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59TH ANNUAL MEETING

August 21, 22, 23, 24, 1972

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EFFECTS OF DEHYDRATION THROUGH THE INTERMEDIATE MOISTURE RANGE ON WATER ACTIVITY, MICROBIAL GROWTH, AND TEXTURE OF SELECTED FOODS

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(Received for publication March 1, 1972)

ABSTRACT

Various food systems were inoculated with *Pseudomonas aeruginosa* and *Staphylococcus aureus* and vacuum-dried to different moisture contents in the intermediate moisture range. Water activity (a_w) values were determined instrumentally with a hygrometer indicator equipped with appropriate hygrosensors. Sorption isotherms and bacterial growth curves at the various water activities were plotted and correlated with food texture. Growth of *P. aeruginosa* was inhibited at a_w test values below 0.98, 0.98, and 0.96 in custard, pea, and beef products, respectively, and growth of *S. aureus* did not occur below a_w test values of 0.94 and 0.96 in custard and ham products, respectively. These results generally agree with earlier work done on model systems. With none of the foods studied could microbiologically stable intermediate moisture products of acceptable texture be produced by drying alone.

The principles and technology underlying fabrication of shelf-stable intermediate moisture foods have attracted considerable interest in recent years (3, 9, 10, 11, 12, 14). Studies on the feasibility of producing intermediate moisture products for human consumption have been encouraged by the successful development of stable semimoist pet foods (2, 4, 8) and by research contracts from the U. S. Army Natick Laboratories (9, 10, 13).

For the most part solutes effective in lowering a food's a_w to the range necessary to inhibit microbial growth impart objectionable taste characteristics and this has seriously limited success in the formulation of intermediate moisture products for human consumption. Exceptions to this exist in the case of foods that are normally sweet and therefore can be formulated with high levels of sugar or glycerol, and with pet foods, which dogs relish despite their sweetness.

Much work has been published concerning growth of organisms of spoilage and public health significance in a_w adjusted model systems (5, 6, 7, 15, 16). Food enzyme activity as a function of a_w also has been studied and found to be operative at a_w levels below which microbial growth is inhibited (1). Less has been reported on growth rates of microorganisms in food products decreased in a_w by partial drying.

The present study was concerned with the growth rates of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in partially dehydrated custard, pea, beef, and

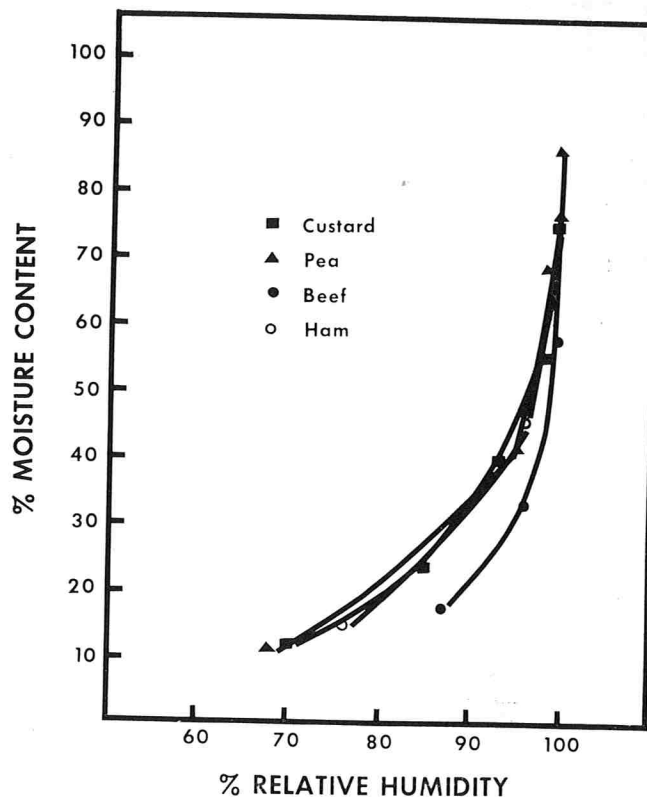


Figure 1. Sorption isotherms of inoculated food products.

ham products. A further objective was to determine the feasibility of controlling these organisms in semimoist textures produced without addition of foreign solutes.

