl Henderson were in attendance. Secretary-Treasurer McCaffrey introduced the new members of the Board from Region II, namely H. H. Vaux, Ken Van Patten, Burdett Heinemann and the three who were re-elected: John Schilling, Earl Wright, and J. C. McCaffrey.

Secretary-Treasurer McCaffrey called for the election of new officers. John Schilling was elected Chairman and J. C. McCaffrey was re-elected as Secretary-Treasurer.

Each member of the Board received a list showing the various hotels and cities which had submitted proposals to host the 1975 meeting. After considerable discussion the meeting was awarded to St. Louis, Missouri. The exact dates will be arranged later.

Chairman Schilling asked for a discussion of new business. Secretary McCaffrey mentioned that no particular recognition had ever been given to individuals who had served as Chairmen of the organization. He suggested that a suitable plaque be purchased and awarded to each of the past chairmen during the 1973 meeting in Des Moines, Iowa. Van Patten moved, seconded by Arledge that McCaffrey be authorized to make all arrangements for the purchasing of the plaques. There being no further new business, the meeting was adjourned at 2:10 p.m.

GE INTRODUCES

(Continued from Page 4)

shredded material is then suspended and whirled around the interior circumference of the combustion chamber wall by the circular motion of the vortex flame where it is burned to a sterile ash. Ash particles are then guided to a cyclone separator where they are collected for disposal.

Vortex I has been demonstrated through the joint efforts of GE, the City of Shelbyville, and the Environmental Health Service's Bureau of Solid Waste Management under Phase I of a two-year, $444,680 Federal program.

Benefits afforded by the new Vorcinerator system were found to include exhaust and ash removal systems which markedly reduce emission of noxious gases, smoke and ash particles to the atmosphere; low maintenance and operating costs; no moving parts in the high-temperature combustion chamber; modular unit construction for ease of portability and installation; and automatic controls for safety and efficiency.

In Phase II of the demonstration program a larger Vorcinerator system (6- to 7-ton per hour capacity) has been built by GE and installed in Shelbyville for further testing. Operation should begin by the end of the year.

The new Vorcinerator system reduces combustible waste materials to a fine ash suitable for industrial applications such as parking lot surfacing material. The inert ash also eliminates the need for large landfill areas and normally does not add to ground water pollution.

For more information on the new Vortex I modular Vorcinerator system, contact your local GE Industrial Sales Representative, or write the GENERAL ELECTRIC COMPANY, Waste Management Systems, 1 Progress Road, Shelbyville, Indiana 46176.

E-3-A ACCEPTED PRACTICES FOR LIQUID EGG AND LIQUID EGG PRODUCTS SPRAY DRYING SYSTEMS

Serial #E-60700

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

It is the purpose of the IAMFES, USPHS, USDA, IAPI and DFISA in connection with the development of the E-3-A Sanitary Standards program to allow and encourage full freedom for inventive genius or new developments. Liquid egg and liquid egg products spray drying systems heretofore and hereafter developed which so differ in design, material, fabrication, or otherwise as not to conform with the following practices, but which in the fabricator's opinion are equivalent or better, may be submitted for the joint consideration of the IAMFES, USPHS, USDA, IAPI and DFISA.

A

SCOPE

A.1

These, E-3-A Accepted Practices shall pertain to the sanitary aspects of equipment for spray drying liquid egg and liquid egg products, and includes all equipment necessary for spray drying liquid egg and liquid egg products beginning with the discharge of the pump which delivers the liquid product to the drying system and terminating at the point the final dried product enters either the packaging systems or storage for further processing. The drying system includes the equipment used for moving and cleaning the air, heating and/or cooling the air, atomizing the liquid, mixing the liquid in the hot air, removing the dry product from the air, additional drying of the product, cooling, conveying, and sizing the product.

A.2

In order to comply to these E-3-A Accepted Practices, equipment in spray drying systems shall comply with the following criteria for design, material fabrication and air supply.

B.

DEFINITIONS

B.1

Product: Shall mean the liquid egg or egg product and dry egg or egg product.

B.2

Air to be Heated: Shall mean processing air to be heated to at least 240°F.

B.3

Air not to be Heated: Shall mean processing air which either will not be heated or will be heated to a temperature less than 240°F.

B.4

Processing Air: Shall mean air prepared by filtration which is intended to be used in contact with the product for such purposes as heating, cooling, drying or conveying or will be used for sealing a bearing or similar purposes.

B.5

Product Contact Surfaces:

B.5.1

Shall mean all surfaces that are exposed to the product or from which liquids and/or solids may drain, drop or be drawn into the product.

B.5.2

Shall mean all surfaces in contact with air which is not to be heated prior to coming in contact with the product commencing at the discharge of the air inlet filter(s) and ending at the first downstream surface in contact with the product.

B.6

Air Contact Surfaces:

B.6.1

Inlet air contact surfaces shall mean all surfaces (1) in contact with air to be heated prior to coming in contact with the product and (2) in contact with heated air, commencing at the discharge of the air inlet filter(s) and ending at the first downstream product contact surface.

B.6.2

Exhaust air contact surfaces shall mean the surfaces of the air ducts, plenum chamber(s) (if provided) and appurtenances from the final product contact surface through the exhaust system.

B.7

Non-Product Contact Surfaces: Shall mean all other exposed surfaces.

B.8

Mechanical Cleaning or Mechanically Cleaning: Shall denote cleaning, solely by circulation and/or flowing chemical detergent solutions and water rinses onto and over the surfaces to be cleaned, by mechanical means.

C.

MATERIALS

C.1

The materials of product contact surfaces of equipment included in the spray drying system for which there are E-3-A or 3-A Sanitary Standards or E-3-A or 3-A Accepted Practices shall comply with the material criteria of the applicable Standards or Accepted Practices.

C.2

All other product surfaces shall be of stainless steel of the AISI 300 series[1] or corresponding ACI[2] types. (See Appendix, Section F.1), or metal that is nontoxic and non-absorbent and which under conditions of intended use is equally corrosion resistant except that:

C.2.1

Plastic materials may be used for scraper blades, sight and/or light glasses, bearings, bushings, short pieces of transparent tubing in dry product areas for observation purposes, short flexible connectors and in sealing applications. These materials shall conform with the applicable provisions of the "3-A Sanitary Standards for Multiple-Use Plastic Materials Used as Product Contact Surfaces for Dairy Equipment, Serial #2000", as amended.

C.2.2

Rubber and rubber-like materials may be used for short flexible connectors, scraper blades and in sealing applications. These materials shall conform with the applicable provisions of the "E-3-A Sanitary Standards for Multiple-Use Rubber and Rubber-like Materials Used as Product Contact Surfaces in Egg Processing Equipment, Serial #E-1800".

C.2.3

Cotton, wool, linen, silk, or synthetic fibers may be used for filtering and/or screening surfaces or entrainment separators, and for short flexible connectors used in dry product packaging areas. These materials shall be non-toxic, relatively insoluble in water, easily cleanable, and shall not impart a flavor to the product.

C.2.4

Alumium alloys conforming to the Aluminum Association[3] designations 5052 and 6061 may be used as a product contact surface for dry product when used as a supporting or reinforcing member in light weight moving parts and other similar applications.

C.2.5

Welded areas and the deposited weld material shall be substantially as corrosion resistant as the parent material.

C.2.6

Heat resistant glass[4] may be used in sight and/or light openings.

C.3

Air contact surfaces, except for those of flexible connectors, fans, burners and dampers, shall be of a corrosion resistant metal that maintains its original surface characteristics under the environment of intended use, or is rendered corrosion-resistant by a coating of corrosion-resistant material other than paint. If the portion of the plenum chamber at the inlet to the drying chamber or the exhaust from the drier is subject to washing, it shall be made of stainless steel.

C.4

Filter Media: Intake air filter media shall consist of one or more of the following: fiber glass with a downstream backing dense enough to prevent fiber glass break off from passing through, cotton flannel, wool flannel spunmetal, activated carbon, activated alumina, non-woven fabric, absorbent cotton fibre, or other suitable materials which, under conditions of intended use, are non-toxic and non-shedding and which do not release toxic volatiles or other contaminants to the air, or volatiles which may impart any flavor or odor to the product. Chemical bonding materials contained in the media shall be non-toxic, non-volatile and insoluble under all conditions of use. Disposable media shall not be cleaned and re-used.
Note: Electronic air cleaners use electrostatic precipitation principles to collect particulate matter and therefore are not included in the preceding

[1]The data for this series are contained in the following reference: AISI Steel Products Manual, Stainless & Heat Resisting Steels, April, 1963, Table 2-1 pp 16-17. Available from: American Iron & Steel Institute, 633 Third Avenue, New York, N. Y. 10017.

[2]Alloy Casting Institute Division, Steel Founders' Society of America, 21010 Center Ridge Road, Rocky River, Ohio 44116.

[3]Aluminum Association, 420 Lexington Avenue, New York, N. Y. 10017.

[4]Glass of a borosilicate type with a coefficient of expansion between 30°C and 300°C of between 3.0 and 3.5 parts per million per degree centigrade.

list of acceptable filter media. This does not preclude their use in spray drying systems.

C.5

Non-product contact surfaces, shall be of corrosion-resistant material or material that is rendered corrosion-resistant. If coated, the coating used shall adhere. Non-product contact surfaces shall be relatively non-absorbent, durable and cleanable. Parts removable for cleaning having both product contact and non-product contact surfaces shall not be painted.

D.

FABRICATION

D.1

The fabrication criteria of equipment included in the spray drying system for which there are E-3-A or 3-A Sanitary Standards or E-3-A or 3-A Accepted Practices shall be those of the applicable Standards or Accepted Practices.

D.2

All other equipment shall conform to the following fabrication criteria.

D.2.1

The product contact surfaces of stainless steel sheets shall be as smooth as a No. 4 mill finish. Seam welds shall be smooth and pit free, and where grinding and polishing is required, such areas shall be at least as smooth as a finish obtained with 80 grit silicon carbide. Intricate fabricated and/or machined components shall be as smooth as a finish obtained with 80 grit silicon carbide, with welds pit free. If stainless steel sheets with No. 2B mill finish are used, they shall be free of imperfections such as chips, flakes or pits. Joints shall be smooth and flush. Permanent joints in metallic product contact surfaces shall be continuously welded.

D.2.2

Product contact surfaces shall be easily accessible for thorough cleaning, either when in an assembled position or when removed. Parts that must be removed for cleaning shall be readily removable and easily dismantled, except that high pressure liquid product lines and such parts as fan wheels, air lock valves, fluidizer valves, conveying mechanisms, and similar parts need only be readily accessible for cleaning.

D.2.3

Product contact surfaces intended for regular wet cleaning shall be self-draining or self-purging except for normal clingage, except where self-draining is not feasible other drying methods including air drying may be used.

D.2.4

Internal angles of 135° or less on product contact surfaces shall have minimum radii of 1/4 inch except where smaller radii are required for essential functional reasons such as those on internal parts of mechanical collectors, collector systems and air lock blades. In no case shall such radii be less than 1/32 inch, except those on atomizing devices and where external welding is necessary. In either case the internal product contact surface must be readily available for cleaning and inspection.

D.2.5

There shall be no exposed threads or crevices on product contact surfaces except where required for functional and safety reasons such as high pressure liquid product lines and atomizing devices, fan wheels, air lock valves, fluidizer valves and conveying mechanisms. The parts for which an exception is made that have exposed threads or crevices on product contact surfaces shall be designed to be mechanically cleaned or shall be readily accessible for cleaning.

D.2.6

Flexible connections having product contact surfaces shall have straight sides without corrugations.

D.3

Air contact surfaces shall be readily cleanable.

D.4

Sheet metal work constructed in accordance with conventional fabricating techniques[5] may be used for portions of the dryer having air contact surfaces.

D.5

The construction of the portions of the spray drying system having air contact surfaces such as sheet metal work, air heating equipment, filtering equipment, pneumatic conveying equipment and exhaust systems, shall be so constructed as to prevent the entrance of unfiltered air.

D.6

Non-product contact surfaces to be coated shall be effectively prepared for coating.

D.7

Sanitary tubing and fittings except those used (1) in high pressure liquid product lines and (2) in dry product conveying piping and equipment shall conform with the design and construction

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[5]Information on sheet metal fabricating techniques will be found in: Paull, James H., *Industrial Sheet Metal Drawing.* 1938. D. Van Nostrand Co., Inc., New York, "Methods of Fastening," Chapt. VII, P. 135.

provisions of the "E-3-A Sanitary Standards for Fittings Used on Liquid Egg and Liquid Egg Products Equipment and Used on Sanitary Lines Conducting Liquid Egg and Liquid Egg Products, Serial #E-0800," and/or "E-3-A Accepted Practices for Permanently Installed Sanitary Product-Pipelines and Cleaning Systems, Serial #E-60500."

D.8

Non-product contact surfaces shall be free of pockets and crevices and shall be easily accessible for cleaning, either when in an assembled position or when removed. Removable parts shall be readily demountable. Panels or doors shall be provided to allow easy access for cleaning of non-product areas of the equipment. They shall be constructed in a manner that will prevent air entrance. Use of hinges, wing nuts, latches and similar easy opening fastening devices are recommended to allow easy access without special tools.

D.9

Gaskets and Gasket Grooves on Product Contact Surfaces: Gaskets shall be removable or permanently bonded. Gaskets retaining grooves for removable gaskets, if provided, shall be no deeper than their width. The minimum radius of any internal angle in a gasket retaining groove shall be not less than 1/8 inch, except that a 3/32 inch radius is permissible where a standard 1/4 inch O-Ring is to be used. Use of gasket positioning grooves or pins, premolded fitted gaskets or gaskets cut from sheet material is recommended.

D.10

When a fan is installed on the downstream side of the intake air filter, it shall be designed and installed in a manner to preclude entrance of air contaminants.

D.11

Fans of the air foil type shall be constructed with blade cavities sealed.

D.12

Any bearing having a product contact surface shall be of a non-lubricated type. Lubricated bearings shall be located outside the product contact surface with at least 1 inch clearance between the bearing and any product contact surface to assure (1) that the product does not contact the bearing or lubricant and (2) lubricants and/or product do not build up between the bearing and any product contact surface. When a shaft or cable passes through a product contact surface, the portion of the opening surrounding the shaft or cable shall be protected to prevent the entrance of contaminants.

D.13

When the exhausts of collectors are connected to the bottom of a plenum whose entire construction does not conform to the criteria for product contact surfaces, (1) the top of the plenum shall be constructed so as to conform to the product contact surface criteria and (2) the collector exhaust connections shall extend upward into the plenum at least 6 inches. This provision does not apply to cloth collector bags.

D.14

A self-closing head shall be installed at the terminal end of all exhaust to atmosphere ducts.

E.

AIR SUPPLY FOR DRYING SYSTEMS

E.1

The location and nature of adjacent structures and the variations of wind and weather shall be considered in selecting the location of the air supply intake opening whether inside or outside a building. It shall be so located that it will reasonably insure that the character of the intake air will be suitable for its intended use.

E.2

Outside intake openings shall be suitably protected against the admission of all foreign objects. Openings should be provided with louvers which can be closed when processing equipment is not in use. Hoods should be used over these openings to minimize the intake of rain, snow, dust or other foreign material. Openings shall be equipped with sturdy screens having openings not larger than 3/4 inch in any dimension.

E.3

The air supply system and/or ducting shall be such that all of the air is caused to pass through air filters properly installed before coming in contact with product contact surfaces of the drying system.

E.3.1

Processing air which will be heated before product contact shall be passed through a properly installed and maintained filter(s), selected to have a minimum average efficiency of 90% when tested in accordance with the ASHRAE Synthetic Dust Arrestance Test[6] when operated at its design face velocity.

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[6]The method of making these tests will be found in the following reference: Method of Testing Air Cleaning Devices, ASHRAE Standard 52-68. Available from the American Society of Heating, Refrigerating and Air-Conditioning Engineers, Inc., 345 E. 47th Street, New York, N. Y. 10017.

E.3.2
Processing air which will not be heated before product contact shall be passed through a properly installed and maintained filter(s), selected to have a minimum average efficiency of 85% when tested in accordance with ASHRAE Atmospheric Dust Spot Method[6] when operated at its design face velocity.

APPENDIX

F.
PRODUCT CONTACT SURFACE MATERIALS

F.1
Stainless steel conforming to the applicable composition ranges established by AISI[1] for wrought products, or by ACI [2] for cast products, should be considered in compliance with the requirements of Section C.2 herein. Where welding is involved the carbon content of the stainless steel should not exceed 0.08%. The first reference cited in C.2 sets forth the chemical ranges and limits of acceptable stainless steels of the 300 series. Cast grades of stainless steel corresponding to types 303, 304, and 316 are designated CF-16F, CF-8 and CF-8M, respectively. These cast grades are covered by ASTM[7] Specifications A296-68 and A351-70.

G.
CLEANING AND SANITIZING PROCEDURES
A cleaning and sanitizing regimen which is effective shall be employed. A description of this regimen shall be available at the drying plant. Because of the possibilities of corrosion, the recommendations of the cleaning compound manufacturer shall be followed with respect to the time, temperature, and the concentration of specific detergents and sanitizing agents. To insure proper strength of solution and to avoid corrosion, the detergent or sanitizer shall be completely dissolved or dispersed prior to use.

The following is adapted from "Special Sanitation Suggestions for Dry Milk Manufacturers" available from American Dry Milk Institute, 130 N. Franklin Street, Chicago, Ill. 60606, ADMI Handbook No. 917.

G.1
High Pressure Supply Pump, Egg Product Lines and Spray Devices

G.1.1
As soon as possible after the drier is shut down, remove the spray nozzles from chamber and place the nozzles in the solution tank. Direct the high pressure lines and/or nozzle pipes to the solution tank.

G.1.2
Remove and manually clean the line through which product is conducted from the high pressure regulating valve at the outlet of the high pressure pump to the inlet of the pump. Hook up lines for the complete recirculation circuit including the preheater, high pressure or supply pump and high pressure lines. Do not put operating pressure on high pressure pump during rinsing and cleaning.

G.1.3
Using clear water at 110-115°F, flush the entire circuit until rinse water is clear. Rinse water should go directly to the drain.

G.1.4
After rinsing, add enough water to the solution tank to avoid sucking air into lines during circulation. Slowly add an alkaline cleaning compound in the amount specified by the supplier. Circulate this solution for the length of time and at the temperature recommended by the cleaning compound manufacturer. To assure full flow through a multiple spray pipe system it may be necessary to circulate groups of pipes, valves and nozzle pipes alternately.

G.1.5
Rinse the alkaline cleaning solution thoroughly from the system and refill circuit with warm water.

G.1.6
Add an acid cleaning solution to the solution tank according to the cleaning compound manufacturer's direction. Circulate for the time and at the temperature recommended by the manufacturer of the cleaning compound. After completion of cleaning, completely rinse the acid cleaning solution from the system.

G.1.7
Disassemble the high pressure pump and check the entire system for effectiveness of cleaning. Allow the high pressure pump to dry before reassembly.

G.1.8
When paper gaskets are used in the preheater or anywhere in the circuit they must be changed daily.

G.1.9
Immediately prior to reuse of equipment, circulate a sanitizing solution through system for 5 minutes and then discharge it to the drain.

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[7]Available from American Society for Testing and Materials, 1916 Race Street, Philadelphia, Pa. 19103.

G.1.10
Circulation time, temperature and strength of cleaning solutions may vary according to amount of egg product processed, temperatures used and water conditions in a particular plant.

G.1.11
Be sure to check spray nozzles daily for cleanliness and wear of cores, orifices, spinning devices, etc.

H.
GAS BURNER MAINTENANCE

It is essential that burners and their controls operate properly to produce a good quality product, and for the prevention of fires. It is suggested that burners be cleaned at least three times a year. If burners are extremely dirty, it is suggested that burners be removed and cleaned in the shop or other area away from the drier.

If in doubt about operation of the burner, the drier manufacturer or a qualified service man recommended by him should be consulted.

At least once a year the burner and controls should be serviced by the manufacturer or a service man recommended by him.

I.
SANITARY ATTIRE AND CLEANING APPLIANCES

I.1
When it is necessary to enter the drier for cleaning:

I.1.1
The cleanup crew should be furnished with freshly laundered outer clothing and cleaned and sanitized multiple use or single service boots to wear while in the drier.

I.1.2
A suitable place should be provided for the storage of laundered outer clothing, cleaned and sanitized boots, unused single service items and cleaning tools and appliances.

I.1.3
A clean place should be provided adjacent to the point of entry to the drier which provides (1) an area to which the clean outer clothing can be carried, (2) an area in which, if required, outer clothing can be removed and stored, (3) an area in which the clean outer clothing and boots for use in the drier can be donned and (4) a clean floor (for example, a covering of clean paper) to maintain the cleanliness of the boots.

I.1.4
Garments and boots worn for interior drier cleaning should be worn only while cleaning the drier and not while performing other tasks. Boots that have been worn while walking outside the drier should be replaced with other suitable boots before reentering the drier.

I.2
Cleaning tools and appliances that are used in the drier should be kept clean and used for no other purpose than cleaning the interior of the drier.

These Practices shall become effective March 6, 1972.

SEMINAR NOTICE

The Department of Food Science and Technology at the University of Massachusetts will sponsor a seminar, "Establishing Thermal Process Requirements for Food Sterilization." The seminar will be held in Chenoweth Laboratory on March 27 to March 30, 1972.

Seminar registrants will be limited to fifty in number. University staff and industry experts will comprise the seminar instruction staff. A registration fee will be charged.

Further details may be obtained from Professor Kirby M. Hayes or Dr. Charles R. Stumbo, Department of Food Science and Technology, University of Massachusetts, Amherst, Mass. 01002.

STAPHYLOCOCCUS AUREUS AND STAPHYLOCOCCAL FOOD INTOXICATIONS. A REVIEW

II. ENTEROTOXINS AND EPIDEMIOLOGY.[1, 2, 3, 4]

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Abstract

Staphylococcal food poisoning is strictly an intoxication which is caused by a water-soluble protein called enterotoxin that is secreted by staphylococcal cells, i.e. an exotoxin. After a general discussion of the properties of the enterotoxins, methods and media employed for producing high toxin yields are detailed. Mechanisms whereby these toxins are secreted differ in that enterotoxin A behaves as a primary metabolite and enterotoxin B acts as a secondary metabolite. Several studies on and explanations of the synthesis of enterotoxins are presented. Assay methods for these toxins that are discussed include bioassay techniques and serological procedures. Finally, some general aspects of the epidemiology of staphylococcal intoxications are described.

Part I of this review (*223*) was concerned with the nature of the staphylococci and highlighted their taxonomy, cultural and physiological characteristics, isolation, and activity in bacteriological media. In this part, we will review some aspects of staphylococcal intoxications, including: (*a*) description and general properties of the enterotoxins, (*b*) production of enterotoxins, (*c*) assays for enterotoxins, and (*d*) epidemiology of staphylococcal intoxications.

While many *Staphylococcus aureus* strains are able to initiate a wide variety of infections in or on the body of man and animals, staphylococcal food poisoning is strictly an intoxication. That is, the food poisoning syndrome is caused by a toxin ingested with a food substance. This toxin, or enterotoxin, is a water-soluble protein which is secreted by staphylococcal cells, i.e. an exotoxin.

