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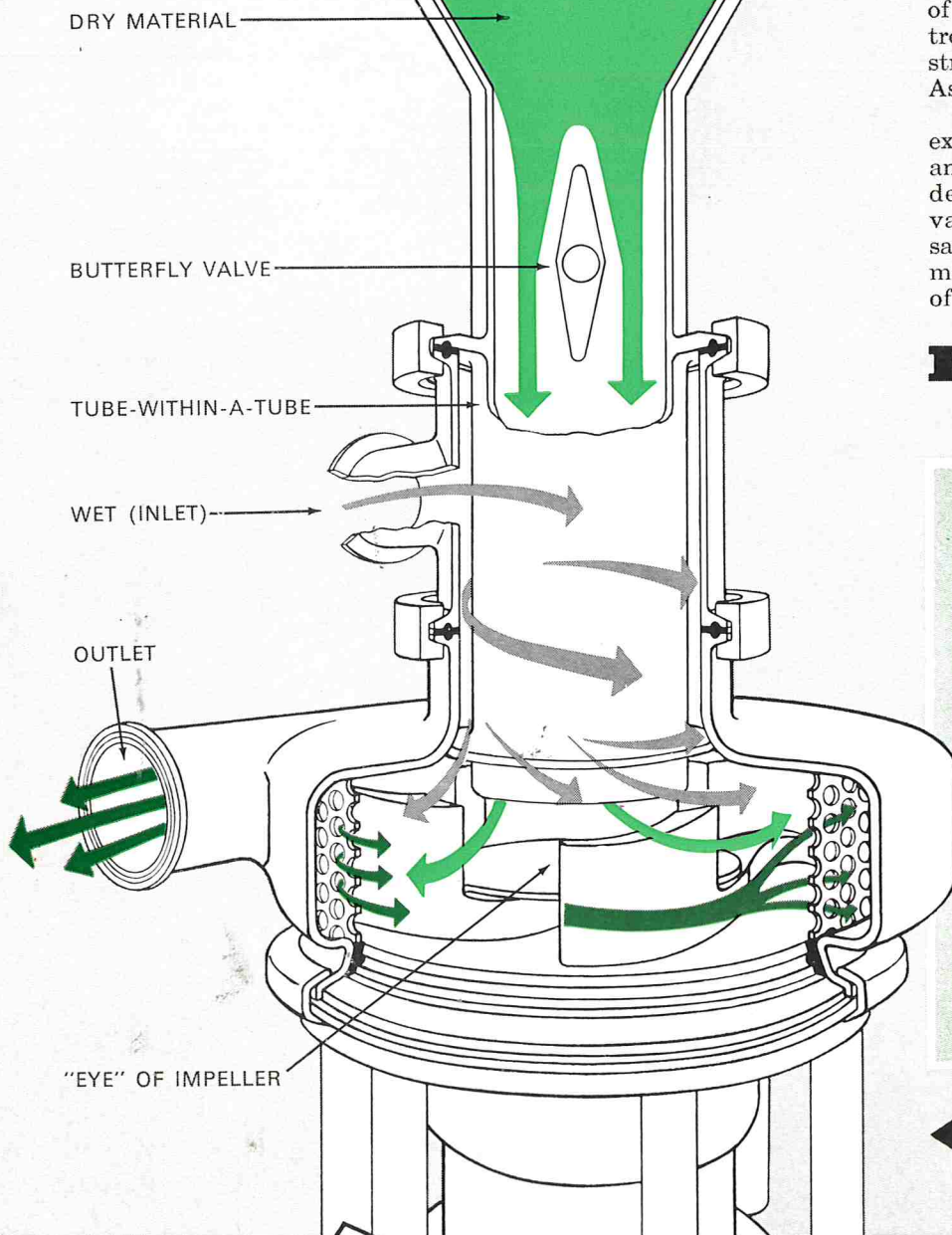
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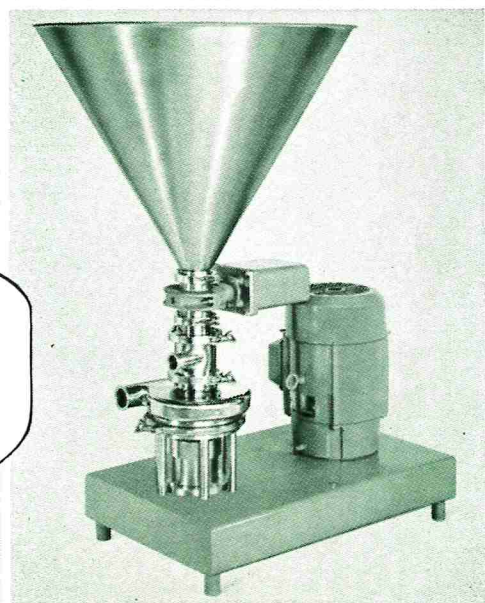
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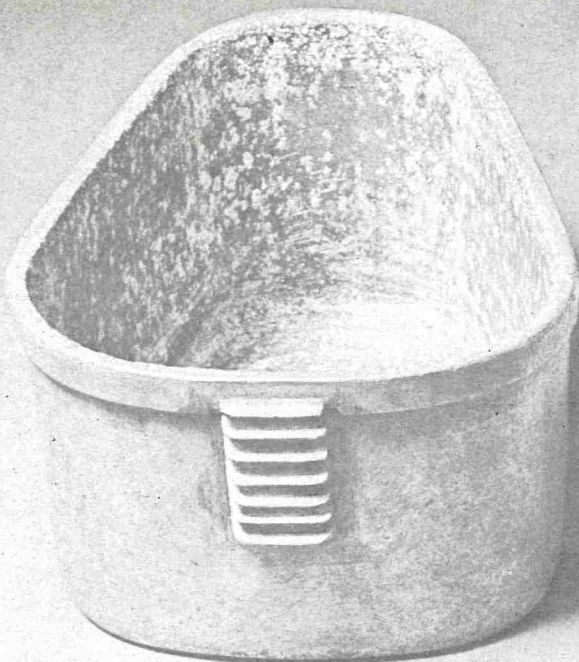


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Journal of

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INCLUDING MILK AND FOOD SANITATION

Official Publication

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

Reg. U. S. Pat. Off.

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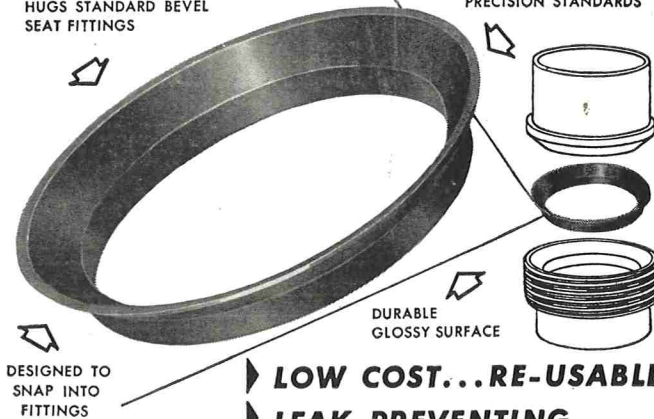
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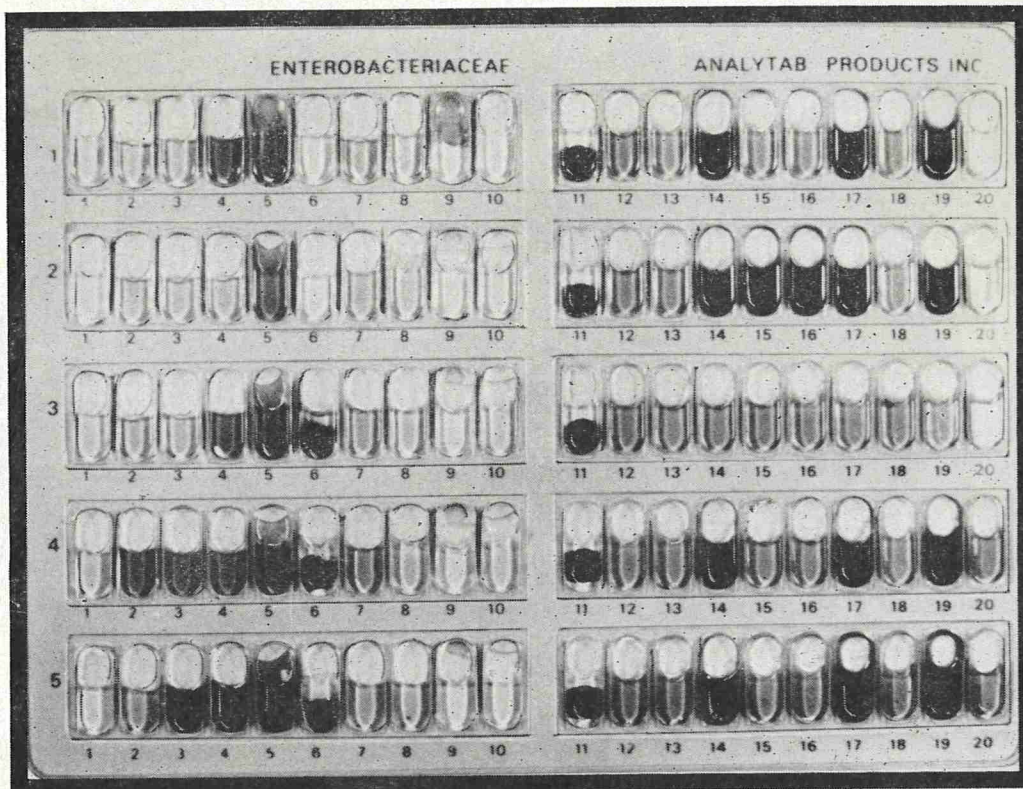
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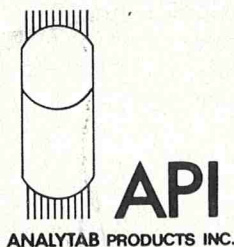
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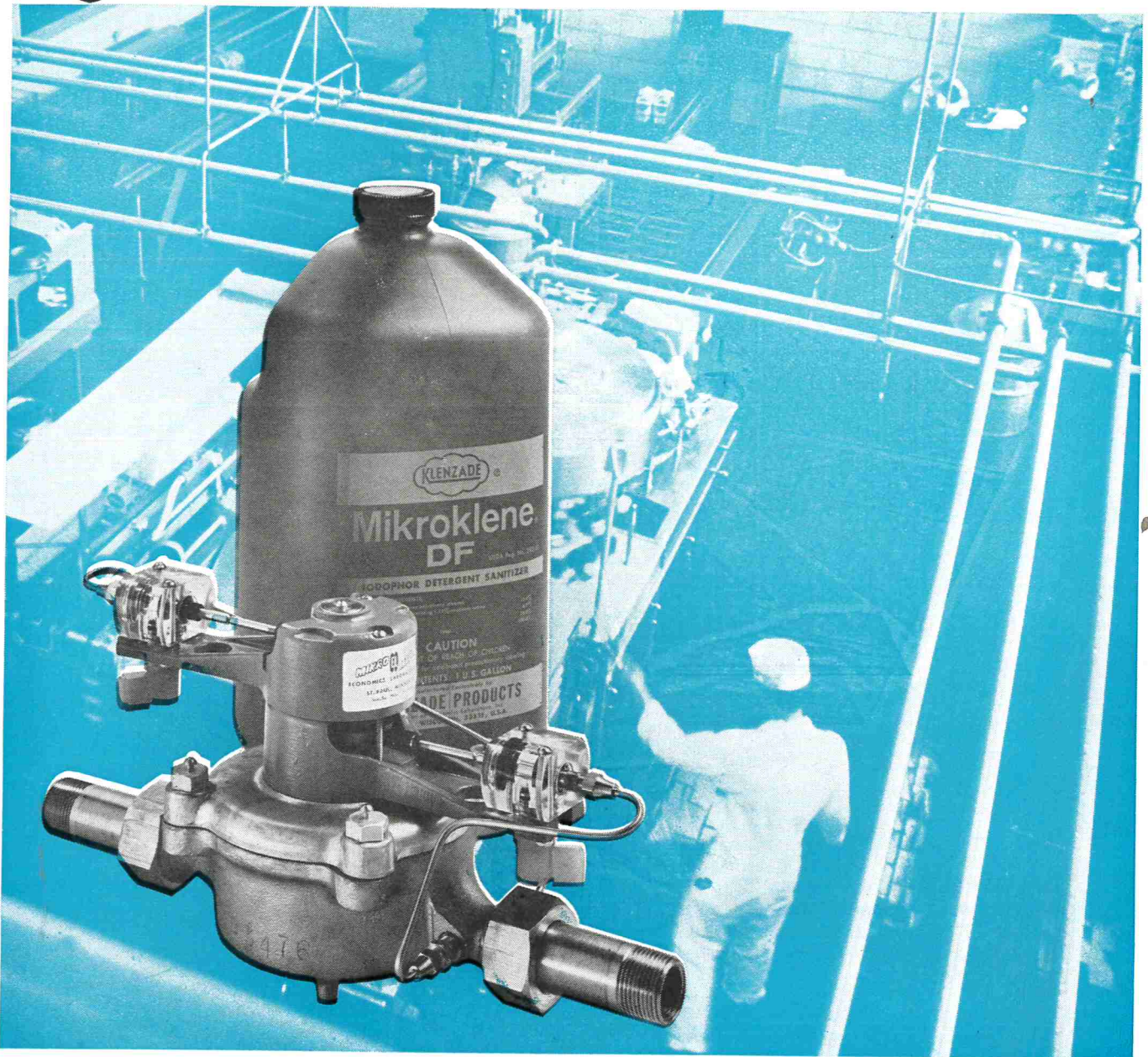
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# NITRATES IN PLANTS AND WATERS<sup>1,2</sup>

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(Received for publication April 14, 1970)

## ABSTRACT

High concentrations of nitrates in waters have been linked to methemoglobinemia in infants, and nitrates in waters and roughage have long been recognized as one cause of animal health problems. The reactions and sources of nitrogen in the environment are reviewed, and factors affecting the nitrate content of edible foods examined. The impact of changed fertilization practices and waste disposal methods on the nitrate levels in ground water requires intensive investigation. The effect of high nitrates in vegetables on infant health, and methods to minimize the nitrate content of fresh and processed vegetable products should be examined.

Nitrate is involved in the mounting concern of possible toxicants in foods and waters, and the nutrient enrichment of surface waters. This review will summarize available information on nitrogen sources in the environment and transformations in soils, waters, and plants, particularly as they influence the nitrate content of potable waters and foods.

## PROBLEMS ASSOCIATED WITH HIGH CONCENTRATIONS OF NITRATES IN THE ENVIRONMENT

Nitrate toxicity to animals was first reported in 1895 by Mayo (37) in Kansas and subsequently verified by reports from other stations (7, 26, 53). Nitrate poisoning in infants was first reported in 1945 by Comly (16) and later by several other workers (12, 15, 19, 51, 52).

It is well known that, toxicologically, nitrite is the main villain in nitrate poisoning problems. The nitrite ion has a high affinity for blood hemoglobin, and the anoxic disease, methemoglobinemia, is induced when methemoglobin, which cannot carry oxygen, is formed on reaction of nitrite with oxyhemoglobin (14, 19). Unborn children and infants not on solid foods are particularly susceptible, presumably because of their small blood volume, deficiency of methemoglobin reductase, and presence of high numbers of coliforms capable of reducing nitrate to nitrite (6, 51).

In farm animals, particularly ruminants, toxicity can range from chronic to acute with symptoms ranging from general loss of appetite, poor performance, inhibition of iodine and Vitamin A metabolism, and abortions, to death within a few hours (54). The extent of toxicity appears to be a function of many factors, including age, diet, adaptation to prolonged nitrate feeding, and species (54). Estimates of the LD<sub>50</sub> in cattle lie somewhere between 160 and 224 mg NO<sub>3</sub>-N per kilogram when nitrate is fed with roughage (54).

High dietary nitrate can arise from either the water or food. With animals, nitrate poisoning from both sources has been documented. However, to date, infant methemoglobinemia has apparently been caused primarily by high nitrates in drinking waters, although certain vegetables (e.g., spinach and beets) which normally contain high levels of nitrate have also been implicated in nitrate poisoning of infants (17). The tolerance limit for nitrate N in potable waters in the U.S.A. has been set at 10 ppm (44 ppm nitrate).

Ensiling plants high in nitrate leads to the production of highly toxic nitrous oxide. These "silo gases" have been the cause of many fatal accidents, although preventive measures have been available to the public for years.

Nitrogen and phosphorus are believed to be the nutrient elements most often limiting the productivity of surface waters (46). Whereas the amount of nitrate in surface runoff waters is generally low, nitrate in ground waters can contribute to the nutrient load of surface waters through interflow (5, 20, 34).

## FORMS OF NITROGEN IN THE ENVIRONMENT

Nitrogen is found in fundamental and sedimentary rocks, humus, living tissue, and the atmosphere. Although the atmosphere is the predominant reservoir source of nitrogen in the hydrospheric and lithospheric nitrogen cycles (there are more than 24 million tons of nitrogen above each square mile of the earth's surface), fundamental rocks contain nearly 98% of the earth's nitrogen (44). The nitrogen in soils and lake sediments is predominantly bound in organic forms (8, 33, 34, 35). Large local deposits of inorganic nitrate occur in caves, caliche, and playa

<sup>1</sup>Published with the approval of the Director, Research Division of the College of Agricultural and Life Sciences.

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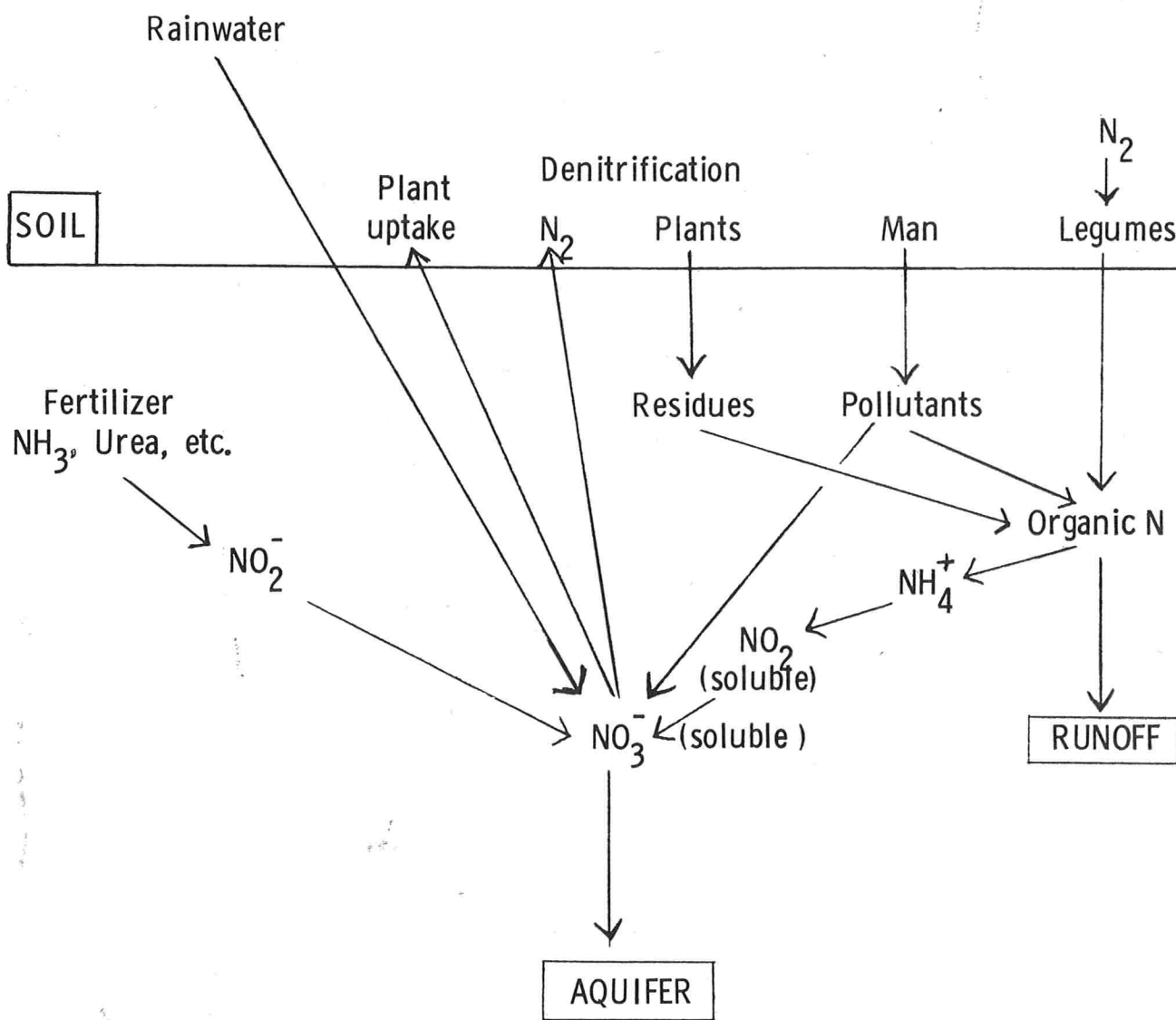
NITROGEN REACTIONS IN SOILS

Figure 1. The nitrogen cycle.

deposits (20). Inorganic nitrogen compounds in biological systems include ammonia, nitrate, nitrite, hydroxylamine, and nitrous acid, whereas numerous organic nitrogen compounds including urea, amino acids, amino sugars, purines, and proteins are important biologically.

Nitrate is seldom found at high levels in lakes and artificial impoundments, either because of its rapid use by the biota or because of denitrification (28). However, streams often contain up to several ppm of nitrate-N (20). Other forms of N in natural waters include ammonium along with trace amounts of nitrite and various organic forms of nitrogen (28).

The inorganic forms of available nitrogen in soils are predominantly ammonium, which is held as an exchangeable cation on soil exchange sites, and nitrate (27). Soils and lake sediments also retain some ammonium within clay lattices (33, 35). This clay fixed ammonium appears to be relatively unavailable to plants and microorganisms (33). Forms of nitrogen in the atmosphere include the various oxides of nitrogen, ammonium, nitrate, and particulate organic nitrogen (20).

It must be emphasized that much of the world's nitrogen is in forms that are not available. The remainder of this review will concentrate on the

sources and transformations of nitrogen that are of practical concern when evaluating a potential nitrate pollution problem.

#### TRANSFORMATIONS OF NITROGEN IN SOILS AND WATERS

A typical schematic of the nitrogen cycle is presented in Fig. 1. It should be recognized that this type of diagram is intended only to represent the possible pathways involved in nitrogen transformations in nature and that the residence time for a nitrogen molecule in a compartment may vary from a few seconds to thousands of years, depending upon the compartment and environmental conditions.

The predominant storage reservoir of nitrogen in soils is the organic matter. Added plant and animal debris are attacked readily by heterotrophic microorganisms with some of the nitrogen being added to the organic matter reserve, and some being liberated as inorganic (predominantly ammonium) waste products. Depending on the supply of available energy (carbon), inorganic nitrogen can be immobilized in microbial tissue which continually adds to the supply of organic matter. Concurrently, soil organic matter is continually depleted through microbial breakdown (2). The dynamic nature of nitrogen turnover in soils does not at first appear to be consistent with the relative stability of soil organic nitrogen. In soils, only about 1 to 3% of the organic nitrogen is available for plant uptake in a growing season (9). The stability of organic nitrogen in soils is generally attributed to formation of heterocyclic nitrogen compounds, clay-organic matter complexes which are resistant to microbial attack, and to lack of sufficient carbonaceous energy material for complete breakdown (9).

The processes leading to formation of nitrate by microbial action in soils and waters can be grouped conveniently into ammonification and nitrification reactions. Ammonification (organic nitrogen to ammonium) is conducted by numerous heterotrophs under a wide diversity of environmental (pH, temperature, moisture) conditions (2, 13, 21). With few exceptions, nitrification (ammonium to nitrite to nitrate) is carried out by strict chemoautotrophs (1). Although several heterotrophic nitrifiers have been identified, their importance in the nitrification process is believed to be minimal except in organic-rich environments such as composts and manure piles (1). Because of its agronomic importance, the nitrification process has been studied widely and reviewed extensively (13, 34, 40). In contrast to ammonification, nitrification is easily inhibited by unfavorable conditions. Optimal nitrification occurs at pH values near 7 and in the presence of adequate oxygen. The nitrification rate increases with temperature to 35 C,

but is inhibited completely at greater than 40 C. Formation of nitrite from ammonium (*Nitrosomonas* spp.) can be inhibited by numerous organic and inorganic compounds. Oxidation of nitrite to nitrate (*Nitrobacter* spp.) can be inhibited by the high pH-free ammonia conditions occurring in the area around urea or anhydrous ammonia fertilizer bands giving rise to phytotoxic nitrite accumulation in some alkaline soils following nitrogen fertilization.

The salts of nitrate are water soluble and nitrate does not react to any great extent chemically or physically with soil constituents to form insoluble products (22). As a result, nitrate in soils is readily available for plant uptake or loss to ground waters by leaching. It is this unique property of nitrate, coupled with its toxicological and nutrient properties, that make it of such concern.

The process of denitrification (reduction of nitrate or nitrite to elemental nitrogen and nitrogen oxides) provides the major pathway whereby nitrogen is cycled back into the atmosphere. Denitrification can occur by two distinct pathways. Biological denitrification occurs when oxygen tensions

TABLE 1. INORGANIC NITROGEN IN SOIL PROFILES AS AFFECTED BY LAND USE

Land use	Number of profiles	Average depth sampled (ft)	Average total content in profiles	
			Nitrate-N	Exchangeable ammonium-N
Colorado (45)				
Alfalfa	13	20	79	—
Native grassland	17	20	90	—
Cultivated dryland	21	20	261	—
Irrigated, not in alfalfa	28	20	506	—
Corrals	47	20	1,436	—
Wisconsin (28)				
Native	6	6.2	70	26
Cultivated	6	8.5	219	30
Barnyard	11	6.2	152	542

TABLE 2. NITROGEN BUDGET FOR A CONNECTICUT DAIRY FARM<sup>a</sup>

Pounds of nitrogen per cow per year			
Input		Output	
Concentrate	180	Milk	70
Fertilizer	110	Meat	10
Fixation	200	Volatilization	155
Rainfall	10		
Total	500	Total	235
NET LOSS: 265			

<sup>a</sup>Taken from Frink (22).

TABLE 3. SOURCES AND AMOUNTS OF AVAILABLE NITROGEN IN CULTIVATED WISCONSIN SOILS (1969)<sup>a</sup>

Source	Available N contribution			
	Dairy farms <sup>b</sup>		Cash crop farms <sup>c</sup>	
	(lb/A)	(%)	(lb/A)	(%)
Fertilizer	12	10	65	54
Legumes	13	11	5	4
Precipitation	8	7	8	7
Soil organic matter decomposition	45	37	42	35
Manure	42	35	0	0
Total	120	100	120	100

<sup>a</sup>Taken from Walsh (50).

<sup>b</sup>Based on a corn-oats-meadow-meadow rotation.

<sup>c</sup>Based on a corn-corn-soybean rotation.