MATERIALS AND METHODS

Food inoculation

Pure cultures of *P. aeruginosa* and coagulase-positive *S. aureus*, obtained from the Cornell University Microbiology Department's culture collection, were grown for 24 hr in nutrient broth at 37 C and then diluted with phosphate buffer of pH 7.0 to obtain an inoculation level of approximately 10^4 cells per gram of food. Products used were commercial strained baby foods. Homogeneity of food product and bacterial inoculum was obtained by thorough mixing for approximately 5 min under sanitary but not aseptic conditions.

Drying procedure

Thirty- to forty-gram food portions then were spread with a straight glass rod over the surface of 12 × 20 cm glass plates provided with 1.6 mm high glass edges to produce a uniform surface and 1.6 mm thickness of food for even

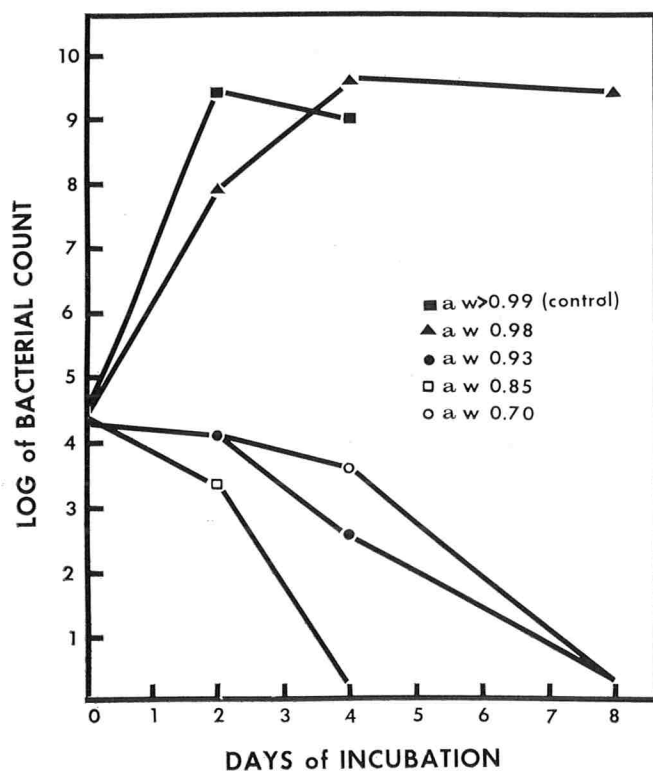


Figure 2. Growth of *Pseudomonas aeruginosa* in a custard product.

drying. Spread plates were placed on shelves of a vacuum oven operated at a vacuum equivalent to 40 mm of Hg and a drying temperature of 40 C. This low temperature was used to minimize destruction of bacteria by heat. Progressively dried samples were removed from the oven, scraped from the glass plates, mixed in a sanitized mortar to further insure homogeneity, and then placed in clean 1/2-pint Ball mason jars for measurement of a_w , moisture content, and subsequent incubation to determine bacterial growth. Uninoculated controls were included with each group of samples studied and showed that chance contamination was minimal and did not invalidate test organism enumeration.

Water activity measurement

All sample jars were incubated for 15 hr at 27 C to allow the food material within the jar to come into moisture equilibrium with the head space of the jar. Measurements of a_w (RH/100) were then made with an electric hygrometer indicator and hygrosensors manufactured by HygroDynamics, Inc., Silver Spring, Maryland. After the appropriate hygrosensor was placed in the mason jar head space via a jar lid adapter, a further 1-hr period was allowed for the hygrosensor to come into equilibrium with the water vapor of the head space. The hygrosensor was then connected to the indicator which had been set in the test position and dial readings, corrected for temperature, were applied to a calibration chart for the particular sensor to obtain the a_w of the sample. Hygrosensors were frequently checked against standard CaCl_2 solutions, with readings within $\pm 1.5\%$ a_w of the solution value being the basis for acceptability.