Description and General Properties of the Enterotoxins

Designation of enterotoxins

Several chemically and serologically distinct forms of enterotoxin have been identified in recent years. In 1960, Casman (*39*) reported that two serologically different types of enterotoxin existed. One of these types, produced by S. *aureus* strain 196E, was designated as type "F" ("food poisoning") and the other, formed by strain 243, was designated as type "E" ("enteritis"). This nomenclature was changed in 1962 (*44*) to conform with the rules for naming other bacterial toxins. Type F became known as enterotoxin A and type E as enterotoxin B. In 1955, Bergdoll et al. (*20*) announced the identification of a new enterotoxin, enterotoxin C, and in 1967, Casman et al. (*46*) named a fourth enterotoxin, designated as D. Recently, Bergdoll et al. (*25*) reported the identification of enterotoxin E.

Some chemistry of enterotoxins

In 1956, Bergdoll et al. (*17*) described a procedure based on column chromatography and extraction techniques whereby enterotoxin could be partially purified. Refined procedures for purification of enterotoxin A (*64, 334*), enterotoxin B (*21, 22, 110, 286*), enterotoxin C (*20, 28*), and enterotoxin E (*25*) have subsequently been published.

Bergdoll et al. (*17*), in their paper of 1956, described a purified toxin preparation as a mixture of water-soluble, heat coagulable proteins with a theoretical molecular weight of 15,000-20,000. The material was separated into two fractions by electrophoresis. One fraction had an isoelectric value near pH 5.0 and the other near 8.5 (the latter fraction retained most of the toxic activity). The toxic material was trypsin-resistant and contained a high percentage of lysine. The toxic fraction was studied by Hibnick and Bergdoll (*145*) and a revised theoretical molecular weight of 24,000 and an isoelectric value of pH 8.6 were reported. Three laboratories simultaneously reported new theoretical molecular weights for enterotoxin B in 1965. Spero et al. (*308*) reported a value of 35,380 and Wagman et al. (*355*) published a figure of 35,300. Bergdoll et al. (*25a*), however, claimed the molecular weight was between 29,000 and 31,000. A revised isoelectric pH of 8.7 was determined by Spero et al. (*308*) and the enterotoxin B molecule was noted to be rich in aspartic acid as well as lysine. Chu (*63*) studied the hydro-

[1]Supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison and by Public Health Service Grant No. FD00009-05 from the Food and Drug Administration.
[2]Part I appeared in a previous issue of this Journal.
[3]Parts III and IV will appear in subsequent issues of this Journal.
[4]References cited in all parts will be listed at the end of part IV.

gen ion equilibria of enterotoxin B, and the properties of this molecule were really resolved when Huang et al. (*154, 155, 156*) announced the complete amino acid sequence. The single poly-peptide chain was reported to contain 239 amino acid residues and the molecular weight was calculated to be 28,496. This latter figure, though, was later discovered to be in error and a true value of 28,366 was determined (Bergdoll, *personal communication*). Bowden (*30*) has reported a method for preparing small quantities of ^{14}C-labelled enterotoxin B.

A freeze-dried preparation of enterotoxin A has been described by Chu et al. (*64*) as a fluffy, snow-white material that is highly hygroscopic and very soluble in water and salt solutions. The active toxin was trypsin-resistant and had a maximum absorption of 277 mμ. Its molecular weight was estimated to be 34,486 and its isoelectric value was 6.8 (compared to 8.7 for enterotoxin B).

Two strains of *S. aureus*, 137 and 361, were involved when enterotoxin C was identified (*20*). Avena and Bergdoll (*11*) showed that the enterotoxins from both strains had identical values for molecular size, sedimentation coefficient, and frictional ratio but possessed different electrophoretic mobilities and isoelectric values. The isoelectric pH for strain 361 was about 7.0 and Borja and Bergdoll (*28*) reported an isoelectric pH of 8.6 for strain 137. A difference in the number of basic groups between the two toxins was demonstrated by Huang et al. (*157*). Bergdoll and his colleagues believe that appropriate designations should be assigned to distinguish between the two forms of enterotoxin C. Strain 137 enterotoxin was tentatively assigned the designation C_1 and strain 361 enterotoxin was named C_2.

Antigenicity of enterotoxins

As early as 1938, Davison et al. (*85*) recognized that enterotoxin was antigenic and demonstrated this phenomenon in monkeys and kittens using subcutaneous injections of toxic filtrates from staphylococcal cultures. In 1944, Dolman (*89*) produced immunity to enterotoxin in cats and humans. It was not until 1952, however, that the property of antigenicity possessed by enterotoxin was demonstrated by an *in vitro* serological procedure. At this time, Surgalla et al. (*328*) observed antigen-antibody reactions in agar and recognized the presence of a number of antigens in the enterotoxin preparation. Cats were immunized by Thatcher and Matheson (*340*) with specific enterotoxic filtrates obtained from different staphylococcal strains. Results of this study suggested to the authors that antigenically distinct enterotoxins might exist.

Bergdoll et al. (*17*), in the 1956 report previously cited, indicated their partially purified toxin preparation contained at least six antigens. Serologically distinct antibodies will, of course, be formed which will be specific for all of the six antigens when such a preparation is administered in an appropriate manner to animals. Since only one of the antibodies so formed will be specific for the toxic moiety of the enterotoxin preparation, presence of the other antibodies interfered with identification of a specific enterotoxin-antienterotoxin interaction. This problem was resolved by Bergdoll et al. (*21*) in 1959. They also provided additional evidence that immunologically distinct enterotoxins existed. With the availability of highly purified enterotoxin preparations and refined techniques, highly potent and specific antisera are routinely produced (*42*).

Consistent with the fact that toxoids (molecules possessing antigenic behavior but little or no toxic activity) can be produced from exotoxins, Bergdoll (*17a*) and Silverman et al. (*301, 303*) were able to form enterotoxoids from enterotoxin by employing a formaldehyde treatment.

Stability of enterotoxins

As early as 1939, Davison and Dack (*83*) recognized the pronounced heat stability of enterotoxin, observing that its potency could only be gradually decreased by prolonged boiling or autoclaving.

Purified enterotoxin A is relatively heat labile compared to enterotoxin B. A decrease of 50% in activity of enterotoxin A was reported by Chu et al. (*64*) when 0.2 mg of toxin per milliliter of 0.05*M* sodium phosphate solution (pH 6.85) was heated at 60 C for 20 min. A 60% decrease in activity occurred with heating at 70 C for 3 min and activity ceased after heating at 80 and 100 C for 3 and 1 min, respectively. Other researchers have reported a much higher heat resistance for enterotoxin A (*87, 146*). Read and Bradshaw (*266*) found that 30 μg enterotoxin B (>99% pure) per milliliter of 0.04*M* Veronal buffer was reduced to less than 0.7 μg active toxin per milliliter in 103, 87, 71, 57, 39, 28, 16, and 12 min, respectively, at temperatures of 96, 99, 102, 104, 110, 116, 121, and 127 C. Crude enterotoxin was slightly more thermostable than purified toxin. Thermal inactivation of enterotoxin B was noted by Satterlee and Kraft (*282*) to be quite rapid in phosphate buffer (pH 7.4) and in 0.85% NaCl at 60-110 C until 65-75% of the activity was lost and then the remaining activity disappeared more slowly. The time required for 50% inactivation was 204 min at 60 C and 12 min at 80 C.

Irradiation of enterotoxin B was studied by Read and Bradshaw (*267*) who determined that a dose of 5 Mrad of γ-irradiation was required to reduce the concentration of 31 μg per milliliter Veronal buffer

to < 0.7 μg/ml.

PRODUCTION OF ENTEROTOXINS

Media and methods suggested for producing enterotoxins in high yields

Several media and different methods have been suggested to obtain high yields of enterotoxin from staphylococcal cultures. Favorite and Hammon (*103*), in 1941, found that a medium composed of acid casein hydrolysate, glucose, vitamin B_1, and nicotinic acid incubated in the presence of CO_2 in a slowly revolving container supported production of enterotoxin. An acid hydrolysate medium (Difco's Casamino Acids) was suggested by Casman (*38*) for producing enterotoxin, but for good growth of staphylococci, it was necessary to add calcium pantothenate, L-cystine, tryptophan, magnesium sulfate, glucose, nicotinic acid, and thiamine (pH 7.2-7.4). Casman and Bennett (*41*) later reported that Brain Heart Infusion broth was the best medium for producing good yields of enterotoxin.

Bergdoll and his colleagues prefer to use a medium that consists of 3% Protein Hydrolysate Powder (Mead Johnson) plus 3% N-Z Amine NAK (Sheffield Chemical) supplemented with 0.00005% thiamine and 0.01% niacin (pH 6.0-6.5) (*181*). Jarvis and Lawrence (*164*) obtained good yields of toxin using either Casein Hydrolysate plus 2% Protein Hydrolysate medium or Heart Infusion broth (pH 6.0). The choice of a medium was found by Reiser and Weiss (*270*) to be critical to yields of enterotoxins A, B, and C. Brain Heart Infusion broth (Difco) was inferior to Fisher Brain Heart Infusion broth, 4% N-Z Amine NAK, or 3% NAK plus 3% Protein Hydrolysate Powder used at initial pH levels of 6.8, 6.0, and 5.3.

According to studies by Surgalla (*326*), enterotoxin was detected in the supernatants of cultures grown in media containing 2-16 amino acids as the source of nitrogen. The simplest medium used successfully to produce enterotoxin contained the amino acids arginine and cystine. Growth of staphylococci did not occur in the absence of glucose in media containing only three amino acids. Both toxin production and growth appeared to be reduced in the simpler media; an observation also made by Mah et al. (*203*). Morse and Baldwin (*230*) reported that glucose repressed the rate of enterotoxin B synthesis by *S. aureus* S6 cells growing aerobically in Casein Hydrolysate medium.

Studies by Wu and Bergdoll (*372*) showed that a relatively large quantity of enterotoxin B (125 μg/ml) was produced in a synthetic medium composed of amino acids, inorganic salts, and vitamins. The medium was based on the utilization of amino acids by *S. aureus* strain S6 throughout its growth cycle. Aspartic acid, threonine, serine, glutamic acid, proline, glycine, and alanine were utilized rapidly, whereas only small amounts of tryptophane, cystine, tyrosine, phenylalanine, and methionine were used. Arginine appeared to be essential for enterotoxin production. In another study (*371*), these authors subjected Protein Hydrolysate Powder (Mead Johnson) to chromatography and collected 18 fractions. Five of the fractions stimulated enterotoxin B production in greater quantity than a basal medium containing Protein Hydrolysate Powder. The stimulatory effect was caused by the presence of peptides in the fractions. Many of the peptides contained large amounts of proline.

Surgalla et al. (*331*) described three methods for producing high yields of enterotoxin in hydrolyzed casein media: shallow cultures in Roux bottles, deep cultures in revolving bottles, and deep aerated cultures. Shake-flask cultures and the cellophane-over-agar method of Hallander (*139*) were utilized by Jarvis and Lawrence (*164*). Casman and Bennett (*41*) employed a cellophane-sac culture method [growth and toxin production are confined within a sac lying on the surface of a fluid medium (pH 7.0)] and have utilized a semi-solid medium (pH 5.3) in standard petri dishes. Donnelly et al. (*95*) employed a sac-culture technique but they placed the nutrient medium inside the sac and the inoculum in a solution surrounding the exterior of the sac.

Enterotoxin B is produced in various amounts depending upon the strain involved, whereas enterotoxin A is formed in relatively uniform amounts between strains. To select isolates able to produce high yields of enterotoxin B, Sugiyama et al. (*323*) plated staphylococci on Amigen agar (pancreatic digest of casein containing amino acids and small peptides, supplemented with niacin and thiamine, pH 7.6) containing rabbit anti-enterotoxin B serum. Enterotoxin production was recognized by halos of antigen-antibody precipitate around the colonies (the size of the precipitate zone correlated well with the quantity of toxin produced). This method has recently been used by Weiss and Robbins (*364*) to determine the standard titers of different lots of antisera.

Effect of pH on production of enterotoxins

Reiser and Weiss (*270*) found an initial pH of 6.8 resulted in higher yields of enterotoxin B and C than did either pH 6.0 or 5.3. Production of enterotoxin A, however, was not materially affected by the initial low pH of 5.3. Growth of an enterotoxin C-producing staphylococcus was reported by Genigeorgis et al. (*125*) to be initiated at pH values as low as 4.00 and as high as 9.83 in 3% Protein Hydrolysate

Powder plus 3% N-Z Amine NAK broth so long as the inoculum contained at least 10^8 cells per milliliter. An optimal initial pH of 6.5-7.0 was reported by Markus and Silverman (*207*) for enterotoxin A production. Enterotoxin B synthesis was observed by Morse et al. (*232*) to increase at pH 6.4 and above and was most repressed in the vicinity of pH 5.0.

Effect of incubation temperature on production of enterotoxins

Segalove and Dack (*292*) found that at least 3 days of incubation at 18 C was necessary before enterotoxin in culture media was detected. At 9 and 15 C, toxin was not produced in 7 and 3 days, respectively. In contrast, 12 hr were required before enterotoxin could be detected in cultures incubated at 37 C.

Few, if any, differences were noted by Scheusner and Harmon (*287*) between the temperature ranges within which enterotoxins A, B, C, and D were produced by cultures grown in Brain Heart Infusion broth on a shaker. Temperatures below 20 C or above 46 C delayed enterotoxin production beyond 3 days or inhibited it completely. Tatini et al. (*336*) reported that production of enterotoxins A, B, C, and D was stimulated in Brain Heart Infusion broth at temperatures above (40 and 45 C) the optimum growth temperature (37 C). Prolonged incubation (48-72 hr) at 37 C was observed by Reiser and Weiss (*270*) to only occasionally result in production of additional quantities of enterotoxins A, B, and C.

Effect of substances in solution on production of enterotoxins

Troller (*345*) reported that enterotoxin B synthesis was markedly affected by a reduction in a_w (water activity). Lowering the a_w from 0.99 to 0.98 in one medium and from 0.99 to 0.97 in another caused a marked reduction in toxin synthesis despite attainment of high numbers of *S. aureus*. Aerobic growth of staphylococci has been observed at a_w values as low as 0.86, although growth at a_w levels below 0.94 is substantially reduced (*291*).

Hojvat and Jackson (*150*) noted that, while *S. aureus* was able to grow in broth containing 12% NaCl between the temperatures of 20 and 35 C, enterotoxin B production was inhibited at temperatures below 35 C when broths contained 4 and 8% NaCl and at all temperatures tested (4-35 C) when the broths contained 12% NaCl. Levels of NaCl up to 10% in Brain Heart Infusion broth were observed by McLean et al. (*200*) to have a relatively slight effect on the total growth of *S. aureus* but at concentrations $>$ 3% NaCl a definite decrease in enterotoxin B production occurred. On the other hand, Markus and Silverman (*207*) reported NaCl concentrations up to 10% did not essentially alter the amount of enterotoxin A produced but higher concentrations inhibited growth of the staphylococcus. Genigeorgis and Sadler (*120*) detected enterotoxin B in broth at pH 6.9 and up to 10% salt and in pH 5.1 broth up to 4% salt.

Genigeorgis et al. (*125*) found growth of an enterotoxin C-producing staphylococcus in 3% Protein Hydrolysate Powder plus 3% N-Z Amine NAK broth decreased as NaCl was added up to a concentration of 12%. Enterotoxin C was formed in broths inoculated with 10^8 cells per milliliter and above in NaCl concentrations of 0, 4, and 8% at respective pH ranges of 4.00-9.83, 4.40-9.43, and 4.50-8.55. In the presence of 10% salt, the pH range supporting toxin production was 5.45-7.30 for an inoculum of 10^8 cells per milliliter and 6.38-7.30 for an inoculum of 3.6×10^6 cells per milliliter. The effect of NaCl on enterotoxin C production followed the same pattern as its effect on enterotoxin B.

Neither $NaNO_3$ in concentrations up to 1000 ppm nor $NaNO_2$ at levels up to 200 ppm (maximum levels permitted in cured meats) appears to affect growth of staphylococci or production of enterotoxin A (*207*) and enterotoxin B (*200*).

Other studies

Aerated cultures produce toxin more rapidly and in larger amounts than do static cultures (*41, 200*). Virtually all methods for producing enterotoxins make a provision for aeration or exposure to atmospheric oxygen.

Genigeorgis et al. (*126*) conducted a factorial design experiment with five strains of staphylococci able to produce enterotoxins A, B, C, and D, and grown in Brain Heart Infusion broth at 30 C. Statistical analysis revealed: (*a*) significant effects by pH, NaCl, and strain on the probability of growth, (*b*) diverse effects of NaCl with various pH levels and strains, (*c*) a linear relationship between NaCl concentration and probability of growth initiation when data for all strains were pooled, and (*d*) the relationship between NaCl concentration and probability of growth initiation varied from linear to sigmoid, depending on the pH of the broth.

Studies on the mechanism of enterotoxin synthesis by S. aureus

McLean et al. (*200*) reported that enterotoxin B production occurred at the beginning of the stationary phase of growth (after 5 hr at 37 C in Brain Heart Infusion broth). Lowering the temperature of incubation decreased the amount of toxin synthesized without affecting the total amount of growth. At 20 C the culture reached the stationary phase of growth between 55 and 60 hr and toxin concentra-

tion was highest after 65 hr (but, the maximal titer at 20 C was 100 compared to 500 in the 37 C control).

Morse et al. (*232*) noted that entertoxin B was formed at a maximal rate after completion of exponential growth and a corresponding increase in pH was observed in the medium. Toxin, however, apparently was not preformed and then released by a change in pH (i.e. toxin or enzymes responsible for its release are synthesized *de novo*). Further investigation led the authors to suggest that toxin synthesis was regulated by catabolite repression.

Data by Markus and Silverman (*205, 206*) showed that although 95% of enterotoxin B produced appeared during the latter part of the exponential phase of growth, growth *per se* was not necessary for toxin synthesis. Non-growing cells collected from several stages of growth could secrete significant quantities of enterotoxin but only if the cells had been grown for at least 4 hr. Non-growing, washed cells harvested from the late exponential phase or stationary phase of growth could produce toxin without an exogenous nitrogen source and with only glucose in the presence of chloramphenicol, indicating the existence of toxin precursors in the cell. The authors suggested that the excretion of enterotoxin B might be an adjustment by the cell to static growth conditions.

Markus and Silverman (*207*) found that enterotoxin A behaved as a primary metabolite (in constrast to enterotoxin B, a secondary metabolite) for it was secreted mainly in the exponential phase of growth (although approximately 20% was secreted as the cells entered the early stationary phase). Chloramphenicol and 2,4-dinitrophenol prevented secretion of enterotoxin but normal amounts of toxin were secreted in the presence of streptomycin and penicillin G by nonreplicating cells.

Data by Friedman (*111*) suggested that the enzymatic system which controls enterotoxin synthesis may require Mg^{++}. In another study (*112*), the author reported that toxin formation was inhibited by Tween 80, oleic acid, sodium deoxycholate, penicillin, D-cycloserine, or bacitracin, without growth being adversely affected (indicating the importance of the cell surface in enterotoxin excretion). Czop and Bergdoll (*77*) demonstrated enterotoxin A production by L-forms (bacteria lacking cell walls) but could not detect toxin production by L-forms of enterotoxin B- or C-producing strains

Rosenwald and Lincoln (*276*) found that streptomycin interfered with the elaboration of enterotoxin by a streptomycin-resistant and a streptomycin-dependent strain of S. *aureus* at drug levels permitting synthesis of proteins necessary for growth and multiplication of the organism. Enterotoxin declined with growth when a streptomycin-sensitive strain was treated with increasing concentrations of the drug.

Genetic studies of enterotoxin B synthesis led Dornbusch et al. (*96*) to consider that toxin synthesis may be an extra-chromosomally-determined character linked to the gene which controls methicillin resistance, both being carried by a single plasmid. Casman (*40*) conferred enterotoxigenicity on a nonenterotoxigenic staphylococcus through lysogenesis of the latter with a temperate phage which was carried by an enterotoxigenic staphylococcus. Genetic studies by Friedman and Howard (*113*) showed that serial exposures of S. *aureus* to N-methyl-N′-nitro-N-nitrosoguanidine, a mutagenic agent, resulted in a 20-fold increase in enterotoxin yield compared to quantities of toxin produced by the parent strain.

ASSAY OF ENTEROTOXINS

Bioassay methods

The best method to determine if a given sample is toxic to humans would be to feed the sample to a group of people. As this is obviously not practical, workers first turned to animals in their search for an assay method for enterotoxin. Of the various animals which have been tested, frogs (*99, 275, 329*), tropical fish (*261*), and nematodes (*54*) have been reported to be unresponsive to enterotoxin. Toxin also had no effect on 170 species of microorganisms tested by Braymen (*32*). Attempts to develop a tissue culture assay were unsuccessful (*220*), but Schaeffer (*283*) reported that the cytopathic effect of enterotoxin B upon mammalian cell cultures is expressed fully only if the cells are grown in human serum-supplemented media. Isolated rabbit gut segments, reliable for detecting toxic filtrates from *Clostridium perfringens* cultures, were unsuitable for detecting enterotoxic filtrates from staphylococci (*6, 182, 274*). Dogs (*92*) and pigs (*151*) have been shown to react to enterotoxin but they lack proper sensitivity.

The most widely used test animals have been the kitten or cat and monkey. The sensitivity of the monkey most closely parallels that of humans. The chimpanzee was reported by Wilson (*367*) to be intermediate between monkey and man in susceptibility to enterotoxin.

The kitten test was developed by Dolman et al. (*92*) in 1936. These workers injected an intraperitoneal dose of about 2 ml of formalinized filtrate from an enterotoxin-producing culture of S. *aureus* into kittens and thus produced vomiting. Kittens are not sensitive enough to enterotoxin orally, whereas monkeys are. A major disadvantage of administering samples to animals by injection is that, in addition

to enterotoxin, staphylococci produce *alpha* and *beta* lysins and other toxic substances which also induce vomiting; necessitating heat treatment, formalization, or other methods of preparation before the sample can be administered (*90, 115*). These treatments do not always completely remove the interfering substances and can be detrimental to the stability of the enterotoxin. The kitten method has worked well in the hands of some (*91, 140, 214*) but others have reported variable to unreliable results (*35, 221*). Monkeys exhibit a wide variability in response to oral doses of enterotoxin (*329*) and become significantly less sensitive to the emetic action of enterotoxin for a period of time following administration of the toxin (*319, 324*).

Serological assay methods

Reliable methods for detection and measurement of enterotoxin did not appear until the property of antigenicity was applied to the problem. Surgalla et al. (*330*), in 1954, used a serological method to enumerate antigens in a partially purified enterotoxin preparation. Casman and Bennett (*43*), in 1965, suggested using serology for routine testing of foods for enterotoxin and developed a reliable assay procedure.

Before describing the serological assay procedures further, it might be well to explain the basis for these tests. If a given antigen like enterotoxin is allowed to interact with its specific antibody of proper titer, a precipitate will form. If antigen and antibody are incorporated into an agar medium, the precipitate will be visible as a white line or band. In actual practice, the reagents are separated one from the other and they either diffuse toward each other (double diffusion) or the antigen diffuses toward the antibody which is immobile (single diffusion). Ouchterlony (*247*) developed a double diffusion technique using ordinary petri dishes to hold the agar containing the antigen and antibody. This procedure was modified by Wadsworth (*354*) who used a glass microscope slide and a thin layer of agar. This allowed her to use microquantities of reagents which were applied to the surface of the agar with the aid of a template equipped with wells. This method, in turn, was modified slightly by Crowle (*76*) and was adopted in this form by Casman and Bennett (*43*). An alternative to the plate and microslide methods is the use of tubes. The double diffusion tube technique employs an agar layer in the bottom of the tube which is seeded with antiserum, an agar layer in the middle which is free of reagents, and a layer at the top which contains the antigen. A band of precipitate is formed for each antigen which is present providing its specific antibody or a cross-reacting antibody is also present. This method is useful to detect impurities in enterotoxin preparations. The single-diffusion tube method also involves a column of agar at the bottom of the tube which contains the antiserum, but the antigen is applied as a solution directly to the surface of the agar column. A precipitate then moves down into the agar as the antigen diffuses downward. This method can be made quantitative because the length of the precipitate band is correlated with the concentration of antigen present. The amount of unkown toxin cannot, however, be compared directly to a known amount of toxin and the presence of other antigens cannot be detected.