TABLE 4. UPTAKE AND RECOVERY OF APPLIED NITROGEN BY CORN<sup>a</sup>

Rate of applied N	Grain yield	Nitrogen removed (grain plus stover)	Nitrogen recovered		
			Total	Each 90 lb nitrogen	
(lb/A)	(bu/A)	(lb/A)	(%)	(%)	(%)
0	29	58	—	—	—
90	110	142	84	93	93
180	133	182	40	69	45
270	128	184	2	42	2

<sup>a</sup>Taken from Gonske and Keeney (25).

reach very low levels (through flooding of soils and utilization of oxygen by decomposition of organic matter) and the heterotrophic microorganisms must utilize other electron acceptors. With few exceptions, carbonaceous material must be present to provide sufficient energy (11). In acid soils and waters, nitrite is extremely unstable and decomposes to yield nitrate and gaseous nitrogen oxides, or reacts with soil constituents with the fixation of nitrogen in organic matter and formation of nitrogen oxides (10, 11). These latter reactions have been termed "chemo denitrification" as they do not involve microbial transformations directly and can occur in arable soils. The importance of denitrification reactions in soils and waters cannot be overstated, and these complex and poorly understood reactions must be evaluated to enable recommendations to be made on methods of biological nitrate removal from soils and waters as well as nitrogen fertilizer rates.

A point worthy of emphasis in the nitrogen cycle is that once nitrogen enters the soils from any source, its identity is lost because of the complex series of transformations it may undergo. Thus, investigations which attempt to pinpoint sources of nitrate polluting water supplies are particularly difficult and, unless properly conducted, subject to many errors.

#### SOURCES OF AVAILABLE NITROGEN

Inputs of nitrogen into surface waters and soils can be through precipitation, from organic wastes and debris, fertilizers, and nitrogen fixed biologically, although the contribution from soil organic matter also must be considered in evaluating sources of nitrate to waters and plants. Because the intervention of man in the nitrogen cycle is of main interest here, the inputs from human activities will be given prime consideration.

In terms of sheer bulk, disposal of organic nitrogenous wastes undoubtedly is the largest single contributor of nitrates and other nitrogenous compounds, as well as to the biochemical oxygen demand in waters (49). In the U.S., about 14.5 million tons of nitrogen was removed from harvested crops in 1967 (3), and this nitrogen essentially is completely transferred back to the environment as plant debris or wastes of some form or other. Obviously, the ultimate solution in waste disposal is the re-use of nitrogen from wastes to support plant growth, but the many economic problems involved appear to make this solution impractical at present.

The shift to intensive agriculture has and will continue to create problems of animal waste disposal and unwise fertilizer use. Farm manures applied to sloping frozen ground may be flushed to streams with the onset of spring rains (28). Several investigations have shown that percolates from feedlots or from soils on which manure has been indiscriminately applied are high in nitrate (18, 24, 28, 42, 43, 45). Data from some of these investigations are presented in Table 1. These data must be interpreted with caution, however, as wide variation in the nitrate-N levels among samplings within each land use category usually are obtained. Also, because the acreage of corrals and barnyards are much less than that of croplands, the magnitude of nitrate contamination would be much greater in the cultivated systems. The high nitrate levels under feedlots, however, obviously present a real threat to the water supplies of farm operations, as these wells usually are shallow and located close to the feeding operation.

An interesting approach to the problem of farm waste disposal was taken by Frink (22). Frink evaluated the inputs and outputs of nitrogen in north-

TABLE 5. ESTIMATES OF NITROGEN ADDED TO THE ENVIRONMENT EACH YEAR FROM VARIOUS SOURCES (CONNECTICUT, 1969)<sup>a</sup>

Source	Tons
Industry	44,000
Automobile	38,000
Domestic wastes	16,500
Animal wastes	13,500
Animal feed	13,500
Agricultural fertilizer	4,600
Non-agricultural fertilizer	2,800

<sup>a</sup>From Frink (22).

TABLE 6. FACTORS AFFECTING THE NITRATE CONTENT OF PLANTS

<i>Plant factors</i>
Species
Variety
Plant part
Stage of maturity
<i>Environmental factors causing an increase in nitrate</i>
Drought
High temperatures
Shading and cloudiness
Time of day (high early, low late in day)
Nutrient deficiencies (P, K, Ca)
Excessive soil N (manure, legume residues, fertilizer)
Plant damage from insects, etc.
Certain weed control chemicals

eastern U.S. dairy farms and found (Table 2) that over one-half of the nitrogen input was lost. This nitrogen ultimately appears in surface and ground waters. The efficiency of nitrogen conversion also was related to the amount of land available per cow, and the results showed that increasing the farm size (acres per cow) from 1.6 to 2.6 resulted in a 3-fold reduction in net nitrogen loss. Frink suggested that improvements in cropping systems, milk production, and fertilizer and manure handling could be used to reduce nitrogen losses in dairy farm operations.

Another obvious and highly visible source of nitrogen to the environment is nitrogenous fertilizers. The switch to intensive cropping systems and concurrent lowering of nitrogen fertilizer prices has resulted in a marked increase in nitrogen fertilizer usage in the past decade. Commoner (17) has implied that nitrogen fertilizers are "destroying" the nitrogen cycle and are the cause of much of the high nitrates in our wells and crops.

Precise experimental data to confirm or refute Commoner's claims are not available. However, much circumstantial evidence can be cited to show that

nitrogen fertilizer usage is, in general, within reasonable limits and not a source of massive pollution.

Nationwide, about one-half of the nitrogen removed by crops was supplied from fertilizers in 1967 (3). Data from Wisconsin (50) shows that the potential for nitrogen fertilizer pollution is dependent on the cropping system (Table 3). Nitrogen fertilizers supply a much higher percentage of the available nitrogen in soils with a cash crop than with a dairy farm system.

The effect of cropping system, soil, type, and amount of nitrogen fertilizer on nitrate in soil profiles has been investigated in Wisconsin (28, 39). These findings show that the amount of nitrate in the soil profile is influenced by the rate of nitrogen fertilization and frequency of the fertilized crop (corn) in the rotation. Very little accumulation of nitrate in the profiles of silt loam soils occurred when up to 100 lb. of nitrogen (the approximate amount needed by a 100-bushel corn crop) was applied, but higher additions of nitrogen fertilizer resulted in nitrate accumulation in the soil. With a sandy soil under irrigation, nitrate was moved below the rooting zone in about two months time. In a medium fertility silt

TABLE 7. NITRATE-NITROGEN CONTENT VEGETABLES

Vegetable	Nitrate-N, ppm dry weight		
	1907 (41)	1964 (31)	1964 (43)
	(Avg.)	(Avg.)	(Range)
<i>Fresh</i>			
Beans, string	52	100	900-1,600
Beets	590	270	1,300-10,000
Broccoli	—	—	900-1,900
Cabbage	44	72	300-3,800
Carrots	15	22	100-1,000
Cauliflower	—	—	200-3,100
Celery	340	635	1,100-7,400
Cucumbers	—	—	0-1,600
Kale	—	—	1,500-11,000
Lettuce	390	150	700-13,900
Mustard	—	—	1,400-14,000
Onions	52	39	—
Peas	11	10	—
Potatoes, Irish	17	24	—
Potatoes, sweet	15	10	—
Radish	410	340	1,900-15,000
Spinach	430	120	700-2,500
Squash	—	—	900-5,100
Tomatoes	12	16	0-1,100
<i>Baby foods</i>			
Beans, green	—	—	400-2,900
Beets	—	—	900-8,400
Carrots	—	—	600-3,000
Corn	—	—	0-100
Peas	—	—	0-200
Potatoes, sweet	—	—	0-400
Spinach	—	—	1,100-8,200
Squash	—	—	100-1,300

loam soil, nitrate-N in the soil solution exceeded the 10 ppm tolerance limit even when nitrogen fertilizer had not been added for at least four years.

Nitrate contamination of ground waters can be minimized in agricultural situations by effective removal of nitrate from the soil by the growing crop. The nitrogen thus is utilized in producing food or fiber and is kept within the soil nitrogen cycle (Fig. 1). When excess amounts of nitrogen are applied, crop recovery of fertilizer nitrogen decreases. This is illustrated by the data in Table 4 (25).

Precipitation supplies generally about 4 to 10 lb. of nitrogen per acre per year, mainly as ammonium and nitrate (20). The sources of atmospheric nitrogen include photochemical decomposition, industrial and auto exhaust emissions, denitrification, and ammonium volatilization. Hutchinson and Viets (29) found that ammonium was evolved from cattle feedlots and could be absorbed directly from the atmosphere by nearby lakes. Frinks (22) indicates that considerable nitrogen is added to the Connecticut environment by combustion processes (Table 5), although calculations show that only about 10% of the nitrogen so added returns by precipitation (C. R. Frink, *personal communication*).

The soil organic matter is a source of nitrate to waters and plants that is often not considered when evaluating nitrogen sources (34). If a soil contains 0.2% organic nitrogen and 1 to 3% of this nitrogen is released by microbial processes per year (8), from 20 to 60 lb. of nitrogen per acre is released. This release is stimulated markedly by cultivation, as exemplified by the fact that the nitrogen content of soils decreases by 30 to 40% on cultivation within a period of a few decades (44). Mere organic matter breakdown caused by cultivation has been implicated as providing the bulk of the nitrate in some profiles (45, 47). Hanway (26) pointed out that many high nitrate wells existed in Iowa during the 1940's, before nitrogen fertilizer usage was at its current high level. Obviously, the fertile, high organic matter soils were the source of much of this nitrate. Considering the slow movement of percolate waters in many areas, the high nitrate levels in some U.S. aquifers may simply reflect the onset of farming more than a century ago.

Nitrogen fixation by legumes in symbiosis with the genus *Rhizobium* (38) contributes significantly to the available nitrogen in soils (see Table 3). A good legume crop will fix up to 200 lb. of nitrogen per year, of which much will be removed by harvesting. Free-living soil bacteria also fix nitrogen (32), although the best estimates indicate that the contribution to the nitrogen balance by free-living bacteria is of little significance agronomically. The potential importance of symbiotic and non-symbiotic nitrogen

fixation to the nitrogen balance of soils has been pointed out by Stout and Burau (47).

Geologic sources of nitrate have been largely ignored (20). As mentioned previously, nitrate can occur in cave, caliche, and playa deposits, and also in sedimentary rocks (20, 34). It would appear that geologic sources of nitrate must be considered when evaluating sources of nitrate to ground waters.

#### NITRATE MOVEMENT IN SOILS

The amount of nitrate-N required to bring a soil percolate to the 10 ppm tolerance limit is about 2.2 lb. per acre-inch of water (34). If deep percolation out of the root zone is about 6 inches of water per year (common in midwestern U.S.), then only 13.2 lb. of nitrate-N per year need be leached to reach the tolerance limit.

The nitrate concentration of the point in the ground water or stream of concern need not be the same as that of the soil solution (23, 34). This is because of the mixing (dispersion) which goes on during the flow process. The dispersion process has been studied in detail theoretically (23), but to date has not been applied to practical problems involving nitrate movement. However, movement of nitrate is quantitatively the same as for chlorides which have been investigated in studies on soil salinity. As a first approximation, it can be stated that nitrate moves with the water, whether water is going into the plant, upward because of evaporation, or downward due to gravity. It is of interest to note that nitrate moved downward in a silt loam profile at the rate of about 16 inches per year (28).

#### NITRATE CONTENT OF PLANTS

As mentioned previously, the available literature on nitrate toxicity hazards indicate that high nitrate foods have not been hazardous to humans, although detrimental effects to livestock fed high nitrate roughage have been thoroughly documented. However, this is not to preclude the possibility that nitrate toxicity, perhaps at the chronic level, may eventually be proven for foods, or that nitrosamines formed from ingested nitrites and secondary amines might be implicated as a causal agent for cancer in man (36). The recent publicity given to high nitrate baby foods (17) behooves us to examine this problem closely.

The nitrate content of plants is a function of a number of plant and environmental factors (Table 6). Some plants, particularly those of the *Chenopodiaceae* family (spinach, kale, beets) are notorious for their high nitrate content (43). The need for nitrogen fertilizer to obtain optimum yields of high value horticultural crops has long been known, and current fertilizer practices would hardly be expected

to change materially the nitrate content of these crops. Data are presented in Table 7 to compare the nitrate content of vegetables in 1907 to that found in recent analyses. These data indicate the wide variability in nitrate content of edible portions of plants and the predominant influence of plant species on the nitrate levels found. It should be mentioned that the shift to mechanical harvesting, which has occurred largely after the data in Table 7 were obtained, may somewhat alter the pattern of nitrate accumulation. For example, tomatoes are forcibly matured by, in part, withholding nitrogen fertilizers toward the end of the growing season, whereas succulent crops such as lettuce are kept in prime condition for harvest by over-fertilizing with nitrogen. It would appear that the influence of cultural practices involved with the changing agricultural scene should be continually monitored with respect to the nitrate content of the edible crops.

Many of the empirical observations on the effects of plant species and environmental factors on the nitrate content of plants are readily explicable on the basis of the activity of nitrate reductase in plants. The literature on nitrate reductase in higher plants has recently been reviewed thoroughly (4). Nitrate reductase is an adaptive, inducible, short-lived (4 hr half time) enzyme. The enzyme contains molybdenum, ferrous iron, and the coenzyme, flavin adenine dinucleotide. The electron donor appears to be, in most instances, nicotinamide adenine dinucleotide. The reduction of nitrate in plants to the ammonium form requires 8 electrons, the energy being supplied as solar energy. It appears that a similar nitrite reductase system operates in plants, although little information is available on this enzyme.

Aside from the fact that the nitrate reductase activity of plant species and varieties varies according to their genetic ability to produce nitrate reductase, the environmental factors listed in Table 6 also influence nitrate reductase activity (4). In fact, any environmental change which reduces plant growth without markedly affecting water uptake will result in higher nitrate levels in plants, because nitrate reductase activity is so closely interwoven with the environment (4).

The effect of weed control chemicals on the nitrate content of plants is of considerable interest. Broad-leafed weeds, cucumbers, and sugar beets will accumulate nitrate if sprayed with 2, 4-D, apparently because of a decrease in nitrate reductase activity, whereas members of the *Gramineae* family (e.g., corn) showed the opposite effect (4). Similarly, simazine treatment of tolerant plants has been shown to increase nitrate reductase activity with resultant increase in growth rates and nitrogen content of the plants (48). Beever and Hageman (4) suggest in

their review that plant breeding programs should be undertaken to obtain varieties high in nitrate reductase activity for optimum utilization of nitrate and maximum yields. If nitrate content of our edible foods also becomes a severe problem, perhaps similar breeding programs in horticultural crops should be established.

### CONCLUSIONS

Whereas the nitrogen cycle in nature is understood fairly well, further information is needed on the impact of changed agricultural practices, and of agricultural, urban, and industrial waste disposal methods on the nitrate levels of ground water supplies. The nitrate content of many vegetables appears to be related to factors difficult to control when economically feasible yields are obtained. Investigations need to be initiated to evaluate the effect of high nitrate foods on humans, especially infants, and on edaphic, genetic, cultural, and processing methods to minimize high nitrates in vegetables.

### ACKNOWLEDGEMENT

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*A Research Note***FUNGI IN FOODS****I. EFFECT OF INHIBITOR AND INCUBATION TEMPERATURE ON ENUMERATION<sup>1</sup>**

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## ABSTRACT

Antibiotics were far superior to acidification for controlling bacteria when counting fungi in foods. Yeast and mold counts from Potato Dextrose Agar containing antibiotics were much higher than those obtained in the acidified medium, and growth of bacteria was never a problem. When incubation temperatures of 32 and 22 C were compared, more colonies appeared at 22 C.

In the classic studies of White and Hood (7, 8) they suggested that a pH of 3.5 be used to selectively inhibit bacteria when enumerating yeasts and molds in butter. However, numerous workers (2, 4, 5, 6) have observed that a pH of 3.5 is often inhibitory to a portion of the fungal population in foods. This inhibition of growth is far more apparent within a naturally occurring population of organisms, as in a food sample, than it is with laboratory cultures (6). Nevertheless, this fact has been largely ignored because of the difficulty of controlling the bacterial contaminants in a sample.

Undoubtedly a number of factors have contributed to the lack of interest in updating methodology for fungal counts. With the present concern over mycotoxins and the impact of an ever changing technology on the selective development of microbial populations in foods, it seems necessary to reevaluate present methodology in this area so as to have available the best possible procedures for enumerating and isolating fungi from foods.

## MATERIALS AND METHODS

*Sources of samples*

Samples listed in Table 1 were obtained from food stores in the Gainesville, Florida area.

*Preparation of samples*

Initial dilutions were prepared by blending 11 g of sample with 99 ml of buffered dilution water (1) for 2 min. Further decimal dilutions were prepared as needed. Two sets of plates were poured with Potato Dextrose Agar (Difco) acidified to pH  $3.5 \pm 0.1$  with sterile 10% tartaric acid (1), and with Potato Dextrose Agar containing 100 mg/l each

of chloramphenicol and chlortetracycline HCl (3). One set of plates was incubated at 22 C, the other at 32 C. The Potato Dextrose Agar to which the antibiotics were added following sterilization was adjusted to pH 7.0 with 1N NaOH before autoclaving. Plates incubated at 22 C were counted after 5 and 7 days, whereas the plates incubated at 32 C were counted after 3 and 5 days.

Periodically, plates were selected at random and all colonies were picked, stained (Gram), and examined for bacterial growth.

## RESULTS

Of the many hundreds of colonies stained from both media, bacterial growth was found only on the acidified medium. In some instances bacterial growth was so pronounced on the acidified medium that meaningful counts could not be obtained and the plates had to be discarded. In no instance was bacterial growth found on the medium containing antibiotic. Except for an occasional sample, yeasts were more predominant than molds.

Table 1 lists the products sampled and the recovery of the organisms according to medium and temperature of incubation. The 7-day counts at 22 C and the 5-day counts at 32 C did not differ greatly from those obtained at the earlier counting period. If anything, the counts exhibited a tendency to be slightly lower at the latter counting periods. This phenomenon was attributed to coalescing of colonies.

In general, highest counts were obtained at 22 C using antibiotics as the inhibitor and lowest counts were obtained at 32 C with the acidified medium.

## DISCUSSION

Other reports in which various conditions have been evaluated for enumerating fungi have not always demonstrated the marked effect observed in this study (5, 7). This is not totally unexpected when one realizes that laboratory cultures were used previously. Laboratory cultures are propagated under ideal conditions, ensuring a vigorous and healthy population of cells, whereas the flora present in a food product may have been stressed by exposure to a number of debilitating conditions such as extremes of

<sup>1</sup>Florida Agricultural Experiment Stations Journal Series No. 3618.

TABLE I. INHIBITOR AND TEMPERATURE EFFECTS ON THE RECOVERY OF FUNGI FROM FOODS

Sample	Incubation temperature			
	22 C		32 C	
	Acidified agar	Antibiotic agar	Acidified agar	Antibiotic agar
	(No./g)			
Mullet fillet	24,000	270,000	10,000	41,000
Flounder fillet	3,400	7,600	<10	<10
Breaded shrimp	16,000	45,000	1,100	3,400
Halibut steak	400	800	100	800
Frozen perch	8,100	27,000	2,700	7,300
Oysters	1,700	5,200	130	1,400
Cottage cheese	260,000	290,000	6,600	8,400
Cheddar cheese	5,400	6,600	400	3,000
Cream cheese	14,000	23,000	14,000	33,000
Butter	1,400	1,700	830	1,300
Raw milk	30	60	10	30
Edam cheese	380,000	790,000	73,000	390,000
Ground beef	31,000	38,000	1,900	18,000
Chicken	38,000	97,000	18,000	63,000
Sandwich steak	800	32,000	200	1,600
Sausage meat	1,500	4,900	300	2,800
Ground pork	19,000	35,000	1,900	5,700
Beef liver	3,500	28,000	800	7,800
Apple	11,000	23,000	4,300	9,200
Peach	16,000	26,000	6,000	5,300
Radish	1,800	4,700	700	3,200
Carrot	7,000	18,000	4,600	9,700
Broccoli (frozen)	800	5,300	500	2,200
Pizza (refrig)	2,800	3,600	2,000	2,600
Frozen orange conc.	1,300	1,500	1,200	1,300
Turkey pot pie	70	900	20	70
Dry spaghetti sauce mix	250	100	<10	<10
Flour	140	160	150	160

pH, salt, temperature, moisture, and competition for nutrients. In addition, processing procedures may have imposed an additional injury during preparation of the food. These factors can bring about a sensitivity to acid not demonstrable in a healthy culture.

There is no evidence to suggest that two distinct populations are being measured with the two inhibitors studied. In fact, most of the organisms studied to date, isolated from the antibiotic medium, have been able to grow at a pH of 3.5.

It is apparent from the data presented that a true measure of the fungal population in foods is not obtained when standard procedures are followed. Both of the factors studied exerted a marked effect on recovery with some samples exhibiting a more pronounced effect than others. Unquestionably other factors not studied at this time influence growth of the organisms. Until these factors are known and controlled, a true picture of the fungal population in foods is not obtainable.

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# ACTIVATION OF BACTERIAL SPORES. A REVIEW<sup>1</sup>

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## INTRODUCTION

Thermal processing of canned foods is employed to produce a wholesome, safe, and stable product. The amount of heating required to attain this goal depends to a large extent upon the heat resistance of the most resistant spoilage or food poisoning organisms likely to be present. Although some thermophiles may require up to several minutes at 80 to 90 C for complete destruction, vegetative cells of bacteria are generally quite easily destroyed. Bacterial endospores vary greatly in heat resistance according to the species of bacterium and conditions during sporulation, but all are far more resistant than the corresponding vegetative cells. Spores of *Clostridium botulinum*, for example, may survive boiling temperatures for several hours, and spores of flat-sour bacteria for over 17 hr (11). To produce a product which is "commercially sterile," the canner must therefore apply that amount of heat which will ensure the complete elimination of bacterial spores which may germinate and grow under normal conditions of storage. In many instances, the severe heating required produces extensive organoleptic and nutritional changes, resulting in a product with little resemblance to the original food. Extensive work has been done to explore the possibility of reducing the severity of heat processes for canned foods by chemical additives (36). As our understanding of the nature of spore dormancy increases, perhaps it will one day be possible to break the dormant state by relatively mild physical or chemical treatment, resulting in a product with greatly reduced thermal processing requirements.

The transformation of a bacterial endospore into a vegetative cell involves at least three sequential processes: activation, germination, and outgrowth.

Activation is a reversible process which results in a spore which retains its typical heat resistance, non-stainability, refractility, and dipicolinic acid (DPA) content, but which is no longer dormant. "Dormant" in this sense means not only that the spore is metabolically inactive, but that it fails to germinate under

apparently favorable conditions. Activation, then, is a process of conditioning the spore to germination. Activation does not occur in all spores, and requirements depend upon the sporulation medium, chemical composition of the spore, its storage history, and the nature of the germinating agent. Present evidence indicates that activation does not involve metabolism, but consists rather of changes in the configuration of macromolecules.

Germination, an irreversible process, results in a cell which has lost the typical characteristics of a bacterial spore. Germination occurs in the presence of inhibitors of macromolecular synthesis, and is therefore a process during which there is no *de novo* synthesis of macromolecules. On the contrary, spore components are broken down and excreted into the medium during germination, so that it is considered to be a process of degradation accompanied by the initiation of spore metabolism. Rapid and complete germination occurs only after activation, and is triggered by specific germination-inducing agents, such as L-alanine.

Outgrowth is a process of synthesis of new macromolecules. During the process of outgrowth new kinds of protein not present in the spore stage are produced, and new structures are formed, ultimately leading to the emergence of a new vegetative cell.

Each stage in the transition from spore to vegetative cell—activation, germination, and outgrowth—has a characteristic response to temperature (Fig. 1). Optimal activation temperatures are generally considerably higher than the optima for germination and outgrowth (34).

Curran and Evans (6, 7) were the first workers to demonstrate systematically that sublethal heat could induce dormant spores to germinate. Later workers found that spores also were activated by high or low pH (12), by reducing agents and low pH (26), by exposure to calcium-DPA solution (29), by dimethylformamide and dimethylsulfoxide (46), by aging (40), by aqueous solvents (21), by water vapor without heating (22), and by ionizing radiation (17). This is by no means a complete listing; numerous combinations of chemical and physical treatments resulting in activation may be found in the literature. Heat treatment is the most studied method of activation;

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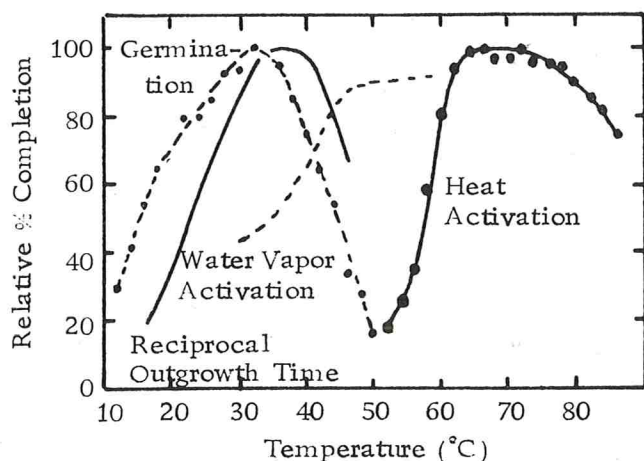


Figure 1. The effect of temperature on spore activation, germination, and outgrowth. Ordinate represents relative per cent completion of event, with 100% completion taken as maximal value attained for each event. (Reproduced with the permission of Levinson and Hyatt, 34).

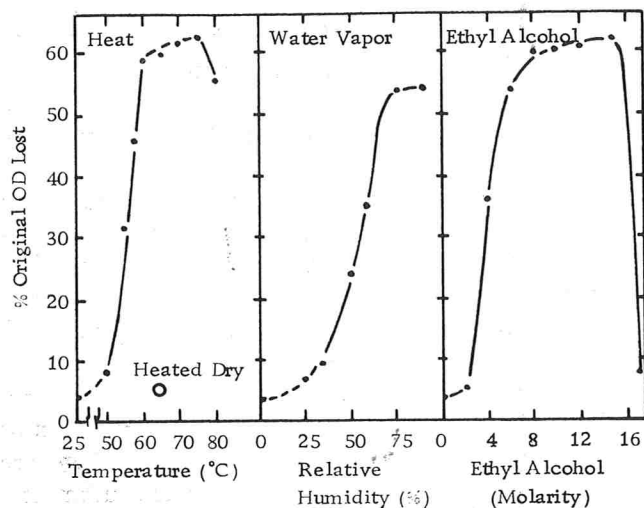


Figure 2. Activation of *Bacillus megaterium* QM B1551 spore germination by heat, water vapor, and aqueous ethyl alcohol. Spores were exposed to heat for 10 min, to water vapor for 120 min at 30 C, or to aqueous ethyl alcohol for 5 min, at 30 C. Germination was determined after incubation for 120 min at 30 C in 25 mM glucose. (Reproduced with the permission of Levinson and Hyatt, 32).

the other procedures generally differ in being less efficient.

#### HEAT ACTIVATION

The conditions required for activation may be very different in spores of different species. Some strains of *Bacillus megaterium* require only a few minutes at 60 C for optimal germination rates, whereas thermophilic organisms such as *Bacillus stearothermophilus* may require up to 115 C for optimal activation (7, 10). Figure 2 shows a typical heat activation curve of *B. megaterium* (32).

A number of environmental factors are important in heat activation. It must be carried out in the presence of water (Fig. 2), a requirement which appears to be common to other methods of activation. Heat activation also is pH dependent. Keynan et al. (27) have shown that activation can be prevented by a low pH; *Bacillus cereus* T spores exposed to a pH lower than 2 at 65 C for 30 min or 1 hr do not germinate in L-alanine or adenosine, both of which are potent germinators, even when their concentrations are increased 100-fold. The spores, however, are still viable and will germinate in a rich nutrient broth. It was found that a short exposure at 65 C at pH 1 rapidly activates spores, with maximum activation attained in 10 min. When incubation is continued, however, the ability to germinate in an L-alanine-adenosine medium decreases as a function of time. Lee and Ordal (29) also have reported that exposure to low pH reverses activation of spores. It appears that while short exposures to low pH induce activation, a longer exposure to low pH blocks the ability of the activated spores to respond to L-alanine-adenosine germination (27). *Bacillus cereus* T spores are not activated at pH values above 8.5 when heated for 1 hr (26). The same spores behave as activated spores when washed, resuspended at neutral pH, and then heated for 1 hr at 60 C, indicating that activation is inhibited at a high pH, without injury to the spores. There is, however, considerable variation among different bacterial species. Gibbs (13) has reported that activation of *Clostridium bifermentans* spores is optimal at high pH and inhibited at low pH.