Moisture determination

Approximately 2-g samples were vacuum-oven-dried for 15

hr at 60 C under a vacuum equivalent to 40 mm of Hg, and moisture was determined by weight loss.

Bacterial enumeration

Plating medium for *P. aeruginosa* consisted of nutrient agar at pH 6.8 ± 0.2 (supplied by BBL) supplemented with 2,3,5-tri-phenyltetrazolium chloride (hydrochloride) to yield a final concentration of 0.05%. This organism reduces the tetrazolium salt and converts it from its leuco form to a reddish pink color. All red colonies were counted and recorded as *P. aeruginosa*. For *S. aureus* enumeration, Staphylococcus medium #110 (BBL) was used. Colonies appearing as large, creamy, and yellow to orange in color were counted as coagulase-positive *S. aureus*.

One-gram samples of previously inoculated and dried foods were removed from mason jars after incubation at 27 C for periods up to 9 days. The samples were appropriately diluted with sterile phosphate buffer. For *P. aeruginosa* pour plates were prepared and for *S. aureus* spread plates were utilized. All plates were incubated inverted at 37 C and counts per gram of product were determined after 48 hr.

RESULTS AND DISCUSSION

Figure 1 shows the sorption isotherms obtained for the custard, pea, beef, and ham products. The almost vertical slope of these curves in the intermediate moisture range reflects a situation where large changes in moisture content are required to produce only small changes in a_w , indicating that a delicate adjustment of a_w by drying alone is very difficult to achieve. This was the prime reason why more uniform and closer spacings of a_w values, seen in subsequent figures, were not obtained with samples drawn from the

TABLE 1. TEXTURE RATINGS OF THE VARIOUS INOCULATED AND DRIED PRODUCTS

| Product | Moisture (%) | Water activity | Texture rating ^a |
|------------------------------|--------------|-------------------|-----------------------------|
| Custard + <i>Pseudomonas</i> | 74.9 | $>0.99^b$ | 1 |
| | 56.1 | 0.98 ^b | 1 |
| | 40.4 | 0.93 | 1.5 |
| | 23.8 | 0.85 | 2 |
| | 13.2 | 0.70 | 3 |
| Pea + <i>Pseudomonas</i> | 87.1 | $>0.99^b$ | 1 |
| | 76.9 | $>0.99^b$ | 2 |
| | 69.0 | 0.98 ^b | 3 |
| | 41.5 | 0.95 | 5 |
| | 12.3 | 0.68 | 5 |
| Beef + <i>Pseudomonas</i> | 76.6 | $>0.99^b$ | 1 |
| | 59.7 | $>0.99^b$ | 2 |
| | 34.5 | 0.96 ^b | 4 |
| | 18.2 | 0.87 | 5 |
| | 6.5 | <0.68 | 5 |
| Ham + <i>Staphylococcus</i> | 78.3 | $>0.99^b$ | 1 |
| | 63.2 | 0.99 ^b | 1.5 |
| | 45.6 | 0.96 ^b | 3.5 |
| | 15.5 | 0.76 | 4.5 |
| | 6.3 | <0.68 | 5 |

^a1 (Undried control) ----- 5 (dry, granular).

^bBacterial growth.