Casman and Bennett (*43*) estimated that a culture of staphylococci containing 15-20 billion organisms per milliliter in aerated Brain Heart Infusion broth could produce 2-4 μg enterotoxin A per milliliter. Since foods contain much lower levels of toxin and the sensitivity of the microslide technique was limited in the beginning to about 1 μg/ml, Casman and Bennett had to concentrate their food samples before they could be tested serologically. In addition, protein substances obscure the antigen-antibody precipitin reaction, therefore these had to be removed. The Casman method then involved three steps: (*a*) separation of the enterotoxin from insoluble constituents, (*b*) separation from soluble extractives, and (*c*) concentration of the extracted and separated enterotoxin so that it can be detected by the microslide gel diffusion technique. The method employs extraction with 0.2*M* NaCl followed by adsorption to carboxymethyl-cellulose and concentration of the eluate by dialysis.

About the same time that the Casman method was introduced, Read et al. (*268, 269*) announced a procedure for recovering enterotoxin from milk and cheese. It differed from the Casman method by the manner in which the antigen was prepared. Still another method became available at this time when Hall et al. (*138*) introduced a procedure, based on the double-diffusion tube method, for detecting enterotoxins in foods. Weirether et al. (*363*) have described a rapid assay method utilizing the single-diffusion tube technique.

According to Hall et al. (*137*), the single-diffusion tube procedure detects as little as 1 μg enterotoxin B per milliliter of food extract or culture filtrate and the double-diffusion tube technique detects as little as 0.05 μg/ml (however, up to 21 days is required to detect this low a level). Casman et al. (*47*) claimed recently they could detect as little as 0.1 μg of enterotoxin A and B per milliliter with the microslide method.

Zehren and Zehren (*375*) made slight changes in

the Casman and Bennett method and then sampled over 4 million lb. of cheese, representing 2,112 vats, for enterotoxin A. They reported that as little as 0.3 μg toxin per 100 g of cheese was detected.

Fung and Wagner (*116*) and Gandhi and Richardson (*118*) have recently described miniature capillary tube gel diffusion methods but, at this time, they appear to lack the sensitivity of other techniques.

Another means for detecting enterotoxin serologically is the fluorescent-antibody test (FAT) (*114, 121, 122, 306, 311, 312*). Antibodies specific for a given enterotoxin are conjugated with fluorescein isothiocynate which is then used to stain smears. Fluorescence on or near cells indicates secretion of toxin by staphylococci. Genigeorgis and Sadler (*122*) claimed that <0.05 μg of enterotoxin per milliliter was detected via this method, but Bergdoll (*19*) believes the sensitivity is about 1 μg/ml.

Johnson et al. (*174*) and Morse and Mah (*231*) have published methods based on a hemogglutination technique. In the procedure of Morse and Mah, formalinized sheep erythrocytes are treated with tannic acid and then sensitized with enterotoxin. In the method of Johnson et al., the enterotoxin is coupled to the formalinized erythrocytes with bis-diazotized benzidine. The sensitized erythrocytes in both methods are then added to enterotoxin-antienterotoxin combinations. If sufficient free enterotoxin is present (i.e. not in combination with its antibody) hemagglutination will occur. The sensitized erythrocytes thus act as an indicator of whether antigen-antibody precipitation has occurred; results are more accurate and sensitive than are those obtained with visual observations.

A reversed passive hemagglutination procedure has been devised by Silverman et al. (*302*) in which formalin-preserved sheep erythrocytes are treated with tannic acid before sensitization with antienterotoxin molecules. Enterotoxin solutions are treated with the sensitized erythrocytes and the degree of agglutination is determined after 2 hr. As little as 0.0015 μg toxin per milliliter has been detected in toxic filtrates. A variation of this method was developed by Salomon and Tew *(281a)* who sensitized latex particles rather than erythrocytes with antienterotoxin preparations.

An antigen-antibody flotation system has been designed by Hopper (*152*) in which enterotoxin B was concentrated and removed from aqueous solution by froth flotation. An anionic wetting agent and anti-enterotoxin B labelled with rhodamine are added to the toxic solution. The dye-toxin complex, located in the top layer of the foam, separates to give a distinctly red-colored fraction. With a slight increase in air pressure, the red fraction is removed. The toxin is identified by taking one drop each of the foam fraction, pH 4.5 buffer, and latex polystyrene suspension and mixing. Within 1 min, heavy agglutination occurs.

Hodoval et al. (*147*) used a radioassay and studied the half-disappearance time of highly purified ^{131}I-labelled staphylococcal enterotoxin B from the blood of male albino rats as a means for assaying for specific staphylococcal enterotoxin B antisera. Gruber and Wright (*134*) studied the antigen-binding capacity of normal and immune rabbit sera for the enterotoxins using an ammonium sulfate coprecipitation technique as a means for measuring antibody.

EPIDEMIOLOGY OF STAPHYLOCOCCAL INTOXICATIONS

Pathology of enterotoxemia

Pathology in animals. Numerous papers in the medical literature deal with the mode of action of enterotoxin in enterotoxemia of animals, however, there is no clear explanation for the changes which occur in the body during staphylococcal intoxication.

Monkeys are often employed in medical studies of enterotoxemia. They develop an acute gastroenteritis which is well developed by 2 hr after intragastric administration of purified enterotoxin B and reaches a maximum at 4-8 hr. The intoxication then rapidly regresses to a state approaching normal by 72 hr (*183, 184*). Pathological changes induced by enterotoxin in animals have also been studied by inoculating monkeys, dogs, cats, rabbits, rats, mice, and other animals with the toxin.

Some similarities between enterotoxin and enterobacterial endotoxin have been noted (*318*). This has been evidenced in altered EEG patterns in the brain (*149*) caused by insufficient blood flow and cardiovascular changes associated with shock (*148, 273*), in pyrogenic responses (*65, 66, 211*), and in the manner by which enterotoxin is cleared from the blood (*263*). Sugiyama (*325*) showed that mice and rabbits pretreated with staphylococcal enterotoxin became more susceptible to the lethal action of enterobacterial endotoxin.

Target sites suggested for binding of enterotoxin in the animal body include the kidneys (*229, 240, 310*), liver (*229*), abdominal viscera (*320*), lungs (*106*), gastrointestinal tract (*6, 142, 184, 188, 274, 284, 285, 298*), various tissues (*264*), and cellular sites (*71, 72, 73, 106, 215, 241, 272, 321, 322*). Shemano et al. (*299*) found enterotoxin-induced diarrhea could not be explained on the basis of increased motility and transport and concluded enterotoxin had no direct action on the intestine.

Symptoms of staphylococcal intoxication in humans. Few data are available on the dose of enterotoxin required to cause emesis in humans. Dolman (*88*), in

1934, fed bacteria-free filtrates from some 200 strains of staphylococci to 42 volunteers but could not produce symptoms of intoxication. Recently, Raj and Bergdoll (*260*) fed a quantity of enterotoxin B corresponding to a dose of 20-25 μg pure toxin to three volunteers. All three showed typical symptoms of intoxication. Bergdoll (*19*) believes that as little as 1 μg or less of enterotoxin A may evoke symptoms of intoxication in humans.

Consuming foods contaminated with sufficient quantities of enterotoxin usually causes onset of symptoms of staphylococcal food poisoning in humans in 2-4 hr, however, the onset time may vary from 0.5 to hr, however, the onset time may vary from 0.5 to more than 7 hr.

The onset of symptoms of the intoxication in humans is marked by salivation, followed in rapid succession by nausea, vomiting, retching, and often diarrhea. Complications can accompany severe attacks and include dehydration, shock, and blood and mucus in the stools and vomitus. Other symptoms noted in cases of varying severity include headache, cramps, sweating, and prostration. Enterotoxin doesn't as a rule elicit elevated body temperatures and subnormal body temperatures may be more common. Recovery frequently follows in 24 hr but several days may be required. The illness is seldom fatal.

General aspects of staphylococcal epidemiology

Since *S. aureus* is predominantly parasitic for man and animals, there must be a reservoir in the animal body in order to perpetuate infections and intoxications. The largest reservoir is currently believed to be man and the nose is the main site for multiplication of these organisms. Staphylococci also survive on the skin and are found in the feces. Scott et al. (*290*) tested feces from 200 hospitalized patients with symptoms of gastroenteritis and found staphylococci to be the predominant or only organisms in the stools of 14 patients. Various kinds of skin eruptions and wounds harbor large numbers of staphylococci and transfer of the organisms from these sites to foods is common when food poisoning outbreaks are caused by food-handlers. Even clothing and objects handled by humans can carry viable staphylococci. These pathogens are also carried by animals which are then able to transfer the infection. The most important of these infections, in regard to food intoxications, is acute or chronic bovine mastitis. Staphylococci are reported to persist in the digestive tracts of flies for over a week, so these and other insects also may be a reservoir (*228*). Finally, these organisms are found in the air where they survive on dust particles and small droplets of moisture.

Every case of staphylococcal food intoxication then is characterized by: (*a*) a source or reservoir of staphylococci, (*b*) a means of transmission for the organism, (*c*) a favorable environment in the food for growth of the staphylococci, (*d*) a sufficient period of time for growth and enterotoxin formation to occur, and (*e*) ingestion of sufficient food by a susceptible person to initiate an enterotoxemia.

Methods of controlling outbreaks of staphylococcal food poisoning must be aimed at one or more of the above steps in the propagation of the intoxication. The only practical areas for control are either minimizing opportunities for transmission of the organism or preventing growth of the organism in food.

When an outbreak of staphylococcal intoxication does occur, the epidemiologist interviews the victims to determine the foods eaten and to ascertain what foods were eaten at the same meals by persons who did not become ill. An attack rate for each food can be calculated and this, along with laboratory analyses for staphylococci and enterotoxins, should implicate a food(s) responsible for the incident. Methods used to prepare these foods and the source (s) of ingredients, etc. then are investigated.

Bacteriophage-typing can be a useful tool to determine the reservoirs and mode of transmission involved in a staphylococcal food poisoning outbreak. Many staphylococci are susceptible to lysis by one

TABLE 1. INCIDENCE OF PRODUCTION OF ENTEROTOXINS A, B, C, AND D BY STAPHYLOCOCCI FROM VARIOUS SOURCES (*46*)

Source of staphylococci	No. of strains	Percent of incidence													Negative ABCD
		A	B	C	D	AB	AC	AD	BC	BD	CD	ABC	ABD	ACD	
Clinical specimens, Washington, D. C.	385	13.8	5.2	2.3	8.6	11.4	1.0	1.3	0.3	2.6		0.3	0.3	0.3	53
Clinical specimens, Atlanta, Ga.	53	3.8			7.5	1.9		1.9		1.9					83
Nasal specimens	144	7.6	1.4	2.8	7.6	1.4	1.4	7.6		0.7			0.7		69
Raw milk	236	1	0.8	0.4	6		0.4	0.4			0.4				90
Mastitic cows	51				2										98
Frozen foods	260	3	1	5	10				2					0.4	70
Food-poisoning outbreaks	80	49	3.8	2.5	7.6	1.25		25	2.5		2.4		2.5		3.8

or more specific bacteriophages. The staphylococci may be "type-specific," i.e. lysed by only a single phage, or they may be lysed by several phages giving rise to a "pattern of lysis." Five groups (I-IV, and a miscellaneous group) are differentiated by the response of the staphylococcus to an international set of typing bacteriophages. Food-poisoning strains are generally associated with Group III. Staphylococci not lysed by the usual phages are termed "nontypable."

Blair and Carr (*26*) found 290 of 539 strains were susceptible to one or more of a series of 25 bacteriophages. George et al. (*281*) found 435 of 746 coagulase-positive staphylococci isolated from bovine udders were phage-typable. Over 50% of 184 isolates from market cheese samples tested by Donnelly et al. (*93*) had phage patterns associated with Group III, whereas 14 of 64 strains isolated from food poisoning samples were typed in Group III.

An alternative to bacteriophage-typing was offered by Martley et al. (*212*). They studied the specificities of the proteinases formed by 136 strains of coagulase-positive staphylococci and found five well-defined groups could be differentiated. In a survey, 63 strains were collected which were phage-nontypable but they could be readily differentiated in terms of their proteolytic activities.

Distribution of enterotoxins in nature

It is widely accepted that the majority of staphylococcal food intoxications are caused by ingestion of enterotoxin A, and are rarely associated with enterotoxin B. The newer enterotoxins will probably become associated with the problem, but it is not known at this time what total impact they have.

Over 1,200 strains of staphylococci were isolated by Casman et al. (*46*) from a wide variety of sources and analyzed for production of enterotoxins A, B, C, and D. The results of this study are detailed in Table 1. Of the staphylococci isolated from food-poisoning outbreaks, 49% produced enterotoxin A and 25% formed both A and D, whereas only 3.8% produced type B. Staphylococci isolated from the human nasal cavity, mentioned previously as a major reservoir for these organisms, also produced primarily enterotoxins A and D.

IN CONCLUSION

The first two parts of this review have supplied information believed to be germaine to a basic understanding of staphylococci and staphylococcal intoxications. The other two parts of the review (*225, 226*) will discuss data which have been published in regard to staphylococci in dairy and other foods.

NATIONAL RESTAURANT ASSOCIATION POLICY STATEMENT ON RESTAURANT INSPECTION PROCEDURES

The National Restaurant Association is opposed to health department inspection procedures that utilize the public posting of inspection reports and the A-B-C- system of grading, re-grading and public placarding. These practices are unnecessary and unfair.

Responsibility for satisfactory protection of food from contamination is threefold. The public's health is a basic responsibility of the establishment owner and manager. The health department has a responsibility to the public to inspect foodservice establishments to ensure safe procedures and practices. Day-by-day observance of proper personal hygiene, food handling and equipment sanitation practices by foodservice workers is essential.

Most foodservice operators are fully cognizant of their obligations to protect their dining customers and of the importance of cleanliness to their business success. It is incumbent on the health department to aid the operator by ensuring that he knows what must be done, why it is necessary for protection of the dining public and how it can be accomplished. Following this, the health department should then inspect the establishment to point out deficiencies that need correcting and follow-up to see that the corrections have been accomplished or, at least, know why they have not and when they will be. In other words, public health departments should educate and evaluate and expect prompt, business-like action to correct deficiencies which are hazardous to health. Food protection and establishment sanitation should be a matter of direct contact between the health department and the foodservice operator. Progressive operators will correct deficiencies promptly. Uncooperative operators will require the application of more stringent enforcement steps.

When an operator fails to comply and major deficiencies exist, stringent enforcement measures are in order and should be initiated promptly. These would include temporary closure for clean-up and

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A Research Note

SOME OBSERVATIONS ON THE MICROFLORA OF DISPOSABLE PAPER HAND-WIPES[1]

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ABSTRACT

A total of 38 samples of disposable hand towels and napkins were analyzed for their microbial content. Total counts on 50% of the samples were <100 organisms per gram of towel, with the remaining counts ranging upward to 7,200 per gram. *Bacillus* species represented 90% of the flora; the remaining organisms were actinomycetes, clostridia, micrococci, molds, and diphtheroids. *Bacillcs cereus* was isolated from 5 samples.

The increasing use of disposable paper goods in the home and industry has raised the question of their expected microbial quality. Many consumers use these disposable paper goods as if they were sterile even though not so advertized. In particular, the wide-spread use of roll towels for wiping surfaces which come in contact with food, and the direct contact of foods with paper towels on which they are laid out to dry or drain, would seem to necessitate making this information available. Therefore a study was undertaken to determine the kinds and numbers of microorganisms present in paper towels and napkins.

Preliminary studies indicated that the predominant organisms found were sporeforming bacilli. No gram-negative organisms were isolated, *Clostridium perfringens* was not recovered, and only on rare occasions were other morphological types isolated. Therefore, the following procedure was adopted in setting up the samples reported in this study.

MATERIALS AND METHODS

Five grams of paper were blended in 245 ml of phosphate buffer *(1)* and four sets of duplicate plates were prepared with plate count agar using 1 ml of the 1:50 dilution per plate as inoculum. Two sets of plates were incubated at 32 C, one aerobically and the other anaerobically (Gas Pak-BBL). The other two sets were incubated at 45 C under the same conditions.

The plates were counted after 48 hr. When growth permitted, five colonies were picked from each incubation condition. When the colonies on the plate exceeded five, the number of colonies picked approximated the square root of the number of colonies on the plate. Colonies from the aerobic plates were transferred to Tryptic Soy Broth and those from the anaerobic plates into Thioglycollate Broth. Each available brand of paper towel was sampled twice, yielding 245 isolates from 38 samples. Samples were obtained from retail outlets in the Gainesville, Florida area and only samples with a protective overwrap were purchased for this study.

Standard microbiological techniques were employed in the identification of the organisms. The *Bacillus* species were identified following the scheme outlined in *Identification Methods for Microbiologists (3)* supplemented by descriptions from *Bergey's Manual of Determinative Bacteriology (2).*

RESULTS AND DISCUSSION

The total counts were generally low (Table 1) with 50% of the samples having 100 organisms or less per gram. The highest count was 7,200 per gram. Clostridia were isolated from 5 samples and on further testing were found not to be *C. perfringens.* Miscellaneous organisms such as molds, diphtheroids, coagulase-negative micrococci, as well as actinomycetes were isolated from 16 samples, usually at low levels. The actinomycetes were found in all four samples representing two brands. In one brand the dye was water soluble and was extracted during the blending process. Napkins yielded low counts, as did the two samples of brown paper hand towels tested. Results between duplicate samples were generally consistent, either both low or both high. Of the 245 isolates, 220 (90%) were sporeforming bacilli, with 5 of the samples yielding *Bacillus cereus.*

Bacillus licheniformis was the most often isolated aerobic sporeforming organism, followed by *Bacillus brevis, Bacillus macerans, Bacillus circulans, Bacillus megaterium, B. cereus, Bacillus subtilis, Bacillus firmus, Bacillus pulvifaciens, and Bacillus polymyxa,* respectively.

It appears that the general microbial quality of these products is good. The assumption by consumers, however, that disposable hand towels are sterile is not correct, and a need for caution is indicated when these products are used in contact with foods.

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TABLE 1. DISTRIBUTION OF ORGANISMS ISOLATED FROM DISPOSABLE PAPER PRODUCTS

Sample	Brand	Counts (No./g)				*Bacillus* species (Percent of total isolates)										Misc. (Percent)	
		Aerobic 32 C	Aerobic 45 C	Anaerobic 32 C	Anaerobic 45 C	*cereus*	*macerans*	*circulans*	*megaterium*	*subtilis*	*polymyxa*	*licheni-formis*	*brevis*	*firmus*	*pulvifaciens*	(non-identifiable)	Clostridia	Mold, Diph., Actino., Micrococci
Brown towels	A	100	100	25	175		33					67						
		100	0	75	150							75		25				
Napkins	B	25	0	50	0							100						
		150	75	25	75			16				54		7		16		7
	C	0	0	0	0													
		0	0	0	0													
	D	25	25	0	0							50						50
		25	25	0	25								33			33		34
	E	25	0	0	25				100									
		0	0	0	0													
Roll towels	F	25	150	25	0							50	50					
		0	50	50	0								100					
	G	50	0	0	0													100
		0	100	0	0							25		75				
	H	25	0	0	0	100												
		50	25	300	0			20				20				40		20
	I	75	175	0	0				12.5	12.5		75						
		75	100	0	0							60						40
	J	700	1100	225	50					5	14	76						5
		850	2100	200	50		10					90						
	K	0	0	0	0													
		0	0	0	0													
	L	1350	25	150	25		27	36				9	9				19	
		675	0	125	25	12.5	25	50				12.5						
	M	4150	3000	450	450		27	23	8			4	12	4		15	4	3
		7200	3500	750	350	3	35	34	3	3						11	11	
	N	125	50	50	100		50					25					25	
		175	175	25	50	12.5				12.5						37.5	12.5	25
	O	0	25	0	0											100		
		0	0	25	0													100
	P	200	350	75	0		22					11	22		33			12
		250	275	0	25		12.5						37.5		25			25
	Q	100	50	0	0								75					25
		125	25	0	0								75			25		
	R	150	200	0	0				40			20						40
		100	225	0	25	17	33		33							17		
	S	0	25	0	0					100								
		0	0	50	0													100

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LACTOSE: ONE OF NATURE'S PARADOXES

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Abstract

Lactose is the major carbohydrate of mammalian milk but its concentration in milks from different species varies considerably. Dietary lactose is responsible for a number of ill-effects in many species including man, both in normal and abnormal conditions of the organism. Although etiologically perplexing, certain distinct nutritional advantages have been attributed to lactose, and technologically we already know how to harness its properties to advantage.

Lactose, or milk sugar, is practically speaking the only carbohydrate in cow's milk and constitutes 4 to 5% of the total weight of the milk. The amount of lactose in milk varies from species to species and reaches levels between 6 and 7% in human milk. This is not in itself nutritionally advantageous, however, because the ability to digest lactose properly varies, not only among different mammalian species, but also among different human races.

Lactose in Foods

Lactose is not a very sweet sugar, always tastes very gritty, and won't dissolve very well. In some of our foods these properties are harnessed to useful advantage. The texture of sweetened condensed milk depends on exactly the right amount of lactose being present. Too much can cause a sandy feeling in the mouth and too little will result in a slimy product. Lactose interacts with milk protein when heated to form brown colored substances. This is useful in toffee manufacture but can be a nuisance in dried milk manufacture, when heat has to be applied in the process. Generally, all those technological difficulties make purification and use of lactose uneconomical and each year, therefore, thousands of tons of waste liquor from dairies are dumped into the sea. Whey contains a lot of lactose, but it is not normally wasted. After concentrating in evaporators, creameries sell it for use in confectionery or pharmaceutical products, or in cruder preparations for animal feeds.

Physiological Characteristics of Lactose

If we want to know why lactose differs from other sugars we must examine it, first at the molecular level and then in terms of its biochemical functions. Molecules of lactose are formed out of galactose joined by a β-D-(1→4) linkage to glucose and before we can absorb milk sugar it must be hydrolyzed in the brush border region of the small intestine, to glucose and galactose, by lactase. The subsequent fates of the glucose and galactose depend on a number of utilizable metabolic pathways and galactose does exist as a permanent feature of many tissues. Brain cells for example contain galactose joined to other groupings, and indeed the brain would not be able to function properly without its galactose. However, it is by no means certain that we need to eat lactose or galactose for this purpose, and probably our bodies convert all the galactose which we absorb into glucose. Galactose is then resynthesized out of glucose whenever it is neded for nervous tissue.

Lactose in Milk

The milk of mammals contains all the essential vitamins, minerals, fats, and proteins which are needed for healthy living. What is not clear, however, is why the milk of so many species should contain over 4% of carbohydrate. More particularly, why should it be lactose?

If we look more carefully at the composition of milk in the different mammalian species we find that there is considerable variation in lactose content not only between species, but also between individuals. Furthermore, although only a limited amount of research has so far gone into it there is also a considerable variation in the amount of the enzyme lactase in the intestine of individuals *(7)*. There may possibly be a relationship between the lactose content of the milk and the lactase content of the intestine. In the California Sea Lion, for example, whose milk contains no lactose, there is no intestinal lactase.