Certain cations may interfere with activation when present during heating. Splittstoesser and Farkas (44) have demonstrated an inhibition of heat activation of *Bacillus popilliae* spores in the presence of potassium ions. Heavy metals such as iron and copper inhibit the activation of spores of *B. cereus* T (39) and of *B. megaterium* (30). Dormant *B. stearothermophilus* spores may be activated by hydrogen ions (35), and *B. megaterium* spores by  $\text{Ca}^{++}$ ,  $\text{Sr}^{++}$ , and  $\text{Ba}^{++}$ . A "trigger reaction" prior to germination of *Bacillus licheniformis* spores was competitively inhibited by  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  (18).

Curran and Evans (2) noted that the composition of the suspending medium determined the degree of activation of their strains. Heat activation of *C. botulinum* type B spores varied from none to about 15 times, depending upon the composition of the sporulation medium (43).

Loss of activation with reversion to the original dormant state is commonly observed when activated endospores are stored for several days (13, 26). This deactivation may be affected by environmental con-

ditions. Deactivation of *B. cereus* spores was slow at 4 C and absent at -20 C, indicating the temperature dependence of the reaction (26). In some instances, deactivation can be chemically induced. Hyatt et al. (22) showed that activated spores of *B. megaterium* were deactivated by absolute alcohol and could subsequently be reactivated by heat.

An interesting phenomenon was observed with two strains of *B. stearothermophilus* spores (10). Maximal activation is usually achieved at temperatures of 110 to 115 C. When spores were heated in distilled water at 80, 90, or 100 C, however, dormancy was increased. This bacterium is of particular interest to the food processing industry because the spore form is extremely heat resistant and the vegetative form is responsible for flat-sour spoilage of low acid canned foods.

#### ACTIVATION BY CALCIUM-DPA

Rieman and Ordal (42) showed that certain *Bacillus subtilis* spores would germinate without heat activation, merely by the addition of equimolar concentrations of calcium and DPA. Keynan et al. (28) made similar observations on spores of *B. cereus* T. Spores of *B. subtilis* 168, on the other hand, were activated by these concentrations of calcium-DPA, but failed to germinate (12). Lee and Ordal (29) were able to isolate refractile spores of *B. megaterium* from a calcium dipicolinate germination solution incubated at 7-10 C; the spores were activated and would germinate spontaneously in distilled water. The activated spores could be deactivated by treatment with acetic acid.

#### ACTIVATION BY LOW pH AND REDUCING AGENTS

It has already been stated that the degree of heat activation is pH dependent. In addition, low pH alone can activate spores for germination. Keynan et al. (26) reported that prolonged incubation of *B. cereus* T spores in a buffer of low pH led to spontaneous germination, and suggested that this may be a significant method of breaking dormancy in nature. It has recently been shown that spores of *B. cereus* and *B. subtilis*, which require 30 min and 3 hr, respectively, for activation in distilled water, were fully activated during 12 min at pH 1.0 at the same temperature (24). This might suggest that low pH activation proceeds through a different mechanism than that of heat activation.

Heat activation of bacterial spores can be imitated by suspending spores in reducing agents. Reversible activation of *B. cereus* T spores has been induced by mercaptoethanol and thioglycolate (26). The process is rather inefficient since no effect was observed in less than 12 hr. Incubation of spores of *C.*

*bifermentans* in the presence of mercaptoacetate, however, did not result in activation; on the contrary, activation appeared to be inhibited (13). The activation of spores in the presence or absence of reducing agents is influenced by pH. Activation between pH 4.5 and 7.2 was potentiated by the presence of reducing agents, whereas at a pH value below 4.5, the pH dependence of spontaneous activation paralleled that of activation induced by reducing agents (26).

#### ACTIVATION BY CHEMICAL TREATMENT

Very polar solvents such as dimethylformamide, dimethylsulfoxide, and concentrated solutions of urea have been shown to activate spores of *Bacillus pantothenicus* at room temperature (46). Activation in 90% dimethylformamide was so rapid even at 4 C that maximum activation was produced in about 1 min. Spores of *B. cereus* T can be activated by D-cycloserine and D-carbamyl-D-serine (14). It was suggested that these antibiotics increase the rate and amount of L-alanine induced germination of spores which contain alanine racemase, by preventing the production of inhibitory D-alanine.

Dormant spores of *B. megaterium* were activated for germination on glucose by treating them with aqueous ethyl alcohol at 30 C (23), or by exposing them to water vapor at room temperature (22, 23) (Fig. 2). The degree of water vapor activation depended upon the time, relative humidity, and the temperature of exposure. Prolonged water vapor treatment also activated spores for germination induced by L-alanine and KI. Spores activated by these methods were deactivated by treatments for either 40 hr over P<sub>2</sub>O<sub>5</sub> or 18 hr in 100% ethyl alcohol at 66 C. Reactivation could be induced by heat, by 5 M ethyl alcohol, or by water vapor.

#### ACTIVATION BY IONIZING RADIATION

The first report of activation of bacterial endospores by ionizing radiation appeared in 1960 (31). Irradiated spores of *B. megaterium* were able to germinate even when damaged to the extent that germinated spores were unable to divide. Spores of *B. cereus* PX exposed to  $\gamma$ -radiation became progressively more activated as the dose increased to 1.08 Mrad (17). Activation by heating and irradiation were similar in that both kinds of activated spores could be deactivated, but differed in that irradiated spores but not heated spores germinated faster than untreated spores in n-dodecylamine.

#### ACTIVATION BY AGING

Spore populations, even when stored at low temp-

eratures and under relatively dry conditions, undergo marked changes with time. These changes are considered to be the result of aging. Aging and heat activation appear to be similar phenomena; long exposure of spores to low but stable temperatures causes activation. Exposure at 5 C for 525 days, for example, activates spores of *B. subtilis* (3). The data obtained in this study indicated that within the range of temperatures studied (5-94 C), the heat activation response obtained at the lower temperatures over extended periods was similar to the response obtained at the higher temperatures during short periods of exposure. The aging effect has been observed even at freezing temperatures. Halvorson and Swanson (19) observed that *B. cereus* T spores that had been frozen and stored for 2 weeks or longer germinated more readily than did freshly harvested spores. Thermodynamically there appears to be no difference, in most instances, between aging and heat activation. Levinson and Hyatt (33), however, reported that the heat activation kinetics of *B. megaterium* spores indicated it would take about 20,000 years to attain a germination rate of 1.0% OD loss/min at 5 C. It seems likely, therefore, that any observed aging in this organism at low temperatures must result from changes in the spores not related to heat.

Activating and aging both terminate the state of dormancy; the primary difference is that activation leads to a reversible loss of dormancy while aging causes dormancy to be irreversibly lost.

#### CHANGES IN SPORES AS A RESULT OF ACTIVATION

Heat activation has been observed to induce at least four changes in spore suspensions. It increases the germination rate, activates enzymes which are dormant in the resting spores, changes germination requirements, and induces changes in morphology, permeability, and spore composition.

The degree to which unactivated spores can be stimulated to germinate varies. Germination can often be induced in a small percentage of a spore population by placing the spores into a rich medium, but it will be slow and unsynchronized, and proceed only after a long lag period. Activation increases both the extent and rate of germination dramatically. Powell and Hunter (41) reported that the rate of germination of preheated spores in 1 mM adenosine was roughly proportional to the temperature of preheating.

The qualitative and quantitative requirements of spores for agents which cause germination are frequently found to have changed after the activation process. Freshly harvested spores of *B. cereus* required either inosine or a mixture of alanine + tyro-

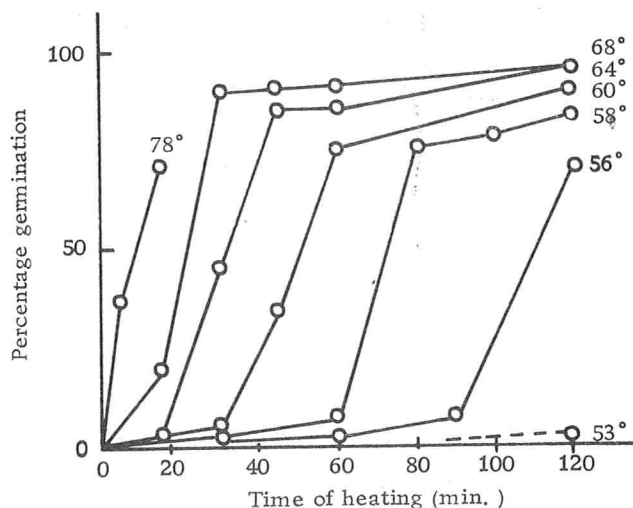


Figure 3. Heat-activated spontaneous germination of *Bacillus megaterium* spores. (Reproduced with the permission of Powell and Hunter, 41).

sine + adenosine for optimal germination; after prolonged storage or a short heat treatment, adenosine alone stimulated rapid and complete germination (41). These same investigators noted an even more dramatic effect of heat activation in spores of *B. megaterium*, which germinated spontaneously after activation (Fig. 3).

Dormant spores do not exhibit any apparent metabolic activity, even in the presence of readily available nutrients (5). Church and Halvorson (4) found that aged spores started to oxidize glucose after activation, even though no germination was observed. In addition to glucose oxidizing enzymes, other enzymatic systems also have been shown to be activated by heating. Bishop and Doi (2) noted that the enzymatic activity of proteases in intact *B. subtilis* spores was increased after heat shocking the spore suspension for 15 min at 60 C. Gould et al. (16) suggested that germination enzymes may be released from a bound form during activation. They postulated attachment to the spore core by weak electrostatic bonds, and demonstrated a release of enzyme by increasing the pH or by raising the solute concentration.

The chemical composition of spores of certain species have been reported to change after activation. Harrell and Mantini (20) observed a release of DPA during the heat activation of spores of *B. cereus* T. The hypothesis was therefore posed that germination ensues only after the endogenous DPA content is first reduced to a critical threshold (28). The release of DPA during heat activation could result from a change in the permeability of the spore coat and the opening of a chemical bond between DPA and the enzymes present in the spores (20).

Falcone and Bresciani (8) suggested that changes in permeability during activation might be responsible for the triggering of germination by pyruvate. Spores of *B. subtilis* germinated in the presence of pyruvate after acid treatment, but water activated spores required L-alanine instead. They assumed that changes in permeability allowed pyruvate to enter through the spore cortex. Gould and Hitchins (15) observed that exposure of spores to mercaptoethanol, a treatment which induces activation, enabled a molecule as large as lysozyme to penetrate the spore coat and reach its substrate located in the cortex.

Morphological and structural changes in activated spores have been reported (37). Electron micrographs of *Bacillus anthracis* spore coats showed that heating at 65 C for 15 min caused a more mottled appearance, and the cytoplasm became more opaque and less granular than in dormant spores.

#### THE MECHANISM OF ACTIVATION

Present evidence points in the direction that the prime event responsible for activation is not metabolic. The kinetics of the reaction resemble melting curves of macromolecules, which indicates that physico-chemical effects are involved. Most workers in this field are of the opinion that the change of the dormant stage to an active one is accompanied by a change in the tertiary structure of some macromolecules of the spores (25).

It has been suggested that activation changes the tertiary structure of a protein responsible for the dormant state of spores, and that activation could be considered as a reversible denaturation of the protein (26). The investigation of temperature dependence of the rate of heat activation of *B. subtilis* spores gave a uniform activation energy ( $\Delta H^\ddagger$ ) of 28 kcal for the range 5-94 C, suggesting that the rate controlling step for activation was the same for this temperature range. The change in entropy ( $\Delta S^\ddagger$ ) for formation of the activated complex was small (6.1 kcal/°C), and it was concluded that the breakage of a few strong bonds rather than many weak bonds must be responsible for activation (3). Most chemicals which bring about activation are known to cause structural changes in macromolecules. Activation at pH extremes occurs in the range at which proteins are denatured. Disulfide bond disrupting agents and reducing agents which induce activation also are known to cause denaturation of proteins. Ionizing radiation also can cause changes in the tertiary structure of macromolecules, either by direct action or by secondary effects of reactive free radicals from water, causing rupture of hydrogen bonds, peptide bonds, or other covalent linkages (1). Spores contain

five times more sulfur than is found in vegetative cells (45). This additional sulfur is concentrated in spore coats as cystine. It is therefore possible that the macromolecules responsible for maintaining the dormant state are coat proteins rich in cystine, stabilized in a specific configuration by S-S linkages. It has been suggested that activation by heat, reducing agents, and low pH may involve a reversible reduction of S-S bonds resulting either in an "unblocking" of an enzyme system, or in a change in the permeability of a structure controlling the dormant state of the spore (26). Figure 4 shows a model constructed by Keynan and Evenchik (25) from the data of Gould and Hitchins (15). A similar mechanism may be involved in activation by ionizing radiation (17).

The mechanism of calcium DPA activation is not known, but Freese and Cashel (12) have advanced the following hypothesis: the solution of calcium DPA used for activation is essentially saturated, so that tiny crystals or complexes of this salt are formed. Such small calcium DPA crystals or complexes may pull individual calcium or DPA ions away from spores. This process may either make sites more accessible that can subsequently react more efficiently with germinating agents such as L-alanine, or the permeability of spores may be increased by the calcium DPA treatment.

Very polar solvents rupture hydrogen bonds, causing changes in the tertiary structures of proteins. Results with polar solvents show that substances which cause denaturation of proteins primarily by H-bond breakage are very effective in producing rapid activation of bacterial spores even at low temperatures (46). While present kinetic evidence suggests that heat activation is more likely the result of breakage of a few strong bonds rather than many weak ones in the structure thought to be responsible for spore dormancy (3), it is not known to what

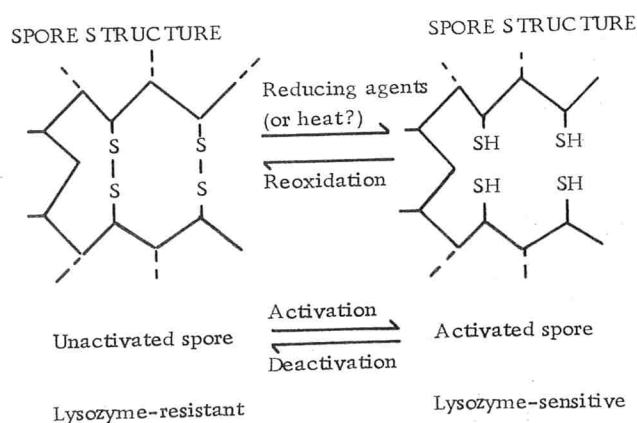


Figure 4. Hypothetical changes in spore structure accompanying activation. (Reproduced with the permission of Keynan and Evenchik, 25).

extent the breakage of H bonds is involved in heat activation. Considerable evidence indicates that it should not be ruled out as a possible important mechanism.

It has long been recognized that activation must be carried out in the presence of water (41). This led Hyatt and Levinson (23) to postulate that, whatever the identity of the activation site, it must be wetted before activation can occur. It is possible that some activation treatments might "open" the spore structure and permit water to reach and hydrate the critical site. Although spores have been found to be freely permeable to water, Murrell (38) has pointed out that present evidence fails to indicate whether a small anhydrous region surrounded by an impermeable barrier exists within the spore. Exposure of spores to aqueous ethyl alcohol or to water vapor resulted in activation of *B. megaterium* spores at room temperature (Fig. 2), while aqueous suspensions were not activated unless heated. Heating or ethyl alcohol may alter the structure of liquid water, permitting it, like water vapor, to reach and hydrate the critical site (23). Heating of liquid water changes its structure so that it becomes more "vapor-like", probably by decreasing the strength of hydrogen bonds (9). Ethyl alcohol may act either by lowering the surface tension of water, or by changing its structure, thus allowing it to enter into critical spore sites. This model could explain the aging effect on the basis that "vapor-like" water present as a small fraction in liquid water could enter into and hydrate a spore site; penetration and consequent activation would increase with time of storage of spores in liquid water (23). The identity of the site of spore activation is unknown, but it may be an enzyme or enzyme system, dehydrated and inactive in the dormant spore, whose activity is required for germination (23).

The various theories offered by different workers, while seemingly opposed to one another, probably are not mutually exclusive, but rather deal with different aspects of the same phenomenon, or reflect differences in the bacterial species studied. The nature of activation will probably only be completely understood when the mechanism of dormancy has been more fully elucidated.

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# THE EFFECT OF PLATE INCUBATION TEMPERATURES ON COUNTS OF SODIUM DESOXYCHOLATE-TOLERANT BACTERIA

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## ABSTRACT

Farm bulk milk samples from 54 producers were analyzed for Standard Plate Counts (SPC), thermoduric counts, and counts of bacteria that can tolerate 0.5% sodium desoxycholate (SDC). For the latter test, plates were incubated at 21, 25, 28, and 32 C for 48 hr. More milk samples had maximum counts at 25 C than at any other temperature used, whereas 32 C yielded the lowest number of colonies per plate. Median counts were 250/ml at 21, 290/ml at 25, 275/ml at 28, and 160/ml at 32 C.

The inadequacy of the Standard Plate Count (SPC) as an index to milk production conditions is widely recognized (2). Consequently, a test that would reflect numbers of organisms which are rarely found in udders would be more valuable. For this purpose a method was suggested (3) to enumerate a group of bacteria, which are recognized to come mainly from neglected milk-handling equipment. The procedure consisted of adding 1 ml of raw milk to 9 ml of 0.5% sodium desoxycholate (SDC) and plating 1 ml of this dilution with SPC agar (1). Organisms that developed visible colonies after 48 hr at 32 C were absent from, or present in extremely small numbers in samples taken from udder quarters (7). Hence their presence in milk in excess of 1,000/ml indicates significant contamination. Since the SDC count purposely excludes Gram-positive bacteria, a Laboratory Pasteurized Count was recommended to detect excessive numbers of thermoduric organisms (3).

At the time of that investigation, it was noticed that higher counts of SDC-tolerant bacteria could be obtained when plates were incubated at temperatures lower than 32 C. However, for practical reasons the recommended temperature (32 C) for plate counts (1) was employed.

Although it is relatively simple to determine the optimum growth temperatures for pure cultures in a given medium, the establishment of a suitable incubation temperature for mixed populations such as exist in raw milk has to be a compromise. Recently, several workers (4, 5) have compared a variety of temperature-time combinations for plate incubation. Temperatures lower than 32 C have generally been reported to yield higher counts. However, 32 C is

still recommended in the 12th Edition of *Standard Methods for the Examination of Dairy Products* (1). Increasing the incubation period to 72 hr, as is common in most countries, has little or no effect on counts of bulk tank samples (6). The purpose of the current study was to evaluate the sanitation of raw milk production and to compare various temperatures for incubating plates for SDC-tolerant bacteria, without adding to the usual incubation time of 48 hr.

## MATERIALS AND METHODS

In May 1970, one sample was taken from the bulk tank of each of the 54 milk shippers of a local dairy plant. Samples collected by the bulk tank truck driver were held at temperatures not exceeding 5 C, taken to the laboratory immediately, and analyzed upon arrival. In addition to SPC and thermoduric counts, the numbers of SDC-tolerant bacteria were determined according to the method of Blankenagel and Okello-Uma (3). In addition to the originally proposed temperature of 32 C for plate incubation for the latter test, duplicate sets of plates also were incubated at 28, 25, and 21 C. After  $48 \pm 0.5$  hr, the colonies were counted and the numbers per plate were compared with those obtained at the other incubation temperatures. The temperature yielding the largest number of colonies per plate was considered to be optimal. With several samples the same maximum counts were found at more than one incubation temperature, in which case all of these plates were considered to have maximum numbers of colonies per plate.

Plate counts were performed according to *Standard Methods for the Examination of Dairy Products* (1), except that all plates with up to 300 colonies were counted.

## RESULTS AND DISCUSSION

Results obtained at the various temperatures are summarized in Table 1. With 26 samples, maximum numbers of colonies developed at 25 C. Median counts also were highest at this temperature. Only 11 samples showed maximum counts at 32 C; in 6 of these, the same numbers of colonies were found at all of the other 3 incubation temperatures (4 counts of  $> 3,000$ /ml, and 2 counts of  $< 10$ /ml). Median counts were lowest at 32 C.

The relative numbers of SDC-tolerant bacteria obtained at plate incubation temperatures of 32 and 25 C are demonstrated in Fig. 1. Only 5 of the 54

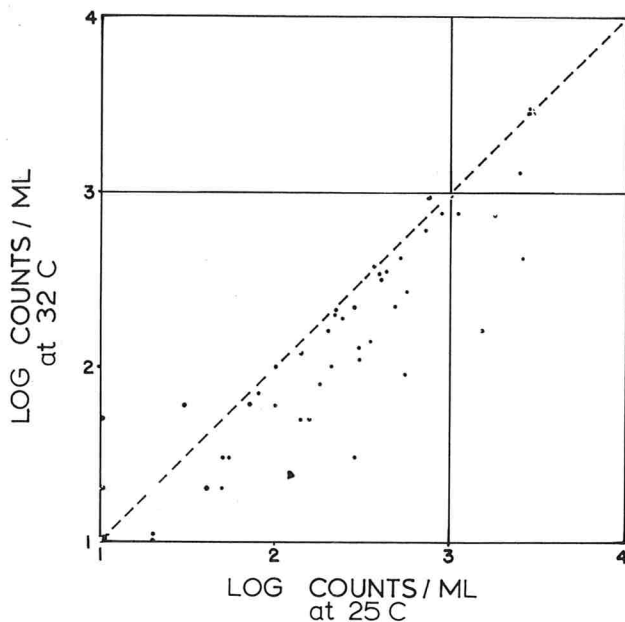


Figure 1. Counts of SDC-tolerant bacteria obtained with plate incubations at 32 and 25 C.

TABLE 1. THE EFFECT OF PLATE INCUBATION TEMPERATURES ON THE NUMBER OF COLONIES PER PLATE

	Plate incubation temperatures (°C)			
	32	28	25	21
Count/ml (Median)	160	275	290	250
Count/ml (Average)	510	610	700	710
Number of samples producing maximum counts <sup>1</sup>	11	20	26	24

<sup>1</sup>In some instances maximum counts appeared at two or more temperatures.

TABLE 2. THE EFFECT OF METHOD AND INCUBATION TEMPERATURE ON THE EVALUATION OF RAW MILK QUALITY

Method of evaluation and count limits	Number of unsatisfactory samples at	
	32 C	25 C
Standard plate count (75,000/ml)	6	
Thermoduric count (1,000/ml)	5	
SDC-tolerant count (1,000/ml)	8	13
Combined SDC-tolerant and thermoduric counts	11	16

samples had higher counts at 32 C than at 25 C, half of those having counts of < 100/ml at both temperatures. It appears that most microorganisms in raw milk have optimum growth temperatures below 32 C, and it may be assumed that a milk flora growing better at 25 C than at 32 C consists mainly of contaminants from unclean equipment, since optimum

temperatures of the natural udder inhabitants are predominantly in the 35 to 40 C range. Bacteria that can tolerate approximately 0.5% SDC are rarely found in the udder (6) and, if present in raw milk, they resulted from contamination. It is reasonable to assume that these contaminants, with the possible exception of those of intestinal origin, grow better at the lower temperatures.

At the present time, microbiological standards for raw milk in the Province of Saskatchewan are based on SPC with maximum permissible counts of 75,000/ml. In Fig. 2 the SPC of the samples are plotted against the counts of SDC-tolerant bacteria when plates were incubated at 32 and at 25 C. Of the 54 milk samples, 6 had SPC exceeding 75,000/ml, and therefore failed to meet the present standard. Assuming an upper limit of 1,000/ml SDC-tolerant bacteria, 8 samples were considered to be unsatisfactory when plates were incubated at 32 C. At 25 C the number of samples with 1,000 or more SDC-tolerant organisms/ml was 13 (Table 2). Since thermoduric counts of > 1,000/ml indicate faulty production procedures, the results of this additional test increased the number of unsatisfactory samples by 3 to a total of 11 at 32 C and by 2 to a total of 15 at 25 C. With one sample the SPC was 8,000/ml (Fig. 2), quite acceptable if the quality is judged on this basis. However, this sample contained > 1,000/ml SDC-tolerant bacteria, indicating improper sanitizing procedures. Unless the SPC is extremely high, it does not accurately reflect the sanitation under which the milk was produced (2).

There were 3 samples with SPC of > 60,000/ml, and SDC-tolerant bacteria counts of less than 500/ml (Fig. 2). Of these, 1 sample had a thermoduric count of > 1,000/ml. Although individual cows may produce milk with SPC of > 60,000/ml (7), such high counts rarely occur in mixed milk from the entire herd unless contamination and/or growth has taken place. No single test can accurately evaluate dairy farm sanitation, and even the combination of two tests is sometimes inadequate. However, the evidence to date suggests that the SDC test, supplemented with a thermoduric count, reflects production conditions better than does the SPC, especially if SDC plates are incubated at 25 C.

#### ACKNOWLEDGMENT

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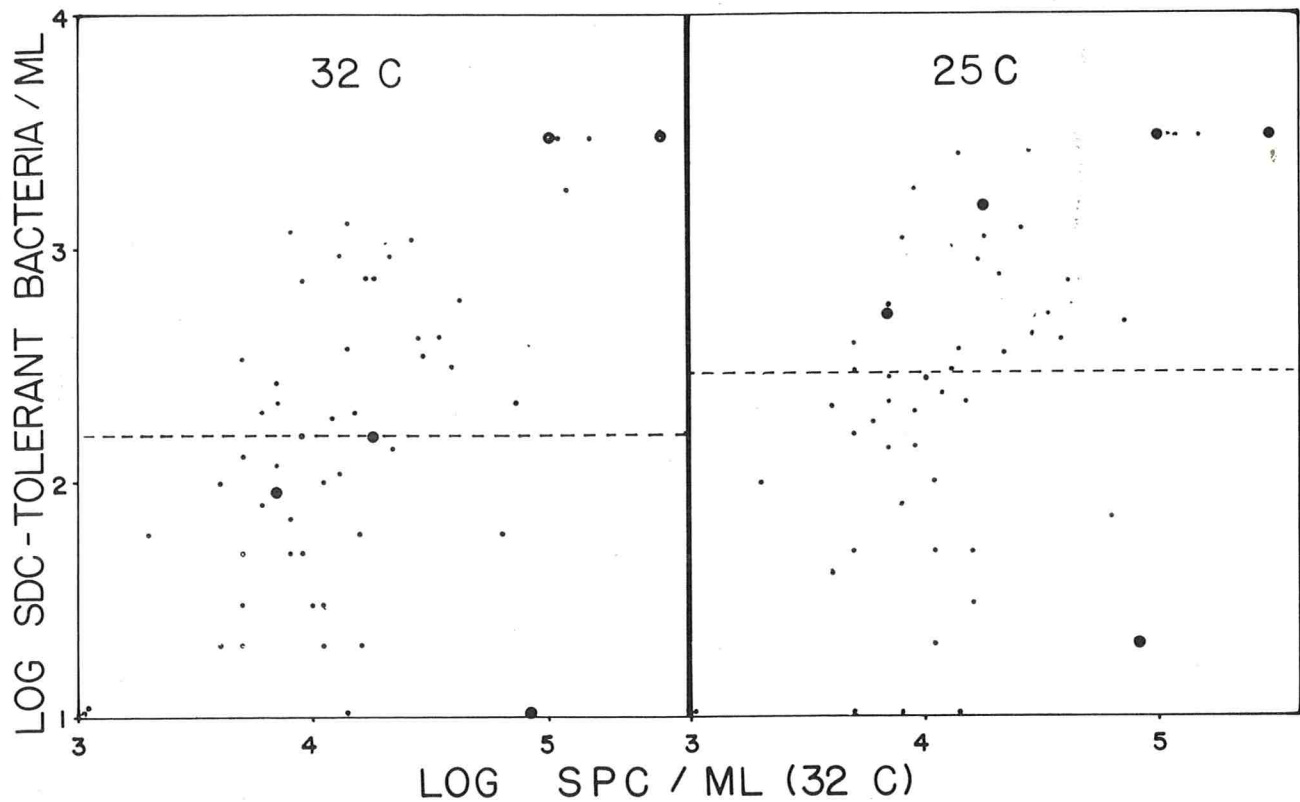


Figure 2. Comparison of SPC (32 C - 48 hr) with counts of SDC-tolerant bacteria obtained at 32 and 25 C. The larger points represent the 5 samples with thermoduric counts of >1,000/ml. The broken lines indicate the median counts at each incubation temperature.