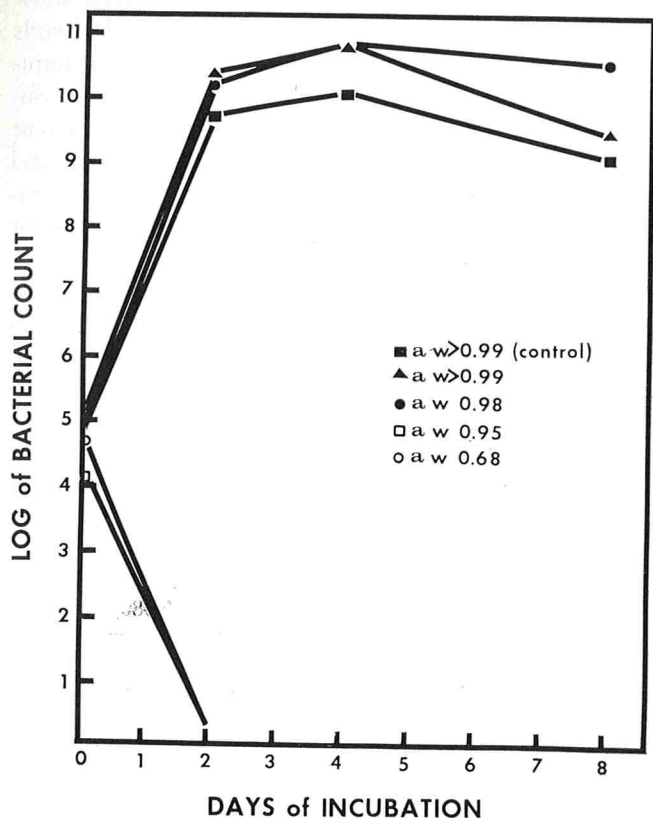


Figure 3. Growth of *Pseudomonas aeruginosa* in a pea product.

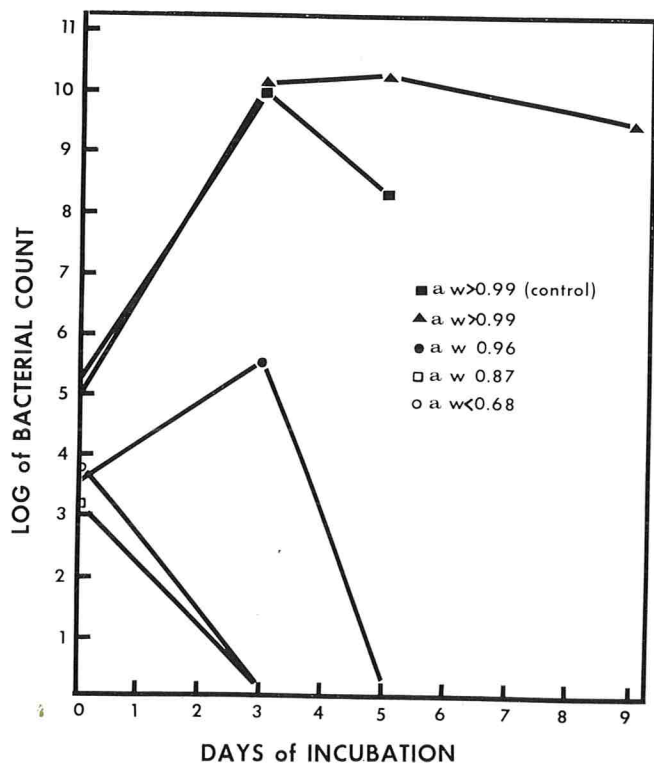


Figure 4. Growth of *Pseudomonas aeruginosa* in a beef product.

vacuum oven at spaced time intervals.

Bacterial growth curves in the different food products dried to various a_w levels are seen in Fig. 2 through 6. Control curves represent undried samples. Because of the sizable spacings between several of the a_w values obtained, only gross ranges for growth and no growth in the different foods were obtained. Actual minimum a_w values for growth cannot be cited. With respect to *P. aeruginosa*, data in Fig. 2 through 4, nevertheless, reveal quite similar a_w requirements for growth in the different foods tested. Thus, with this organism growth failed to occur below a_w test values of 0.98, 0.98, and 0.96 in the custard, pea, and beef products, respectively, and growth at 0.96 in the beef product was meager before a rapid decline in viable cell count. This agrees with literature values on other species of *Pseudomonas*, generally found not to grow at water activities below about 0.94 in model systems (16). It will be noted in Fig. 2 through 4 that slopes are only approximations where final counts below 10^1 were obtained. In Fig. 5 and 6, growth of *S. aureus* occurred at a_w test values of 0.94 and 0.96 in the custard and ham products, respectively, but did not occur at 0.85 in the custard or at 0.76 in the ham. These data also generally agree with previous findings in model systems (15), where *S. aureus* ceased to grow at an a_w below 0.86.