Problems When Milk is Consumed

During the past decade it has become clear that the majority of the world's adult population cannot very easily drink milk and this may be caused by the absence of the enzyme lactase in their intestines, so that they cannot digest lactose. As a result adults who drink milk develop cramping pains, sometimes within minutes of drinking the milk, followed frequently by flatulence and diarrhea *(2)*. In several surveys 70 - 90% of adult Africans, Thais, Chinese, and other Oriental races were found to suffer from

the complaints, which are typical of many rare food sugars *(1)*. In such individuals the unhydrolyzable lactose (or other sugar) draws water osmotically from the walls of the intestine, and is attacked by gut microflora producing acids. Fortunately for most of us in the U.K. and U.S.A. only 5 - 10% of the population or less suffer in the same way. Why should this racial difference exist? The answer is still obscure and scientists are divided in their opinion over whether the difference is genetic or environmental. In other words either we inherit the ability to digest lactose normally, or we may develop the inability to do so by failing to drink enough milk after weaning, so that the level of the enzyme lactase decreases in the intestine.

Babies and children may also suffer from the same type of symptoms after drinking milk and the collective syndrome is referred to specifically as *lactose intolerance*. In surveys among Bantu populations doctors have fed lactose to children and produced very violent intestinal disturbances, so that in some instances death has resulted following the tests *(5)*.

Another way of proving lactose intolerance is to feed lactose to the patient and subsequently to take blood tests every few minutes for the next hour. If the blood sugar level rises normally, as after the ingestion of sucrose, the patient is normal. If, however, the blood sugar level does not rise, this means that the patient is lactose intolerant. Consequently the lactose in the intestine is not digested, bacteria will attack it, and all the usual symptoms of cramping pains, flatulence, and diarrhea will result.

Lactose intolerance is encountered all over the world, but a rarer and much more deadly disease is galactosaemia. This disease is inherited but only affects about one baby out of 40,000 due to the lack of the enzyme glucose 1-phosphate: galactose 1-phosphate uridyl transferase. Galactose builds up in the liver (as its phosphate) because it cannot be converted to glucose, and the babies become very ill and frequently die *(11)*.

Almost all surviving galactosaemics are mentally retarded and the only way to treat the disease known to doctors is to recognize it quickly and remove all galactose and lactose from the babies' diet. Obviously, since babies drink only milk, we cannot do this without special dietary preparations being made available in the hospitals to meet such emergencies. Fortunately food manufacturers in the developed countries are already able to produce substitute milk powders sweetened with substances such as corn sugars or fructose. There are several products of this type now available *(6)* and the demand for similar commodities tailored for clinical purposes will probably increase in the future. A typical preparation for use in cases of galactosaemia, lactose intolerance, or gastroenteritis is shown in Table 1, but these substitute milks are not complete foods and should therefore only be used under medical supervision.

TABLE 1. COMPOSITION OF SUBSTITUTE MILK POWDERS

Approximate analysis	Dry food (g per 100g)
Vegetable fat	22.3
Unhydrogenated cocount oil	15.0
Unhydrogenated maize oil	7.3
Protein (washed casein)	22.3
Carbohydrates (liquid glucose)	50.2
Mineral salts	3.0
Moisture	2.0
Calcium	0.720
Phosphorus	0.480
Lactose (approx.)	0.098
Meso-inositol	0.313
Choline chloride	0.067
Calories	503

As well as the inability of some humans to digest lactose properly there are several known cases of animal species which are also unable to tolerate milk sugar in quantity as a nutrient. If weanling rats, for example, are fed lactose at a level of 30% of their diet, in addition to diarrhea they rapidly (within a few days) develop cataract *(8)* or opacity of the lens of the eye. This must result from the galactose part of the lactose, because if rats are fed either galactose or human milk alone, they also develop cataract, but they do not if they are fed glucose alone. Galactose-cataract formation in rats is not reversible. A more alarming experiment along these same lines has recently been carried out at the Johns Hopkins Hospital, Baltimore *(9)*. Every single member of a colony of rats fed yogurt as an exclusive diet developed cataract. Evidently galactose, part of the lactose structure, constitutes 22 to 24% of the caloric value of yogurt. Chicks also develop a peculiar syndrome if they are fed galactose or lactose, which is characterized by shivering and shaking and general debilitation *(10)*.

FURTHER USES FOR LACTOSE

Since some human beings cannot tolerate lactose as a food we are left with the problem of harnessing some of the lactose which we produce for some other useful purpose. One which has already been discovered is to use it as a fermenting medium for penicillin production, as the sugar is fermented slowly and conveniently by penicillin moulds.

It must not be thought that because lactose has certain drawbacks for some human beings that the majority of us in the western world cannot enjoy it as a normal food component. It is clearly of great use as a health food in special diets when decreased

sweetness is required and some have claimed that diabetics can tolerate lactose levels far in excess of what might be expected compared with sucrose or glucose. Some accepted diabetic ice-creams have relatively large levels of lactose. In normal health lactose may exert considerable nutritional benefit in the diet. In a recent experiment it was shown that when lactose was ingested by normal adult volunteers the fecal and urinary calcium and phosphorus fell, with a striking improvement in calcium and phosphorus balance. In lactose intolerant patients no such improvement in mineral balance occurred *(4)*.

In most food products lactose probably exists as milk or milk products rather than as the refined sugar. Lactose is a large component of dry coffee cream preparations, for example, and concentrated whey is used in many of our chewy confections. Whey lactose interacts with the milk protein, casein, and on heating, brown substances are formed with attractive flavors that are essential ingredients in toffee making *(3)*. Sogginess in pie crusts can be reduced by applying a lactose solution wash to the surface before baking. Potato chips and French-fried potatoes can be given a deeper, more uniform golden color if they are dipped into a lactose solution before cooking in deep fat. For more of us lactose taken as a normal balanced constituent of milk products in our diet is probably a beneficial nutrient, encouraging us not to hunt for immoderate levels of sweetness which may result in obesity and related dietary problems. Probably the racial differences in regard to lactose intake described in this paper reflect acquired dietary needs, resulting from generations of national food preferences. Provided that we take care to note any physiological disorders recurring after a particular food intake and seek medical advice where necessary, we may with some safety, accept the old adage 'a little of what you like is good for you.' As in many other aspects of our diet it appears that Nature has deliberately concealed her purpose in providing lactose as the milk sugar of so many different species. We must be careful how we use it, so that we may be sure its applications can be extended further.

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NATIONAL RESTAURANT ASSOCIATION

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corrections, or suspension of the operator's permit in flagrant cases of non-compliance.

Grading and public placarding or public posting of inspection results, is grossly unfair to the business establishment—especially when it becomes a substitute for adequate counseling, inspections and application of enforcement procedures.

The customer certainly has a right to expect that the foodservice operator and health department are both doing their job with respect to the protection of the customer from contaminated food and possible foodborne illness. The customer should not have to face a posting of sanitation deficiencies as a prelude to enjoying a meal in a given establishment. It would be unreasonable and unfair to offend the sensibilities of the customer and to jeopardize the business success of all establishments by requiring the posting of inspection sheets or the frequently-misunderstood A-B-C-rating placards.

The NRA, speaking for its combined membership of foodservice industry leaders, reaffirms its basic policy, which is:

A. To safeguard the health and welfare of its patrons in providing the best in foodservice.

B. To see that food is prepared under the highest standards of sanitary handling and,

C. To guarantee that meals will be served under conditions and in surroundings maintained with such constant supervision as assures service of food in attractive, sparkling clean establishments.

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SPRAY DRIED MILK OF THE SAKHA PLANT

I. QUALITY CONTROL STUDIES

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Abstract

Fifty samples of freshly spray-dried non-fat milk were taken from the Sakha plant. The quality of this powdered milk was assessed by bacteriological, chemical, and physical tests. Bacterial counts showed the following averages per gram: total viable counts, 13 $\times$ 10^6 at 30 C and 6.8 $\times$ 10^6 at 37 C; thermoduric, 1.6 $\times$ 10^6; psychrophilic, 4.5 $\times$ 10^5; non-pathogenic staphylococci, 4 $\times$ 10^5; Group D streptococci, group I comprising *Streptococcus faecium, Streptococcus durans* and *Streptococcus bovis*, 5.3 $\times$ 10^5 and group II comprising *Streptococcus faecalis* and its varieties *zymogenes* and *liquefaciens*, 9.3 $\times$ 10^4; and coliforms, 64. $\times$ 10^2. Saccharolytic anaerobes were found in 40% of the samples examined while proteolytic anaerobes and β-hemolytic bacteria were not detected. The chemical and physical tests showed the following averages: 1.9% fat, 0.21% acidity, 3.25% moisture, and 1.1 ml solubility index.

Bacteriological, chemical, and physical studies on powdered milk have been carried out by many investigators *(4, 15, 19, 20, 21, 27, 28, 30, 32)* in order to assess the quality of this product. In the U.A.R. it was not until 1962 that a plant for manufacturing dried milk was established at Sakha in Kafr-El-Sheik. The capacity of this plant is 20 tons of whole raw milk per day. As drying milk is a recent industry in our country, no attempt has been made to study the quality of the product. Therefore the present investigation was carried out to study the bacteriological quality, and some chemical and physical properties of this product. Results of this study constitute the material of another paper *(31)*, in addition to the present one.

Type of equipment and processing conditions at the Sakha plant were as follows: *(a)* on reception, milk is filtered and cream separated; *(b)* pasteurization is at 85 C for 15 sec; after rapid cooling to 4 C, milk flows through a balance tank to the pre-heater; *(c)* pre-heating is at 90 C for 20 sec just prior to vacuum concentration; the preheater is equipped with a flow diversion valve; preheated milk is delivered to a small balance tank feeding the vacuum evaporator; *(d)* the pre-heated milk is concentrated to about 40-45% solids by means of a double-effect climbing film evaporator (60 C then 40 C) connected to an indirect barometric condenser and a vacuum pump; *(e)* concentrated milk is withdrawn from the evaporator by pumping, and passes through the atomizer feed tank via a rotating disk atomizer to the spray drying chamber (inlet air 150 C - outlet air 96 C); and *(f)* the resulting powder is discharged from the main chamber and cyclone separators into a conveyor which delivers it to a sifter; The final sifted powder is discharged from sifter outlets into suitable containers.

Materials and Methods

Samples

Fifty samples of spray-dried non-fat milk produced at the Sakha plant were collected aseptically in sterile and tightly closed containers. They were immediately transported to the laboratory where they were tested.

Methods

Samples were examined bacteriologically for total viable counts at 30 and 37 C; thermoduric, psychrophilic, and β-hemolytic bacteria, adopting the methods given in *Standard Method for the Examination of Dairy Products (3)*. Staphylococcus counts were determined on S 110 medium (Difco). Differentiation between the typical colonies of the pathogenic and the non-pathogenic staphylococci was carried out according to Evans *(11)*. Proteolytic anaerobes were detected following the method reported by Mackie and McCartney *(25)*. Saccharolytic anaerobes were determined according to the method described by Chalmers *(9)*. For coliform counts the dilution frequency method (Most Probable Number, MPN) was adopted and the MPN was calculated on the basis of positive tubes using McCrady's Table as indicated in *Standard Methods for the Examination of Dairy Products (3)*. Group D streptococcus counts were determined on the modified thallous acetate tetrazolium glucose agar medium recommended by Barnes *(5)* who divided the streptococcal colonies that would grow on this medium into: Group I: *Streptococcus faecium, Streptococcus durans, Streptococcus bovis*, and a number of unclassified strains and Group II: *Streptococcus faecalis* and its varieties *zymogenes* and *liquefaciens*.

Moisture content and titratable acidity were determined according to the methods given in Richmond's Dairy Chemistry *(35)*. Fat content was estimated using the method given by Ling *(24)*. Solubility index was determined according to the method of Crossley as described by Davis *(10)*.

TABLE 1. BACTERIOLOGICAL ANALYSIS OF SPRAY DRIED MILK POWDER

	No. of positive samples[1]	Incub. temp. C	Minimum	Maximum	Average
Total count	50	30	4×10^5	80×10^6	13×10^6
	50	37	3.2×10^5	70×10^6	6.8×10^6
Thermoduric counts	50	30	3.4×10^4	9×10^6	1.6×10^6
Psychrophilic counts	50	7	3.6×10^4	5×10^6	4.5×10^5
Staphylococcus counts	50	37	3.1×10^4	3×10^6	4×10^5
Coliform counts	10	37	0(not detected)	5.4×10^3	6.4×10^2
Group D streptococcus counts					
group I	50	37	12×10^3	15×10^6	5.3×10^5
group II	47		0(not detected)	4.9×10^5	9.3×10^4

[1]Total samples, 50

RESULTS AND DISCUSSION

Bacteriological analysis (Table 1)

Total viable counts. As seen in Table 1, the total viable counts ranged from 4×10^5 to 80×10^6 with an average of 13×10^6 per gram at 30 C and from 3.2×10^5 to 70×10^6 averaging 6.8×10^6 per gram at 37 C. These results clearly show that incubation at 30 C for 5 days gave higher colony counts than incubation at 37 for 2 days. This confirms the data of Mattick et al. *(27)* and Hiscox *(19)*. These workers attributed this phenomenon to the inability of microbacteria, that occurred most frequently in powdered milk, to grow at 37 C. But as the pre-heating temperature used at the Sakha plant was 90 C, thus surpassing the destructive limits for microbacteria mentioned by Hiscox *(19)*, therefore the difference between the counts at 30 C and 37 C is attributed to the presence of psychrophilic bacteria, detected in high numbers (Table 1), unable to grow at 37 C *(39)*. Other types of micrococci, such as *Micrococcus varians* which has also been found in the samples studied, also are unable to grow at 37 *(2)*.

It is also quite clear that counts obtained in the current work were similar to the findings of Anderson and Stone *(4)* and Hobbs *(20)*. However, they generally were distinctly higher than those reported by other workers *(15, 28, 30)*.

High counts obtained in the current work are attributable to more than one factor, such as the bacteriological quality of the raw milk used *(27)*, and to inadequate plant cleanliness and sterility *(26, 27)*. This is also supported by the findings of Abd-el-Ghani *(1)*. During a three-year study (1967-1970) he obtained average counts of 86×10^8/ml at 30 C and 64×10^8/ml at 37 C for raw milk used for processing at the Sakha plant. He showed that unsatisfactory conditions of cleanliness led to contamination during processing and to the subsequent high counts of the finished product.

Thermoduric counts. Thermoduric counts ranged from 3.4×10^4 to 9×10^6 with an average of 1.6×10^6 per gram. The high thermoduric counts obtained in the present work are probably due to the relatively large numbers of heat-resistant organisms in the raw milk used *(1, 13, 27)* as well as to contamination during processing *(1)*. Inefficient cleaning of condensers and feed tanks were shown to be additional sources of contamination *(12)*

Psychrophilic counts. The psychrophilic counts obtained ranged from 3.6×10^4 to 5×10^6, averaging 4.5×10^5 per gram. Water supply is considered the main source of psychrophilic bacteria in the dairy industry and a second, perhaps more frequently encountered source of contamination, is improperly cleaned equipment and utensils *(40)*. However, since these organisms are destroyed by pasteurization *(9)* and by successive heat treatments during processing, their presence in the fresh dried milk samples examined could only be attributed to contamination after heating. This indicates unsatisfactory sanitary conditions in the plant, particularly lack of efficient cleaning and sanitization of equipment surfaces involved ahead of the drying chamber *(1)*.

Staphylococcus counts. The staphylococcus counts were in the range of 3.1×10^4 and 3×10^6, and averaged 4×10^5 per gram. None of the samples examined revealed the presence of typical colonies of the pathogenic types *(11)*. The presence of colonies of the non-pathogenic types of staphylococci, being non-heat resistant, is due to contamination after processing caused by improper management and cleaning *(18)*.

β-hemolytic bacteria. In none of the fifty samples examined could β-hemolytic non-sporing organisms be detected.

Proteolytic anaerobes. No proteolytic anaerobes were found in any of the samples examined.

Saccharolytic anaerobes. Stormy fermentation was observed in 40% of the samples. Presence of these organisms in dried milk was reported by Mattick et al. *(27)* and Parson and Fraser *(33)*. Saccharolytic anaerobes were derived from raw milk and being in

the highly resistant spore form, they were unaffected by the process of spray-drying *(27, 33)*.

Coliform counts. The coliform counts ranged from 0 (not-detected to 5.4×10^3, with an average of 6.4×10^2 per gram. These organisms were detected in 20% of the samples. Since the isolated coliforms proved to be non-heat resistant strains *(31)*, it can be safely assumed that coliforms initially present in raw milk used for processing were readily destroyed during the manufacturing process *(1, 27)*. Their presence in the dry milk samples examined is therefore due to postheating contamination *(27, 29)*. The same conclusion was arrived at by Abd-el-Ghani *(1)* when evaluating the bacteriological changes occurring at each stage of manufacture of spray-dried milk at the Sakha plant during 1967-1970.

Group D streptococci. Counts of group I organisms ranged from 12×10^3 to 15×10^6 with an average of 5.3×10^5 per gram and those of group II, from 0 (not detected) to 4.9×10^5, averaging 9.3×10^4 per gram. Group I organisms were present in all the samples examined (100%), while those of group II were detected in 94%. The fact that the counts of group I were much higher than the corresponding counts of group II indicates that S. *faecium*, S. *durans*, and S. *bovis*, were present in higher numbers than S. *faecalis* and its varieties. Similar results were reported by Hashimoto *(16)* and Hashimoto et al. *(17)*. The presence of Group D streptococci in spray-dried milk may be due partly to their incidence in raw milk used for processing *(1, 8)*. Because of their relatively high resistance to unfavourable conditions like heat treatment *(38)* and drying *(6)*, they may survive processing. However, recontamination after pasteurization and during processing can not be excluded *(8, 16)*.

Comparing the results regarding Group D streptococci with those of coliforms it is obvious that the counts and numbers of positive samples of the former were much higher than those of the latter. Thus all the samples that revealed presence of coliforms were also positive for Group D streptococci but the reverse was not the case. This could be attributed to the heat treatments used during processing destroying the coliforms but not all the Group D streptococci. This means that negative coliform results in the finished product do not always ensure safety from the hygienic point of view. It also indicates greater accuracy of Group D streptococci as fecal pollution indicators, at least in dried milk. This point of view has been stressed by Larkin et al. *(23)* and Raj et al. *(34)*. Buttiaux *(7)*, in discussing the value of the *Escherichiae* and Group D streptococci as indicators of contamination, regarded Group D streptococci as affording a more sensitive test for fecal contamination of food than the coli-aerogenes bacteria. Taking into consideration the heat stability *(38)* of the former, a character not possessed by the latter, the presence of coliforms will point to post-heating contamination, which is also an important criterion from the hygienic standpoint. Therefore, the presence of Group D streptococci associated with the coli-aerogenes bacteria will justify the diagnosis of fecal contamination in powdered milk.

TABLE 2. PHYSICAL AND CHEMICAL ANALYSIS OF SPRAY-DRIED MILK POWDER

	Minimum	Maximum	Average
Moisture (%)	1.33	5.90	3.25
Solubility index (ml)	0.3	2.5	1.1
Fat (%)	1.30	4.00	1.90
Acidity (%)	0.13	0.23	0.21

Physical and chemical analysis (Table 2)

Moisture content. As seen in Table 2, the moisture content ranged from 1.33 to 5.90% with an average of 3.25%. These results coincide with those reported by Napoli *(32)*.

Solubility index. The solubility index varied from 0.3 to 2.5 ml, averaging 1.1 ml. Similar results were obtained by Ibrahim *(21)*.

Fat content. The fat content ranged from 1.3 to 4%, with an average of 1.9%.

Titratable acidity. The samples examined showed acidity values ranging from 0.13 to 0.23%, averaging 0.21%. Comparing these results with those reported by Gould and Skiver *(14)* it can be seen that the current results were higher. This could be attributed to the low bacteriological quality of raw milk used for processing *(1)*. In this respect, several investigators *(22, 37)* reported mean acidity values of 0.17% and 0.18%, respectively, for locally produced raw milk.

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(Continued from Page 34)

The NRA and its individual members work cooperatively with public health officials, organizations concerned with the health and welfare of our citizens and with other groups concerned with food sanitation to develop and improve procedures, the use of which will result in the foodservice industry maintaining a high level of sanitation excellence.

As part of its ongoing program of assistance to the foodservice operator, the Association develops and distributes booklets, charts and audio-visual programs for foodservice employees on the subjects of food pro-

(Continued on Page 44)

EFFECTS OF INCUBATION TEMPERATURE ON THE SALT TOLERANCE OF *SALMONELLA*

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ABSTRACT

Salt has been shown effective in preventing growth of salmonellae in foods. Many of the studies reported in the literature have been on the lethal action of high levels of salt as used in curing brines. Little information is available on the interaction of incubation temperature and low levels of salt on the growth of salmonellae. The growth of *Salmonella heidelberg, Salmonella typhimurium,* and *Salmonella derby* in nutrient broth containing 0 to 8% added NaCl (in 0.5 or 1% increments) has been tested by shake cultures at 8, 12, 22, and 37 C. In addition, *S. heidelberg* has also been tested in 0 to 9% added NaCl at 39, 41, 43, and 45 C. At 8 C, growth of *S. heidelberg* took place in 1 and 2% added NaCl; *S. typhimurium* increased in numbers in 1% added NaCl; and *S. derby* failed to increase. When incubated at 12 C, the three serotypes were all able to increase in numbers in the range of 0 to 4% NaCl. At 22 C, this range increased from 0 to a maximum of 5 to 8%. When incubated at 37 C, the organisms were able to increase in numbers in up to 7 to 8% NaCl. The salinity of the medium was not found to increase the maximum growth temperature of *S. heidelberg* as has been reported in the literature for other organisms. Low levels of salt were found to stimulate growth of salmonellae. This stimulation was more pronounced at low temperatures than near the optimum for the organisms. Since salt is used to preserve foods, these data are important in the preservation of perishable foods. Salt concentrations preventing growth of salmonellae at low temperatures may not be sufficient to prevent growth of these pathogens at higher temperatures.

Salmonellae are frequently associated with foods of animal origin and with foods handled or contaminated by man. Small numbers of salmonellae may increase to infective levels in these foods if proper processing and storage conditions are not maintained.

Salt has been shown effective in preventing growth of salmonellae in foods. The effect of salt is often similar to that of drying, and the effects can be equated in terms of the activity of the water in the system *(10)*. The lethal action of salt, like that of other disinfectants, is known to be reduced at low temperatures, but the influence of temperature on the inhibitory (as distinct from lethal) action of salt is not well documented *(5)*.

Shipp *(13)* reported that *Salmonella enteritidis* is rapidly killed at room temperature in curing brines, but survives for weeks at the lower temperatures (5 C) which occur in curing cellars. Similar results were reported by Koelensmid et al. *(6)*, who showed that salmonellae may survive for up to 70 days at 5 C but are rapidly destroyed at 20 C. These authors also studied growth of salmonellae at 5 C and 20 C in media containing salt. They reported no growth in any concentration at 5 C, and this is in accord with the general observation that the minimum growth temperatures for *Salmonella* species lie above 5 C *(8)*. Growth at 20 C was observed in 6% but not in 8% NaCl. Other authors have also indicated a maximum tolerance between 6% and 8% NaCl *(5, 12)*.