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# NON-LETHAL INJURY AND LIMITATIONS OF RECOVERY OF COLIFORM ORGANISMS ON SELECTIVE MEDIA<sup>1</sup>

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## ABSTRACT

Cell injury as a factor in the enumeration of coliform bacteria with selective media was evaluated. Non-lethal injury reduced the ability of cells to produce outgrowth. *Escherichia coli* and *Aerobacter aerogenes* were equally sensitive. Brilliant green lactose bile broth, desoxycholate lactose agar, and violet red bile agar were similar in inhibitory effect.

Cell injury occurred with sub-lethal heat treatments, exposure to chlorine, exposure to sodium chloride, and freezing and thawing cycles. Circumstances to inflict injury are found in common environmental conditions of the food industry. Presence of injured cells may provide a count with a selective medium that is only 10% of the total count obtained with standard plate count agar. The limiting factor for recovery in selective media was associated with the surfactant.

An understanding of these limitations in the use of selective media in quality control and public health applications of the tests should contribute to proper interpretation of results.

Tests for coliform organisms are used in the dairy food industry, because the presence of these organisms indicates post-pasteurization contamination and certain species may indicate fecal contamination. A standard of < 10 per milliliter in pasteurized milk (17) indicates the degree of control that has been attained in the dairy industry.

Routine coliform counts on freshly pasteurized Grade "A" milk show numbers commonly recorded as < 1 per milliliter. Such low counts, however, are not as common after the product has been stored for a few days. Quality control laboratories most often check products immediately after pasteurization, whereas regulatory laboratories check the product later. Considerable anguish is caused when quality control laboratories of dairy plants get negative results which are then contradicted in a few days by regulatory laboratories. This variability is primarily attributable to the extremely low counts, but other factors may be involved.

The problem of evaluating samples with low numbers of coliform organisms is confounded by the presence of cells having been exposed to various degrees of stress. The degree of stress and the alterations in behavior of the cells must be considered in recovery in certain test media (5, 13). Sub-lethal heat

treatments increase the nutritional requirements for recovery (11, 14) and reduce the tolerance to selective agents in the recovery medium (2, 4, 12). Cold stress resulting from either sudden exposure (6, 15) or prolonged exposure below growth temperatures (3) reduces the ability of cells to recover. Prolonged storage under frozen conditions increases the sensitivity to inhibitory agents in the recovery medium (7) and increases the nutritional requirements for recovery of the injured cells (10, 16). Sub-lethal injury of cells caused by exposure to chlorine increases their sensitivity to nutritional substances in the recovery medium (9). Thus, there are many conditions causing stress that may influence the recoverability of cells on test media.

Conditions for stress and injury of coliform organisms are common in the food industry. Media for recovery and enumeration of these organisms contain inhibitors intended to limit growth of other microorganisms yet may be antagonistic to coliform cells. Thus, an exploration of cell injury resulting from treatments simulating common practices of the food industry and the effect on recovery with common test media (1) for coliforms was undertaken.

## MATERIALS AND METHODS

### Cultures

Pure cultures of two strains of *Escherichia coli* and one of *Aerobacter aerogenes* were propagated by incubation at 32 C for 24 hr and held at 3-5 C for storage. Since the results for the three strains were the same, only data for *E. coli* will be given. To obtain young cells, cultures were incubated only 6 hr at 32 C. Old, arrested cells were obtained from cultures in storage at 3-5 C for 7-30 days.

Cultures were propagated in nutrient broth (Difco). Selective media were violet red bile agar (Baltimore Biological Laboratories and Difco), desoxycholate lactose agar (Difco), and brilliant green lactose bile broth (Difco). In each experiment the three selective media were used. Media were prepared according to directions of the manufacturer, except that heat treatment was varied for certain trials as reported in the section on results.

A selective medium of nutrient agar and Nacconol with surface plating was used for comparative purposes (8).

The plating operation for comparing media was done with minimum delay and designed so that a small delay between treatment and addition of the plating media would not contribute to differences in the results.

### Procedure for injury of cells

The menstruum for the heat injury process was reconsti-

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TABLE 1. THE EFFECT OF HEAT INJURY ON THE COMPARATIVE RECOVERY OF ARRESTED *E. coli* CELLS ON STANDARD PLATE COUNT AGAR AND ON VIOLET RED BILE AGAR

Heat exposure (min)	Count per ml	
	Standard plate count agar	Violet red bile agar
0	140	130
5	140	120
8	39	8.6

TABLE 2. THE EFFECT OF HEAT INJURY ON THE COMPARATIVE RECOVERY OF YOUNG *E. coli* CELLS ON STANDARD PLATE COUNT AGAR AND ON VIOLET RED BILE AGAR

Heat exposure (min)	Count per ml	
	Standard plate count agar	Violet red bile agar
0	380	360
5	230	100
7	23	2

TABLE 3. THE EFFECT OF CHLORINE INJURY ON THE RECOVERY OF YOUNG *E. coli* CELLS ON STANDARD PLATE COUNT AGAR AND ON VIOLET RED BILE AGAR

Chlorine exposure (min)	Count per ml	
	Standard plate count agar	Violet red bile agar
0	80	60
5	49	32
10	33	12

TABLE 4. THE EFFECT OF EXPOSURE TO SODIUM CHLORIDE ON THE COMPARATIVE RECOVERY OF *E. coli* ON STANDARD PLATE COUNT AGAR AND ON VIOLET RED BILE AGAR

NaCl exposure time	Count per ml	
	Standard plate count agar	Violet red bile agar
0 min	130 x 10 <sup>6</sup>	110 x 10 <sup>6</sup>
10 min	41 x 10 <sup>6</sup>	5.1 x 10 <sup>6</sup>
20 min	220 x 10 <sup>5</sup>	4.8 x 10 <sup>5</sup>
2 hr	76 x 10 <sup>5</sup>	3.6 x 10 <sup>5</sup>
24 hr	200 x 10 <sup>5</sup>	9.7 x 10 <sup>5</sup>

tuted nonfat milk solids, 10% w/v, which was autoclaved (15 min at 121 C). The inoculum consisted of 1 ml of culture added to 99 ml of reconstituted sterile nonfat milk solids in a dilution bottle. The sample was heated quietly in a water bath at 65 C, thus allowing highly varying heat treatments for the population as a whole. The holding period was chosen to obtain approximately a 99% kill of the total population.

To obtain cell injury by freezing and thawing, cultures were grown in nutrient broth in 20 x 150 mm test tubes, which constituted the containers for subsequent treatments. Freezing was by quiescent storage at -3 to -7 C. Thawing was by exposure of the test tubes to running tap water, approximately 12 C.

Chlorine injury was obtained by exposing bacterial cells to autoclaved (15 min at 121 C) 1% w/v reconstituted nonfat milk solids to which calcium hypochlorite had been added. Through trial and error, sufficient calcium hypochlorite was added to obtain approximately a 50% destruction of the cell population in 10 min. Sodium thiosulfate was added to arrest the action of the chlorine and appropriate dilutions and plating were made immediately.

For observations on the destructive effect of sodium chloride, heat sterilized 1% w/v nonfat milk solids served as the basal medium to which NaCl was added to provide 5% w/v. An inoculum of 1% of culture was made as required. Subsequent dilutions and platings were made in accordance with the time of exposure desired.

## RESULTS

### *Recovery of uninjured cells*

In the exploratory phases of the work reported herein, comparative counts of coliform bacteria were made with standard plate count agar and with selective media. The mean count with selective media was approximately 95% of the mean count with standard plate count agar. These results are in agreement with those reported in the literature. It was later realized, however, that such close agreement only was attained when the cells were near the maximum growth phase and with only limited storage in the arrested state.

### *Cell injury by heat*

When a culture was heat-treated to give a widely varying degree of exposure to the individual cells, numbers recovered on selective media were considerably less than on standard plate count agar. Results of an average of three replications with arrested *E. coli* and violet red bile agar are given in Table 1. With an increased heat treatment, there was a reduction in the total population when measured with standard plate count agar and a still greater reduction when measured with selective media. For example, the results in Table 1 show that after 8 min, 72% of the cells were killed as judged by the standard plate count. Of the surviving cells, 78% had received a non-lethal injury that prevented their recovery on violet red bile agar.

Young, growing cells subjected to similar heat injury showed greater sensitivity to heat as measured

TABLE 5. THE EFFECT OF HEAT INJURY ON THE COMPARATIVE RECOVERY OF *E. coli* ON STANDARD PLATE COUNT AGAR AND ON SURFACE PLATING WITH A MEDIUM CONTAINING A SURFACE ACTIVE AGENT

Heat exposure (min)	Standard plate count	Count per ml		
		Surface plate method		
		0.1% surfactant	0.2% surfactant	0.3% surfactant
		(x 10 <sup>7</sup> )		
0	170	170	190	190
5	130	140	130	130
8	24	11	7.6	5.9

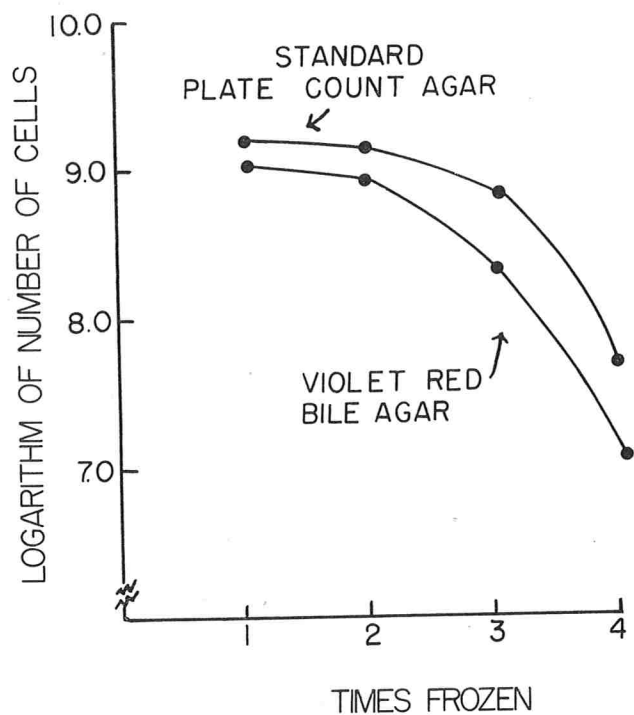


Figure 1. The effect of repeated freezing and thawing of *Escherichia coli* cells on the comparative recovery on standard plate count agar and on violet red bile agar.

by plate count agar and by selective media. Results representing an average of three replications are shown in Table 2. Approximately 94% of the cells were killed in 7 min, whereas 91% of those surviving had received a non-lethal injury which prevented their recovery on violet red bile agar.

Heat injury caused a similar behavior for *A. aerogenes* and *E. coli*. The three selective media (violet red bile agar, desoxycholate lactose agar, and brilliant green lactose bile broth) were equally limiting in recovery of injured cells. Steaming or autoclaving of the two solid media gave similar results.

#### Cell injury by chlorine

When chlorine was used to injure cells, there was a progression of kill and non-lethal injury with 5 and 10 min exposures. The average results of three replications with young cells are given in Table 3. When 59% of the cells were killed, 64% of the survivors received a non-lethal injury that prevented their recovery on violet red bile agar. Similar results were obtained with arrested cells.

Approximately equal limitation of recovery of injured cells was obtained with *A. aerogenes* and *E. coli* using any one of the selective media.

#### Cell injury by sodium chloride

When cells were exposed at room temperature to 5% w/v NaCl and 1% w/v nonfat milk solids, there was a moderate reduction in numbers as determined by standard plate count. Many cells, however, received a non-lethal injury that prevented their recovery on selective media. An example of the results representing four replications is given in Table 4. At zero time of exposure, the destructive effect was limited and of questionable significance. After 10 min, 67% of the cells had died as judged by their inability to recover on standard plate count agar. Of these remaining cells, 88% had received non-lethal injury which prevented their recovery on the selective medium. After 20 min exposure, 82% of the cells had died and of those remaining, 98% had received a non-lethal injury. Further exposure had minor additional effect in the destructive process. Cell injury limited recovery of either *A. aerogenes* or *E. coli* to approximately the same degree on any of the selective media.

#### Freezing and thawing injury

The effect of repeated freezing and thawing on cell destruction and comparative recovery was determined. Average results of three replications are given in Fig. 1. Each freezing and thawing cycle killed some cells. It was also apparent that each freezing and thawing cycle was accompanied by an increase in the extent of non-lethal injury which prevented recovery on selective media.

*Aerobacter aerogenes* and *E. coli* showed similar response to injury through freezing and thawing and subsequent recovery on selective media.

It is interesting to note, although no data are given at this point, that the well known phenomenon of cold shock did not produce a detectable number of non-lethally injured cells by our method of evaluation.

#### Inhibitory constituents of the selective media

To determine the critical factor(s) limiting recovery of non-lethally injured cells, media to simulate violet red bile agar were prepared except that certain inhibitory constituents (bile salts, neutral

red, and crystal violet) were deleted. These results showed bile salts to be the primary antagonistic constituent to recovery.

Observations were made to determine if the antagonism was from physio-chemical effects of surface activity or a direct biological inhibition. Comparative recovery of arrested *E. coli* after heat injury was determined by use of a surface plating technique developed in our laboratories (8). The inhibitory constituent was Nacconol, a surface active agent. Average results of three replications are given in Table 5. With the progression of heat injury there was an increased number of cells with non-lethal injury that prevented their recovery on the medium with surface active agent. Effectiveness was concentration dependent. Results indicate antagonism and associated limitation of recovery to be a physio-chemical phenomenon.

#### *Diluent as a factor in selective recovery*

Since the extent of dilution of test samples may be variable and milk solids influence surface active agents, observations were made using phosphate buffer and sterile skim milk as the diluent. There was no apparent difference in results obtained with the two diluents. Thus, the extent of dilution of samples did not appear to be a factor in the selective recovery process.

#### DISCUSSION

The preceding data are in agreement with general concepts that coliform bacteria are recovered on selective media. This agreement, however, holds only for test cultures of uninjured cells. When cultures contain injured cells, some of these are not able to grow on selective media. In commercial conditions opportunities for injury are common. Thus, non-lethal injury should be considered in quality evaluations. Recognition of the limitations of selective media should clear some of the confusion that has arisen in quality control and regulatory use of coliform tests.

Injury of *A. aerogenes* and of *E. coli* was inflicted by different methods and of the same general magnitude. Injury reduced recovery of cells with brilliant green lactose bile broth, desoxycholate lactose agar, or violet red bile agar. It would appear therefore, that the results are from a common mechanism of injury. Further understanding of the mechanism of the limitation of recovery might lead to improved methods. Some work along this line is now in progress.

#### ACKNOWLEDGEMENTS

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## SANITATION BY DESIGN<sup>1</sup>

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My association with the milk industry has been limited to plant inspections. During these inspections I have become aware of two facts regarding this industry. First, this industry is among the minority of food processors who have the highest degree of limitations concerning the equipment they may purchase. Secondly, the milk industry is among the majority of food processors who are operating in old buildings. My general inspection work has shown me that most of us operate in old structures constructed in a by-gone era. The problem facing us is how to upgrade, where necessary, and maintain these facilities to meet ever rising sanitation standards. I chose the title "Sanitation By Design" to convey the image that this objective can be met both from the standpoints of structural renovation and pest control. The task of upgrading may seem hopeless where little or no effort has been exercised in these areas for some time, but I am going to mention a lot of ideas for your consideration and hope that you will find at least some of them helpful.

### PLANT IMPROVEMENT

Much of this discussion on building improvement centers around one of our old facilities that was constructed in approximately 1910. We began upgrading at this location 8 years ago. Although we are not through yet, the "Superior" rating awarded this plant by the American Institute of Baking after its last inspection in 1969 gives you an idea of what can be accomplished.

#### *Roofing and paving*

Reroofing and ground paving work should be among the first of outside repairs. Concerning roofing materials, I'll only say gravel roofs or the like are the poorest types to use because of difficulty in cleaning. Where outside ground surfacing is concerned, asphalt seems to work best in most instances. We expect a minimum of 6 years life from asphalt when it is properly maintained. A sealer is required where heavy traffic and oil leaks are a possibility.

#### *Exterior of buildings*

With very few exceptions our building exteriors are concrete or brick. In preparing to resurface the structures, it is necessary to thoroughly sandblast concrete wall exteriors to remove old paint, loose patching, and other materials not well sealed to the cement. Brick requires sandblasting, but a balanced job is one which removes most loose material without taking excessive mortar. Window repairs and replacement next need attention. Mortar joints should be repaired and patching done as required.

Epoxy paints have been generally satisfactory for both concrete and brick exteriors. The hardening type is used on concrete, whereas a non-hardening epoxy is best for brick—best because it allows the brick to "breathe." Where major tuckpointing is required, ample curing time is necessary prior to application of non-hardening epoxy.

#### *Improved rodent control*

Mingled with all the repairs and paving that are done on the outside is a concern for better rodent control. We do not allow rodenticides in our buildings, and because of this must rely very heavily on preventive measures. Adequately protecting entry ways is not easy. Pedestrian doors are probably easier to rodent-proof than dock, dock shelter, and rail doors. Elevated access ways may be protected by a variety of methods. Where wide docks are concerned, protection very often may be obtained at the steps by use of rodent proof gates. Truck doors that are flush with the outside walls usually require only a thin gauge stainless steel strip approximately 12 inches wide, and long enough to extend out beyond the width of the doors. This strip should be sealed to the wall about 2 ft off the ground. We often use the "Hawser Guard" technique where ledges are involved. This is merely a relatively heavy gauged sheet metal that is cut to fit the ledge and wall so as to prevent rodents from using the ledge as a pathway.

Field rodents can be controlled effectively with a program of weed cutting, gassing holes, and carefully attended bait stations. Of course, equipment and trash cannot be left lying about.

<sup>1</sup>Presented at the 57th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Cedar Rapids, Iowa, August 17-20, 1970.

### *Equipment storage*

Regarding outside equipment storage, pallets should be used to keep equipment off the ground. Polyethylene sheeting can be used for protecting both pallets and equipment from bird infestations and as a container for fumigation.

### *Birds*

Problems created by birds around food plants are many. Because each problem is unique, I will not spend much time suggesting control methods. I have heard much ridicule against some highly effective techniques. However, I attribute most failures to lack of knowledge concerning mode of action and misunderstanding of the problem. Revolving lights, metal arc lights, high frequency sound, pastes, etc., are all effective, but special skill is required to select the correct technique for a particular situation.

### *Refurbishing walls, floors, and ceilings*

Inside wall, floor, and ceiling refurbishing follows the outside upgrading. Our own plant personnel do as much of this work as possible. It has been our experience that extra attention is given to details by plant personnel, and the result is a higher quality finish than one provided by an outside contractor.

With tongue and groove wood flooring, a careful evaluation is required to determine what must be replaced and what can be repaired. Once this basic work is done, an epoxy filler works well to fill cracks and recesses. Power sanders will bring the floor to a smooth finish in preparation for the gym sealer and finish. Caulking around equipment that passes through the floors and adding sanded safety strips, where helpful, is all that remains to be done.

Wood ceilings also must be examined to determine what must be replaced and repaired prior to sanding. Holes should be caulked, though sanding is sufficient for most gouges. The purpose is to eliminate recesses where insects may harbor and dust can collect. A latex primer and an epoxy finish will give the wood ceiling an excellent appearance.

Close examination of concrete floors and ceilings will reveal chips and cracks that may be filled with epoxy patch. Larger holes require concrete patches. We suspended some equipment from the ceiling rather than use the standard floor support legs. By so doing, we reduced the number of obstacles to sweep around. Suspending heavy equipment from the ceiling requires drilling holes through the floor above, setting bolts, and filling the holes with con-

crete patching. Patches and rough spots in the ceilings need only be smoothed or feathered out prior to painting with an epoxy coating. Concrete floors should be ground prior to being finished with an epoxy product.

Painted brick walls probably become the least attractive of all types as coats of old paint build up over the years. However, with adequate preparation, one can bring them to the same fine, finished appearance attained in wood and concrete. Wire brushing is required. Power wire brushing is most helpful for good preparation, but some hand brushing also may be necessary, particularly after filling small cracks or recesses with caulking compound. An excellent caulking compound is one with an epoxy base. A latex/cement mixture will serve very well as a primer, whereas epoxy paint will give the wall an excellent long life and smooth finish. Epoxy grouting also may be used at floor/wall junctures, no matter what materials were used in construction of the walls and floors.

### KEY POINTS FOR SUCCESS

As you can visualize, a plant that has been upgraded is much easier to maintain from all standpoints. Several key points regarding structural upgrading we feel are conducive to maximum success in this type of venture. They are:

- (a) Avoid crash programs. The basic decision to upgrade should be made early enough so that rapid progress is not necessary.
- (b) Lay out, and follow closely a priority area list and give top priority to those areas requiring maximum effort to upgrade or areas where product zones are most exposed.
- (c) Use plant personnel where possible for the reasons mentioned earlier.
- (d) Exercise great caution in the total job, particularly where surface coatings are concerned. The majority of failures in this area of work result from poor knowledge, preparation, and application of materials.
- (e) Now that you have a priority list of areas to be upgraded (see item *b*), don't plan on complete renovation with a short deadline. The estimated cost for the total renovation not only tends to discourage one from starting, but the urgency to meet the deadline tends to reduce quality.

# NUTRIENT REQUIREMENTS AND GROWTH CONDITIONS FOR PRODUCTION OF LACTASE ENZYME BY *SACCHAROMYCES FRAGILIS*<sup>1</sup>

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## ABSTRACT

Lactase enzyme was produced by *Saccharomyces fragilis* NRRL Y-1109 grown in deproteinized Cheddar cheese whey. Nutrients and growth conditions required for maximum lactase production were determined. Lactose concentration, sources of growth factors, temperature of incubation, and pH of the whey were the primary factors affecting lactase production. Increased levels of lactose in whey stimulated the yeast to produce higher levels of lactase activity per gram of dried yeast. Corn steep liquor was the best source of added growth factors. Yeast grown under optimum conditions, pH 4.0 to 4.7 and 28 C, yielded 175 units per gram of yeast and 1300 units per liter of whey.

Lactose, the major constituent of milk solids-not-fat, limits the concentrations of milk solids-not-fat that can be used in ice cream, milk concentrates, pasteurized processed cheese spreads, and numerous other food and feed products. Lactose utilization is limited by its low solubility, lack of sweetness, and its laxative effect if consumed at high levels. Hydrolysis of lactose by lactase enzymes will overcome some of these limitations and permit greater usage of lactose and lactose-containing products, such as whey.

Lactases now available in commercial quantities have low activity and are not widely used. Preparations of higher activity for hydrolyzing lactose could be used advantageously by the food industry.

The primary sources of commercial lactases are calf intestines and microorganisms. Lactase enzyme preparations have been obtained from *Escherichia coli* (5), *Neurospora* (6) and various strains of lactose-fermenting yeasts including *Saccharomyces fragilis*, *Torulopsis sphaerica*, *Zygosaccharomyces lactis*, *Torula utilis*, and *Candida pseudotropicalis* (11). Lactases from various sources, however, differ considerably in their characteristics and activity (12). Lactase from yeast readily hydrolyzes lactose in milk and milk products (13).

Methods of producing lactase by *S. fragilis* were described by Caputto et al. (3) and Van Dam et al.

(16), and some factors affecting its ability to form lactase were listed by Davies (4). Extensive studies have been made on optimum growth conditions for *S. fragilis* to produce maximum yeast protein in whey (8, 9, 17, 18). Maximum protein production was obtained when yeast was grown in whey containing 4.5% lactose with 1.0% corn steep liquor added to supply growth factors and with phosphoric acid and ammonia added to control pH (8). The optimum temperature range for growth was 28 to 35 C. (18).

Young and Healey (19) stated that conditions for maximum growth of *S. fragilis* were not necessarily the same as those for maximum lactase enzyme production. They reported yields of 120 to 142 units of activity per liter of medium when this yeast was grown in whey containing about 0.06% available nitrogen with an aeration rate of 1.0 to 2.0 volumes per volume per minute.

It was the purpose of this study to determine the nutrient requirements and growth conditions necessary for maximum lactase production by *S. fragilis*.

## MATERIALS AND METHODS

### Organism

*Saccharomyces fragilis* NRRL Y-1109, a lactose-fermenting yeast, was carried on slants of deproteinized whey containing 2% agar. Surface growth was washed from slants with 5 ml of sterile water, and 1.5 ml of this suspension, containing about 0.05 g of wet cells, was added to each 100 ml of whey for propagation. The wet cells contained 20 to 22% solids (10).

### Propagation

The whey used to propagate yeast cultures was reconstituted dried Cheddar cheese whey deproteinized by heating at 93 C for 5 min at pH 4.5 and filtering to remove the coagulated protein. Supplementary nutrients added, unless otherwise listed, were 0.5% (v/v) corn steep liquor, 0.3% (w/v) K<sub>2</sub>HPO<sub>4</sub>, and 0.3% (v/v) 5N NH<sub>4</sub>OH. The pH was readjusted to 4.5 with H<sub>2</sub>SO<sub>4</sub>. This whey medium was heated to 65 to 70 C, held for 30 sec, transferred to sterile flasks, cooled to 28 C, and inoculated.

All yeast cultures were grown in shake flasks with volumes of whey adjusted to give an oxygen absorption rate of 0.2 millimole of O<sub>2</sub> per liter per minute (2). Flasks were shaken in a reciprocating water bath shaker (Research Specialties Model 2156) at a temperature of 28 C.

### Harvest

When maximum growth was reached, as measured by absorbance of the medium at 650 mμ (8), yeast cells were

<sup>1</sup>Published with the approval of the Director of the Research Division, College of Agricultural and Life Sciences, University of Wisconsin.

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harvested in an International Model V centrifuge at 500 x g for 5 min. A lactase-active, zymase-inactive preparation was obtained by washing the sludge of yeast cells in distilled water, filtering, and drying them at 60 C for 4 hr (14). The dried yeast cells were stored at 5 C until assayed.

#### Assay

Lactase activity was estimated from the weight of monosaccharides liberated during the hydrolysis of the lactose contained in 40 ml of 30% total solids skim milk by 10 mg of dried yeast. To insure uniformity, the skim milk was obtained by reconstituting non fat dry milk from a single supply. The non fat dry milk was prepared from skim milk obtained from the University of Wisconsin Dairy Plant. The skim milk was heat treated at 62.8 C for 30 min, concentrated under vacuum to 45% total solids and spray dried on the University of Wisconsin spray dryer (1). Hydrolysis was over a period of 4 hr at 50 C, and a pH of 6.6. Monosaccharides were determined by the method of Tauber and Kleiner (15).