Texture ratings on the products studied, corres-

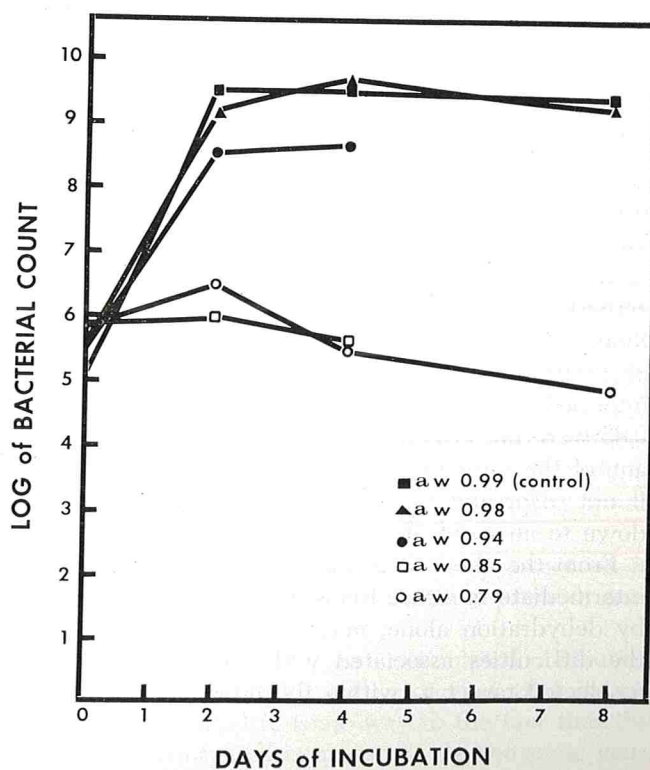


Figure 5. Growth of *Staphylococcus aureus* in a custard product.

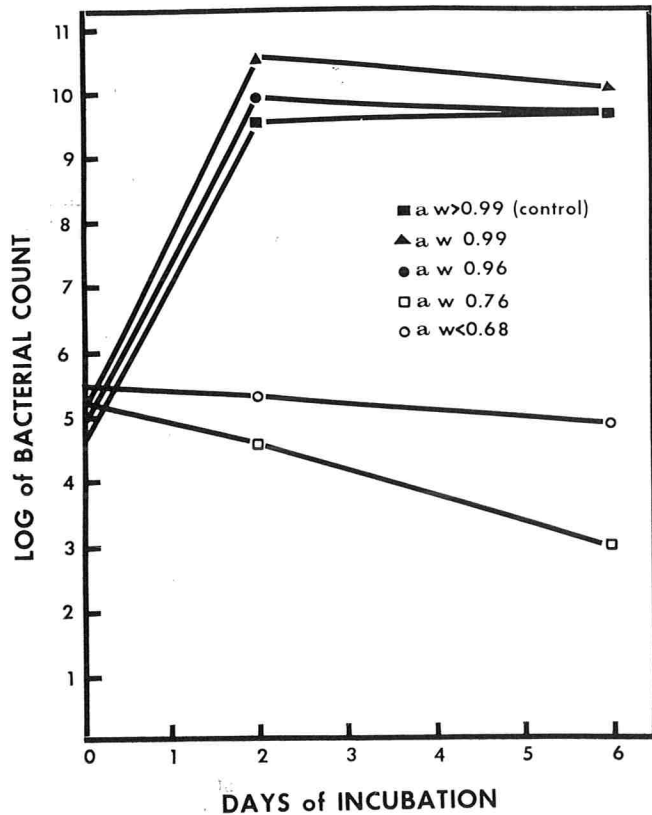


Figure 6. Growth of *Staphylococcus aureus* in a ham

product.

ponding to their various water activities and abilities to support bacterial growth are given in Table 1. A texture rating of 1 to 5 was used, with 1 being the most desirable or normal moist control texture, and 5 being the least desirable resembling a dehydrated granular product. It will be seen that with the pea, beef, and ham products, water activities necessary to inhibit bacterial growth resulted in dry and granular textures. With the custard product, effective water activities produced a taffy-like consistency. Even though these consistencies were beyond the degrees of dryness that could be considered acceptable in intermediate moisture textures, water activities above 0.85 were not effective in inhibiting mold growth on any of the samples beyond a one-week period. This is not surprising since many molds will grow slowly down to an a_w of about 0.70.