Most authors have reported that bacterial growth occurs over the widest range of salt concentrations near the optimum temperature for each species tested. There have, however, been reports of growth in higher salt concentrations at low temperatures near the minimum rather than at the optimum, and it has also been suggested that the temperature optimum and maximum temperature for growth may be raised when an organism is grown in the presence of salt.

Since many foods contain salt at levels which are sublethal for *Salmonella,* it was felt important to determine the potential for *Salmonella* growth in presence of salt at various temperatures.

MATERIALS AND METHODS

Medium

Nutrient broth was used in these studies. The medium was prepared by dissolving the nutrient broth (dehydrated) and sodium chloride in distilled water and diluting to volume with distilled water to give the desired concentrations. Volumes were carefully tested before and after autoclaving to ensure that the salt concentrations had not increased due to loss of water during sterilization.

Test organisms

Salmonella heidelberg, ATCC 8325; *Salmonella typhimurium,* ATCC 6994; and *Salmonella derby,* ATCC 6966, were used in these studies because information was available on the growth of these organisms at low temperatures *(7, 8, 9)*.

Inoculum

The inoculum in all instances was a nutrient broth culture without added salt, incubated at the temperature to be used in the test. Overnight incubation was used for 37 C samples, but several days' incubation was required for inoculum cul-

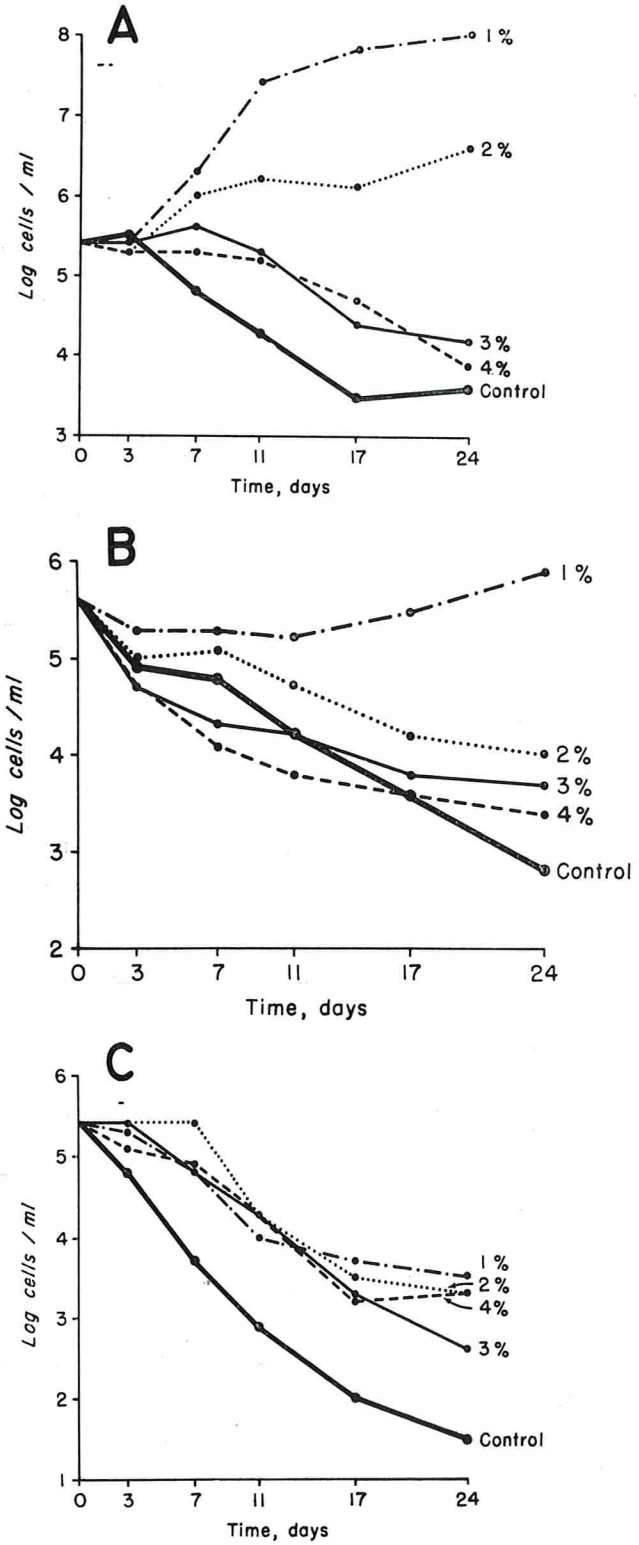

Figure 1. Growth of *Salmonella heidelberg* (A), *typhimurium* (B), and *derby* (C) at 8 C in 0 to 4% NaCl.

tured at 8 C. Cultures were diluted to 55% transmittance at 660 nm with a Bausch & Lomb Spectronic 20 spectrophotometer, yielding approximately 10^8 cells/ml. Serial decimal dilutions were prepared, using 0.1% peptone water, and the test medium was inoculated with 0.1 ml, yielding approximately 5×10^6 cells/ml. The large inoculum was used so that absorbance readings would increase when cell numbers began to increase.

Growth test

In these studies salmonellae were inoculated into 20 ml of nutrient broth containing the desired levels of sodium chloride in 50-ml Erlenmeyer flasks fitted with side arms. The flasks were incubated at the test temperatures in refrigerated and/or heated water bath shakers. Increases in numbers of cells in samples incubated at 8 C were determined by the drop plate method. Duplicate or triplicate 0.1 or 0.01 ml volumes of the appropriate dilutions from each sample were inoculated onto the surface of trypticase soy agar. The plates with duplicate or triplicate samples were incubated at room temperature until counted.

Increases in number of cells in samples incubated at 12, 22, 37, and 41 C were determined by turbidity measurements at 660 nm, using a Bausch and Lomb Spectronic 20 spectrophotometer. Absorbance was expressed as the negative log of transmittance.

RESULTS

Growth of salmonellae in a medium containing added sodium chloride between 0 and 8% is greatly influenced by the incubation temperature. The slowest growth and the lowest salt tolerance are expressed at the low incubation temperatures. As the incubation temperature is increased, the organisms are able to grow in the presence of higher levels of sodium chloride. At 8 C, *S. heidelberg* was able to increase in only 1 and 2% sodium chloride during 24 days' incubation (Fig. 1a). At this temperature, the rate of growth is slow in the nutrient broth test medium. At the higher salt concentrations (3 and 4%), the organisms were unable to increase in number during the 24 days' incubation, and the number of cells actually decreased. *Salmonella typhimurium* was able to increase only in the medium containing 1% sodium chloride (Fig. 1b), and *S. derby* was unable to increase in any of the concentrations of salt tested between 0 and 4% at 8 C for 24 days (Fig. 1c).

It is interesting that growth was obtained with both *S. heidelberg* and *S. typhimurium* in low levels of salt, but no growth was obtained in the controls without added salt. In fact, the overall rate of decline was greater for the three organisms in the control medium than in the media containing salt. This does not appear to be an acquired ability to tolerate salt, but rather an expression of the organisms of a specific osmotic requirement or a requirement for sodium chloride. The effects of lithium or other cations in satisfying this requirement were not tested.

At 12 C, growth was obtained at higher salt concentrations and growth was much more rapid than that obtained at 8 C (Fig. 2). Both *S. heidelberg* and *S. derby* increased in number in salt concentrations up to 5% but not above (Fig. 2 a, c). *Salmonella*

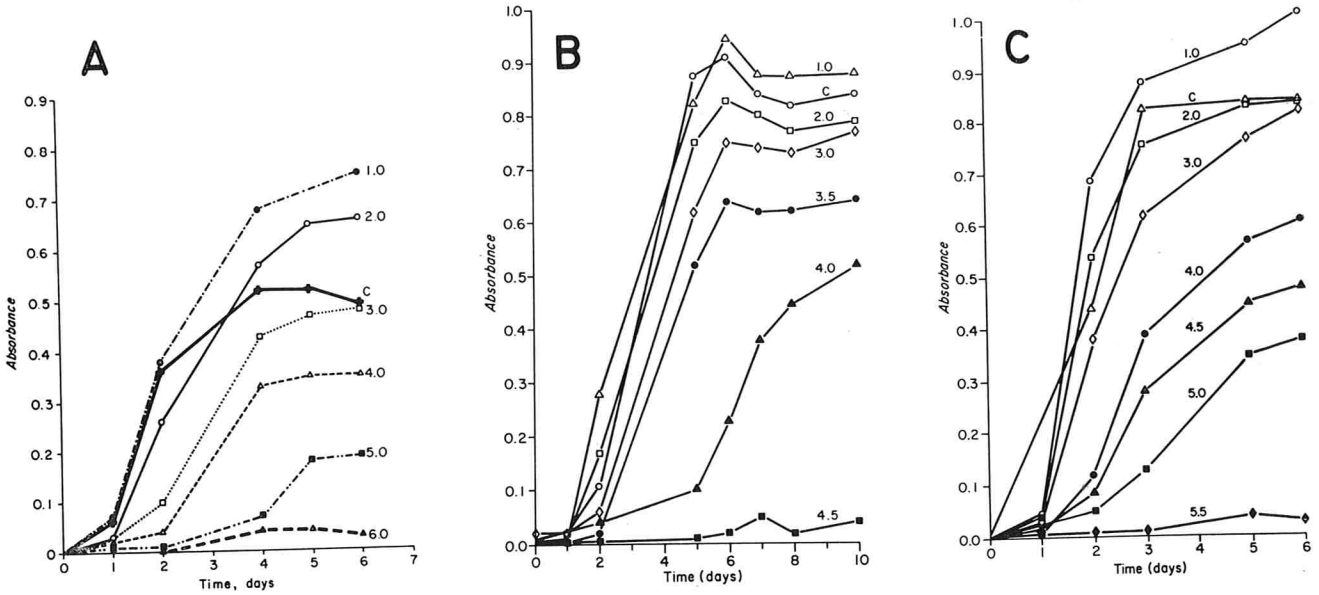

Figure 2. Growth of *Salmonella heidelberg* (A), *typhimurium* (B), and *derby* (C) at 12 C in 0 to 6% NaCl.

typhimurium (Fig. 2b) increased in salt concentrations only up to 4%. The salt requirement or growth stimulation exhibited at 8 C is also evident at 12 C for S. *heidelberg* but less pronounced for S. *derby* and S. *typhimurium*.

As the salt concentrations are increased, the rates of growth decrease and the length of lags are increased. Conversely, as the incubation temperature is increased, the rates of growth increase and the lengths of lag decrease for the same salt concentration. This is evident at 22 C for the organisms tested (Fig. 3). The most rapid growth was obtained with S. *heidelberg* (Fig. 3a), followed closely by S. *derby* (Fig. 3c). *Salmonella heidelberg* increased in numbers in medium containing 8% salt after a lag of 25 hr. *Salmonella derby* increased in numbers in the medium containing up to 7.5% salt, but only after a 48-hr lag at 7.5% salt. *Salmonella typhimurium*, which increased in 5% salt and below (Fig. 3b), failed to increase in salt concentrations between 5.5 and 8% for the duration of the 112-hr incubation period, and increased in 5% salt only after a 22-hr lag. Only concentrations between 5.5% and 7.5% were tested with S. *derby*, but concentrations between 0 and 8% were tested with S. *heidelberg* and S. *typhimurium*. The salt requirement of the organisms evident at 8 and 12 C also show up at 22 C for S. *heidelberg* and S. *typhimurium* (Figs. 3a, b) but is not evident for S. *derby* (Fig. 3c) because salt levels between 3 and 5% were not tested.

The ability of salmonellae to tolerate higher salt concentrations as the incubation temperature is increased, which shows up at 12 and 22 C, is even more pronounced at 37 C. At this higher temperature the lag period was greatest in samples containing the highest salt concentrations, while the rate of growth and number of cells obtained were greatest at the lower salt concentration. *Salmonella heidelberg* tested in salt concentrations ranging from 0 to 8% (Fig. 4a) increased at all levels of salt tested. The rate of growth and the final number of cells attained decreased with an increase in salt concentration. Growth at 8% salt did not take place until 38 hr after incubation. *Salmonella derby* (Fig. 4c) was also able to increase in salt concentrations up to 7.5% salt, but not at 8%. The rate of growth between 0 and 3% salt were very similar; however, above 3% the rates of growth decreased.

Salmonella typhimurium (Fig. 4b) showed similar growth patterns, but the rate of die-off after maximum cell numbers were obtained was more rapid than with the other two serotypes.

The temperature optimum and the maximum temperature for growth reportedly are raised when halophilic or marine organisms grow in the presence of high concentrations of salt *(3, 14)*. *Salmonella heidelberg* was grown at 41 C in the presence of salt ranging between 0 and 9% (Fig. 5). The rate of growth at this temperature, as measured by change in absorbancy/time, was not as rapid as that obtained at 37 C. As a further test of the reports that the optimum temperature for growth and maximum growth temperature are raised at high salt concentrations, S. *heidelberg* was grown in nutrient broth containing 0 to 9% salt and incubated at 37, 39, 41, 43, and 45 C. The increase in cell number, as measured by increased absorbancy readings, was followed for 70 hr. At each of the five incubation temperatures, the greatest increase in cell number took place in samples containing 0, 1, and 2% NaCl. As the salt concentrations

were increased beyond 2%, at each of the incubation temperatures, the increase in number of cells became less; thus the optimum temperature for *S. heidelberg* was not increased above 37 C by increasing salt.

DISCUSSION

It is obvious from the literature that in high concentrations of salt, as used in curing brines (i.e. 10 to 30% NaCl), the effects of temperature on the survival of *Salmonella* is considerable, and there is a possibility that some of these pathogens might survive at low temperatures. At higher temperatures, salmonellae are rapidly destroyed. The interaction of low or inhibitory salt concentrations and incubation temperatures on growth of salmonellae and other non-halophilic organisms has not been well defined.

The experiments described in this report show that at the lower non-lethal salt concentrations (<10%) the maximum salt concentrations in which growth occurs are greater at higher incubation temperatures near the optimum. At lower temperatures, the salt concentrations and temperatures interact to increase the length of the lag phase and decrease the rate of growth. This becomes more evident as the salt concentration is increased and as the incubation temperature is decreased.

The rate of growth of bacteria in media containing salt or other solutes is a function of the water activity and generally not of the concentration of a particular solute or solutes *(10, 11, 16)*. As the solute concentration is increased, the water activity (a_w) is decreased. With a decreased a_w, the lag phase of the organism increases and the rate of growth decreases. A lowering of the incubation temperature from the optimum, while not materially affecting the a_w, at a given salt concentration *(1)* does raise the lower limit of a_w for growth. Wodzinski and Frazier *(16, 17, 18)* reported that both *Pseudomonas fluorescens* and *Aerobacter aerogenes* exhibited the greatest tolerance for low a_w at the temperature which was nearly optimal for rate of growth of the organisms. The reverse was true with *Lactobacillus viridescens*, which showed the greatest tolerance to low a_w at the temperatures below the optimum. Lactic acid bacteria isolated from curing brines also tolerated the highest sodium chloride concentrations at the optimum temperature for growth *(2)*. However, as the temperature was increased, the optimum sodium chloride concentrations also increased. Growth was not obtained with *Lactobacillus* and *Pediococcus homari* at 40 C unless sodium chloride was added to the medium, but growth was obtained at 30 C without the addition of sodium chloride. No other substances were found that would replace the NaCl requirement at higher incubation temperatures.

The salinity of the growth medium was also found to have a marked effect on the maximal growth temperature of four bacteria isolated from the marine environment *(14)*. Two strains of *Vibrio marinus* and unidentified strains of a gram-positive coccus and a gram-negative bacillus were found to possess increased maximum growth temperatures as the salt concentrations were increased from 0 to 3.5%. *Vibrio marinus* MP-1 had a salt requirement of at least 0.7% and when grown in a defined medium a decrease in the maximal growth temperature was observed at both low and high concentrations of NaCl.

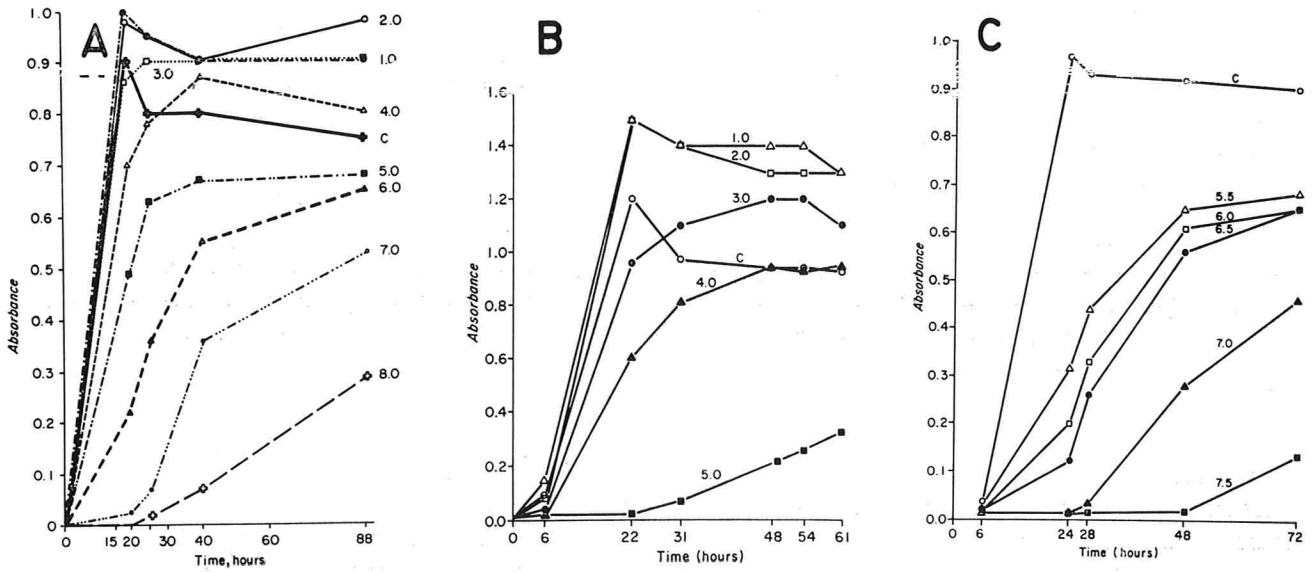

Figure 3. Growth of *Salmonella heidelberg* (A), *typhimurium* (B), and *derby* (C) at 22 C in 0 to 8% NaCl.

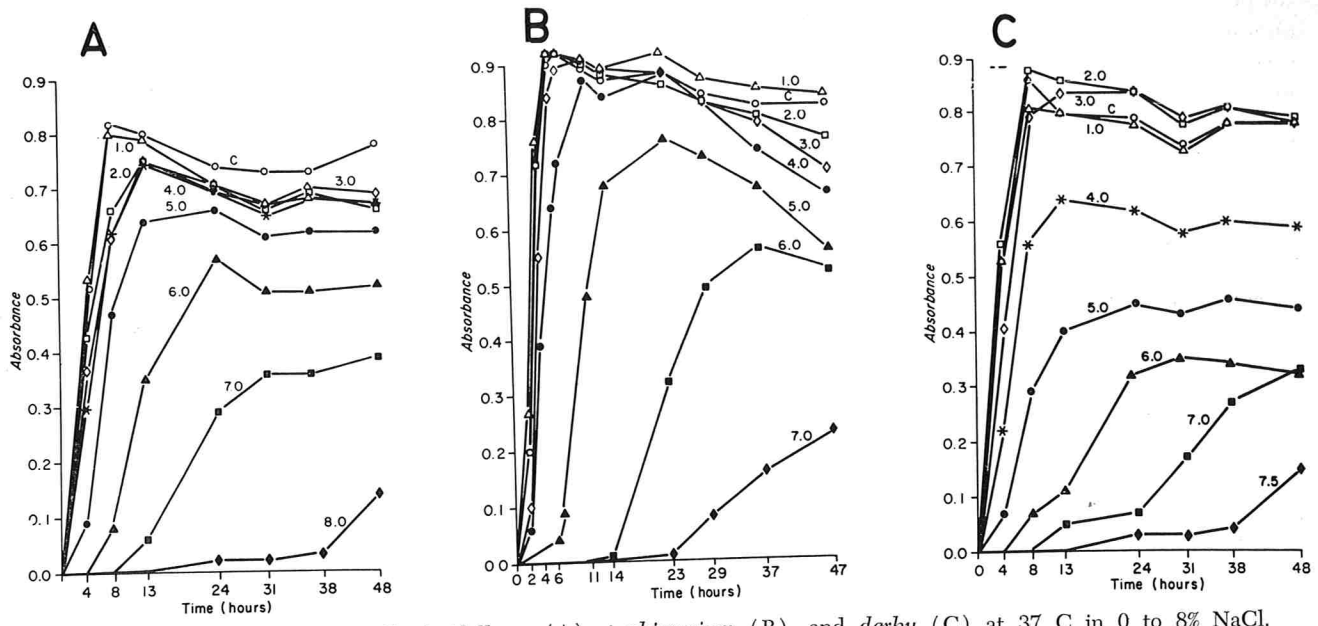

Figure 4. Growth of *Salmonella heidelberg* (A), *typhimurium* (B), and *derby* (C) at 37 C in 0 to 8% NaCl.

As a rule, relatively low concentrations of salt will stimulate microorganisms, while higher concentrations inhibit them *(5)*. This stimulation has been borne out with S. *heidelberg*, S. *typhimurium*, and S. *derby*, used in this study. At the lower suboptimum temperatures below 37 C, the organisms appear to grow more rapidly when low levels of NaCl are present. This effect is evident in salt concentrations ranging from 0.5 up to about 2.5% salt. In most instances the final number of cells attained is also higher at these salt concentrations than in the control samples, as measured by absorbance. This stimulation, which shows up at the lower temperatures, diminishes as the incubation temperature approaches optimum for the organisms and is not evident at temperatures above optimum. The growth of *Escherichia coli* in a dilute medium was reported to be greatly stimulated by low concentrations of sodium chloride *(15)*. The test organism decreased in number when grown in media containing 0 to 0.2% NaCl, but growth was stimulated at NaCl levels of 0.4% and 0.8%, with maximum stimulation at 2.8% NaCl.

Halophilic bacteria often incubated at 25 C have considerably higher optimum temperatures in solutions of high salt concentration. This same general phenomenon has been reported with osmophilic yeasts on high sugar media *(4)*. A shift in optimum temperature was not evident when the incubation temperature of S. *heidelberg* was raised from the optimum of 37 C to 41 C or above in the presence of high and low NaCl concentrations. The rate of growth at 41 C and above was, in fact, slower than at 37 C. Growth was rapid at both temperatures in salt concentrations up to about 5%, but rapidly decreased thereafter, indicating that the temperature optimum for S. *heidelberg* remains close to 37 C. When S. *heidelberg* was incubated at temperatures between 37 and 45 C in the presence of 0 to 9% NaCl, a shift in optimum temperature could have been de-

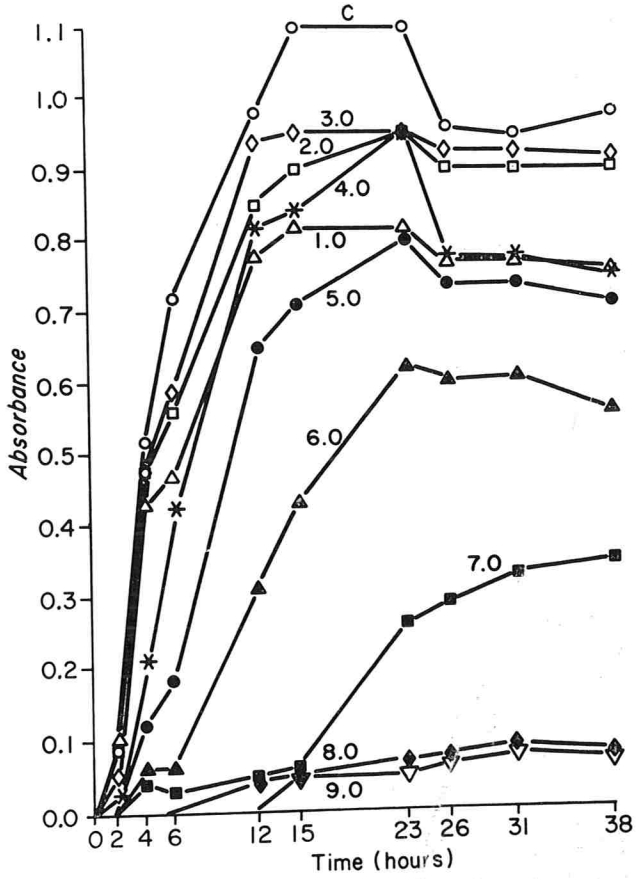

Figure 5. Growth of S. *heidelberg* at 41 C in 0 to 9% NaCl.

tected. Since a shift did not take place, it is reasonable to assume that under the test conditions this organism does not possess a higher optimum temperature in the presence of higher levels of salt. Work reported in the literature was done with marine isolates or halophilic bacteria, and it is possible that this phenomenon is peculiar to these organisms.