Units of lactase activity were defined as the number of grams of lactose hydrolyzed by the lactase in 1 g of dried yeast. Total units of activity recorded per liter of whey are equal to the lactase units per gram of dried yeast times the grams of dried yeast produced per liter of whey.

Lactose in whey was determined by a modification of the phenol sulfuric acid method (7).

## RESULTS

### Lactose

The effect of lactose concentration on lactase production by *S. fragilis* was determined in two series of trials by adjusting the lactose content of whey medium to various levels in the range of 4.9 to 21%. In the first series, lactose was adjusted by varying concentrations of whey solids; in the second, by adding lactose powder.

In the first series, the lactose content ranged from 4.9 to 15%; the highest level was assumed to be the maximum limit of utilizable sugar in growth media. No additional nutrients were added to the whey. In the second series, the concentration of whey solids was held constant at 12.5%, with the exception of the 6.6% lactose level, which was made by diluting the whey with water from 12.5 to 9.2% whey solids. The lactose level was adjusted from 6.6 to 21%, by addition of crystalline USP lactose. Supplementary nutrients were added to the whey.

Results in Fig. 1 show that lactase activity per gram of dried yeast increased with higher levels of lactose in the whey with varied whey solids. In whey with 4.9% lactose, the yeast contained 50 lactase units per gram; with 15% lactose, 115 units per gram. Growth was maximum in whey containing 8 to 11% lactose, where 6.0 g of yeast per liter of whey were produced; with 4.9% lactose in whey, 4.3 g of yeast were produced. In the whey containing additional nutrients, lactase activity was increased greatly over the activity of unfortified whey. Lactase units per gram of yeast increased progressively to the highest levels of lactose, which were actually above the max-

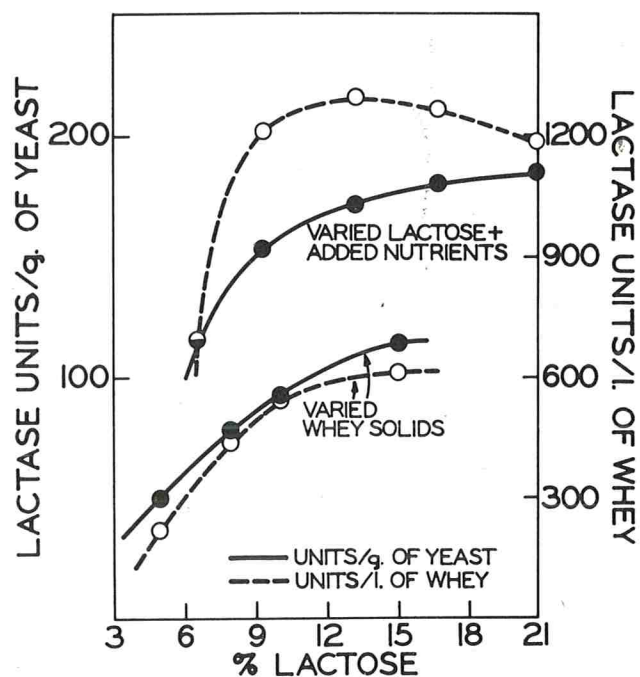


Figure 1. Effect of lactose content of whey on lactase production.

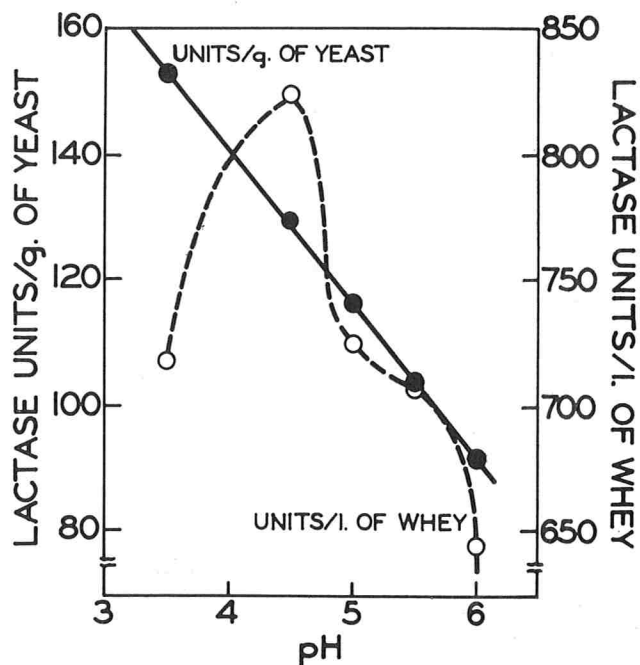


Figure 2. Effect of pH on lactase production.

imum amount assumed to be usable by the yeast. However, the lactase activity per liter of whey leveled off or decreased when the lactose exceeded 13.2%. The yeast did not ferment more than the 15% lactose in whey, since appreciable amounts of lactose remained in the spent whey which originally contained 16.6 and 21% lactose.

### Nitrogen and phosphorus

Urea,  $(\text{NH}_4)_2\text{SO}_4$ ,  $(\text{NH}_4)_2\text{HPO}_4$ , and aqueous am-

TABLE 1. EFFECT OF 0.5% GROWTH FACTOR SOURCES ON LACTASE PRODUCTION

Source	Grams of yeast per liter of whey	Lactase units per gram of yeast	Lactase units per liter of whey
None	3.1	91	283
Yeast extract	5.5	71	390
Peptone	5.0	74	370
Casein digest	4.1	120	492
Corn steep liquor	6.4	135	864

monia were added at the rates of 0.5 and 1.0% (w/v) to whey containing 5% lactose. None resulted in greater lactase activity in the yeast; consequently, aqueous ammonia was chosen as the source of added nitrogen because it handled easily and cost less.

Variations in amounts of aqueous ammonia had little effect on lactase production by *S. fragilis*. The optimum approximated 0.3% (v/v) 5N  $\text{NH}_4\text{OH}$ . This amount of nitrogen plus the available nitrogen in 12.5% solids whey furnished approximately 0.06% total available nitrogen.

Variations in the level of  $\text{K}_2\text{HPO}_4$  added to whey containing 9.0% lactose had little effect on the production of lactase except to make it more uniform from batch to batch. The addition of 0.3%  $\text{K}_2\text{HPO}_4$  was optimum. In whey containing 6.0% lactose or less, 0.5%  $\text{K}_2\text{HPO}_4$  was needed to provide maximum growth and enzyme production.

#### Growth factors

Sources of growth factors used were corn steep liquor, casein digest, yeast extract, and peptone, which were added to whey containing 8.0% lactose at a rate of 0.5% (w/v). Supplementary nutrients added were 0.5%  $\text{K}_2\text{HPO}_4$  and 0.3% 5N  $\text{NH}_4\text{OH}$ . As shown in Table 1, casein digest and corn steep liquor increased lactase activity to 120 and 135 units per gram of yeast, respectively. Yeast extract and peptone addition resulted in a reduction of lactase activity per gram of yeast by 20% from that of the control, which contained 91 units per gram. All sources of growth factors promoted cell production over that of the control and, thereby, produced more lactase units per liter of whey. Corn steep liquor added to whey containing 8% lactose, at various levels ranging from 0.1 to 1.0% (w/v), yielded yeast containing 120-135 units per gram. The optimum level was 0.5%, which yielded 135 units per gram of yeast.

#### Other sugars

The effects of end products of lactose fermentation by *S. fragilis* on lactase formation were studied by

fermenting a synthetic broth containing 1.0%  $(\text{NH}_4)_2\text{SO}_4$ , 0.5%  $\text{K}_2\text{HPO}_4$ , 2.0% casein digest, and either 5% galactose, 5% glucose, or 5% lactose. Yeast grown in lactose and galactose broth both produced 130 units per gram, whereas the yeast grown in glucose broth exhibited no lactase activity. The yield of the yeast cells was slightly higher with lactose than galactose.

Studies on how lactase activity was affected by adding various levels of glucose and ethanol to whey were also made. With 2% glucose or ethanol added, lactase activity decreased only 10%. As additional amounts of glucose were added, the decrease was only slightly more than with 2%. For example, with 8% glucose added, activity per gram of yeast was reduced only 16%. However, with 4% ethanol present, total activity was reduced 77%.

#### pH of medium

The pH of the deproteinized whey containing 7.5% lactose plus supplementary nutrients was adjusted to various levels in the range of 3.5 to 6.0 by adding  $\text{H}_2\text{SO}_4$ . As shown in Fig. 2, lactase activity per gram of yeast decreased gradually as the pH level increased. Growth at pH 3.5 was considerably less than at higher pH values, so that maximum production of lactase per liter of whey was obtained at pH 4.0 to 4.7 and decreased when the pH reached 4.8. Growth of the yeast and lactase activity were completely lacking at pH 3.0.

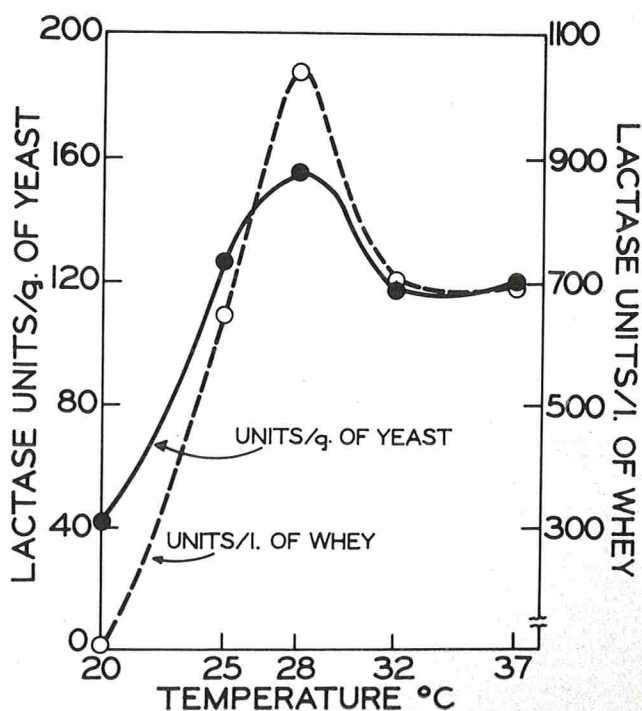


Figure 3. Effect of propagation temperature on lactase production.

Five acids,  $H_3PO_4$ ,  $HNO_3$ ,  $H_2SO_4$ ,  $HCl$ , and lactic, were used to adjust the pH of whey to 4.5 before heating in the deproteinizing and pasteurization treatment. Yeast grown on the  $H_2SO_4$ -treated whey produced the highest lactase activity, whereas lactic acid-treated whey produced the least.

#### Temperature of incubation

Fermentations of whey, containing 7.5% lactose and supplementary nutrients, were carried out at 20, 25, 28, 32, and 37 C. Figure 3 indicates that temperature was critical for maximum production of lactase enzyme; a change of 3° from the optimum temperature of 28 C decreased the lactase activity per gram of yeast by 20% and the lactase activity per liter of whey by 30%. Maximum growth was reached sooner at higher temperatures because of the shorter lag phase. Growth was inhibited to some extent and activity per gram of yeast was greatly reduced at 20 C.

#### DISCUSSION

From the results shown, we can conclude that optimum conditions for lactase production differ considerably from those optimum for growth. Lactose was a primary factor regulating lactase formation in the yeast cell. Previously it was felt (11) that maximum amounts of lactase could be produced by *S. fragilis* by fermentation of whey containing 0.5 to 5% lactose. In this study, higher levels of lactose stimulate the yeast to form substantially larger yields of lactase per gram of yeast. Total maximum lactase activity was obtained when whey for fermentations contained 10 to 15% lactose. Lactase was also formed when galactose served as the substrate, but yeast growth in a medium containing galactose was somewhat less than in a lactose medium. The presence of 2% glucose or ethanol reduced lactase yields slightly, but this level was higher than would normally accumulate in fermentations.

The fermentation medium which produced maximum lactase yields consisted of: whey containing 10 to 15% lactose, 0.5% corn steep liquor, 0.3%  $K_2HPO_4$ , and 0.3% 5N  $NH_4OH$ . Corn steep liquor was extremely effective in stimulating lactase production.

Maximum lactase production occurred at pH levels of 4.0 to 4.7. The pH during the fermentations stayed in this range without further buffering. Fermentations were conducted at pH 4.5 using sanitized equipment and non-sterile media without significant contamination from lactose-fermenting bacteria or other microorganisms.

Studies are being made on aeration, dissolved oxygen, and consumed oxygen and their effect on growth and lactase production.

#### ACKNOWLEDGMENTS

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## CONSOLIDATED COOPERATIVE'S ROLE IN QUALITY ASSURANCE<sup>1</sup>

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Unfortunately, we have a cancerous disease in our industry today . . . reciprocity between State Agencies . . . a disease which has constantly eaten away at our endeavors toward uniformity and quality assurance. We have too long been the victims of jealousies and petty actions resulting when one state agency hits another if that agency is using the United States Public Health Service Ordinance and Code.

Task Force VII received two quite concernable problems this past July at a meeting of the National Interstate Milk Shipments (IMS) Conference and two outstanding recommendations were made. Neither recommendation passed but they are worthy of mentioning because they show the concern of several areas of the country. These were as follows:

(a) That the IMS Conference require by July 1, 1973, the *delisting* of all shippers from the IMS list from any state or municipality which does not have reciprocal inspection agreements or does not abide by the conference agreements for all IMS rated supplies, and that the IMS Executive Board refer this matter to the proper task force or a special committee for implementation.

(b) That the IMS Conference require by July 1, 1973, the *delisting* of all shippers from the IMS list from any state or municipality which does not accept the accreditation program for brucellosis and tuberculosis as outlined in the USPHS Grade A Pasteurized Milk Ordinance, 1965 Recommendations, and that the IMS Executive Board refer this matter to the proper task force or special committee for implementation.

Even though these two problems submitted to Task Force VII were defeated after the action of Task

Force VIII, which was adopted with a slight amendment will look very closely into these and other problems of reciprocity and report back to the conference in 1971.

Problem 5 of Task Force VIII asked for ways to eliminate duplicate inspections including possible formation of a study committee. The action taken by the conference by a 28-12 affirmative vote was that: "It is recommended that the Conference Chairman appoint a committee of five, charged with the responsibility of receiving reports of *lack of reciprocity*, investigating such reports and taking, in respect to the facts determined, warranted action within the powers of the conference Agreements and finally that the committee submit to the 1971 Conference a resume of its function with recommendations."

As you know, there were some who were not wholly in accord with all of the thinking in these recommendations. Any step forward of this type will pose problems to some which will have to be ironed out. But we must take these steps if we are to have the quality which our industry must have to survive.

Steps necessary toward obtaining nationwide quality assurance in our industry are going to be costly ones for all of us. But it is well for us to remember that in the minds of the consumer, the bitterness of poor quality lingers long after the sweetness of cheap price is forgotten. Our future, versus that of the substitute markets which are making such strong inroads, depends on this.

It is high time that we as an industry begin to practice what we preach and work seriously toward quality and uniformity both as individuals and as a group without regard for selfish *momentary* monetary interests. When we have perfected the quality aspect of our industry, we will have reached a plateau

<sup>1</sup>Presented at the Fifty-Sixth Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Inc., Louisville, Kentucky, August 17-21, 1969.

# EFFECT OF MAGNESIUM CARBONATE AND SODIUM PHOSPHATE ON THE EXTRACTION OF CHLOROPHYLL-LIKE PIGMENTS AFTER THERMAL PROCESSING OF SPINACH PUREE

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## ABSTRACT

Because buffers are important in maintaining green color in vegetable purees, a study was undertaken to determine if sodium phosphate and/or magnesium carbonate would effect extraction of some or all of the chlorophyll-like pigments in spinach puree and thus interfere with their analysis. Samples were prepared with and without addition of the above additives and pigment analyses were carried out for chlorophylls, chlorophyllides, pheophytins, and pheophorbides. It was found that addition of sodium phosphate and/or magnesium carbonate prior to blanching and processing caused chlorophyll b, pheophytin a, pheophorbide a, and pheophorbide b to be bound to some extent. Pheophytin b was unaffected. Chlorophyll a, chlorophyllide a, and chlorophyllide b were present in such small quantities that no conclusions could be drawn about their extractability.

This phenomenon is an important consideration in the analysis of chlorophyll-like pigments in buffered systems and also might supply a suitable means for removal of chlorophyll-like pigments from foods and beverages where the color they impart is undesirable.

Many attempts have been made to stabilize the green pigments of thermally processed green vegetables with varying degrees of success. Elevated pH conditions created in the food by addition of approved alkaline substances have enjoyed some success (1, 2, 3, 7, 9, 10).

Another approach has been the use of High-Temperature Short-Time processing as evidenced by the work of Epstein (5), Tan and Francis (11), Gupte and Francis (7), and Luh et al. (8).

Still another has been the conversion of chlorophylls to chlorophyllides with or without addition of magnesium carbonate as summarized by Clydesdale and Francis (4). This work arose from further investigations into green pigment stability in spinach puree being carried out by Fleischman (6). In this work chlorophylls were converted in part to chlorophyllides and further stabilization was attempted by addition of magnesium carbonate and/or phosphate buffer systems. Pigment analyses were carried out by the method of White et al. (12) as modified by Clydesdale and Francis (4). Using this analytical

technique it was found that the total amount of pigments (Chlorophylls, chlorophyllides, pheophytins, and pheophorbides) in the control sample remained basically constant during the entire study while with addition of magnesium carbonate and/or sodium phosphate to the spinach puree, the total amount of pigments decreased during blanching and processing.

This work was carried out to determine if magnesium carbonate and/or sodium phosphate were effecting extraction of pigments. Further, an investigation was done to determine if these agents were specific for chlorophylls, chlorophyllides, pheophytins, or any combination of these when all were present within the vegetable tissue. This was an interesting area to explore since in certain food materials it is desirable to remove green pigments during processing and this method might prove to have some application in these areas. It also is an important consideration in any analysis of buffered green vegetables for chlorophyll-like pigments.

## MATERIALS AND METHODS

All pigment analyses in this study were done according to the method of White et al. (12) as modified by Clydesdale and Francis (4). The spinach puree used was made from spinach purchased at a local retail outlet and comminuted cold. In all packs subsequently described each was de-aerated, packed in glass baby-food jars (201 x 210, 3.5 oz), and given a still cook at 115.6 C with an  $F_0 = 4.9$ .

In order to determine if anhydrous sodium phosphate changed the amounts of pigments which were extracted, a study was undertaken using sodium phosphate in spinach puree with and without the addition of magnesium carbonate. Samples were mixed with increasing amounts of sodium phosphate, so that the final result, in each instance was buffered at pH = 8.0. Following this, the samples were blanched at 68 C for 20 min in order to convert the chlorophylls to chlorophyllides (4), then processed at 115.6 C and analyzed for pigment content.

To determine specificity of absorption a study was undertaken with a sample of spinach puree alone and a sample which contained 0.35% magnesium carbonate and 1.0% sodium phosphate buffered at pH 8.0. Both were blanched at 87.8 C, processed, and analyzed for pigment content. This study was undertaken to determine if any one pigment or pigments were affected more than others.

<sup>1</sup>Current address: Hunt-Wesson Foods, Fullerton, California



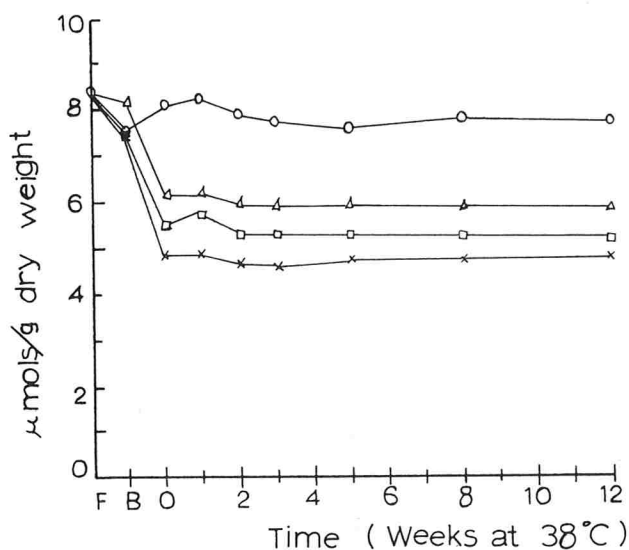


Figure 1. Total concentration of a pigments (chlorophyll, chlorophyllide, pheophytin, and pheophorbide) in spinach puree: The treatments are as follows: O blanched at 87.8 C for 5 min and processed at 115.6 C; Δ mixed with 0.35% magnesium carbonate, blanched at 68 C for 20 min, and processed at 115.6 C; □ mixed with 0.35% magnesium carbonate, buffered at pH 8.0 with 1.0% sodium phosphate, blanched at 68 C for 20 min and processed at 115.6 C; and X mixed with 0.35% magnesium carbonate, buffered at pH 9.0 with 1.0% sodium phosphate, blanched at 68 C for 20 min, and processed at 115.6 C. F: Fresh; B: After mixing and blanching.

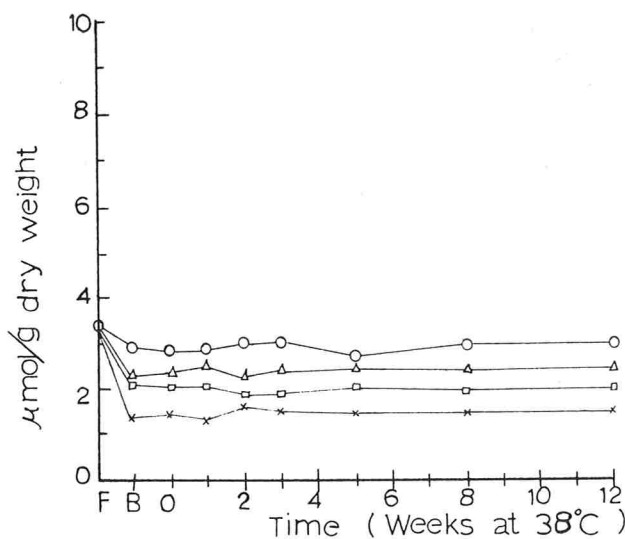


Figure 2. Total amounts of b pigments (chlorophylls, chlorophyllides, pheophytins, and pheophorbides) in spinach puree. The treatments are as follows: O blanched at 87.8 C for 5 min and processed at 115.6 C; Δ mixed with 0.35% magnesium carbonate, buffered at 68 C for 20 min, and processed at 115.6 C; □ mixed with 0.35% magnesium carbonate, buffered at pH 8.0 with 1.0% sodium phosphate, blanched at 68 C for 20 min, and processed at 115.6 C; and X mixed with 0.35% magnesium carbonate, buffered at pH 9.0 with 1.0% sodium phosphate, blanched at 68 C for 20 min, and processed at 115.6 C. F: Fresh; B: After mixing and blanching.

## RESULTS AND DISCUSSION

Figures 1 and 2 are examples of results obtained from pigment analysis of total a and b pigments (total pigments include chlorophylls, pheophytins, chlorophyllides, and pheophorbides), respectively, during the early stages of this work when the major concern was pigment stability. From these results it is apparent that magnesium carbonate and/or sodium phosphate creates a situation whereby some or all of the green pigments present and their normal degradation products were not being extracted from the spinach puree. This was verified by the observation that all samples which showed a decrease in total pigments yielded a filter cake that after repeated extractions with acetone had a greenish brown cast to it.

There was a small decrease in total pigment in the control sample as well as the others after blanching. However, the decrease in the control sample was within the experimental error of the method which was found to be 3-8% by Clydesdale and Francis (4). However, after processing and before storage the control samples did not show any decrease in total pigments while the others did.

Table 1 shows the effects of the addition of increasing amounts of sodium phosphate on the amount of total a and b pigments extracted. From data in this table it is apparent that an increase in sodium phosphate caused a decrease in the total pigment extracted up to a maximum, and then the amount of pigment unaccounted for remained constant with an increase in the amount of sodium phosphate.

Table 2 shows the effect of the addition of both magnesium carbonate and sodium phosphate. Magnesium carbonate caused an additional decrease in the amount of pigment extracted but not to the extent that sodium phosphate did.

Apparently the sodium phosphate alone and in combination with magnesium carbonate affected some pigment or pigments and prevented extraction. This was a linear relationship up to a point and then levelled off with addition of an increasing amount of sodium phosphate. Thus, the amount of pigment extracted was a function of the amount of sodium phosphate or magnesium carbonate added, up to a maximum concentration and then became a function of some pigment or pigments, since the latter represented the limiting factor above a certain concentration of sodium phosphate. Controls in Tables 1 and 2 differ because different batches of spinach were used. The pigment content of spinach can easily vary by  $\pm 20\%$ .

Results of the specificity study may be seen in Table 3. The pigments which were affected were chlorophyll b, pheophytin a, pheophorbide a, and

TABLE 1. EFFECT OF SODIUM PHOSPHATE ON THE AMOUNT OF PIGMENTS EXTRACTED FROM PROCESSED SPINACH PUREE BUFFERED AT pH 8.0 WITH SODIUM PHOSPHATE, BLANCHED AT 68 C FOR 20 MINUTES, AND PROCESSED AT 115.6 C.

Per cent sodium phosphate	Amount a pigments	Amount b pigments
Pigment content (micro mols/g dry weight)		
0.00	6.32	2.52
0.5	5.70	1.79
1.0	5.12	1.66
2.0	4.72	1.37
3.0	4.70	1.36
4.0	4.69	1.35

TABLE 2. EFFECT OF SODIUM PHOSPHATE AND MAGNESIUM CARBONATE ON THE AMOUNT OF PIGMENTS EXTRACTED FROM PROCESSED SPINACH PUREE MIXED WITH 0.35% MgCO<sub>3</sub>, BUFFERED AT pH 8.0 WITH SODIUM PHOSPHATE, BLANCHED AT 68 C FOR 20 MINUTES, AND PROCESSED AT 115.6 C.

Per cent sodium phosphate	Amount a pigments	Amount b pigments
Pigment content (micro mols/g dry weight)		
0.0	5.87	1.90
0.5	5.20	1.60
1.0	4.30	1.30
2.0	4.02	1.21
3.0	3.76	1.25
4.0	3.81	1.20

pheophorbide b. However it should be noted that chlorophyll a, chlorophyllide a, and chlorophyllide b were not present in large enough quantities to determine if there was any effect. The effect noted occurs only during processing. Chlorophyll a and chlorophyllide a are very heat labile and are reduced to a very low level by processing (4). Chlorophyllide b is more heat stable but very little was produced during this process. Therefore conclusions cannot be drawn about its extractability. However pheophytin b was present in large enough quantities to conclude that it was not affected.

Further studies were carried out to free the pigments. Use of various mild acids and bases did not release the pigments nor could a greater yield of pigments be obtained when the filter cake was resuspended in acetone and acid or base was added.

It is felt that results of this study are important because of their potential influence on analytical techniques. A great deal of work has been and is

being done on the use of buffering systems to stabilize green pigments of vegetables. Care should be taken when analyzing for such pigments that the buffering systems employed are not effecting extraction of the pigments.

On the positive side, this procedure might be useful for removing such pigments where their presence is undesirable.

#### ACKNOWLEDGMENTS

Contribution from the University of Massachusetts Agricultural Experiment Station, Amherst, Massachusetts. Supported in part by a grant from the U.S.P.H. Service, F. D. 0079-06, and the Glass Container Manufacturers Institute, New York, New York.