From the above it is concluded that production of intermediate moisture foods of the types investigated, by dehydration alone, may not be feasible. Besides the difficulties associated with controlled drying to a selected target a_w within the intermediate moisture

range, texture ratings and bacterial growth data show that to inhibit growth of the test organisms the foods had to be dehydrated to a point well beyond semi-moistness. These results, including mold growth observations, support the relevance of various current approaches to the problems of developing safe and acceptable intermediate moisture foods. These include infusion on non-aqueous edible materials or aqueous solutions high in solutes into previously dehydrated products to maintain texture, similar infusions into moist raw or cooked products, product development through formulation with selected ingredients of low water activity, and use of antimicrobials.

REFERENCES

1. Acker, L. 1969. Water activity and enzyme activity. *Food Technol.* 23:1257.
2. Bone, D. P. 1968. Method of preparing a solid semi-moist marbled meat pet food. U. S. Patent 3,380,832.
3. Bone, D. P. 1969. Water activity—its chemistry and applications. *Food Product Development* 3(5):81.
4. Burgess, H. M., and R. W. Melletin. 1965. Animal food and method of making the same. U. S. Patent 3,202,514.
5. Christian, J. H. B., and W. J. Scott. 1953. Water relations of salmonellae at 30°C. *Australian J. Biol. Sci.* 6:565.
6. Christian, J. H. B. 1955. The influence of nutrition on the water relations of *Salmonella oranienburg*. *Australian J. Biol. Sci.* 8:75.
7. Christian, J. H. B. 1963. Water activity and the growth of microorganisms. *Recent Adv. Food Sci.* 3:248.
8. Hallinan, F. J., E. J. Czarnetzky, and A. I. Coombes. 1963. Pet food and method of packaging same. U. S. Patent 3,115,409.
9. Hollis, F., M. Kaplow, R. Klose, and J. Halik. 1968. Parameters for moisture content for stabilization of food products. Tech. Rept. 69-26-FL. U. S. Army Natick Laboratories, Natick, Mass.
10. Hollis, F., M. Kaplow, J. Halick, and H. Nordstrom. 1969. Parameters for moisture content for stabilization of food products (Phase II). Tech. Rept. 70-12-FL. U. S. Army Natick Laboratories, Natick, Mass.
11. Kaplow, M. 1970. Commercial development of intermediate moisture foods. *Food Technol.* 24:889.
12. Labuza, T. D. 1968. Sorption phenomena in foods. *Food Technol.* 22:263.
13. Lampi, R. A. 1963. Infiltration of porous foods with high caloric non-aqueous, edible materials. Repts. 1, 2, 3 Quartermaster Contract DA 19-129-AMC-84 (N) (OI 9056).
14. Potter, N. N. 1970. Intermediate moisture foods: principles and technology. *Food Product Development.* 4(7): 38.
15. Scott, W. J. 1953. Water relations of *Staphylococcus aureus* at 30°C. *Australian J. Biol. Sci.* 6:549.
16. Scott, W. J. 1957. Water relations of food spoilage microorganisms. *Advan. Food Res.* 1:83.

HEAT ACTIVATION OF *BYSSOCHLAMYS FULVA* ASCOSPORES¹

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ABSTRACT

Activation of *Byssochlamys fulva* ascospores was influenced by temperature and the suspending medium. At 60 C maximal activation occurred in hydrochloric and nitric acid solutions. Both the concentration of hydrogen ions and the type of anion were critical; little activation was achieved above pH 1.6, and pH 1 solutions of sulfuric and phosphoric acid were not stimulatory. Storage of activated spores as aqueous suspensions at 32 C resulted in about a 50% reversion to a dormant state.