The amount of water in a food necessary to allow growth of spoilage and pathogenic bacteria varies with the types of organisms present. Each organism has a specific water requirement. The effects of temperature on these water requirements for *Salmonella* as regulated by the amount of salt present are large, especially at low temperatures far below the optimum for the organisms. At higher temperatures these effects are not as great with Salmonella growing over a wider range of salt concentrations.

From a practical point of view, application of these data to the storage of perishable food products is important. Foods with a low salt content may not support the growth of *Salmonella* or other pathogens at low temperatures. If, however, storage temperatures are raised, conditions may become favorable for growth of these pathogens.

ACKNOWLEDGMENT

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NATIONAL RESTAURANT ASSOCIATION

(Continued from Page 38)

tection, personal hygiene, pest control and establishment and equipment sanitation. The NRA works with technical organizations in the fields of public health and safety to ensure that new developments of importance to the foodservice industry are evaluated and reported through bulletins, seminars and programs.

The foodservice industry has been represented in the development of the Public Health Service Food Service Sanitation Manual and will continue to be represented in the development of revisions to this manual, in order that the food protection and sanitation guide will be reasonable, applicable and will be beneficial to both public health departments and to the restaurant industry as a pattern for local codes and ordinances.

State and local restaurant associations are encouraged to promote the adoption and the use of the model code to the maximum extent appropriate for state and local application, excepting those provisions which pertain to the grading, regrading and placarding of establishments.

SPRAY DRIED MILK OF THE SAKHA PLANT

II. IDENTIFICATION OF PREDOMINATING MICROORGANISMS

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ABSTRACT

The bacterial groups isolated at 30 C and 37 C from spray-dried milk proved to be streptococci, micrococci, microbacteria, and spore-formers in decreasing order. These bacteria were identified to species.

Initiation of the dry milk industry in U.A.R. has emphasized the importance of not only counting the microorganisms but also of determining their types. Enumeration of organisms belonging to the different groups was undertaken by Taha et al. *(21)*, while the complete identification is the subject of this report. The possibility of reconstituted milk being held for some time before use, and the implication of milk-containing foods in food poisoning of the enterotoxin type made it important that the types of surviving organisms which grow readily after reconstitution should be known.

Mattick et al. *(12)* found that spray dried milk contained streptococci, micrococci, and spore-formers in decreasing order. According to Thiel and Pont *(22)* spray-dried milk contained almost enclusively streptococci and only a few micrococci. Many fresh powders, especially spray-dried skim-milk, gave positive coliform tests with 2 g samples, whereas 0.1 g samples were almost uniformly negative. Richter *(18)* reported the absence of coliforms in 109 out of 155 samples of spray-dried milk. Cihova and Saxl *(5)* stated that enterococci (*Streptococcus faecium*) were the major component of the total microflora of 126 samples of dried milk. They added that *Bacillus subtilis, Bacillus licheniformis, Bacillus pumilus, Bacillus cereus*, and *Bacillus megatherium* occurred most frequently among the sporulating flora.

MATERIALS AND METHODS

Bacterial identification

After recording the total viable counts at 30 C and 37 C for 35 nonfat spray-dried milk samples examined *(21)*, prevalent colonies were isolated, purified, and identified to species by the following schemes: *(a)* streptococci and microbacteria according to Bergey's Manual *(4)*; differentiation between *Streptococcus faecalis* and S. *faecium* according to Barnes *(3)*; *(b)* micrococci according to the scheme of Abd-el-Malek and Gibson *(2)*; *(c)* aerobic spore-formers according to Smith, Gordon, and Clark *(20)*; *(d)* coliforms as outlined by the Coliform Sub-Committee *(16)*.

RESULTS AND DISCUSSION

Predominance of bacterial groups (Table 1)

For the detailed study of the bacterial flora of spray-dried milk produced at the Sakha plant, 598 cultures were isolated. Of these 296 were taken from plates incubated at 30 C and 302 from plates incubated at 37 C. Results showed that the bacterial flora was comprised of streptococci, micrococci, microbacteria, and aerobic spore-forming bacilli in decreasing order. Out of the 296 cultures isolated at 30 C, 199 (67.2%) were found to be streptococci, 58 (19.6%) micrococci, 22 (7.4%) microbacteria, 10 (3.4 %) spore-forming bacilli, and 7 (2.4%) miscellaneous which were identified as sarcinae. Of the 302 cultures isolated from plates incubated at 37 C, 220 (72.9%) proved to be streptococci, 39 (12.9%) micrococci, 27 (8.9%) microbacteria, and 16 (5.3%) spore-forming bacilli.

The predominance of streptococci in spray-dried milk powder agrees with the findings of such investigators as Mattick et al. *(12)*, Thiel and Pont *(22)*, and Cihova and Saxl *(5)*. This could be due to the high pre-heating temperature (90 C) used at the Sakha plant. Also, Hiscox *(10)* and Mattick et al. *(12)*

TABLE 1. PERCENTAGE DISTRIBUTION OF THE BACTERIAL GROUPS ENCOUNTERED IN SPRAY DRIED MILK POWDER WHEN PLATES WERE INCUBATED AT 30 C AND 37 C

	30 C		37 C	
Microorganisms	No. of isolates	%	No. of isolates	%
Streptococci	199	67.2	220	72.9
Micrococci	58	19.6	39	12.9
Microbacteria	22	7.4	27	8.9
Spore-forming bacilli	10	3.4	16	5.3
Miscellaneous	7	2.4	—	—
Total	296	100.0	302	100.0

TABLE 2. PERCENTAGE DISTRIBUTION OF STREPTOCOCCI ISOLATED FROM SPRAY DRIED MILK WHEN PLATES WERE INCUBATED AT 30 C AND 37 C

Streptococci	30 C		37 C	
	No.	%	No.	%
Enterococcus group	119	59.8	142	64.8
S. faecium	(106)	(89.1)	(142)	(100.0)
S. faecalis	(13)	(10.9)	(–)	(–)
Viridans group	73	36.7	65	29.7
S. bovis	(47)	(64.4)	(14)	(21.5)
S. thermophilus	(26)	(35.6)	(51)	(78.5)
Lactic group				
S. cremoris	7	3.5	12	5.5
Total isolates	199	100.0	219	100.0

TABLE 3. PERCENTAGE DISTRIBUTION OF MICROCOCCI ISOLATED FROM SPRAY DRIED MILK WHEN PLATES WERE INCUBATED AT 30 C AND 37 C

	30 C		37 C	
	No.	%	No.	%
Dairy micrococci	51	87.9	30	76.92
M. luteus	(38)	(74.5)	(30)	(100)
M. varians	(13)	(25.5)	(–)	(–)
Staphylococcus sub-group D	4	6.9	6	15.4
Intermediate group	3	5.2	3	7.7
Total	58	100.0	39	100.0

attributed the predominance of streptococci at both 30 C and 37 C to the high pre-heating temperature used. They found that at 87.7 C and 93.3 C, streptococci tended to predominate over other types of organisms.

Micrococci were second in order to streptococci, as also reported by Hiscox *(10)* and Mattick et al. *(12)*.

Microbacteria, the next most prevalent group, were round in comparatively lower numbers. Similar results were obtained by Hiscox *(10)* and Mattick et al. *(12)*. The presence of microbacteria in relatively low numbers could also be attributed to the high pre-heating temperature (90 C) used at the Sakha plant, which is apparently near the limit of their heat resistance and consequently few survived the heating process *(10)*. The presence of microbacteria on plates incubated at 37 C had been previously shown by Hiscox *(10)*, Nashif and Nelson *(15)*, Thomas and Thomas *(23)*, Sabbour *(19)*, Robinson *(17)*, and Jayne-Williams and Skerman *(11)*. The latter authors concluded that the inability of microbacteria to grow at 37 C was not suitable for differential purposes. In this respect a taxonomic study of the genus *Microbacterium (Microbacterium lacticum, Microbacterium flavum,* and *Microbacterium liquefaciens)* carried out by Robinson *(17)*, verified that these bacteria grow in the range 30 C and 37 C, and also certain strains grow well at 39 C.

Aerobic spore-forming bacilli were less frequently encountered and were isolated in small numbers in 16.7% and 8.5% of the samples at 30 C and 37 C, respectively. These low figures possibly resulted from testing the samples within a few hours of their manufacture, since Crossley and Johnson *(6)* found that on prolonged storage, spore-forming bacilli tended to predominate.

Identification of microorganisms

Streptococci (Table 2). At 30 C, 199 cultures were isolated. The enterococcus group predominated and 119 cultures (59.8%) were identified as *Streptococcus faecalis* and *S. faecium (3)*. The latter was represented by 106 cultures (89.1%) against 13 cultures (10.9%) of the former. The viridans group comprised 73 cultures (36.7%) of which 47 (64.4%) were identified as *Streptococcus bovis* and 26 (35.6%) as *Streptococcus thermophilus*. The lactic group was far less common only comprising 7 cultures (3.5%) which were identified as *Streptococcus cremoris*.

At 37 C, 219 cultures of streptococci were isolated. Of these, 142 cultures (64.8%) were enterococci identified as *S. faecium*. The viridans group comprised 65 cultures (29.7%) of which 51 (78.5%) were identified as *S. thermophilus* and the remaining 14 (21.5%) as *S. bovis*. The lactic group was far less frequently encountered, comprising 12 cultures (5.5%), identified as *S. cremoris*.

It is clear that the streptococci isolated at 30 C and 37 C belonged to the enterococcus, viridans, and

lactic groups in descending order. The enterococci predominated, and S. *faecium* was predominant over S. *faecalis*. Similar results were obtained by Hashimoto *(7)* and Hashimoto et al. *(8)* who attributed this phenomenon to the comparative thermostability of S. *faecium* over S. *faecalis*.

Streptococcus thermophilus and S. *bovis*, representing the viridans group, were isolated in fewer numbers than those of the enterococcus group. This is in accordance with the work of Naguib *(14)*. The presence of S. *thermophilus* in dried milk was reported by Crossley and Johnson *(6)* and Higginbottom *(9)*.

Far less frequently encountered was S. *cremoris* representing the lactic group. Similar results were reported by Naguib *(14)*.

Micrococci (Table 3). Results showed the predominance of the dairy micrococcus group over the other two groups, namely the staphylococcus and the intermediate groups.

At 30 C, out of the 58 isolates, 51 cultures (87.9%) were identified as dairy micrococci of which 38 (74.5%) were found to be *Micrococcus luteus* and 13 (25.5%) *Micrococcus varians*. *Staphylococcus* subgroup D was represented by 4 cultures (6.9%), and the intermediate group by 3 cultures (5.2%). This clearly shows the predominance of *M. luteus* over the other species encountered.

At 37 C, 39 cultures of micrococci were isolated. The dairy micrococci comprised 30 cultures (76.9%) and were identified as *M. luteus*. Six cultures (15.4%) were identified as *Staphylococcus* subgroup D and 3 cultures (7.7%) as intermediate. The presence of micrococci in milk powder was also shown by Mattick et al. *(12)*, Hiscox *(10)*, Thiel and Pont *(22)*, and Naguib *(14)*.

Microbacteria. Twenty two cultures were isolated at 30 C and 37 cultures at 37 C and were identified as *M. flavum*.

Spore-forming bacilli. Ten cultures were isolated at 30 C, of which 6 (60%) were *Bacillus brevis* and 4 (40%) *B. pumilus*. At 37 C, 16 cultures were isolated. Out of these 10 (62.5%) were *B. pumilus* and the remaining 6 (37.5%) were identified as *B. brevis*. The presence of *B. pumilus* in dried milk was also shown by Naguib *(14)* and Cihova and Saxl *(5)*.

Coliforms. All the 180 cultures of the coli-aerogenes group isolated during this investigation were identified as *Escherichia coli* type 1. The presence of coliforms in dried milk was also shown by Thiel and Pont *(22)* and Richter *(18)*.

In view of the fact that all strains isolated proved to be of the non-thermoduric type (resistance to 63 C for 30 min) their presence in the dried milk is an indication of post-heating contamination *(1, 12, 13)* resulting from improper cleaning and sterilization of post-heating equipment *(1)*.

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REPORT OF THE EDITOR JOURNAL OF MILK AND FOOD TECHNOLOGY 1970-1971

Review of Volume Thirty-Three

Publication of the December, 1970 issue of the *Journal of Milk and Food Technology* completed volume 33. Volume 33 established new records for the Journal. It was the largest volume (688 pages) ever published and represented a 10% increase in size over volume 32 and a 34% increase over volume 30 published in 1967. The single largest issue (68 pages) ever published appeared in volume 33; in fact, four issues of this volume each contained 68 pages. More papers of all kinds (104) and more research papers (66) were published than appeared in previous volumes. The number of research papers published in 1970 represents an increase of approximately 40% over 1969 and 120% over 1967. The number of technical general interest papers in volume 33 also was greater than in recent years and occupied approximately 14% of the total pages in the journal. Twenty non-technical general interest papers appeared in volume 33. This number compares well with that of recent years. Publication of a greater number of research papers means that readers are better informed on current developments in the food and dairy fields. Details on the composition of volume 33 and a comparison to other volumes appear in Table 1.

As in the recent past, a wide variety of topics were considered by papers which appeared in this volume of the journal. Rather than to enumerate all the subjects, it perhaps is more meaningful to indicate that approximately 41% of the papers dealt with subjects related to dairy foods, 42% discussed topics dealing with other foods, and 17% were concerned with environmental or other matters.

Present Status of Volume Thirty-Four

The first six issues of volume 34 contained 36 research papers, 11 technical general interest papers, and 4 non-technical general interest papers. This compares with 25, 7, and 8 papers in the same catagories for the first six issues of volume 33. In addition, the March and June issues each contained 14 pages of 3-A or E-3-A standards.

On July 15, 1971 there was a backlog of 42 papers awaiting publication. This included 20 research papers, 16 technical general interest papers, and 6 non-technical general interest papers. In addition, on July 15 there were 12 research papers, 1 technical general interest paper, and 1 non-technical general interest paper being reviewed or revised. The backlog is somewhat less than in 1970 at this time because somewhat fewer pages in volume 34 than in volume 33 have been devoted to standards and 36 additional pages over those in volume 33 have thus far appeared in volume 34.

Review Papers

Volume 33 contained review papers on a number of timely topics including: detection of microorganisms in foods, perfringens food poisoning, the salmonella problem, egg processing technology, textured vegetable proteins, nitrates in plants and waters, activation of bacterial spores, microorganisms in raw plant foods, and environmental carcinogens.

Thus far, review papers on the following subjects have appeared in volume 34: microbiology of poultry products, changing patterns in food production and processing, solid waste disposal, bacteriological testing of milk, and fecal contamination of fruits and vegetables. In addition, review papers awaiting publication deal with the following topics: 50 years of progress in the cheese industry, guidelines for production of non-carcinogenic food additives, *Vibrio parahaemolyticus*, staphylococcus food poisoning, sodium in foods and beverages, and perfringens enterotoxin.

A number of scientists have expressed either an interest in or willingness to prepare review papers on the following topics: lead in food and the environment, the propionic acid bacteria, ecology of the lactic acid bacteria, process cheese, mercury in food and the environment, Swiss cheese flavor, machine milking and udder health, effects of abnormal milk on processing, *Bacillus cereus* food poisoning, thermal properties of foods and heat processing, and heat resistant molds of importance in foods. It must be remembered, however, that people who agreed to prepare reviews are all busy with their daily tasks. Hence, some time may elapse before all of the reviews become available for publication.

Editorial Board

The Editorial Board has been expanded to include 41 scientists in university, industry, and government laboratories. The following persons were added to the Editorial Board in 1970 or early in 1971: J. A. Alford, F. L. Bryan, W. J. Dyer, H. R. Groninger, N. F. Insalata, R. T. Marshall, S. A. Matz, E. M. Mikolajcik, R. L. Olson, Z. J. Ordal, J. W. Pence, H. J.

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EFFECT OF pH ON LOW TEMPERATURE GROWTH OF *SALMONELLA*[1]

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Abstract

The combined effects of pH and temperature on growth of three serotypes of salmonellae are reported. *Salmonella heidelberg* grew on the surface of agar over the pH range of 5.0 to 9.0. Minimum growth temperatures of 5.3, 5.2, and 5.3 C were obtained at pH values of 6.0, 7.0, and 8.0, respectively. In broth, *S. heidelberg* increased in number over the pH range of 6.0 to 8.0, with the lowest growth temperature of 6.0 C obtained at pH 7.0. *Salmonella typhimurium* increased in number in broth at pH values of 6.0 to 9.0. A time-temperature effect was shown by this organism after 16 days. *Salmonella derby* grew over the pH range of 6.0 to 8.0 in broth, with the lowest growth temperature of 9.0 C obtained at pH 6.0 and pH 7.0. The results indicate that food-poisoning *Salmonella* serotypes grow only over a narrow pH range at low temperatures.

Several reports have been published on the minimum growth temperatures of salmonellae in foods. Prescott and Geer *(8)* and Prescott and Tanner *(9)* reported that no growth of salmonellae occurred in foods held below 5 C. This was later confirmed by Angelotti et al. *(2)*, who also indicated that minimum growth temperature may vary with the foodstuff. Thus, there was no growth between 4.4 and 10 C in custard and ham salad, but growth did occur within 5 days in chicken a la king. Similarly, Matches and Liston *(6)* reported that salmonellae would not grow below 11 C in picked crabmeat but would grow at 8 C on fish fillets. These results suggest that properties of the food as a milieu for growth affect the minimum temperature at which salmonellae will grow. The pH of foods varies widely either as a result of natural composition or because of deliberate addition of acid or alkaline flavoring or preserving agents. Surprisingly little has been published on the influence of pH on salmonellae growth. In an early paper, Dernby *(5)* reported on the pH range which permitted 40 different organisms to grow, apparently at their optimum temperatures. *Salmonella typhosa* grew between pH 6.2 and 7.6; *Salmonella paratyphi* between 4.5 and 7.8; and *Salmonella schottmuelleri* between 4.5 and 8.0. Chung and Goepfert *(4)* studied the growth of salmonellae on medium adjusted with different acidulants and reported growth as low as pH 4.05. Ayres *(3)* reported that salmonellae were rapidly destroyed in lemon juice, pH 2.3, and lime juice, pH 2.5. He also reported that tomato juice, pH 4.3, was bactericidal to these organisms. Alford and Palumbo *(1)* reported growth of 23 strains of *Salmonella* at pH 5.8 and 6.5, but only one strain at 5.0, in broth containing 2% salt, incubated at 10 C.

Nothing seems to have been published concerning the minimum growth temperatures of salmonellae over the pH range occurring in foods. Yet the combined effects of low temperature (refrigerated storage) and lowered pH are commonly used to preserve foods. This paper reports results of a study on the ability of three common *Salmonella* serotypes to grow at low temperatures over the pH range 4.0 to 9.0.

Materials and Methods

Test organisms

Salmonella heidelberg, ATCC 8326; *Salmonella typhimurium,* ATCC 6994; and *Salmonella derby,* ATCC 6966, were used in these studies because they were among the serotypes most frequently isolated from human sources *(10)* and because data were available on their minimum growth temperatures.

Inoculum

The inoculum for the temperature-gradient incubator was 18 hr trypticase soy broth cultures which were spread on the agar surface by means of a sterile cotton swab.

The inoculum for the polythermostat was standardized by adding 18 hr trypticase soy broth cultures of the test organisms to 0.1% peptone water to obtain 55% transmittency at 660 nm in a Bausch and Lomb spectrophotometer, yielding 10^8 cells/ml. Appropriate serial decimal dilutions for inoculation were prepared in 0.1% peptone water, chilled in ice, and added to flasks of chilled sterile trypticase soy broth. The chilled suspension was added to 26 × 55 mm screw-cap tubes (12 ml/tube) and held chilled until placed in the polythermostat.

Medium

The pH of media used in both the temperature-gradient incubator and the polythermostat was adjusted with HCl or NaOH prior to sterilization so that the desired pH of 4.0 to 9.0 was obtained after sterilization. Medium adjusted to a different pH was used in each of the channels of the temperature-gradient incubator and in the tubes in each of the channels of the polythermostat.

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The temperature-gradient incubator (7) consists of an aluminum block with six longitudinal channels (1 × 1 1/4 × 30 inches) machined into one surface. The channels are filled with agar, each adjusted to a different pH, and the surface inoculated with the test organism. The temperature-gradient incubator was converted into a polythermostat by placing screw-cap tubes into the channels and filling the channels around the tubes with 2% agar in water.

The incubator and polythermostat were cooled at one end by circulating refrigerant and warmed at the other end by circulating water. During operation a linear temperature-gradient was obtained along the length of the aluminum block. Temperature was monitored constantly by thermocouples placed at intervals along the gradient and recorded on a Brown 6-point recording potentiometer.

Enumeration and detection

The minimum growth temperature of *Salmonella* on the surface of agar in the temperature-gradient incubator was determined by visual observation of the point at which growth could no longer be seen, and calculation of the actual temperature on the basis of the gradient. *Salmonella* counts were determined by the drop-plate method. Duplicate or triplicate 0.1 or 0.01 ml volumes of the appropriate dilutions from each tube in the polythermostat were inoculated onto the surface of trypticase soy agar. The plates, with duplicate or triplicate samples, were incubated at room temperature (about 22-23 C) and the colonies counted after 16-24 hr with the aid of a dissecting microscope. The organisms grew rapidly at room temperature, with a generation time of approximately 1 hr. At higher incubation temperatures, growth was more rapid and colonies grew together, making counting difficult. Therefore, room temperature incubation was used and the small colonies, although easily seen, were more easily distinguished with the 12× dissecting microscope.

RESULTS

The growth of S. *heidelberg* on the surface of trypticase soy agar in the temperature-gradient incubator was determined after 7 days of incubation over a temperature range of –1 to 15.5 C. Experiments of longer than 7 days duration were not conducted because moisture evaporated from the warm end of the temperature-gradient incubator and condensed on the cold end. The average results of three experiments are shown in Fig 1. After 7 days of incubation at pH 4.0, no growth of *Salmonella* could be detected even at the warm end of the incubator. On agar adjusted to pH 5.0, S. *heidelberg* was able to grow, but the minimum growth temperature at this pH was only 9.5 C. As the pH of the medium was adjusted closer to neutrality, the minimum growth temperature began to drop. At pH values of 6.0, 7.0, and 8.0, the minimum growth temperatures were 5.3, 5.2, and 5.3 C, respectively. The minimum growth temperatures then increased to 6.4 C at pH 9.0.