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TABLE 3. EFFECT OF SODIUM PHOSPHATE AND/OR MAGNESIUM CARBONATE ON THE EXTRACTION OF SPECIFIC PIGMENTS FROM SPINACH PUREE

Pigment	Control <sup>a</sup>	Buffered <sup>b</sup>	Per cent bound
Pigment content (micro mols/g dry weight)			
Chlorophyll a	0.00	0.00	—
Chlorophyll b	0.75	0.53	29.4
Chlorophyllide a	0.09	0.10	—
Chlorophyllide b	0.08	0.10	—
Pheophytin a	2.71	2.03	25.1
Pheophytin b	0.22	0.24	—
Pheophorbide a	1.38	1.13	18.1
Pheophorbide b	0.42	0.14	66.6
Total	5.54	4.43	20.05

<sup>a</sup>Control: Blanched at 87.8 C for 5 min and processed at 115.6 C.

<sup>b</sup>Buffered: Mixed with 0.35% magnesium carbonate, buffered at pH 8.0 with 1.0% sodium phosphate, blanched at 87.8 C for 5 min and processed at 115.6 C.

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## CONSOLIDATED COOPERATIVE'S ROLE

(Continued from Page 455)

which will automatically boost us toward the monetary plateau for which we are all striving.

### EFFECTS OF LACK OF QUALITY AND UNIFORMITY

Let's take a quick look at just a few of the effects of the lack of quality and uniformity on our industry.

- (a) *Consumers* are confused and confusion leads to mistrust which affects sales and profits.
- (b) *Producers* lose confidence in the basic sanitation problems because of confusion, and this is something *no* industry can long afford without being hurt both internally and externally.
- (c) *Processors* have taken advantage of special gimmicks to protect their local sales and territories. At the moment it seems like shrewd business, but in the long run their practices have promoted inefficiencies and high costs too great to survive when they finally were forced to compete with other groups.

Now . . . how do we combat these problems? The greatest single factor, in my opinion, that has come about in this decade to erase the problems of quality and uniformity, as well as others affecting our industry, has been the move toward merging or consolidating cooperatives. The unified efforts of groups covering large territories have erased countless headaches originally encountered via local and state boundaries. We must face the fact that in today's hard-nosed era of business and progress, our industry can get only what it rightly deserves through greater power . . . a greater bargaining power that comes only through the consolidated voices of dairymen united through mergers.

### CONSOLIDATION OF COOPERATIVES

Through consolidation of cooperatives we are able to virtually eliminate the headaches of state and local boundary lines. Through merging we have develop-

ed a stability of operation which does away with the uncertainties that haunt single organizations in market supplies and sales . . . competition with neighboring cooperatives . . . yes, and countless of the headaches of reciprocity that have had such an enormous effect on quality and uniformity.

Efficiency of operation through consolidation also proves a great asset to the quality program. Through more efficient organization, the industry has greater economy which allows for increased quality control programs. Using my own Dairymen, Inc. as an example, we now have a full-time quality control program coordinated between the eight Divisions of our organization. Plans are now in the making for a centralized laboratory which will not only take care of our present needs but which will go into an outstanding research program to benefit the future of our entire industry.

It would seem in order here to indicate that although we point accusing fingers at local ordinances as prime culprits in milk sanitation regulations, there are other factors which have profound even though indirect effects.

#### *Pricing of the raw product.*

Unfortunately there are still a number of states which have milk control laws either heavily dominated by one pressure group or the other which tends to breed inefficiencies and to create confusion. Hopefully, these are becoming fewer all the time.

#### *The problem of state laws.*

Our industry is plagued with some states which have not accepted, in its entirety, the United States Public Health Service Milk Ordinance and Code. Oh, they say they do. But lip service comes easily to many, and in reality they don't accept it. In one of our neighboring states there is still an antiquated law to the effect that the milk house must be at least 50 feet from the barn. Ridiculous in these days of modern sanitation.

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# BACTERIAL CONTAMINATION IN PROCESSING RABBIT FRYERS<sup>1</sup>

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## ABSTRACT

Bacterial examinations were made of skin surfaces of rabbit carcasses following several methods of processing. There was a correlation between high bacterial counts and shelf life. Prolonged pre-chilling shortened shelf life. The value of adding ice to chill water was not indicated but 15 ppm chlorine lowered bacterial counts and extended shelf life one day. The types of organisms encountered were micrococci, coliforms, gram-positive bacilli, and pasteurellae.

A search of the literature reveals no study reported on the number and types of microorganisms ordinarily recovered from commercially processed rabbit fryers. Such studies on chickens and turkeys have been reported by Drewniak et al. (1), Gunderson et al. (2), May (3), and Walker and Ayers (4, 5).

The body heat of freshly eviscerated poultry fryers is usually removed by submerging the carcasses in chilled water. The current practice of many processors is to hold these carcasses for 1 to 24 hr. Gunderson et al. (2) found that storing poultry carcasses in chill tanks beyond the cooling period caused an increase in surface bacterial contamination. The present study was undertaken to determine bacterial contamination and shelf life as influenced by several methods of processing.

## METHODS AND MATERIALS

### Experiment 1

Thirty-six rabbits weighing approximately 4.5 lb. each were processed in a commercial processing plant. Each group of six animals was handled in a slightly different manner.

*Group 1-A.* One man killed, skinned, and eviscerated all six rabbits. No water was used during this processing. The rabbits were then placed in a tank of running tap water for 1.5 hr.

*Group 1-B.* One man did all of the processing. The man rinsed his hands and the knife after skinning the rabbits. Rabbits were thoroughly washed after skinning, and again after evisceration. Carcasses were then placed in a tank of running tap water for 1.5 hr.

*Group 1-C.* One man did all of the processing. The man rinsed his hands and the knife after skinning the rabbits. Rabbits were thoroughly washed after skinning and again after evisceration. Carcasses were then placed in a tank of running tap water with ice for 1.5 hr.

*Group 1-D.* One man killed and skinned the rabbits.

Another man eviscerated the rabbits. Carcasses were thoroughly washed after skinning and again after evisceration and were then held for 1.5 hr in a tank of running tap water.

*Group 1-E.* One man killed and skinned the rabbits. Another man eviscerated the rabbits. Carcasses were thoroughly washed after skinning and again after evisceration and were then held for 1.5 hr in a tank of ice with running tap water.

*Group 1-F.* These were handled as in Group E, except that 15 ppm chlorine was added to the tap water.

Dressed carcasses from each group were trayed together and placed into a cooler. Forty-eight hours following processing, carcasses were again weighed in groups and then transported to the laboratory where bacterial counts were made every day until the carcasses were considered spoiled.

### Experiment 2

With a commercial processing plant line operating at a rate of approximately 1,200 rabbits per hour, 30 carcasses were selected at random. It can be reasonably assumed that all the products were processed in a similar manner. Following processing the carcasses were divided into five groups.

*Group 2-A.* Carcasses were chilled in running tap water for 1.5 hr.

*Group 2-B.* Carcasses were chilled in running tap water for 0.5 hr with the water being air-agitated by a circulating air pump.

*Group 2-C.* Carcasses were chilled in ice water for 1.5 hr. The water was air-agitated by a circulating air pump.

*Group 2-D.* Carcasses were chilled in ice water for 1.5 hr.

*Group 2-E.* Carcasses were chilled in ice water for 1.5 hr. In addition, 15 ppm chlorine was added to water, which was then air-agitated by the use of a circulating air pump.

A circulating pump was attached to a Y-shaped length of pipe that was fitted into the bottom of each chill tank. There were small holes drilled in the pipe to permit the passage of a stream of air.

Following the chill period, carcasses were trayed together and placed in a cooler. Approximately 48 hr following processing, carcasses were transported to the laboratory where bacterial counts were made. Coliform counts were not made during this study.

### Experiment 3

While a commercial processing plant line was operating at a rate of approximately 1,200 rabbits per hour, 30 carcasses were selected at random from the line. Following processing, the carcasses were divided into five groups and weighed.

*Group 3-A.* Carcasses were trayed together and placed in a cooler for air chilling.

*Group 3-B.* Carcasses were chilled in running tap water for 30 min, then trayed and placed in a cooler.

*Group 3-C.* Carcasses were chilled in running tap water for 1 hr, then trayed together and placed in a cooler.

*Group 3-D.* Carcasses were chilled in running tap water for 1.5 hr, then trayed together and placed in a cooler.

*Group 3-E.* Carcasses were chilled in running tap water for 2 hr, then trayed together and placed in a cooler.

<sup>1</sup>The research was accomplished while the author was located at the U. S. Rabbit Experiment Station (closed June 1965), ARS, USDA, Fontana, Calif.

TABLE 1. CARCASS MOISTURE GAIN

Group	Weight (lb) after evisceration	48 hours later	Gain (%)
1-A	11.62	12.25	5.4
1-B	11.31	11.81	4.1
1-D	11.56	12.00	3.8
Avg.			4.4
1-C (ice)	11.81	12.37	4.2
1-E (ice)	12.12	12.50	3.0
1-F (ice)	12.12	12.50	3.0
Avg.			3.4

Forty-eight hours after processing, carcasses were weighed in groups and transported to the laboratory where bacterial counts were made every other day until the carcasses were considered spoiled.

In all three experiments, bacteriological samples were obtained from the skin surface of the rib cage by firmly rolling a sterile swab over a 6 cm<sup>2</sup> area. The swab was then broken so that the cotton portion was placed directly in a test tube containing 10 ml of a 0.1% peptone broth. Total counts were made on Tryptose Agar with incubation at 32 C for 48 hr; coliform counts were made on Violet Red Bile Agar with incubation at 32 C for 48 hr. Total counts were made to determine the bacterial population. Samples were taken every other day until the carcasses were considered spoiled (by odor and slime). During the time these cultures were made, carcasses were held at 4 C in home-type mechanical refrigerators.

## RESULTS AND DISCUSSION

The total moisture pickup of each group of rabbits in Experiment 1 is indicated (Table 1). Each group was weighed immediately after processing and again 48 hr later, just before packing and transfer to the laboratory. Carcasses in Groups 1-A and 1-D, those without ice in the tap water, had a total percentage moisture increase of approximately 35% greater than those groups in which carcasses were washed in tap water plus ice. While this increased weight has value to the processor, the increase in tissue moisture may adversely affect the shelf life of the product.

Averaged bacterial counts from Experiment 1 are shown (Table 2). Generally, the bacterial counts were relatively low following killing. There was a correlation between high bacterial count and shelf life as indicated by those carcasses in Groups 1-E and 1-F compared with Group 1-A. However, there was no correlation with the numbers of bacteria required to produce spoilage. In Group 1-C spoilage accompanied a bacterial count of 34,000 per 6 cm<sup>2</sup> while in Groups 1-E and 1-F spoilage was found when counts were 8,500 or less. While the numbers involved are few, some tentative conclusions can be made from the data. The use of one man to kill and skin and another man to eviscerate rabbits is bene-

ficial as indicated by the increased shelf life in Groups 1-D, 1-E, and 1-F as compared with Groups 1-A, 1-B, and 1-C. Thorough washing of the carcass also contributed to a longer shelf life, which is indicated in Groups 1-B and 1-C as compared with Group 1-A. The highest total bacterial count and shortest shelf life was found in those carcasses processed by one man who killed, skinned, and eviscerated and used no water during processing. The value of chilling in ice and water is not clearly indicated. Total bacterial counts and shelf life for both Groups 1-D and 1-E were comparable. Final day coliform counts varied with the method of handling. When numbers of coliforms are compared with total bacterial populations, the coliforms do not appear to be a prime factor in spoilage. Spoilage occurred when the coliforms made up 7% and 30% of the bacterial count, but the day on which spoilage took place was later with the lower count.

Using colonial appearance and gram stain morphology as the only criteria for identification, some of the types of microorganisms recovered from the skin surfaces could be described as micrococci, coliforms, gram-positive bacilli, and organisms resembling pasteurellae. In order of incidence, the micrococci were most frequently noted.

Averaged bacterial counts from Experiment 2 are shown in Table 2. These counts were relatively low following killing, and then increased with each following day. As indicated in the previous study, the value of chilling in ice and water is not clearly indicated. Total bacterial counts and shelf life for Group 2-D are comparable to those of Group 2-A. Air agitation appeared to adversely affect both total bacterial count and shelf life. This appears most evident in Groups 2-A and 2-B where chilling was done in running tap water only. Adding ice to the air-agitated tap water extended shelf life from 14 days found in Group 2-C to 16 found in Group 2-D. The reduction in bacterial population resulting from adding 15 ppm chlorine to the tap water is illustrated in Group 2-E. Day 2 total bacterial counts were lower in Group 2-E than in any other group in this study, and low counts continued through Day 6. In other lots, total bacterial counts became too many to count as early as Day 6. Shelf life, however, does not appear to be extended as a result of the addition of chlorine to the chilled water as spoilage took place on Day 16 for Groups 2-A, 2-D and 2-E.

Following the air agitation cycle in the processing procedure, the pipes were removed from the bottom of the chill tanks, disassembled, and then examined. A considerable amount of organic material was found in the contents of these airlines. Presence of such material could well explain the high bacterial counts noted in those groups wherein the water was aerated

TABLE 2. BACTERIAL CONTAMINATION IN PROCESSING RABBIT FRYERS: AVERAGE COUNTS PER 6 CM<sup>2</sup> WHEN HELD AT 4 C FOLLOWING KILLING

Group <sup>a</sup>	Counts at day <sup>b</sup>					Coliform count	Day spoiled	Day extended <sup>c</sup>
	2	4	6	8	10			
<i>Experiment 1</i>								
1-A	22,600	TM,S	---	---	---	5,830	4	0
1-B	1,230	6,900	S,Day 5(17,700)	---	---	6,810	5	0
1-C	1,160	7,250	34,000,S	---	---	2,830	6	0
1-D	1,040	5,500	20,200	S,Day 7(TM)	---	5,600	7	0
1-E	2,050	---	4,600	S,Day 7(8,700)	---	2,160	7	0
1-F	3,460	1,600	7,300	7,750,S	---	500	8	0
<i>Experiment 2</i>								
2-A	3,910	5,300	19,300	TM	---	---	16	8
2-B	6,330	20,250	TM	---	---	---	14	8
2-C	1,320	7,160	TM	---	---	---	14	8
2-D	1,700	3,500	20,250	TM	---	---	16	8
2-E	240	1,050	5,700	28,900	TM	---	16	6
<i>Experiment 3</i>								
3-A	3,250	3,000	1,500	3,500	5,160	---	20(D)	6
3-B	3,370	2,750	5,660	TM	---	---	18	10
3-C	3,660	6,120	6,710	TM	---	---	18	10
3-D	1,900	2,700	6,170	TM	---	---	14	6
3-E	2,870	9,170	TM	---	S	---	10	4

<sup>a</sup>Experiment 1: A, one man processing, no water used; B, one man processing, hands, knife, and carcasses rinsed with water; C, one man processing, hands, knife, and carcasses washed after skinning and evisceration; D, one man killed and skinned and 1 eviscerated, carcasses washed after skinning and evisceration; E, same process as D, but ice was added to chill water; F, same process as E, but 15 ppm chlorine was added to water.

Experiment 2: A, carcasses were chilled in running water for 1.5 hr; B, same process as A except water was air-agitated; C, same process as B except ice was added to water; D, Carcasses were chilled in ice water for 1.5 hr; E, same process as D except water was air-agitated and 15 ppm chlorine was added to water.

Experiment 3: A, carcasses were air-chilled; B, carcasses were chilled in running water for 30 min; C, carcasses were chilled in running water for 1 hr; D, carcasses were chilled in running water for 1 1/2 hr; E, carcasses were chilled in running water for 2 hr.

<sup>b</sup>TM = >35,000; S - spoiled; D - discarded, dehydration, not spoiled.

<sup>c</sup>Days shelf life extended past day bacterial counts became too numerous to count.

mechanically. Bacteriologically contaminated water was probably circulated in the chill tanks in Groups 2-B, 2-C and 2-E. The presence of chlorine in the wash water in Group 2-E undoubtedly accounts for the lower bacterial growth.

The total moisture pickup of each group of carcasses from Experiment 3 is indicated in Table 3. As might be expected, those carcasses that were air-chilled in the cooler lost weight, whereas those held in chill tanks gained moisture. The percentage of moisture gained was in ratio to the time these carcasses were held in the chill water. A relationship between immersion in chill water and shelf life was found. Those carcasses held for 30 min and 1 hr in chill tanks had a shelf life of 18 days, whereas those carcasses held for 1.5 hr and 2 hr had a shorter

shelf life of 14 days and 10 days, respectively. Bacterial counts (Table 2) remained lowest in Group 3-A, those animals chilled in the cooler. Fourteen days' storage was required for the colonies to become too numerous to count. These carcasses were discarded on Day 20, however, not because of spoil-

TABLE 3. CARCASS MOISTURE GAIN WEIGHT (LB)

Group	After evisceration	48 Hours later	Gain (%)
3-A	14.8	13.0	-12.2
3-B	13.2	13.0	- 1.5
3-C	13.8	14.2	+ 2.9
3-D	13.0	13.6	+ 4.6
3-E	12.0	12.6	+ 5.0

age, but because of dehydration and appearance. Shelf life decreased in proportion to the duration of immersion in the chill water. Bacteriological counts in Group 3-E, those animals held longest in the chill water, were the first to reach numbers too numerous to count. The results of this study indicate that bacterial contamination and shelf life of a rabbit carcass are affected by prolonged pre-chilling in tap water. It would appear that optimum gains, both in shelf life and carcass weight, are achieved by a 1 hr period of pre-chilling. The number of days shelf life could be extended past the day bacterial counts became too numerous to count was greatest in Groups 3-B and 3-C. Air chilling, while effective in holding down bacterial contamination and prolonging shelf life, cannot be considered because of dehydration affecting the appearance of the product.

#### CONSOLIDATED COOPERATIVE'S ROLE

(Continued from Page 459)

Many states are still not accepting reciprocity, as I mentioned earlier. They insist upon having their own inspectors check a product before it can move across the state line, even though it has been checked in its home state. And, they can do a pretty good job on this point in order to protect their own interests. We must remember that this is an age of jet-propelled transportation, on the ground as well as in the air, and with modern transportation it is now possible, and should be profitable, to move our milk across many state lines without the continuous delays of repetitive examinations.

##### *Local ordinances*

Local ordinances also can become major bottlenecks in our industry. In this general area, though not a part of Dairymen, Inc., there exists a major market with a Grade A ice cream law. A little ridiculous in my estimation since people will travel to a point where they can buy a product of good quality, such as is true with ice cream that is *not* made with Grade A milk. Historically there has been no real public health significance shown for the need for Grade A milk in ice cream. Where the Public Health Code is not needed for ice cream, however, Grade A cottage cheese does make sense. There is a greater problem from the standpoint of quality and a greater need. But again, some municipalities have used this general area as a means of protecting their market . . . or at least they think they are protecting their market.

#### ACKNOWLEDGEMENTS

Appreciation is expressed to Dr. I. P. Blum, Veterinarian, Bureau of Poultry Inspection, California State Department of Agriculture for his advice and assistance.

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#### LABELING OF PRODUCTS

Problems also facing us include the marketing trickery of labeling products . . . and . . . equipment standards to mention a few. In their own way, all of these problems have an effect on the quality program needed in our industry. By the same token, all of these problems can be better met through the powerful voices of dairymen united under cooperative operations.

#### DAIRY AND INDUSTRY POLITICS

Some time ago I was asked if I was trying to get the dairy industry into politics. My answer was . . . and is . . . "you're darned right I am". Local, state, national . . . any form of politics where the voice of the dairyman can be heard loud and strong through the action of the cooperatives.

Now and then, many of you are probably saying to yourself, "We're aware of this, but we've come a long way *without* all of this consolidation of cooperation bit.

Have we? I ask you . . . have we really come a long way or have we drifted into what has come naturally. Recently someone placed an article on my desk which appeared in a farm magazine in 1923 warning the dairy industry and the general public about the dangers of dairy substitutes. Here we are, almost half a century later, wringing our hands about this problem, and believe me it is a problem. We sometimes joke about our wives and tend to picture them as worshipful, dependent souls. Wor-

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## NEW PRODUCT DEVELOPMENT—INDUSTRY APPROACH<sup>1</sup>

WILLIAM F. STOLL

*Green Giant Company  
Le Sueur, Minnesota*

### ABSTRACT

Vitality of our economy today stems in part from industry's activity in new products. Industries content to rest on past accomplishments have found themselves in an unfavorable competitive position. In our changing society we find changing needs. What is good for today would not have met the needs of an earlier time nor will it be sufficient for a future day. This is the philosophy which is necessary for corporate survival in our rapidly changing society.

New product activity may be merely innovation of old product concepts but sufficiently new to warrant research and development activity and development expenditures. As new technology develops, new product concepts which were unfeasible become reality.

Organization within a company, necessary for successful development of new products, is complex. Marketing, accounting, production, and research and development efforts must be coordinated to implement the introduction of a new product. New product activity is hazardous for there are many pitfalls. Failures are common in the market place; however, the rewards are great for the creator of a successful products.

In the beginning one can make an obvious statement. Product development or new product development is an important activity within our economic society. New has become a contagious thing, perhaps best epitomized in our auto industry where some few years back one could identify without much difficulty the model and year of a vehicle. Today one is fortunate to recognize the model, i.e. Ford, Chevrolet, etc. let alone the year. We are caught up in a situation where change is essential for economic survival.

### NEED FOR CHANGE

Having had a background in the Dairy Industry, I can make some observations concerning this industry which can illustrate the necessity for change. Here is a segment of the food industry which has done a tremendous job in the production and quality aspects of the food product area. However, evidence indicates the Dairy Industry has lost some of its marketing advantage, particularly with butter. In 1960 butter consumption was 9.1 lb per person, where today consumption is less than 5 lb per person. Are these problems of the Dairy Industry because

they have too long neglected the marketing aspects, including new product development? The American Dairy Association is doing a very creditable job in this area today. New products, based on dairy ingredients, are becoming more prevalent—the growth of chip dips and yogurt are examples. Presently I am using dehydrated cultured products; these are also new.

### WHAT IS A NEW PRODUCT?

Before proceeding further I think we should agree in thinking: "What constitutes a new product?" In most instances what is termed a new product is merely an innovation of an existing or familiar product. For example, in March of 1968 the Green Giant Company introduced a line of Pork and Bean items, Brown Sugared and Molasses Ovenscrock in three markets. Here is an old product but new to the Green Giant line. It was new because it was an innovation of an existing product. Rarely is a new product so uniquely new as to fit the pure definition of the term. Therefore, a new product might be considered as one in which there is sufficient innovation to warrant development cost and can make a spot for itself in the market place.

In recent months and years, development of highly nutritious products from inexpensive raw material has received considerable attention. It is becoming increasingly apparent that this is one area of new product development which must be considered if we are to solve problems of world hunger or even hunger and malnutrition in America.

### *Criteria for new product success*

There are several criteria which a product must meet on the road to becoming a new product in the market place. Most importantly, is there a market need? Green Giant defines the seven criteria of new product success factors as: profitability, distinctiveness, adaptability, trade acceptance, consumer acceptance, volume, and areas of competition.

Reference has been made from within our company that we are "marketing oriented." I would like to rephrase this to say we are "market needs oriented". To illustrate—suppose we would develop new products which were designed to use existing plant facilities with no thought of the marketability of the finished product. Probably the product would

<sup>1</sup>Presented at the Fifty-Sixth Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Inc., Louisville, Kentucky, August 17-21, 1969.



fail after expenditure of large sums of development monies. A point here can be made. A successful new product is not so different that the consumer cannot relate it to past experience and present need. Many of the successful new products we see today would not have succeeded ten years ago.

#### *One company's position on new products*

It would be helpful to review briefly the Green Giant Company's position on new products and also to reflect somewhat on the industry's attitude. One-third of the Green Giant Company's sales growth each year is to come from new product introductions. This responsibility falls to a new products marketing group and a new products development group. Approximately 1% of sales is set aside for research and development (R and D) activities. Stated in the Company's 1969 report, "The Green Giant Company recognizes that corporate growth and vitality depend more and more on development of new products and organizational steps were taken during the past year to strengthen this effort".

The company has in the past year completed a 38,000 ft<sup>2</sup> product research facility which houses offices for product development staff; 33,000 ft<sup>2</sup> of pilot plant and storage area and product development kitchens.

#### *New product activity in food industry*

Concerning new product activity in the food industry as a whole, in 1966 there were over 7,000 new items introduced. Obviously finding space in the store for new products is becoming an increasing problem. Therefore, new product introductions are becoming more difficult. Long term new products are rare and the lead time that a company has with a new product is about 2 years.

#### *Life cycle of new product*

The life cycle of a new product may be compared to the bacterial growth curve. Booz, Allen, and Hamilton categorized the life cycle of a new product into: introduction, growth, maturity, saturation, and decline periods. Many factors influence the shape of the curve. In the period 1961-1966, 48% of the products used had a primary life cycle of less than 2 years, 15% over 3 years. It is the job of marketing and R and D to design and promote products in such a way that they have a rapid growth rate and a long saturation phase.

#### *System for new product development*

For a company to be successful in the new product development business, it must have a system which coordinates the necessary activities which will bring the product into the market place. I suppose that each company has its own system which works best

for their situation. In any event, any system must integrate R and D, production, and marketing efforts. One without the other results in inefficiencies which place a new product at a disadvantage from the beginning.

I will discuss somewhat the Green Giant Company's system for new product development. Much of this material has been discussed by Dr. John Jackson at the recent 17th Annual Food Technology Conference at Columbia, Missouri.

To start, the President of the Company defines the corporate "fields of interest". Marketing then identifies market needs within "fields of interest" and translates needs into concepts. After sufficient evaluation, the Product Planning Committee takes action. This committee is made up as follows: Vice-President of R and D who serves as Chairman, Director of Marketing Development, Director of New Product Marketing, Director of Product Development, Director of Plant Production Planning and Services, Director of Profit Planning, Director of Quality Assurance, Controller, and New Products Coordinator who serves as Secretary.

The development of a new product at Green Giant moves through five stages. The responsibility for development of a product through the various stages rests with the Product Development Team, composed of a member from New Product Marketing and a member from Product Development. This team reports at various stages to the Product Planning Committee which acts upon the Team's evaluation of the product at each stage of development. The activities of the team are coordinated by the New Products coordinator. At Stage I, the parameters of a new product are developed. These parameters serve as guidelines for the R and D group to begin building a prototype. It serves to alert the Product Planning Committee that development is beginning in the area.

At Stage II, evaluation of a prototype of the product has been developed along with a production cost analysis. Stage III involves consumer testing and evaluation of the product. Stage IV involves development of production plans in which long range capital requirements are developed. On the basis of Stage III and IV results, the decision is made to enter test markets with the product (Stage V). Assuming a successful performance in test market, the product enters the regular line. The necessary activities to move a new product through the various stages is generally coordinated by a critical path schedule. This schedule is designed to take a product to the market test stage.

I should dwell just a moment on the economics involved. Before a prototype is accepted by the Prod-

uct Planning Committee, it must meet Company profit objectives or if this should not be possible, contribute sufficiently to corporate objectives to justify its existence. Charged against the selling price are such items as marketing costs, processing and packaging costs, and distribution costs. Somewhere in this cost package is included those intangible items called indirect costs.

With each new product developed there also develops a new set of problems relating to the product. Early in the stages of development, product specifications are written covering the in-going materials and the finished product. Quality Assurance personnel develop those tests which they feel will give them adequate quality evaluation of the product. Processing procedures are developed in detail. From these beginnings flow diagrams and processing equipment are developed.