Molds of the genus *Byssochlamys* can survive the thermal process given many acid foods and have been responsible for spoilage outbreaks of commercially canned fruits and fruit products (6, 7). It has long been known that their resistant structures, ascospores, possess a dormancy that can be broken by heat shock (3). Recently it was observed that the medium in which the spores were heated affected activation in that a grape juice menstruum afforded higher viable counts than did distilled water (8). This report describes further studies on some of the factors affecting activation.

MATERIALS AND METHODS

Ascospore production

Byssochlamys fulva H-36, isolated by us from the surface of a mechanical grape harvester, was used in this study. The procedure for producing crops of ascospores has been described in detail (8). Briefly, it consisted of growing the culture as a pellicle over 15° Erix Concord grape juice for 28 days at 32 C. The mat and broth were then blended in a Sorvall Omni-mixer until a homogeneous mixture was obtained, usually 3 min. After washing in distilled water, the homogenate was suspended in 85% ethanol (final concentration) for 20 min to destroy viable conidia and hyphae. Spores were stored in water at 5 C or frozen at -23 C.

Typical suspensions contained about 10^8 asci per milliliter as determined with a haemocytometer. Some heated suspensions gave viable counts that were higher than the direct microscopic ascus counts, indicating the presence of free ascospores. The exact number of single ascospores that might be present could not be determined with the microscope because they could not be differentiated from conidia.

When suspensions of only free ascospores were desired, the asci were shaken briefly in a Braun MSK cell homogenizer. Treating 5 ml of washed asci with 5 g of 0.11-mm glass beads for 5 to 10 sec resulted in almost 100% breakage of the

asci. The ascospores were harvested by repeated mixing and decanting of the suspension in sterile distilled water.

Heat activation

The standard procedure was to add 0.5 ml of washed spores to a tube containing 4.5 ml of the activation medium. The tube then was placed in a water bath for the desired time period. When the heating was to be brief, under 1 hr, the volume usually was reduced to 1 ml per tube to minimize the time required for temperature equilibration. Following heating, tubes were placed in an ice bath where they were held until plating was completed.

Viable counts

Approximate decimal dilutions in water were plated on Difco potato dextrose agar acidified to pH 3.5 with tartaric acid. Colonies were counted after an incubation of 3 days at 32 C.

RESULTS AND DISCUSSION

Our search for the substance(s) in grape juice that stimulated ascospore activation led to the finding that activation was enhanced even more when spores were heated in a solution of 0.1 N HCl. The interaction between suspending medium and activation temperature is illustrated by data in Fig. 1. At 60 C little or no activation in water could be detected even after heating for 2 hr. Whereas grape juice afforded a significant increase in the viable count (often about ten-fold), maximal activation occurred in the 0.1 N HCl. At 70 and 80 C the nature of the menstruum during heating was less important since, at these temperatures, rapid activation occurred in all three media.

In addition to activation, some lethal effects were evident at the higher temperatures in that the viable count decreased with extended heating (Fig. 1). Spores suspended in hydrochloric acid were affected most; 2 hr at 80 C reduced the viable population of this suspension below 10^4 per milliliter. The small-

est reduction in viable count at 80 C occurred in grape juice indicating that this medium exerted some protective effect.

The finding that HCl also stimulated activation of free ascospores (Fig. 1D) indicated that its action was not related in some way to the fact that the spores were contained in an ascus. In the same vein, microscopic enumeration of asci before and after heating in HCl showed no change in count, indicat-

¹Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 1938.