When *Salmonella* was grown in broth in the polythermostat over a temperature range of 2 - 12 C, the incubation period was extended for as long as 28 days. At each sampling time the numbers of organisms were determined in each tube by the drop-plate method. An increase in numbers of cells was not considered significant and recorded as an increase unless the number of cells had increased by 0.5 log.

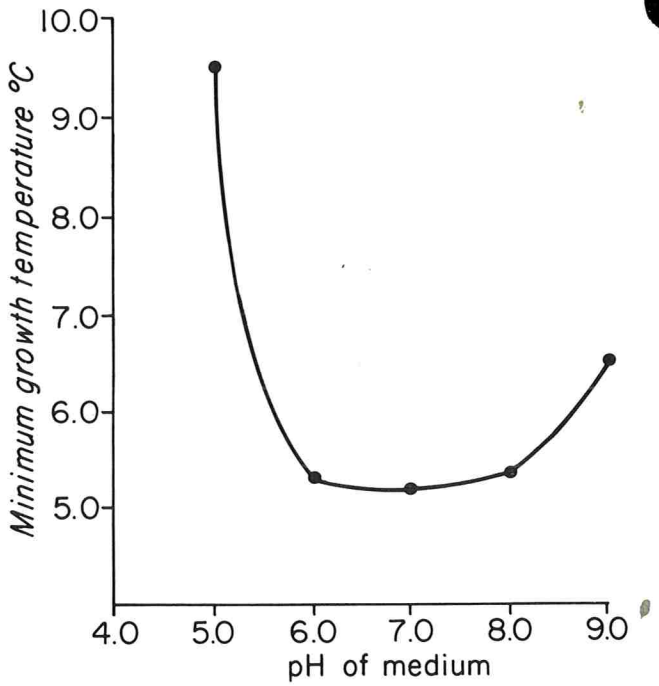

Figure 1. Minimum growth temperatures of *Salmonella heidelberg* after 7 days' incubation over the pH range of 4.0 to 9.0.

The minimum growth temperature of each organism for each pH value was determined at intervals for 28 days. The average results for two experiments are shown in Table 1. Increases in number of cells were obtained in all tubes incubated at temperatures higher than those given in Table 1. The actual minimum growth temperature may fall between the recorded temperature in the tube showing growth, and the temperature of the next colder tube which does not show an increase in numbers of cells.

When grown in broth at pH 4.0 and 5.0, the inoculum for the three serotypes decreased throughout the 28-day incubation period from an initial count of 10^4 to 10^6 cells/ml to fewer than 10 cells/ml.

Salmonella heidelberg showed greater pH effects in broth than on an agar surface. At pH 4 a decrease in cell numbers from 10^6 to <10/ml was obtained at all temperatures tested over the incubation period. An increase was not obtained at pH 5.0, but the number of cells remained almost constant throughout the incubation period. Increases in numbers of cells were obtained at pH 6.0, 7.0, and 8.0, and the minimum growth temperatures obtained at these pH values were 6.5, 5.9, and 7.7 C, respectively. At pH 9.0 the inoculum decreased from 16^6/ml to <10/ml in 6 days.

TABLE 1. INCUBATION TIME AND MINIMUM TEMPERATURE AT WHICH GROWTH OF SALMONELLA WAS DETECTED AT EACH pH VALUE.

pH	*S. heidelberg*		*S. typhimurium*		*S. derby*	
	Days incubation	Minimum temperature (°C)	Days incubation	Minimum temperature (°C)	Days incubation	Minimum temperature (°C)
5.0	No Growth		No Growth		No Growth	
6.0	6	6.5	2	12.0	5	10.0
	13	7.1	6	10.4	12	9.0
	20	7.1	16	7.2	19	9.0
	27	7.1			26	9.0
7.0	7	6.5	2	12.0	6	9.0
	14	5.9	6	9.6	13	9.0
	21	6.5	16	7.2	20	9.0
	28	6.5			27	9.0
8.0	7	7.7	2	11.2	6	9.5
	14	7.7	6	8.8	13	9.5
	21	7.7	16	6.4	20	9.5
	28	7.7	23	6.4	27	9.5
9.0	No Growth		16	8.0	No Growth	
			23	8.0		

At pH values of 6.0, 7.0, 8.0, and 9.0 an increase in the numbers of viable S. *typhimurium* cells was obtained. This increase showed a time-temperature effect with a longer lag at the lower temperature. The lowest minimum growth temperature, 6.4 C, was obtained at pH 8.0. This minimum increased to 7.2 C at pH 6.0 and 7.0 and to 8.0 C at pH 9.0.

The growth pattern obtained with S. *derby* was similar to that obtained with S. *heidelberg*. The numbers of viable cells decreased throughout the incubation periods at pH 4.0, 5.0, and 9.0. Increases in numbers of cells were obtained at pH 6.0, 7.0, and 8.0, and the minimum growth temperatures obtained at these pH values were 9.0, 9.0, and 9.5 C, respectively. The minimum growth temperature for S. *derby* over the pH range was higher than that obtained for either S. *typhimurium* or S. *heidelberg*.

DISCUSSION

The use of HCl and NaOH to adjust pH inevitably causes production of NaCl in the medium. However, the quantities used in these studies were insufficient to raise the NaCl content of the medium by even 0.5%. We have shown in a separate study (to be published in this Journal) that there is little effect on minimum temperature of growth of *Salmonella* of NaCl concentrations in the range 0.5 to 3%. NaCl may thus be ignored as a factor in the pH investigation.

The minimum growth temperature-pH relationship for S. *heidelberg* on solid medium (Fig. 1) indicates the pattern of events for the three serotypes tested. In all instances the pH range permitting growth at minimum temperatures was pH 6 to pH 8. The actual minima observed under these conditions are in good agreement with values reported earlier *(7)*. A pH of 5 seemed to represent a break point in the ability of salmonellae to grow at temperatures in the refrigerated food range, although Chung and Goepfert *(4)* reported the growth of salmonellae as low as pH 4.05 at 25 and 32 C. Cells held at pH 4 died off rapidly at temperatures below 10 C. The organisms showed somewhat marginal growth at pH 9 when temperatures were below 10 C but the high pH seemed, not unexpectedly, to have less effect than pH values in the acid range.

Of the three serotypes tested, S. *heidelberg* showed the greatest tolerance of varied pH conditions. This organism has also shown the lowest minimum growth temperature and may in fact be somewhat more psychrotrophic than other *Salmonella* serotypes. It is probable that the difference in response of this organism when grown on the surface of solid media and in liquid media simply reflects a greater availability of oxygen on the solid medium. Chung and Goepfert *(4)* were able to get growth of *Salmonella senftenberg* at a lower pH with aeration than without. Obviously, physiologically important factors such as temperature, acidity, and oxygen availability interact in affecting the growth of microorganisms.

The combined effect of pH conditions and low temperature on the outgrowth time of salmonellae is of interest in relation to control of these organisms in foods. Where growth occurred at the extreme pH values of 5 and 9, it was greatly delayed. However, only S. *typhimurium* showed a marked time-temperature growth effect in the pH range permitting growth at below 10 C.

On the basis of these studies it appears that three of the more common food-poisoning serotypes of

Salmonella grow only over a narrow pH range between 6 and 8 at temperatures close to 5 C. The results reinforce earlier published conclusions that foods held at temperatures below 5 C will not support growth of *Salmonella.*

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REPORT OF THE EDITOR JOURNAL OF MILK AND FOOD TECHNOLOGY

(Continued from Page 48)

Peppler, G. H. Richardson, R. L. Saffle, F. M. Sawyer, C. E. Swift, B. A. Twigg, C. Vanderzant, H. B. Warren, H. Wistreich, and E. R. Wolford. One member of the Editorial Board, Dr. B. J. Liska, resigned during 1971 because he became Scientific Editor of the *Journal of Food Science.*

The following persons not on the Editorial Board have reviewed papers during the first one-half of 1971 and their help is gratefully acknowledged: C. H. Amundson, R. L. Bradley, Jr., A. L. Branen, C. L. Duncan, W. Gjomerac, J. M. Goepfert, R. V. Lechowich, D. B. Lund, N. F. Olson, T. Richardson, H. Sugiyama, and J. H. von Elbe.

E. H. MARTH
Editor
Journal of Milk and Food Technology

TABLE 1. SUMMARY OF CONTENTS OF *Journal of Milk and Food Technology,* 1967-1970

Item	Volume 30 (1967)	Volume 31 (1968)	Volume 32 (1969)	Volume 33 (1970)
1. Total pages, including covers	512	540	624	688
2. Research papers				
a. Number	30	32	47	66
b. Pages	137	142	205	280
c. Percent of total pages	26.7	26.3	32.9	40.7
3. General interest papers-technical				
a. Number	11	16	14	18
b. Pages	47	74	87	99
c. Percent of total pages	9.2	13.7	12.2	14.3
4. General interest papers-non-technical				
a. Number	23	14	26	20
b. Pages	72	65	91	64
c. Percent of total pages	14.1	12.0	14.6	9.3
5. Association affairs				
a. Pages	64	68	62	49
b. Percent of total pages	12.5	12.6	9.9	7.2
6. News and events				
a. Pages	51	42	36	23
b. Percent of total pages	9.9	7.8	5.8	3.4
Percent of pages-technical material	35.9	40.0	45.1	55.1
Percent of pages-non-technical material	36.5	32.4	30.3	20.0
Percent of pages-covers, standards, index, advertising, etc.	27.6	27.6	24.6	24.9[a]

[a]Includes 48 pages of E-3-A and 3-A Standards in the March and April issues.

CHARACTERISTICS OF MILKING CENTER WASTE EFFLUENT FROM NEW YORK STATE DAIRY FARMS

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Abstract

Waste profile studies were carried out on milking center (milkhouse and milking parlor) wastes from 24 New York State dairy farms selected in 20 different counties. Data show that farm-generated milking wastes should not be confused or compared with diluted aqueous sewage typical of effluents from dairy processing plants. Presence of high levels of settleable solids explain the rapid failure and fouling of traditional tile field waste systems. While many sanitarians have speculated that milking center sewerage line plugging has occurred because of milk solids and biological sludge accumulation, this field experiment suggests that manure, feed, bedding, and hoof dirt are the principal solids in milking center wastes.

This study was made to measure volumes and concentration of milk center wastes in order that one can treat them intelligently. In 1950, Roadhouse and Henderson (3) stressed the need for milkhouse convenience. The authors completed their review of milkhouse and parlor activities by merely commenting that the waste line should have a tile cleanout trap just outside the building, in the event of plugging. Two decades later, the literature is still sparse with data that define milking center effluents.

One pertinent study in 1969 by Loehr and Ruf (2), which dealt with an aerobic lagoon treatment of parlor wastes in Kansas, is remarkably substantiated by this investigation in regard to pounds of B.O.D. per cow per day. Gallons of waste produced per cow per day in the milking parlor are also within reasonable agreement.

Materials and Methods

From May through June, 1971 State County Extension specialists were visited to establish guidelines for a milking center waste study. Waste handling training sessions were held in five areas of New York State to instruct the participating county agents on how best to obtain reliable waste samples.

Composite samples were collected from the total milking center wastes produced from single and double milkings at selected farms in plastic 8 ft in diameter by 20 inches high children's swimming pools with a capacity of approximately 640 gal. Two-quart portions in jugs were taken from impounded well mixed waste material and then packed with ice in styrofoam picnic baskets for transporting to the Cornell campus laboratories where testing was done. Biological and chemical analysis followed the procedures outlined in the 1969 F.W.P.C.A. *Methods for Chemical Analysis of Water and Wastes.* The waste volumes were physically measured on farms by actually filling and then dumping 10-gal cans.

Results

Volume and strength

With the exception of one large farm sampled, the numbers of animals per dairy farm were typical of New York State milking operations. Table 1 states that the average gallons of waste per cow per day in this study was 4.05. The pounds of B.O.D. per cow per day produced in milking centers approximate an amount equal to a population equivalent of 1 (human waste load taken to be 0.17 lb. per day).

It was quite evident by observation and smell that the collected waste composite samples are diluted manure fluids. One could see a brown greenish liquid with settled feed materials such as corn and grain together with bits of straw mixed with mud and sand.

Not only were the settleable solids high, as indicated in Table 1, but the percentages of total solids and centrifuged residues suggest that substantial amounts of debris will also be carried in presettled

Table 1. Milking center waste volumes and B.O.D. pounds—determined from 24 New York State farms—June 1971[1, 2]

	Highest individual farm	Lowest individual farm	Average for all farms	Total sampling
Number of cows	396	36	100	2401
Total gallons of waste generated	1608	111	405	9713
Total pounds of B.O.D./day	93.4	0.57	12.7	305.5
Settleable solids, ml/l	200	trace	49.5	1189
Gallons of waste/cow/day	16.8	1.8	4.05	—
Pounds of $B.O.D._5$/cow/day	0.38	0.01	0.127	—

[1]Odors from effluent varied from slight feed to strong manure. Colors varied from brown to olive green.
[2]If the data from the two extreme farms of highest and least number of cows are discarded, the average gallons of waste/cow/day are identical with a slight reduction in average pounds of $B.O.D._5$ to 0.107/cow/day.

TABLE 2. MILKING CENTER WASTE VOLUMES FROM 24 NEW YORK STATE FARMS GROUPED AS TO HERD SIZE. JUNE 1971

Cows/herd	No. of farms in group	Total cows in group	Avg. gal waste/farm in group	Avg. lb. B.O.D./farm in group	Avg. gal waste/cow in group
0 - 49	5	209	302	6.3	7.2
50 - 99	10	675	284	7.6	4.2
100 - 149	4	446	491	15.2	4.4
150 - 199	4	675	445	13.5	2.6
>200	1	396	1608	93.4	4.1
Total	24	2401			

milking center waste.

Generally, a linear relationship existed between the number of animals milked and the gallons of waste produced at the farm. However, Table 2 indicates that locations are sufficiently different in operation to be variable as illustrated by the slope of the line in Fig. 1 when data are plotted on normal probability grids. The measure of variation is the slope of the line where the steeper slope means variation and the flatter slope denotes less.

Nitrogen and phosphorus

It seems reasonable to expect that nitrogen and phosphorus content in milking center waste should vary as organic load levels change. Data in Tables 3 and 6 show that relatively small differences occurred in soluble phosphorus and nitrogen compounds when compared with animals per farm or with pounds of B.O.D. produced in the milking center. The high percentage of results falling into similar bands of concentration levels may indicate that some solubility or buffering control mechanism regulates the chemical system for these compounds.

The data on nitrate and nitrite are shown in Table 4 and indicate that amounts of these substances are no greater than the levels in sewage-treatment-plant effluent. The summary in Table 5 shows the enormous amount of total solids present in milking center waste effluents. Even centrifuging waste at 37,000 RCF failed to remove most of the solids which will cause waste handling problems.

TABLE 3. NITROGEN AND PHOSPHORUS LEVELS IN MILKING CENTER WASTES FROM 24 NEW YORK STATE DAIRY FARMS. JUNE 1971

Micrograms/ml[1]	Soluble PO_4P	Soluble P	Soluble NH_4-N	Soluble Kjeldahl N
	(Number of farms)			
<49	9	9	7	1
50 - 99	13	12	11	5
100 - 149	1	2	3	9
150 - 199	1	1	2	3
200 - 249	0	0	0	3
>250	0	0	1	3
Total	24	24	24	24
	(Micrograms/ml)			
Highest result	183	179	625	736
Lowest result	6	8	5	40
Average result	57.6	61.8	132.1	186.6

[1]Micrograms/ml approximates ppm

TABLE 4. NITRATE AND NITRITE LEVELS IN MILKING CENTER WASTES FROM 24 NEW YORK STATE DAIRY FARMS. JUNE 1971.

Micrograms/ml[1] Soluble	Soluble NO_3 & NO_2	NO_2
	(Number of farms)	
<0.5	1	10
.5 - 1.0	12	9
1.1 - 1.5	4	1
1.6 - 2.0	1	2
2.1 - 2.5	2	2
>2.5	4	0
Total	24	24
	(Micrograms/ml)	
Highest result	6.5	2.5
Lowest result	0.3	<.5[2]
Average result	1.6	1.1[3]

[1]Micrograms/ml approximates ppm
[2]Lowest test sensitivity
[3]Average of data for 0.5 and above

DISCUSSION

Evidently milking center wastes do not resemble typical milk plant fluid sewage. The most comprehensive review of dairy food plant wastes was recently made by Harper *(1)* who presented data from 697 plants in 38 states or about 11% of the total industry plants. The suspended solids from milk plants are mostly coagulated milk, fine particles of cheese, and residues of by-products such as nuts or fruits from ice cream manufacturing. Usually the settleable solids in fresh dairy plant wastes are present in trace amounts which differ markedly from the average of 49.5 ml/liter, as shown in Table 1. Wastes generated from milking centers are more like animal manures with large amounts of feed, bedding, and hoof dirt.

While the data show a wide variation in the high and low waste ratios, 50% of the 24 farms sampled produced approximately 4 gal of waste per cow per day from milking center operations. This also is the mean figure in the survey.

It is interesting to note that 60% of the experimental sites sampled showed a sewage load of <10 lb. of

TABLE 5. SOLIDS CONTENT IN MILKING CENTER WASTES FROM 24 NEW YORK STATE DAIRY FARMS. JUNE 1971

Grams/liter	Total solids	Centrifuged solids[1]
	(Number of farms)	
0 - 2.9	4	15
3.0 - 5.9	11	7
6.0 - 8.9	8	2
9.0 - 11.9	1	0
Total	24	24
	(Grams/liter)	
Highest result	10.4	7.8
Lowest result	0.8	0.2
Average result	5.0	2.7

[1]Centrifuged 15 min at 37,000 RCF, 10 g approximate 1%

TABLE 6. NITROGEN AND PHOSPHORUS LEVELS IN MILKING CENTER WASTE SOLIDS FROM 24 NEW YORK STATE DAIRY FARMS. JUNE 1971.

Component (Percentage)	Nitrogen in total solids	Phosphorus in centrifuged residue[1]
	(Number of farms)	
0 - 2.9	4	5
3.0 - 5.9	17	13
6.0 - 8.9	1	4
9.0 - 11.9	0	0
12.0 and above	2	2
Total	24	24
	(Percent)	
Highest result	17.7	20.2
Lowest result	2.3	2.0
Average result	4.9	5.5

[1]Centrifuged 15 min at 37,000 RCF.

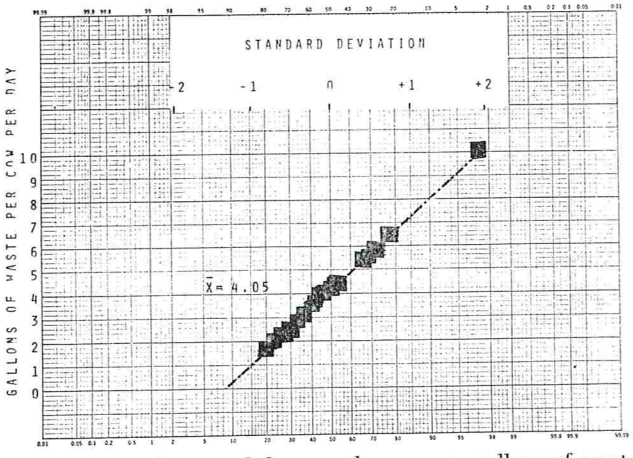

Figure 1. Distribution of data on the average gallons of waste per cow per day determined at 24 New York State farms—June 1971.

B.O.D. per day per farm. This amount is small for conventional industrial waste handling systems. If one agrees that a one-half acre lagoon can accept 10 lbs of B.O.D. per day as a manageable waste loading amount for the area, then the lagoon concept could be an attractive method for treating milking center wastes.

The question also might be raised that perhaps parlor wastes should be part of animal solids or liquid manure systems and that the milkhouse liquid wastes might best be handled in the former traditional manner. Failure and rapid plugging of cesspools and tile fields or similar systems are explained by high amounts of settleable solids, total solids, and centrifuged solids.

Further work is now underway to explore the value of separating milking center wastes into categories. These include: *(a)* milking parlor wastes separated to be discharged to solid or liquid manure handling systems; *(b)* combine aqueous milkhouse wastes with parlor sewage to be handled in traditional tile fields or cesspools; and *(c)* apply lagoon waste handling methods for treating either combined or segregated portions of milkhouse-milking parlor wastes.

The data on total solids content are presented in Table 5 and show that enormous amounts of solids can be present from milking centers. In addition, if approximately 50% of the solids cannot be settled even at 37,000 RCF then household 500-gal to 1,000-gal septic tanks currently being used for settling tanks can only provide minimal solids separation benefits at best.

It does seem reasonable that 5% levels of nitrogen and phosphorus in the waste solids (see Table 6) may make a land application of these wastes attractive, however, should the soluble material seep into water ponds then algal growth could be stimulated.

While this project is not a complete in depth study of milking center wastes, it has disclosed some interesting facts. The Agricultural Engineer may wish to review the grate sewers and trench drains in parlors to reduce the commingling of manure, hoof dirt, and feed with liquid washing solutions. Surely feed losses to sewers may be high enough to discourage animal feeding in this location. It seems that the pounds of B.O.D. per farm are generally small enough to be easily handled effectively on farm lands without elaborate waste systems if proper techniques are followed.

ACKNOWLEDGEMENT

N. Y. S. Cooperative Extension Agents volunteered both time and effort to measure and sample milking center waste.

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PREPARATION OF *ALPHA*$_{S1}$- AND *BETA*-CASEIN RELEASED DURING PREPARATION OF *KAPPA*-CASEIN FROM BUFFALO MILK

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(Received for publication August 2, 1971)

ABSTRACT

Methods were modified to improve preparation of α_{s1}- and β-casein from the precipitate obtained when *k*-casein was prepared by Hill's method. A concentration of 3.3 *M* urea was used instead of 4.6 *M* urea to precipitate all the α_{s1}-casein. β-Casein was precipitated from the second-cycle casein fraction P at pH 4.9 as described by others, the gummy precipitate was washed with alcohol, and then air dried. Starch-gel electrophoresis showed that α_{s1}- and β-casein prepared by this method followed by further purification through redissolving and reprecipitating, was essentially free from specific fractions.

Fractionation of casein had to be repeated with buffalo milk and new techniques developed to obtain individual casein components. Previously methods to prepare α_{s1}- and β-caseins were given by Warner *(12)*, Hipp et al. *(4)*, and von Hippel and Waugh *(10)*. These methods formed the basis for further modifications by Payens *(6)*. Schmidt and Payens *(8)* prepared α_{s1}-casein by using a combination of procedures described by Waugh and von Hippel *(13)* and Zittle and Custer *(14)*. They found by addition of Ca++ to the α-casein solution, two fractions resulted which appeared to be highly heterogeneous when tested by starch gel electrophoresis. Melnychyn and Wolcott *(5)* found that addition of polyphosphate to milk retarded or reversed the migration of *k*-casein and this reaction facilitated resolution of α_{s1}-casein in a high state of purity. Aschaffenburg *(1)* modified the method of Hipp et al. *(4)* for preparing β-casein.

In the present work a combination of methods has been used to prepare α_{s1}-casein and β-casein released when *k*-casein was prepared from buffalo milk by Hill's method.