In many instances the new product requires new technology, or at least new technology within the company. It is not uncommon that the first apparent need arises only after the product is in production. It was for this reason that the Green Giant Company provided pilot plant facilities in conjunc-

tion with new product development. Previously expensive factory time was needed to test out the necessary processing steps for the new product. The transition from hand made prototype samples to pilot plant and then factory samples can produce some puzzling and costly results.

Ingredients for formulated products can also produce results which are unexpected. Despite all of our sophistication in science, I believe there is much which we do not know about products with which we deal. Variation in character of ingredients going into a product can produce unanticipated results. Although it is the job of the new product development scientist to anticipate and solve all of these problems before a prototype is presented, a realistic evaluation of the odds makes this improbable. All that can be done is to bring to bear all the facts and knowledge that the individual can accumulate. *A fundamental and practical background in food technology is invaluable in this respect.*

In conclusion the development of a successful new product demands the best skills which a corporation can bring to bear—marketing, research, production, accounting, and quality assurance.

#### CONSOLIDATED COOPERATIVE'S ROLE

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shipful they may be, we hope. But dependent they are not. Though we may be running the business, thousands of faceless women are running us and we can't sell them short. They're shopping economists. They cannot be fooled about the behind-the-scenes problems, such as quality, which we try to hide from them. The resentment in these ladies caused by the knowledge that they are being deceived in so many ways . . . by packaging . . . by paying higher prices because of the many side effects of the lack of uniformity and quality . . . that their tax dollars paid out are higher because of lack of proper legislation in our field regarding uniformity . . . that resentment of the female consumer—and that passed on to her husband—is going to unleash a hornets' nest in short order.

#### SUBSTITUTES

In fact, there's a breed of hornets buzzing all around our heads right now that the housewife is becoming more and more enamored with. It's that 1923 item I mentioned a few moments ago which has grown into quite a swarm . . . dairy substitutes. You can find them on every grocer's shelf in the most inviting and economical packages you've ever

seen. You can hear them advertised actually contrasting them with real dairy products so that the real dairy product seems like a dirty word. Our smart female consumer still realizes that dairy products offer her more in taste, cooking quality, and nutrition. But we must face the fact that with the average consumer the purse string is stronger than the taste bud, and our industry cannot afford to slip in any direction.

Today, through our cooperatives, we are able not only to advance our quality control programs, but, hopefully to advance the story of quality to the public. This is an age for moving forward . . . for consolidating into positions of strength that cannot be defeated. The first fellow to break his leg will be the guy looking back over his shoulder to see where he's been instead of keeping his eyes on the road ahead to see where he's going.

#### PROGRESS

I am a firm believer in the old proverb, "blessed are those who are not satisfied with things as they are: to them we owe all the progress that the world has made." Be proud of your past . . . but turn loose of it in favor of a more progressive future. The small cooperative which sits in its tiny corner of this

(Continued on Page 479)

## FIFTY-SEVENTH ANNUAL MEETING OF IAMFES

Cedar Rapids, Iowa  
August 17-20, 1970

Nearly 400 members and guests attended the 57th annual IAMFES meeting at the Roosevelt Hotel in Cedar Rapids, Iowa. The meeting was hosted by the Iowa Association of Milk, Food, and Environmental Sanitarians. Participants at the meeting had opportunities to attend one or several committee meetings, listen to papers on different timely topics, learn about the status of their Association at the business meeting, attend the annual awards banquet, go on tours, visit hospitality rooms, and attend a horse show.



The IAMFES Executive Board for 1970-71. Left to right: Milton E. Held, Professor Richard P. March, Orlowe M. Osten, Dick B. Whitehead, Elmer E. Kihlstrum, and Walter Wilson.

### EXECUTIVE BOARD MEETINGS

The IAMFES Executive Board began its deliberations on Sunday (August 16) afternoon and continued until Monday evening (August 17). Representatives from the local arrangements committee described in detail the events which were planned for the meeting which began on Monday evening.

The Executive Board moved to reduce the "communication gap" between it and the Affiliate Council. According to the IAMFES constitution, the two past-presidents on the Board are members of the Council. This requirement will be implemented more fully in the future and the Chairman of the Affiliate Council will be asked to attend meetings of the Executive Board.

Dr. E. H. Marth presented his report as Editor of the *Journal of Milk and Food Technology*. Marth indicated that: (a) a backlog of papers exists and larger issues of the journal will be needed to minimize the delay in publication of papers, (b) changes are needed in the editorial board of the journal, (c) the regular use of editorials will not be pursued since most



U. S. Congressman Fred Schwengel speaking at the opening session.

members of the Editorial Board exhibited little interest in preparing them, and (d) the possibility exists that some mutually beneficial arrangements for publication of papers can be worked out with the *Journal of Dairy Science*. The Executive Board voted to seriously explore this possibility provided such arrangements would not result in elimination of papers which presently appear in the *Journal of Milk and Food Technology*.

H. L. Thomasson presented his report and recommended that: (a) a membership committee be appointed which would work to expand IAMFES membership among persons in the food industry, (b) a



Members of the local arrangements committee and speakers at the opening session. Left to right: Farris Biggart, Roy Roedel, Don Jaeger, U. S. Congressman Fred Schwengel, and Mayor (of Cedar Rapids) Don Canney.



A visitor from Scandinavia. Dr. Irene von Bockelmann, Lund Institute of Technology, Alnarp, Sweden.

committee be appointed to work on securing more advertising for the journal, and (c) plans be made to hire a successor for the current Executive Secretary and Managing Editor since Mr. Thomasson plans to retire in a few years.

The possibility of forming a single organization for sanitarians was discussed briefly but since no firm proposals were pending, no action was needed nor could it be taken. The Board voted to reaffirm that the past position of IAMFES still prevails and that an interest in further negotiations still exists. No steps, however, will be taken until firm proposals are received from the National Environmental Health Association (NEHA). President Held indicated that IAMFES has received an invitation to attend the 1971 annual meeting of NEHA in Portland, Oregon.

John Fritz reported on activities of the Sanitarian's Joint Council. He indicated efforts would be made to determine if changes are needed in the registration act and that attempts will be made to develop a more meaningful definition of "sanitarian." Fritz offered to prepare a position paper on development

of the term "sanitarian" and agreed to make the paper available for publication in the *Journal of Milk and Food Technology*.

According to A. K. Saunders, the Dairy Farm Methods Committee will have a new subcommittee to deal with problems of liquid manure handling and with pollution caused by manure from feed lots. Saunders also indicated that a summary of 15 years of work by the Committee has been prepared for publication. E. H. Marth, H. L. Thomasson, A. K. Saunders, and J. C. Flake were asked to work out details for publishing this document.

A. E. Parker described activities of the National Mastitis Council and indicated that M. W. Jefferson will serve as an alternate IAMFES representative to the Council. Dr. K. G. Weckel reported for the 3-A Symbol Council and indicated that: (a) bylaws were changed to permit appointment of an assistant secretary-treasurer, (b) a successor for C. A. Abele is needed, and (c) procedures for handling applications and for mail ballots must be clarified.

Future IAMFES meetings are scheduled as follows: (a) 1971; August 15-19; San Diego, California; headquarters hotel will be the Ramada Inn; the regional meeting of the National Mastitis Council is scheduled for August 16, 1971 at the adjacent Travel Lodge Motel; and (b) 1972; August 20-24; Milwaukee, Wisconsin; the Pfister Hotel will be the headquarters for the meeting.

The following committee was designated to ar-



Professor E. O. Wright, Co-Chairman of Local Arrangement Committee and Master of Ceremonies, (left), the Iowa Dairy Princess, and Ivan Parkin at the Awards Banquet.



Celebrities at the IAMFES meeting. Left to right: Helen Biggart, Farris Biggart, Nina Held, and Milton Held.



Some of the ladies gathered for the start of a tour.



Conversation between sessions.

range for a replacement when H. L. Thomasson retires: Milton Held, Orlowe Osten, and Elmer Kihlstrum.

#### AFFILIATE COUNCIL MEETING

The Affiliate Council, with Ben Luce as Chairman, met on Monday evening, August 17th. The opinion was expressed that locations for annual IAMFES meetings should be designated at least 3 years in advance. Professor R. P. March agreed to explore the possibility of meeting in Rochester, New York in 1973.

Luce reported that a questionnaire was prepared and submitted to all 25 affiliates. Responses were

received from 21 and results will be summarized and reported at a later date.

Some time was devoted to a discussion of affiliate members who do not belong to IAMFES. It was indicated that in some states all affiliate members belong to IAMFES but in others only a portion hold both memberships. Affiliate representatives were asked to encourage local members to join IAMFES. Some affiliate representatives felt the *Journal* was useful for securing new IAMFES members.

Affiliate representatives were encouraged to have their local organizations: (a) recommend (by December 1, 1970) speakers for the 1971 annual IAMFES meeting and (b) nominate persons for the Sanitarian's Award.

A committee composed of Dr. R. M. Parry, Pat Dolan, Karl Jones, and Leon Townsend will develop criteria for the Shogren Award which is scheduled to be presented annually to the affiliate with the most outstanding record of achievement.

Officers of the Affiliate Council for 1970-1971 are: Chairman, Ben Luce; and Secretary, Leon Townsend.

#### TECHNICAL SESSIONS

This year seven papers were presented in the General Sessions. Topics covered include: frozen food microbiology, sanitation in the food industry, solid waste handling, sanitation in other countries, and environmental problems and policies. Eight papers were presented in the Milk Sanitation section. These papers considered topics such as milk flavor, sterilized dairy and imitation products, plant quality assurance, manure disposal systems, testing milk for cleanliness, bacteriological testing of milk, recruiting personnel, and the fieldman in 1970.

The Food and Environmental Sanitation section offered six papers on subjects such as enzymes in the food industry, control of water pollution, microorganisms on flies, rodents in the environment, food protection research at the Food Research Institute, and health care facilities.

Three papers which dealt with inspection of flour, microbiology of poultry products, and sanitation by design were presented in the Food Industry Sanitation section.

Abstracts of nearly all papers presented at the meeting appear elsewhere in this issue of the *Journal*. Most papers presented at the annual meeting will be published in subsequent issues of the *Journal*.

#### BUSINESS MEETING

The annual business meeting was called to order by President Milton Held on Wednesday, August 19, 1970. The membership heard reports from: (a) H. L.



Some of the reports at the business meeting were given by: Dr. Frank Bryan, upper left; C. A. Abele, upper right; A. K. Saunders, lower left; and B. Luce, lower right.

Thomasson on his activities on behalf of the Association and on the financial condition of IAMFES, (b) E. H. Marth on the status of the *Journal of Milk and Food Technology*, (c) C. A. Abele on the 3-A Symbol Council, and (d) Ben Luce on the Affiliate Council. Reports also were presented by the following persons on behalf of their respective committees: Dr. A. R. Brazis—Applied Laboratory Methods, Karl Jones—Food Equipment Sanitary Standards, Dick B. Whitehead—Sanitary Procedures, Erwin Gadd—Frozen Food Sanitation; Dr. Louis King—Baking Industry, William Hickey—Food Protection, A. K. Saunders—Dairy Farm Methods, and Dr. Frank Bryan—Communicable Diseases Affecting Man. Most of these reports will appear in later issues of the *Journal of Milk and Food Technology*.

President Held announced that: (a) Walter Wilson was elected as the second vice-president, and (b) Professor R. P. March was elected as secretary-treasurer.

The Resolutions Committee consisting of S. O. Noles (chairman), C. A. Abele, and Roy Ginn presented the following resolutions which were unanimously adopted by the membership.

#### Resolution No. 1

Whereas: The Iowa Association of Milk, Food, and Environmental Sanitarians has hosted the 57th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, held at the Roosevelt Motor Hotel August 17-20, 1970; and

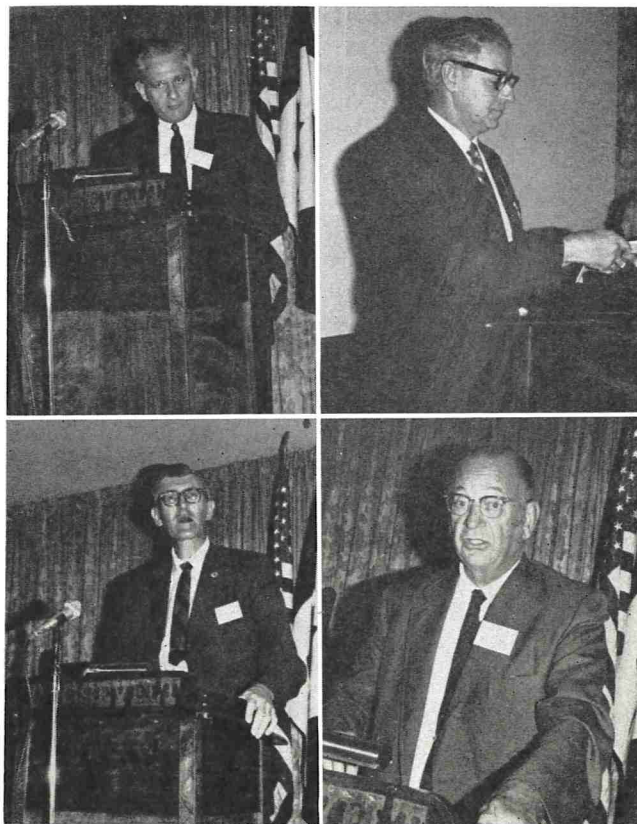
Whereas: The Local Arrangements Committee of the Iowa Affiliate and Management of the Roosevelt Motor Hotel provided excellent facilities and detailed plans for the conduct of the meetings, as well as exceptionally fine entertainment for the members and their families; and

Whereas: The Merchants' National Bank generously financed the meals for the ladies' tours; and

Whereas: The Chambers of Commerce of Cedar Rapids and Marion, Iowa, added a touch of beauty by providing the lovely flowers for the ladies at our Awards Banquet;

Be It Therefore Resolved: That we, the members of International Association of Milk, Food, and Environmental Sanitarians, express our grateful thanks to the Iowa Association, officers, committees, members, and friends who have contributed so unselfishly of their time, financial contributions, and individual efforts to make this Annual Meeting an outstanding success; and

Be It Further Resolved: That copies of this resolution be forwarded to the President of the Iowa Affiliate, Chairman of the local arrangements committee, the Merchants' National Bank, and the



Some of the reports at the business meeting were given by: Dr. E. H. Marth, upper left; Dr. A. N. Myhr, upper right; Dr. A. R. Brazis, lower left; and Dr. L. A. King, lower right.

Chambers of Commerce of Cedar Rapids and Marion, Iowa.

**Resolution No. 2**

Whereas: Dr. Elmer H. Marth during his three-year tenure as Editor of the *Journal of Milk and Food Technology* has accomplished such meritorious success in publication of the most informative, outstanding Journal of its kind;

Therefore Be It Resolved: That the members of this Association join the Executive Board in expressing our commendation to Dr. Marth for his untiring efforts in contributing to the constant education of the membership and our gratitude for his willingness to continue this inspiring leadership as Editor of our Journal, and that a copy of this resolution be forwarded to him as a token of our appreciation.

**Resolution No. 3**

Whereas: The International Association of Milk, Food, and Environmental Sanitarians has for a number of years expressed its concern about the continuing high incidence of food-borne illness in this country; and

Whereas: The ultimate control of food-borne disease is dependent upon the application of adequate prevention control measures; and

Whereas: This Association recommended in Resolution No. 6 at the 56th Annual Meeting of 1969 that there be a National Food Protection Conference sponsored by the United State Public

Health Service to bring nationally recognized experts together to work toward resolution of the many existing and new food protection problems facing the nation; and

Whereas: The Food and Drug Administration has responded in a commendable manner to this resolution by contracting with the American Public Health Association to organize and conduct such a National Conference scheduled to be held in Denver, Colorado, during the month of April 1971;

Therefore Be It Resolved: That the International Association of Milk, Food, and Environmental Sanitarians go on record as heartily commending the Food and Drug Administration and the American Public Health Association for making this National Conference possible; and

Be It Further Resolved: That this Association offer to involve its membership in this worthwhile and essential endeavor; and

Be It Further Resolved: That this Association urges the Food and Drug Administration and American Public Health Association take necessary steps to assure that the conference deliberations be primarily preventive in nature to accomplish a maximum of return in public health benefits from endeavors of participants; and

Be It Further Resolved: That copies of this resolution be forwarded to the Commissioner of the Food and Drug Administration and the President of the American Public Health Association.

## BARNUM AND PARKIN RECEIVE AWARDS AT 1970 IAMFES MEETING



Harold J. Barnum, left, receives the Honorary Life Membership Award from Dr. A. N. Myhr.

The committee on Recognition and Awards selected Harold J. Barnum as the 1970 recipient of the Honorary Life Membership and Ivan E. Parkin of the Citation Award. The Sanitarian's Award was not given this year. Dr. A. N. Myhr served as Chairman of the committee. Other members were: Karl

K. Jones, John H. Fritz, Ivan E. Parkin, and Virgil N. Simmons.

### HONORARY LIFE MEMBERSHIP AWARD TO HAROLD J. BARNUM

The Honorary Life Membership is awarded annually to one or several IAMFES members who have given long and faithful service to the association. Members in this class have all distinguished themselves by the very substantial contributions they have made over the years to the furtherance of the objectives of this Association. This year the honor went to Mr. Harold J. Barnum.

Mr. Barnum obtained his B.S. degree from Montana State University and the M.S. degree from Michigan State University. In 1929 he began his career as a milk inspector for the City Health Department in Detroit. Over the years he became recognized as both a professional sanitarian and able administrator. He was made Chief of Milk Sanitation Services for Denver City and County in 1947. Within a few years, Mr. Barnum had reorganized his office, promoted industry and public cooperation in joint sanitation programs, and had raised Denver's milk sanitation rat-

ings to commendably high levels. Mr. Barnum also devoted a great deal of effort to furtherance of sanitation on the national scene. He was elected to the Executive Board of IAMFES and served as President of the Association in 1952.

Since then Harold has served on many of the Association's committees and has been Chairman of the National Conference on Interstate Milk Shipments, Secretary of the National Products Labeling Committee, Executive Secretary of the Dairy Products Improvement Institute, and a member of the Advisory Committee for the 1965 USPHS Grade A Milk Ordinance and Code. He received the coveted IAMFES Sanitarian's Award in 1957.



Ivan Parkin, left, receives the Citation Award from Dr. A. N. Myhr.

#### CITATION AWARD—IVAN E. PARKIN

The Citation Award is presented annually to a member of IAMFES who has contributed substantially to the growth, professional advancement, and status of the Association. The 1970 winner of the award was Mr. Ivan E. Parkin.

Ivan obtained his B.S. degree in Agriculture from the University of Connecticut and for many years was on the faculty of Pennsylvania State University as Dairy Extension Specialist. He was elected to the Executive Board of IAMFES in 1951 and was President of the Association in 1954-55.

Ivan has commanded the respect and admiration of his fellow sanitarians during the many years he has participated in the affairs of this Association. He has always held strong views on most questions brought before the membership and has particularly enjoyed entering into discussions of controversial matters. He has served with distinction on the Committee on Sanitary Procedures, the Farm Methods Committee, and many others. In 1965 this Associ-

ation recognized his many years of faithful service by awarding him Honorary Life Membership.

Mr. Parkin retired from Pennsylvania State University in 1963 after which he returned to his home state of Connecticut. His retirement, however, did not diminish his interest in IAMFES and he has continued uninterrupted attendance at the annual meetings. He has thereby continued to make himself available to the Executive Board and other members for consultation and advice and has willingly performed numerous assignments, often on short notice. As an example, last year when it became known that the late Roy Fairbanks had become incurably ill, Ivan did not hesitate to accept the invitation to serve as Acting Secretary-Treasurer for 1969-70. For the past several years, he has ruled as a firm but fair parliamentarian at our annual business meetings. Each year sanitarians in attendance at the Awards Banquet have marvelled at the professional manner in which he has delivered the invocation.

Ivan holds, and is not afraid to manifest, a genuine affection for his fellowman. This is the main reason why he is so universally liked by those who are privileged to know him. In his 1970 Invocation he said, "Sanitarians should enter into controversy without bearing hate or rancor." Those words typify the true nature of Ivan E. Parkin.

#### SANITARIAN'S AWARD

This award is presented to the member of IAMFES who, in the opinion of the Committee, has made the greatest contribution to the field of public health sanitation during the preceding 7 years. The award consists of a plaque and \$1000.

The award has been made annually since 1952 with the exception of 1964 when it was not presented because insufficient nominations were received to provide meaningful competition. The Committee on Recognition and Awards regretted that the same situation prevailed again in 1970 and therefore the Sanitarian's Award was not presented.

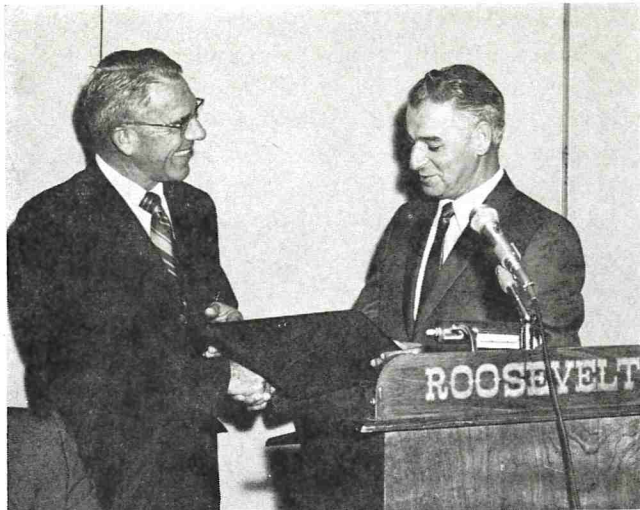
The award is sponsored jointly by three commercial chemical companies: the Diversey Corporation, Klenzade Products (Economics Laboratory), and the Pennwalt Corporation. Although these companies are the sponsors, the award is administered by IAMFES.

#### COMPLETE LIST OF AWARD RECIPIENTS

##### *Honorary Life Membership*

- 1957—Dr. J. H. Shrader
- 1958—H. Clifford Goslee
- 1959—Dr. William H. Price
- 1961—Sarah Vance Dugan
- 1963—Dr. C. K. Johns and Dr. Harold Macy





S. O. Noles, left, receives the Past-President's Award from Dr. A. N. Myhr.

- 1964—C. B. Shogren and A. L. Shogren
- 1965—Fred Basselt and Ivan Parkin
- 1966—Dr. M. R. Fisher
- 1967—C. A. Abele and Dr. L. A. Black
- 1968—Dr. M. P. Baker and Dr. W. C. Frazier
- 1969—John Faulkner
- 1970—Harold J. Barnum

#### *Citation Award*

- 1951—Dr. J. H. Shrader and William B. Palmer
- 1952—C. A. Abele
- 1953—Clarence Weber
- 1954—Dr. C. K. Johns
- 1955—Dr. R. G. Ross and A. W. Fuchs
- 1956—Dr. K. G. Weckel
- 1957—Fred C. Basselt
- 1958—Dr. M. R. Fisher
- 1959—John D. Faulkner
- 1960—Dr. Luther A. Black
- 1961—Harold S. Adams
- 1962—Dr. Franklin W. Barber
- 1963—Dr. Merle P. Baker
- 1964—W. K. Moseley
- 1965—H. L. Thomasson
- 1966—Dr. J. C. Olson, Jr.
- 1967—William V. Hickey
- 1968—A. K. Saunders
- 1969—Karl K. Jones
- 1970—Ivan E. Parkin

#### *Sanitarian's Award*

- 1952—Paul Corash
- 1953—Dr. E. F. Meyers
- 1954—Kelley G. Vester
- 1955—B. G. Tennent
- 1956—John H. Fritz
- 1957—Harold J. Barnum
- 1958—Carl A. Mohr

- 1959—William Kempa
- 1960—James C. Barringer
- 1961—Martin C. Donovan
- 1962—Larry Gordon
- 1963—R. L. Cooper
- 1964—No Recipient
- 1965—Harold R. Irvin
- 1966—Paris B. Boles
- 1967—Roger L. Stephens
- 1968—Roy T. Olson
- 1969—W. R. McLean
- 1970—No recipient

## PRESIDENTIAL ADDRESS<sup>1</sup>

MILTON E. HELD

*Food and Drug Administration  
Public Health Service  
U. S. Department of Health, Education, and Welfare  
San Francisco, California*



Milton Held giving the presidential address.

I deeply appreciate the privilege and honor afforded me this morning to, on behalf of the Officers and Executive Board, welcome you to the Fifty-Seventh Annual Meeting of the International Asso-

<sup>1</sup>Presented at the 57th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Cedar Rapids, Iowa, August 17-20, 1970.

ciation of Milk, Food, and Environmental Sanitarians. We appreciate the cooperation of the Iowa Association of Milk, Food, and Environmental Sanitarians in hosting this sure to be successful and profitable meeting. I also would like to interject a personal note of appreciation in being able to come home to Iowa at this time to relive some of the experiences I have had here since becoming a charter member of the Iowa group in 1943.

We are here to spend three days with a number of purposes in mind. The extension of technical and administrative knowledge and capabilities is certainly of prime importance in all our minds. The opportunity afforded us to meet together, renew old acquaintances, and meet other people with like or related professional interests is important. Finally, I am sure that the Iowa group will see to it that a proper balance of entertainment for the men and their ladies becomes an important part of this meeting.

#### THEN AND NOW

In 1911, thirty-five dedicated sanitarians met in Milwaukee, Wisconsin, and thus the "International" had its beginning. There is no question in my mind that these people and the added ranks, as the years progressed, played a vital role in the development of milk sanitation programs to be in the forefront of all food sanitation programs. Institution of detailed requirements, beginning in the 20's and continuing to this day, aptly illustrates this situation and has led to quality and safety of fluid milk supplies in this country which are unsurpassed.

#### ACHIEVEMENTS OF INTERNATIONAL

From that time to the present, the "International" has played an important role in a great many developments in the interest of high quality milk and food supplies. It would be impossible to note all of these in the span of time allotted me, but I would like to recall some of these highlights with you. The *Journal of Milk and Food Technology* enjoys a reputation as a technical and scientific publication on a progressively enhanced basis and is available as a reference in libraries throughout the world. We are proud of it and the salutary effect it plays in directing attention to our Association. The "International" publication, *Procedures for Investigation of Foodborne Disease Outbreaks*, has been a "best seller" with thousands of copies in circulation. I am sure that we all agree that this wide acceptance has stimulated and improved epidemiological techniques and procedures in the investigation of foodborne disease incidents. Beginning in 1944, a joint venture of the

"International," the Dairy Industry Committee, and the Public Health Service in promulgating 3-A Sanitary Standards was launched. At the outset, it was confined to dairy processing equipment, but more recently has been expanded to include other food processing equipment. In 1950, the "International," and its members as individuals, joined in sponsoring a "National Conference on Interstate Milk Shipments." This Conference, a voluntary affiliation of regulatory and industry representatives, in the interest of high quality milk supplies has grown immensely in its scope and coverage. The most recent *Interstate Milk Shipper's List* includes 1,509 shippers of pasteurized milk and milk products from 49 States, 63 non-fat dry milk sources from 23 States, 151 single-service container manufacturers from 33 States, 151 certified State milk sanitation rating officers from 49 States, and 63 certified State milk laboratory survey officers from 43 States. This publication is widely distributed and serves as a major governmental and institutional procurement source. Your Association, in 1960, was instrumental in the formation of the National Mastitis Council; whose primary objective is to correlate and stimulate research and education for the control of bovine mastitis. We, along with the American Public Health Association, and the National Association of Sanitarians, instituted the Sanitarian's Joint Council. Among other accomplishments, this Council was responsible for formation of the American Intersociety Academy for Certification of Sanitarians which is dedicated to the recognition of the professional quality and achievement of the sanitarian.