MATERIALS AND METHODS

Preparation of α_{s1}- and β-casein

Whole casein was precipitated from 2 liters of skimmilk with 1 N HCl at pH 4.6, the precipitate was filtered through a Buchner funnel, washed with warm water, re-dispersed with the aid of 1 N NaOH, and precipitated again at pH 4.6. The precipitate was used for preparing *K*-casein by Hill's method *(3)*. The precipitate (second cycle Ca-caseinate) remaining after preparation of *k*-casein was used to prepare α_{s1}- and β-casein. It was suspended in water and Ca++ was removed from the suspension by addition of 200 ml of 1.5 *M* potassium oxalate and 0.2 *M* oxalic acid to maintain the pH at 7.0. The calcium oxalate was removed by centrifugation and filtration. The filtrate (second cycle casein fraction P) contained a_S- and β-casein which were precipitated by reducing the pH to 4.6, filtered off, dispersed in water, and 6.6 *M* urea was added to give a final concentration of 4.6 *M* urea. An oily precipitate was formed which consisted mainly of crude Ca-sensitive α_S-casein. The supernatant containing the β-casein was filtered through Whatman No. 1 filter paper, the pH of the filtrate adjusted to 4.9, and the β-casein precipitated by the method of Aschaffenburg *(1)*. The oily floccules of β-casein were then centrifuged and further purified by dispersion in water, addition of NaOH, and re-precipitation at pH 4.9. The crude Ca-sensitive (α_S-casein) was washed with 4.6 *M* urea, and dissolved again in 6.6 *M* urea solution with 2.12 g NaCl added per 100 ml. It was re-precipitated by diluting to 3.3 *M* urea to obtain α_{s1}-casein. Both α_{s1}- and β-casein precipitates were washed with alcohol and ether, dried in air, and finally crushed to a fine powder.

Starch-gel-electrophoresis

The procedure of Wake and Baldwin *(11)* and a modification of Schmidt's *(7)* method for using 2-mercaptoethanol were used to prepare the gel. A further modification was made in the present work, in which 13% starch hydrolysates were used instead of 12.2% since the 2-mercaptoethanol had a weakening action on the gel strength.

Application of the sample

The technique of Ganguli and Majumder *(2)* was used in which a solution of sample in urea was prepared according to the procedure of Aschaffenburg *(1)*. Strips of Whatman 3 mm wide filter paper 10 mm long were impregnated with pro-tem solution and then inserted into the gel bed.

Buffer systems

A discontinuous buffer system was used. The tris-citrate-urea buffer at pH 8.6, containing 0.02 *M* 2-mercaptoethanol was used to prepare the starch gel. The buffer system of 0.3 *M* boric acid titrated to pH 8.6 with NaOH was used for the electrode vessels.

Electrophoretic test

To allow solubilization of proteins into the gel bed, the electrophoresis was started 30 min after insertion of strips. Electrophoretic tests were carried out using a constant voltage of 200 for 16 hr at room temperature.

Staining and washing

The procedure described by Smithies *(9)*, was used.

RESULTS AND DISCUSSION

The method of preparing α_{s1}- and β-casein described in this paper depends on the nearly complete removal of *k*-casein and the use of the precipitate (second cycle Ca-caseinate). Since *k*-casein is insensitive to

[1]Visiting scholarship under Indo-U.A.R. exchange program at National Dairy Research Institute, Bangalore, India. Present address: Statens Forsøgsmejeri, Hillerød, Denmark.

Ca ion concentration, a high concentration of $CaCl_2$ (4 *M*) can be used, as described by Hill *(3)*, for complete precipitation of the second cycle Ca-caseinate and to be sure that all *k*-casein remains in the supernatant. Moreover, the crude *k*-casein was centrifuged at high speed so that the recovery of α_s- and β-caseins is as complete as possible. The removal of Ca^{++} from the precipitate of second cycle Ca-caseinate gave a supernatant, second cycle casein fraction P, as described by Waugh and von Hippel *(13)*. For this reason, potassium oxalate in sufficient amount was used to precipitate all the Ca^{++}, and the calcium oxalate was removed by centrifugation. The supernatant was dialysed against distilled water to insure removal of excess potassium oxalate. As shown by Hipp et al. *(4)*, the property of differential solubility in urea of the casein components can be utilized for preparative purposes. Meanwhile, fractionation is achieved by step-wise dilution of a solution of all casein components. Therefore, by using this method for second cycle casein fraction P, with some modifications as reported in the present work, more effective separation of α_{s1}- and β-casein can be obtained. A concentration of 4.6 *M* urea has been used for precipitating all α_s-casein from the second cycle casein fraction P, and the β-casein remains in the supernatant of 4.6 *M* urea. The α_s-casein was redissolved in 6.6 *M* urea and diluted to 3.3 *M* urea instead of 4.6 *M* urea, with the addition of 2.12% sodium chloride solution, to precipitate all the α_{s1}-casein. Using 3.3 *M* urea improved precipitation of α_{s1}-casein over the original method of Hipp et al. *(4)*. The supernatant of 4.6 *M* urea with the β-casein, was filtered, and the pH of the filtrate adjusted to 4.9, to precipitate the β-casein as described by Aschaffenburg *(1)*. Figure 1 shows the electrophoretic densitometer tracing of α_{s1}- and β-casein samples. The bands have been numbered as to their relative position in the gel. Distance from the starting slot to front has been set at 1.00. Figure 1-a shows the peak in the position of 0.8 to 0.9 corresponding mainly to α_{s1}-casein, and very minor peaks in the position of 0.03 to 0.24 corresponding to *k*-casein. At the same time a very faint band appeared in the position of β-casein. Figure 1-b shows the peak in the position of 0.47 to 0.55 which corresponds to β-casein, and slower moving material which corresponds to *k*-casein was noted in the position of 0.13, 0.19, and 0.26.

Generally, starch-gel electrophoresis showed that α_{s1}- and β-casein prepared by this method, and after further purification by redissolving and reprecipitating, to be free from major impurities.

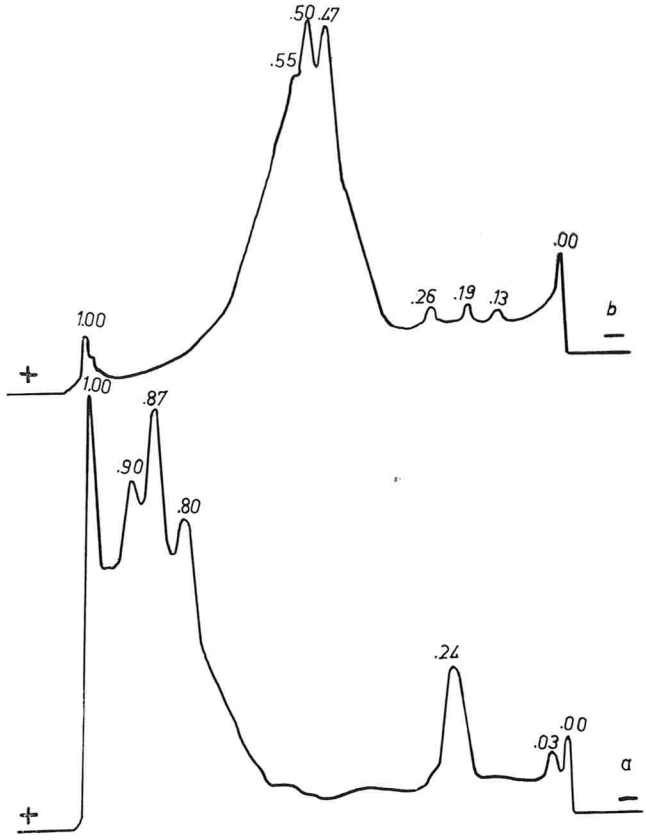

Figure 1. Densitometric tracing of electrophoretic patterns of α_{s1}-casein and β-casein. Starch gel electrophoresis conditions: 0.76 *M* Tris-citrate buffer pH 8.6; 7 *M* urea; 0.02 *M* 2-mercaptoethanol. a: α_{s1}-casein; b: β-casein.

ACKNOWLEDGMENT

The first author is grateful to the Council of Scientific and Industrial Research for awarding a Fellowship. Thanks also go to the National Dairy Research Institute, Bangalore, India, for providing facilities for this investigation.

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ASSOCIATION AFFAIRS

ANNOUNCEMENT CONCERNING THE SANITARIANS AWARD FOR 1972

Announcement is made that nominations will be accepted for the annual Sanitarians Award until June 1, 1972, and the members of the International Association of Milk, Food and Environmental Sanitarians, Inc. are requested to give consideration to the nomination of individuals whose professional work in the field of milk, food, or environmental sanitation has been outstanding.

The Award consists of a Certificate of Citation and $1,000 in cash, and is sponsored jointly by the Diversey Chemical Corporation, Klenzade Products, Inc., and Pennwalt Corporation. It is administered by the International Association of Milk, Food and Environmental Sanitarians, Inc., and is presented annually. The next presentation of the Sanitarians Award will be made at the 59th annual meeting of the Association which is to be held at Milwaukee, Wisconsin, in August 1972.

The Executive Board of the Association has established the following rules and procedures governing the Sanitarians Award.

Eligibility:

1. *General Criteria*

   To be eligible for nomination the Sanitarians Award offered annually by the International Association of Milk, Food and Environmental Sanitarians, candidates must:

   a. Have been a member of IAMFES in good standing for a period of five years prior to the date when the Award is to be presented;

   b. Be a living citizen of the United States or Canada who, at the time of nomination, is employed as a professional sanitarian in the field of milk, food, and/or environmental sanitation by a county, municipality, state or federal government provided that in the odd years beginning with 1969 the Sanitarians Award will be limited to state and federal employees and the even years to county and municipal employees.

   Members of the Executive Board, members of the Committee on Recognition and Awards of the International Association of Milk, Food, and Environmental Sanitarians, and industry members shall not be eligible for the Award. Race, sex or age shall not enter into the selection of the Award recipient.

   c. Have made a meritorious contribution in the field of milk, food or environmental sanitation, to the public health and welfare of a county, counties, district, state or federal government with the United States or Canada.

   d. Have completed the achievements and contributions on which the nomination is based during the seven-year period immediately preceding January 1, of the year in which the Award is to be made.

2. *Additional Criteria*

   a. Co-workers are eligible for nominations if both have contributed equally to the work on which the nomination is based and each independently meets the other qualifications for nomination.

   b. Where co-workers are selected to receive the Award, each shall receive a certificate and share equally in the cash accompanying the Award.

   c. No person who has received, or shared in receipt of the Award, shall be eligible for renomination for this Award.

Nominations

Nominations of candidates for the Sanitarians Award may be submitted by the Affiliate Associations of the IAMFES, or by any member of the Association in good standing except members of the Executive Board, members of the Committee on Recognition and Awards, and employees of the

sponsoring companies. Nominations from persons who are not members of the Association cannot be accepted. No member or Affiliate may nominate more than one candidate in any given year.

Each nomination must be accompanied by factual information concerning the candidate, a resume of his work and achievements, evidence supporting his achievements and if available, reprints of publications. A form for the submission of nominations may be obtained upon request from H. L. Thomasson, Executive Secretary, International Association of Milk, Food and Environmental Sanitarians, Inc., P. O. Box 437, Shelbyville, Indiana 46176.

Submission of Nominations

The deadline for submission of nominations is set annually, and all nominations and supporting evidence must be postmarked prior to midnight of that date. The deadline this year is June 1, 1972. Nominations should be submitted to Milton E. Held, Chairman, Commitee on Recognition and Awards.

Selection of the Recipient

The Committee on Recognition and Awards of the International Association of Milk, Food and Environmental Sanitarians, Inc., has full responsibility for selecting from among the candidates nominated the recipient of the Sanitarians Award. In judging the contributions of each candidate, the Committee will give special consideration to (*a*) originality of thought, mode of planning, and techniques employed, (*b*) the comprehensive nature of the candidate's achievements, and (*c*) their relative value as they affect the health and welfare of the area served by the candidate. The Committee will give consideration also to the efforts of the candidate to establish professional recognition in the area in which he serves, as well as to his research, administrative development, program operation and educational achievements. Additional information or verification of submitted information will be requested when considered necessary by the Committee. Testimonial letters in behalf of a candidate are not desired.

If after reviewing the nominations and supporting evidence, the Committee decides that the work and achievements of none of the candidates have been significantly outstanding, the Award shall not be made. In this connection, it is fundamental that if meritorious professional achievement cannot be discerned the Award shall be omitted for a year rather than to lower the standards for selections of a recipient.

Milton E. Held,
910 Upper Lupin Way,
San Carlos, Calif. 94070

MINNESOTA SANITARIANS ASSOCIATION ANNUAL MEETING AND AWARDS BANQUET

Left to right: H. Erickson and Roy Ginn

Left to right: A. J. Sjowall and Roy Ginn

Left to right: C. Holcombe and Roy Ginn

A successful meeting of the Annual Sanitarians Conference at the University of Minnesota was highlighted by the annual awards banquet with attendance of over 112 members and friends.

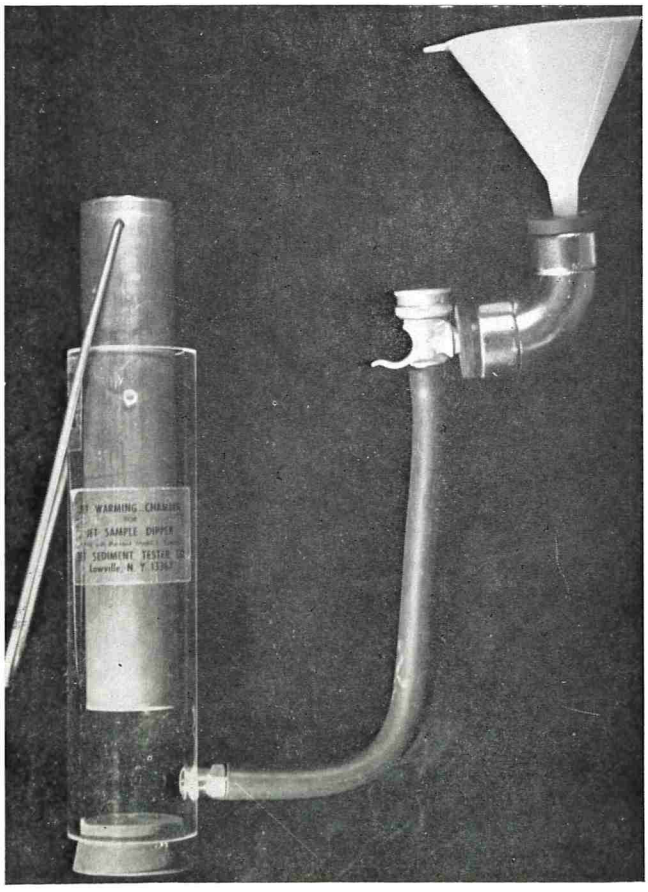
Omer Majerus, Universal Milk Machine, Albert Lea, was Master of Ceremonies, and Dr. Orville Young, South Dakota State University, was the main speaker. He gave a very interesting slide presentation on the dairy industry in Brazil.

Roy Ginn, Chairman of the Awards Committee, presented Honorary Life Membership Certificates to Charles Holcombe, Minnesota Dept. of Agriculture and H. E. Erickson, St. Paul Bureau of Health. The Certificate of Achievement Award went to A. J. Sjowall, in recognition of his outstanding contributions to the Association and to the sanitarian profession. Al has served in a number of capacities in education, industry and, more recently, as Executive Secretary of the Quality Control Committee. He was the Sanitarians Association second President and served two years (1946, 1947) one of two men to be thus distinguished.

There was only one note of regret. This year, after eleven years of distinguished and faithful service, Orlowe M. Osten resigned as Secretary-Treasurer of the Association in order to devote more time to his post as President of IAMFES.

VERNAL S. PACKARD, JR.
Secretary-Treasurer

NEW ITEM RELEASE



An inexpensive and practical invention makes possible rapid on-the-spot testing of farm bulk tank milk for cleanliness. Sample warming and testing time is about two minutes. The patented tester, weighing only about one and half pounds, is of a water aspiration type and connects to the cold or hot water supply in a milkroom. Its discharge end can be attached to a plastic warming chamber into which a stainless steel dipper eleven inches long and containing the one-pint test sample of milk is inserted for warming prior to testing. A minimal amount of flowing hot water is used and rapid warming of the sample results because of the elongated feature of the dipper. If desired, warming of the sample can be temporarily halted at 60 degrees F. for lactometry determination of the milk. The combination of tester, sample dipper and warming chamber is ideally suited for sanitarian or milk hauler use. It is a handy tool for dairymen to use in monitoring their own milk cleanliness.

U. S. Patent 3,267,724 and Canada Patent 781,148 have been issued for this tester. Sediment tests 0.4" in diameter are standard and the insertion of portable adapters convert the tester to other size tests such as 0.8" or 0.2" diameters, the latter being for the testing of 4-ounce universal milk samples.

This invention has many other practical applications, such as the testing of filtrable or liquefiable foods, gathered cream, waters, steam condensates, food and equipment rinses and the like in cleanliness determinations.

For further information, write to: Jet Milk Cleanliness Tester Co., Lowville, N. Y. 13367.

NOTICE TO MEMBERSHIP

In accordance with our Constitution and By-laws which requires our Second Vice-President and Secretary-Treasurer to be elected by mail ballot, you are hereby notified that President Orlowe M. Osten, at the annual meeting in San Diego, California, August 1971, appointed A. E. Parker, Chairman, Pat Dolan, Harold Barnum, Fred Uetz and Roy Ginn to the nominating committee for 1972.

Nominations for the office of Second Vice-President and Secretary-Treasurer are now open and any member wishing to make a nomination should send a picture and biographical sketch of his nominee to A. E. Parker not later than March 1, 1972.

A. E. PARKER, *Chairman,* Nominating Committee
Multnomah County Oregon,
Division of Public Health,
104 S. W. 5th Ave.,
Portland, Oregon 97204.

INTERNATIONAL SYMPOSIUM ON HEAT AND MASS TRANSFER PROBLEMS IN FOOD ENGINEERING

The International Union of Food Science and Technology and the European Federation of Chemical Engineering are organizing a symposium on *Heat and mass transfer problems in food engineering*, which will take place in Wageningen, the Netherlands, from October 24-27, 1972.

Papers will be presented in the following sections: steady and non-steady state heating or cooling, steady and non-steady state mass transfer and simultaneous heat and mass transfer. Write for more information to the International Agricultural Centre, P. O. Box 88, Wageningen, the Netherlands.

ANNOUNCEMENT

A National Symposium on Costs of Water Pollution Control will be held in Raleigh, N. C. on April 6 and 7, 1972. Sponsored by the Research Triangle Universities and several national societies, the program will include sessions on: Economic Implications of National Goals for Water Pollution Control Cost Effectiveness of Comprehensive Planning, Design and Construction, Information and Monitoring Systems, Economic Incentives for Pollution Control Economics of Industrial Waste Management. For further information, please contact: F. E. McJunkin, Associate Director, Water Resources Research Institute, North Carolina State University, 124 Riddick Building, Raleigh, N. C. 27607.

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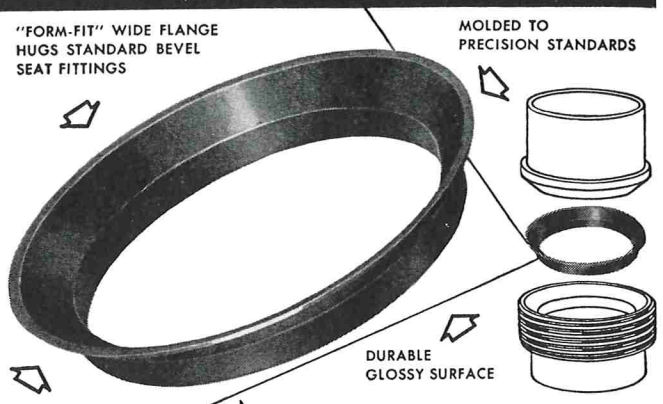

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Dairy authorities speak out on better cow milking.

R. R. Hacker / University of Guelph / Canada

Prime the udder for maximum milk let-down

Proper pre-milking stimulation is the first and a most important step in the milking operation. It increases milking rate, cuts labor cost and brings about maximum milk ejection which helps maintain udder health.

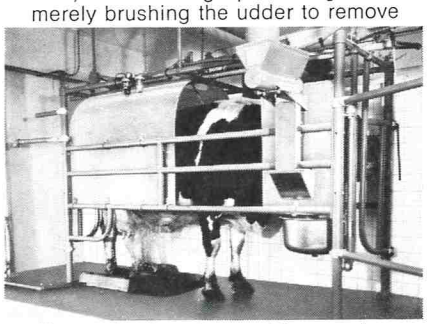
Production of high quality milk demands that the udders be cleaned and sanitized. Fortunately, the cleaning process can accomplish both sanitation and stimulation.

Washing or massaging the udder causes a stimulation of the nerve endings, releasing a hormone called oxytocin from the pituitary gland. In the udder, oxytocin causes a contraction of the muscle tissue thus expelling milk into larger ducts and cisterns. A recent experiment demonstrated that the sounds of milking do not cause adequate release of oxytocin to achieve milk let-down.

It has been determined that the response to oxytocin will begin 13 to 50 seconds after washing and massaging the udder and will last for 2 to 10 minutes, depending on the quantity released. The act of stimulating should be performed one or two minutes before the milking machine is attached. This interval is of the utmost importance. If a cow is stimulated 5 to 10 minutes before the milking machine is attached, most of the effect of oxytocin will be lost. Results of work at Kansas have shown that when milking was delayed 8 minutes after stimulation, yields decreased by 5% and machine time increased by 14%.

A milking machine should not be placed on a cow until her teats are plump and fully distended from the pressure of the milk. Teat cups will draw in flabby teats and loose udder tissue when milk pressure is low. If this happens, the teat can be injured or partially blocked and milking time will be longer.

A cow in the early lactational phase will often only require a 10 second stimulation period to achieve full let-down, whereas cows in the latter half of lactation will often require 50 seconds or more. When all cows are compared, the higher producers generally require less stimulation. However, even with high-producing cows, merely brushing the udder to remove



dirt or simply using a strip cup does not prove adequate to stimulate maximum let-down. In a New Zealand study, one identical twin in each of several pairs was stimulated for 30 seconds before milking. The unstimulated twin cows exhibited a rapid decline in milk production after 50 days, allowing the stimulated twins to produce 32% more milk and fat.

From a milking rate study, conducted by Dr. J. D. Sikes and the author at the University of Missouri, it was learned that total parlor time decreased by approximately 2 hours per day when 79 cows were properly stimulated by an automatic stimulator-washer. The results of this experiment are shown in the table.

PERCENTAGES OF COWS THAT MILKED OUT IN VARIOUS INTERVALS

	Manual (15 Sec.)	Automatic (30 Sec.)
0-3 min.	0	15.3
3-4 min.	9.8	45.8
4-5 min.	33.3	26.4
5-6 min.	16.7	9.4
+6 min.	40.3	2.8

Milking time per cow decreased with the use of the stimulator-washer. Cows prepared with the stimulator-washer ranged from 2.02 to 7.47 minutes, while cows prepared manually required 3.40 to 14.7 minutes for milking. On the average, cows that were stimulated for 30 seconds gave 76% of their milk in the first two minutes as compared with only 51% with a 15 second pre-milking stimulation. Milk yield in the first 30-60 seconds of milking serves as a measure of completeness of milk ejection.

In this experiment, an automatic stimulator-washer was used. It should be noted, however, that careful and thorough hand-stimulation can achieve the same results.