#### FOOD SANITATION

You undoubtedly have noted that much which has been said refers primarily to "International" accomplishments and leadership in the field of milk sanitation. This is not to imply that little has been done in the field of food sanitation, but rather to try to equate relative emphasis. We have, on an ongoing basis, generally interested ourselves in a rather narrow phase of food sanitation with primary emphasis on food service. I believe we should review what is happening in the foods field with the thought of implementing a wider and stronger stance in this direction. The meat, potatoes, vegetables, and bread philosophy is vanishing. In its place, we now purchase and enjoy semi-processed or processed foods which may more often be complex compounds rather than single item foods. These complex foods now may travel long distances from processor to consumer and various ingredients may enjoy wider travel, even to the extent of coming from other parts of the world. Excluding commercially canned food, we must reckon with raw, semi-processed, processed,

frozen, dehydrated, and refrigerated products which are not sterile and thus possess the potential to become health hazards. An important consideration is the recognition that every person is a food consumer, be it in the home, the restaurant, the factory, the office, the school, etc.

With the foregoing in mind, coupled with the recognition that we are living in an age of consumerism or consumer protection, it is my firm belief that this Association should strongly consider utilizing the full talents of its membership to launch a vigorous food protection program from source to consumer. Such a program would include the stimulation or development of health and sanitation standards for food production, processing, transportation, and distribution. I do not consider this to be of a revolutionary nature, but rather one of encouraging the extension of recent developments in our organization. Two years ago, we instituted a "Food Industry Sanitation Section" at our St. Louis annual meeting to provide a forum for a sizeable number of food industry representatives who are members. This was well attended and we have continued this practice. Based upon this experience, and in consideration of previously noted expanded 3-A Standards development, I believe that a concerted effort in apprising the food industry of our interest in this rapidly developing and highly important field would result in the "International" gaining expanded status and additional members.

#### RECOGNITION OF MEMBER'S EFFORTS

This report would not be complete if the unsung heroes of our Association were not recognized. I am, of course, referring to the membership of the standing committees, special committees, and representatives to other action groups. I wonder if we truly realize the impact of these people's efforts on the professional growth and status of the "International," as well as their contribution to the field of health and sanitation with the consumer as the beneficiary. Committees are active on Applied Laboratory Methods, Baking Industry, Communicable Diseases Affecting Man, Dairy Farm Methods, Food Equipment Sanitary Standards, Food Protection, Frozen Food Sanitation, Environmental Health, Journal Management, Nominations, Resolutions, Professional and Educational Development, Recognition and Awards, and Sanitary Procedure. In addition, we are represented, on the Intersociety Council on Standard Methods, the National Mastitis Council, the Potable Water Committee of the Conference of State Sanitary Engineers, the National Sanitation Foundation, and the Sanitarians Joint Council. You will find the roster in the current (July, 1970) issue of the *Journal* for your

review. I recommend your reviewing this "Who's Who" and, if you desire to contribute to a committee's productivity, please indicate your interest to the Chairman.

#### AFFILIATE COUNCIL

Another vital cog in the affairs of the Association is represented by the Affiliate Council. Our Constitution and By-Laws state that the Council shall act as an advisory body to the Executive Board; serve as the means for the interchange of ideas and recommendations on programs, activities, and procedures among and between the Affiliate Association and the Executive Board; aid in putting into effect policies and programs authorized by the Association and by the Executive Board, convey information on the activities of the Association to the respective Affiliate Association, and report on its activities to the Executive Board at the annual meetings. I am pleased to tell you that a questionnaire was developed and certain information was solicited from our Affiliates. The returns have been analyzed by the Council Chairman and you will be hearing from him concerning this at our business meeting tomorrow. As far as I know, this is the first serious attempt to obtain detailed information of this nature and I feel sure that the report will be of great interest to the membership.

#### THE MERGER

During the past few years, you have been kept informed, by means of the President's Annual Reports and the Special Task Committee Reports, of the details and results of discussions relating to the possibility of forming of a single new sanitarian's association. Your President, last year, stated that negotiations had been terminated and that our task committee had been retained on a standby basis pending possible future developments. This situation still maintains and it would, at this time, be impossible to predict such future developments.

#### EXECUTIVE-SECRETARY

Finally, on behalf of the Executive Board, I have the privilege of reporting to you a matter of business which we considered to be one of the more important problems which we faced and upon which action, long delayed, needed to be taken. It involved our Executive-Secretary and Managing Editor, "Red" Thomasson. I would like to take you back 19 years, a time when the "International" was at a low ebb, particularly from a financial viewpoint. Action was taken by the Executive Board whereby "Red," who as you all know is a Past-President, agreed to resign

his position with the Indiana Board of Health in order to assume the duties of Executive-Secretary. Those of us who have known "Red" during this period can attest to his dedication and capable management of Association affairs during these last 19 years.

In his report to you last year, he listed net current assets of \$23,561.54, as of June 30th, compared with \$17,475.13 in 1968, an increase of \$6,086.41. The ratio of current assets to current liabilities was 6.9 as of June 30th and the CPA's report stated that a ratio of 2.0 is usually considered to be satisfactory. The year's operations showed a net addition to the contingency reserve of \$6,116.98.

A retirement plan for Red had been discussed periodically over the years. Your present Board felt that this matter could no longer be postponed and some action would have to be taken to reward him for his many years of devoted service. Accordingly, suggestions and plans were solicited from companies and from similar types of Associations. After reviewing the matter and acknowledging the fact that delayed action had complicated the situation, the Board,

by unanimous vote, entered into a deferred compensation agreement through the Participating Annuity Life Insurance Company, a subsidiary of Aetna Life Insurance Company. This standard type agreement provides for four annual payments of \$6,120.00 to provide for monthly payments in the range of \$250.00 upon retirement at the end of this period. Safeguards relating to Association liability, preservation of its assets versus liabilities relationships, and special clauses in case of death or severance of employment are incorporated in the plan. We feel sure that you will agree that this action will result in a well deserved reward for "Red's" devotion to the "International." We only wish we could have done more. The first payment has been made and it is the Board's opinion that the remaining payments will be possible with the maintenance of financial stability.

In closing, I wish to express my deepest appreciation to all those members who contributed to the conduct of the affairs of the "International" during the past year. It has been my privilege and honor to serve as your President.

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### ABSTRACTS OF PAPERS PRESENTED AT 1970 IAMFES MEETING

Abstracts of most papers given at the 1970 IAMFES meetings appear below. Some speakers elected not to prepare abstracts because the subject matter was not suitable for this purpose. Most papers presented at the annual meeting will appear in subsequent issues of the *Journal of Milk and Food Technology*.

**MILK FLAVOR, THE TRUE TEST OF QUALITY.** *D. K. Bandler.* Department of Food Science, Cornell University, Ithaca, N. Y. 14850.

Since the consumer's appraisal of a glass of milk is based solely on taste, flavor and keeping quality are of paramount importance to maintaining consumption of fluid milk and by-products.

For the past 5 years, the N.Y. State Milk Flavor Program has identified the off-flavor problem areas to the industry, particularly with regard to the farm supply. There has been a marked improvement where companies and the college have cooperated. The emphasis now is being placed on getting the finished product protected to the highest degree in an effort to improve shelf life. The program is carried out primarily by the Quality Control staffs currently employed by industry. This force is assisted by the extension staff in the Department of Food Science at Cornell in the following ways: (a) individual plant seminars for each plant or company participating in the program; (b) follow-up sessions to help solve special

problems, to evaluate progress, and to maintain interest and enthusiasm; and (c) direct contact with top management to evaluate shelf life performance, route returns, relationships with non-owned outlets, and promotion of extra high quality dairy products.

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**MANURE HANDLING SYSTEMS AND ENVIRONMENTAL CONTROL FOR CONFINED DAIRY HOUSING.** *Donald W. Bates.* Department of Agricultural Engineering, University of Minnesota, St. Paul, Minnesota 55101.

There is no single best manure handling system. Odor and appropriate time of disposal are two of the most difficult waste problems facing dairymen. Systems may range from a gutter cleaner and daily hauling with a manure spreader to extended storage in concrete tanks whose contents are pumped and spread periodically. Daily hauling requires the lowest investment in equipment but has the disadvantage of possible higher labor costs and the hazard of encountering unfavorable weather, soil, or crop conditions. Slat floors in warm free-stall barns, or gutters with grated bottoms in conventional stall barns, both with under-the-building manure storage, offer suitable systems for manure handling with a minimum of labor. Carefully planned ventilation systems of high capacity must be provided for all confined units. Research shows that manure containing little bedding deposited in the end of a 30 x 90 x 8 ft external flat-bottom tank will distribute itself



Some of the speakers who presented papers. Upper left, Dr. C. K. Johns; upper right, L. W. Penn; lower left, Dr. J. L. Buckley; and lower right, Dr. G. W. Reinbold.

under its own weight. Waste heat from the dairy barn ventilation system is used to prevent freezing.

ENVIRONMENTAL TOXICOLOGY AND NATIONAL POLICY. *John L. Buckley*. Office of Science and Technology, Executive Office of the President, Washington, D. C.

Classical toxicology has dealt with acute or relatively short-term exposures, usually to single insults. Its concerns have largely been with exposures in the work place or substances reaching man in food, water, or medicine. In recent months there have been reports of undesirably high levels of mercury and DDT in fish, mercury poisoning from meat fed on treated grain, and restriction of use of the herbicide, 2, 4, 5-T, based on evidence of teratogenicity in experimental animals. The public is increasingly concerned about longer-term effects, lower levels of dosage, new kinds of substances, and combinations of these.

It is up to us scientists to devise more sophisticated methods of assessing chemical hazards. We need to find ways to predict long-term effects without waiting for epidemiological evidence. We need to learn more of the effects of chemicals on other living things—as individuals, populations, and ecosystems. We need to learn more of the functioning of ecosystems and the movement, accumulation, and degradation of chemicals in these systems. The mercury and DDT situations in the Great Lakes are illustrative of our need.

The Federal government is responsible for regulating drugs, food additives, and pesticides, and with the States for controlling air and water pollution. The organization of the Federal government in relation to environmental pollution now, and as proposed in Reorganization Plan 3 submitted to Congress on July 9 will be described.

STERILIZED DAIRY AND ANALOGUE PRODUCTS. *Ernest Glaser*. Avoset Food Corporation, 80 Grand, Oakland, California.

The article discusses changes taking place in processing, packaging, and distribution of sterilized dairy products. The origins and purposes of present governmental controls are reviewed in the light of these changes. The impact of substitutes for dairy products is discussed, and the author stresses the need for a new approach to regulating dairy products in a manner which would reflect the changes taking place in the industry.

THE INDUSTRIAL SIGNIFICANCE OF LOW TEMPERATURE MICROBIOLOGICAL PRESERVATION OF FOODS. *Nino F. Insalata and Inara Raab*. General Foods Corporation, Post Division, Microbiological Research, 275 Cliff Street, Battle Creek, Michigan 49016.

The U. S. food industry grosses approximately 100 billion dollars annually and is serviced by more than 25,000 firms. By 1975, the frozen food portion of this industry will represent approximately 8% of the total retail sales. The microbiological stability of low temperature foods is contingent on variables such as: raw materials, processing conditions, packaging and storage conditions, and consumer use and abuse.

Inevitably, a design in governmental regulatory control becomes apparent. Steps which may be taken by industry to protect the public include the establishment of: a Quality Assurance program; Microbiological Referee Task Force; Vendor Assurance program; the statistical application of test designs; the implementation of valid, rapid microbiological test methods; and the management decision on the degree of risk inherent in any statistical plan with a course of action should a potential health problem arise.

WHAT CAN WE LEARN ABOUT SANITATION FROM OTHER COUNTRIES? *C. K. Johns*. Lazarus Laboratories, Inc., Long Island City, N.Y. and Lazarus Laboratories (Canada) Ltd., Toronto, Ont.

The value of regulatory standards for foods in improving sanitation and protecting the consumer is illustrated with reference to various products. The importance of educating and training plant personnel in sanitary practices is stressed. Simplified procedures for cleaning and sanitizing equipment are described.

In determining the sanitary quality of milk, the need for a lower incubation temperature is indicated, and reference is made to a number of new tests recently described. In mastitis control the encouraging results obtained from teat dipping, combined with dry-cow treatment, are mentioned, together with the value of marker dyes in intra-mammary antibiotic preparations and of a simple test for inhibitory substances in milk.

MICROBIOLOGY OF POULTRY PRODUCTS. *Allen A. Kraft*. Department of Food Technology, Iowa State University, Ames, Iowa 50010.

Changes in poultry processing and marketing over the past

few decades have resulted in production of many convenience food items. These products are subjected to much handling and require that strict attention be given to sanitation measures.

Many types of microorganisms are present on poultry products as a result of contamination from feathers, feet, and intestinal contents of the birds. Equipment and personnel on processing lines also contribute to the spread of bacteria. The bacterial flora may be significant in causing spoilage, or may represent a public health hazard unless controlled by proper sanitation and cooking or low temperature. Trends in bacterial numbers during processing vary with different plant practices and the adequacy of plant sanitation; examples of these differences are described. Several investigations are reviewed on microorganisms present on poultry from the farm through the finished product, including retail store practices. Sources and control of salmonellae and other potential pathogens are discussed.

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RECRUITING PERSONNEL FOR A DYNAMIC INDUSTRY IN THE SOARING SEVENTIES. *C. Bronson Lane, Jr.* Department of Dairy Science, University of Florida, Gainesville, Florida.

The dairy industry and milk regulatory agencies are suffering from a severe manpower shortage. Recent surveys show that enrollment in agricultural colleges is up, but that the dairy science curriculum is not drawing its share of the new student crop.

Suggestions for rectifying our manpower shortage include: changing the image of agriculture in general; involving regulatory and industry people in a one to one career guidance situation with potential recruits; increasing incomes and inducements for dairy-related professions; and establishing innovative training programs and curricula to interest a wider segment of our young people in dairying and regulatory professions.

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EMOTIONALISM AND THE ENVIRONMENT—LET'S PUT THE PROBLEM IN ITS PROPER PERSPECTIVE. *Norman Myrick.* International Paper Company, 220 East 42nd Street, New York, N. Y. 10017.

Rapid industrialization, growing populations, and an ever expanding technology have generated a new and well founded concern with the quality of our environment. Currently some 360 million tons of solid waste must be collected and disposed of each year. Methods of accomplishing this huge task are still in their infancy. Some 70% of municipal disposal facilities are open dumps. Only 6% of so-called sanitary landfills meet official standards. Collection techniques are still labor oriented. Nevertheless, some remarkable technological developments are taking place which suggests that methodology and technology in this area will cease to be a problem in the near future. The big factors, yet to be resolved, are the economic problems and the people problems. The lessons learned and the progress achieved by milk and food sanitarians, pioneers in environmental control, lead one to believe that they have much to contribute in achieving solutions to these aspects of the broad ecological problem. The lesson appears to be this: despite the sensationalism that is characteristic of modern communications media, a vast number of energetic and talented people are quietly formulating methods and programs which will produce reasonable solutions to these great issues.

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URBAN RAT CONTROL. *L. A. Penn.* Milwaukee Health Department, 841 North Broadway, Milwaukee, Wisconsin 53202.

Although two species of rats have become thoroughly domesticated and have lived parasitically in man's environment for several centuries, organized urban rat control programs are of comparatively recent origin. A brief history of commensal rats and their relationship to man is presented. Some early urban control programs are summarized.

Recent social developments in cities have focused attention on rat control programs and have intensified efforts to free urban areas of rats particularly in poverty-stricken neighborhoods. An effective community rat control program depends upon motivation and education of citizens as well as enforcement of wisely written rat control ordinances and related solid waste disposal rules. A good rat control ordinance should require rat-proofing of buildings, elimination of rat harborages and sources of food for rats, as well as rat extermination on all premises.

For the future, not only will more sophisticated means of citizen motivation be needed, but further studies of the role of sewers and other heretofore neglected areas of rat control in the urban environment.

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IS BACTERIOLOGICAL TESTING OF RAW MILK AND OF DAIRY PRODUCTS STILL ADEQUATE? *George W. Reinbold.* Department of Food Technology, Iowa State University, Ames, Iowa 50010.

Bacteriological testing of raw milk and processed dairy products is not adequate for public health and quality control purposes. Bacteriological test results are not highly correlated with farm production conditions. Routine bacterial quality control tests are not always adequate for recovery of microorganisms injured during processing. Present systems and trends are discussed.

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MILK CLEANLINESS TESTING. *Michael H. Roman.* N.Y.S. Dept. of Agriculture and Markets, Bldg. 8, State Campus, Albany, N. Y. 12226.

Clean milk production is a requisite for high quality and a continuing problem with generally the same sources where sediment testing is practiced. Milk can be kept clean if milking preparation and procedures as outlined in regulatory codes are followed. The mixed sample sediment test of producer's milk is indicative of production practices and should be renamed "Cleanliness of Production Test".

Unclean milk can be only partially "cleaned" by filtration due to fragmentation and solubility of some filths. Disks used for testing are likely to be fouled by highly abnormal milk thus the test serves a dual purpose. Yellow color observed on back side of disk has much significance. Before-straining testings will indicate degree of sediment contamination. Thorough washing and drying of teats with single service material with no reuse of solution cannot be over-emphasized. Universal milk sample taken of each milk collection is desirable for monitoring cleanliness. A mixed milk four-ounce sample and 0.2 inch diameter test using as acceptable a standard of less than 1.5 mg/gal basis is practical. Milk cleanliness testing should be regulated as voluntary programs are subject to many variables. Producers should assume responsibility for clean milk production by conducting the mixed sample test using Lintine disks.

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ROLE OF ENZYMES IN THE DAIRY AND FOOD INDUSTRY. *Khem M. Shahani.* Department of Food Science and Technology, University of Nebraska, Lincoln, Nebraska 68503.

In general, this presentation will include the discussion of

the role and significance of enzymes that are inherently present in milk and other food products and that are added during the manufacture of foods. Initially, the major classes of enzymes and their reactions will be discussed. Also, the discussion will include how processing inactivates or modifies the enzymes to improve the keeping quality or nutritive value of foods and how the presence or absence of certain enzymes is checked for quality control or regulatory purposes. For example, the presence of phosphatase in milk signifies under-pasteurization. The significance of rennet and rennet-substitutes in the manufacture of cheese will be discussed. Role of lipase in the development of rancidity will be included in the talk, as well as the role of various lipases in development of specific flavors in the Blue and Italian cheeses. Also, studies will be reviewed showing how butteroil or cream can be enzymatically modified to accentuate butter flavor in baked goods and confectionery.

systems of this country, which are expanding every day in an effort to improve the delivery of health care to individuals. Only a small number of Sanitarians now working in this field are knowledgeable of the systems and the future it holds for professional Sanitarians. The enactment of Medicare and Medicaid legislation not only spurred new efforts in this field, but it has also focused attention on a multitude of problems that have been plaguing the health care delivery systems for many years; one of which is the physical environment of hospitals and nursing homes. The professional Sanitarian, because of his broad training and experience in the field of environmental health, is uniquely qualified to make tremendous contributions to the improvement of the physical environment in health care facilities. The Sanitarian's contributions in this field can be as significant in the years ahead as has been his contributions in the conventional environmental health field in years past.

THE FUTURE OF ENVIRONMENTAL HEALTH IN HEALTH CARE FACILITIES. *Darold W. Taylor*. U. S. Public Health Service, 5600 Fisher's Lane, Rockville, Maryland.

For many years the term "Sanitarian" and the duties performed by Sanitarians have been closely associated with conventional environmental problems in the fields of water, sewage, milk, food, radiation, solid waste, etc. It is time the Sanitarian became active and involved in the health care delivery

SOME APPROACHES TO WATER POLLUTION CONTROL. *C. M. Widmer*. Penick & Ford, Limited, Cedar Rapids, Iowa 52406.

Need for defining and limiting problem effluents, through use of water balance and process review. In-plant treatment vs. cooperative municipal-industry facilities. Retreatment methods. Brief review of primary, secondary, and tertiary treatment schemes, including some of the problems which are encountered.

## CONSOLIDATED COOPERATIVE'S ROLE

(Continued from Page 466)

country and says, "we've done alright so far," has gone as far as it can.

We have to face facts. We're living in a mobile age . . . one where activities and interests can no longer be confined to our own small circle of service. Compare our industry to a chain, if you will . . . a heavy chain! We build a strong link right here in our own area, but if the link attached to it is a weak one, then there is no strength in the entire chain. We must have strength. This is the key factor in obtaining the needed quality, the economy, and the integrity of our entire industry.

I've done a lot of finger pointing in this discussion. That's what you expected me to do, because the problem of quality assurance is such that we cannot discuss it without pointing fingers. I sincerely hope that I have thoroughly disturbed any feeling of apathy on your part. Without question, the consolidation of cooperatives into major groups of power will, by virtue of a newfound strength, both financially and vocally, play a major role in solving the prob-

lems of quality assurance. But these cooperatives must have the backing and strength of each individual member . . . of each interested part of the dairy industry. Not one of us can rest on our laurels as long as there is one speck of dirt in the milk bucket. The time to say, "let George do it," is gone. We've run out of Georges.

But while I am intentionally stirring an issue, let me say this loud and clear. So long as there is a choice, I will not be a part of any other industry in the world. There is none better. My feeling for my industry, and the country which my industry is privileged to serve, is expressed somewhat in a statement made by Dean Butz, a fine agriculturist and educator at Purdue University. Dean Butz said, "Tonight in many nations around the world, two people in three will go to bed with a prayer on their lips, 'Lord, give me enough food to last through tomorrow.' Tonight, in the United States, two people in three will go to bed with a prayer on their lips, 'Lord, give me courage to stay on my diet tomorrow.' What a privilege is ours to live in this blessed land of abundance!" Let's keep our industry living up to this privilege!

## COURSE IN FOOD MICROBIOLOGY

The Cincinnati Facility of the FDA Training Institute is offering a course in Food Microbiology in Cincinnati, Ohio March 22 to April 2, 1971.

The course presents advanced technical information for laboratory and supervisory personnel actively involved with the bacteriological examination of food. It is designed to enable the trainee to participate in surveillance programs to determine the sanitary quality of foods and to examine food implicated in foodborne disease episodes. Instruction prepares the trainee to perform selected analyses and to interpret the results of such analyses.

The laboratory work includes Aerobic Plate Count, Coliform Group MPN, *E. coli* MPN, Salmonellae, Shigellae, Staphylococci, *C. perfringens*, *C. botulinum* including mouse assay for toxin, and Examination of suspect food sample. Emphasis is on the detection, isolation and enumeration of these organisms from foods.

In addition to lectures on each organism studied in the laboratory, other topics include Sampling procedures and sample preparation, Staphylococcal enterotoxins, Time-temperature relationships in food processing, Relationships among *C. botulinum* types, Viruses in foods, Surveillance sampling and Marine versus botulinal toxins.

The course is limited to 20 students. All applications must be received by December 1. There are no registration or course fees. Send applications to: O. W. Kaufmann, Senior Microbiologist, Cincinnati Training Facility, Training Institute, FDA, 1090 Tusculum Avenue, Cincinnati, Ohio 45226.

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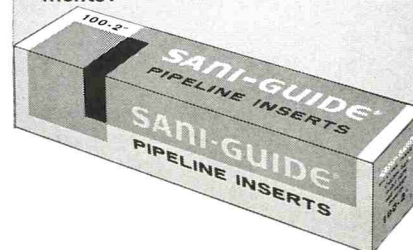
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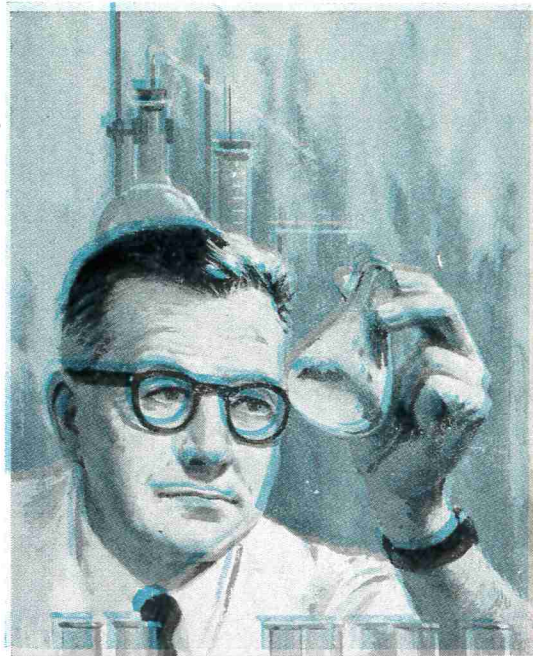


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Gerald

Billy

Eldridge

A Surge Double-six Sawtooth Parlor is the nucleus for the new dairy installation on the Marshall Brothers' farm near Buchanan, Virginia. They changed their operation from one they described as "dawn to dusk drudgery" to a Surge Parlor, free stall set-up. They expanded their herd at the same time.

The Marshalls had been milking 75 to 80 head in two herds on separate farms in stanchion barns.

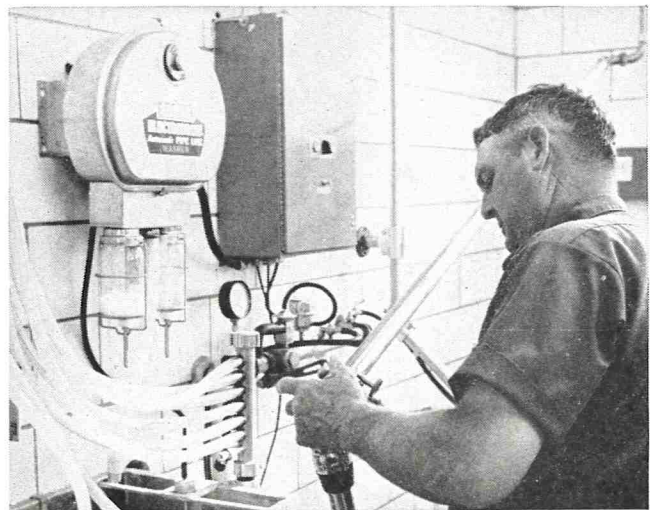
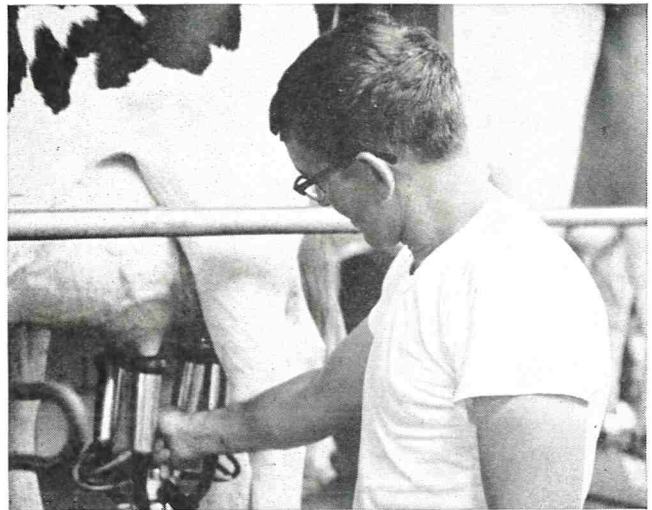
"It took all three of us plus our full time tenant just to get the milking done and we had to be out there at 5 A.M. every day," explained Eldridge Marshall.

This spring the Marshalls expanded their herd to 120 and put in an entirely new facility. Free stall housing, mechanized feeding, liquid manure handling and a new Surge Parlor make up the unit which is designed to accommodate more cows with less work.

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