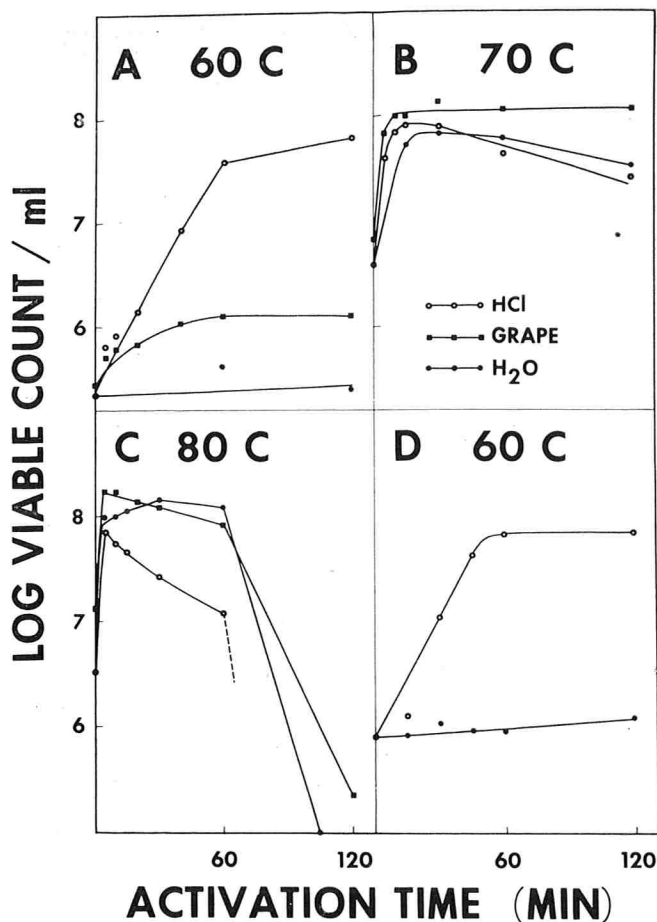


Figure 1. Activation at various temperatures in distilled water, 0.1 N HCl, and 15° Brix Concord grape juice. (A-C) suspensions of asci, (D) free ascospores.

ing that the rupture of asci with the release of free spores was not partly responsible for the increase in viable count.

The effect of pH was studied by heating asci in HCl-NaCl (0.1 N each) solutions adjusted with 6 N NaOH. Relatively small differences had a major effect (Fig. 2); raising the pH only 0.4 unit, from 1.0 to 1.4, resulted in almost a 90% decrease in the number of spores activated. Above pH 1.6, viable counts were similar to water controls indicating that activation no longer was benefited.

Stimulation provided by Concord grape juice can not be explained merely on the basis of hydrogen ion concentration since the juice always had a pH above 3.0, thus above that found to have an effect in the studies with HCl. Results with other mineral acids also indicated that factors in addition to pH were involved. When spores were heated in solutions of nitric, phosphoric, and sulfuric acids, all carefully adjusted to pH 1, only the nitric acid improved activation (Table 1). The difference here between nitric and hydrochloric acid may be significant since in a number of trials nitric acid gave the higher

count.

Trial 156 (Table 1) illustrates further that activation was influenced by the anion as well as by the concentration of hydrogen ions. Here the addition of chloride to pH 1 solutions of sulfuric and phosphoric acids resulted in better than a 20-fold increase in the viable count. In other experiments both sodium chloride and potassium chloride afforded similar increases indicating that the cation was not a factor. We conclude from these results that activation in a mineral acid requires a high concentration of hydrogen ions plus the presence of specific anions.

The response of *Byssoschlamys* ascospores to hydrogen ions reveals a similarity with *Bacillus cereus* (5), *Clostridium bifermentans* (2), and perhaps other bacteria whose spores are activated by a low pH. Just how similar remains to be established. It is not known, for example, whether small changes in pH (Fig. 2) would have such a marked effect on bacterial spores. It appears that *Byssoschlamys* ascospores are considerably more resistant to hydrogen ions than spores of *B. cereus* in that the latter rapidly lost their capacity to germinate when heated longer than 10 min at 65 C in a pH 1 medium (5).

The fact that reducing compounds activate certain bacterial spores (4) caused us to study the effect of glutathione, thioglycolate, and L-cysteine on *B. fulva* ascospores. Some twenty trials in which pH, temperature, time, and concentration were varied failed to show that activation was improved by their presence. This is illustrated by results obtained when 0.1 N HCl was supplemented with the compounds (Table 1). In this particular experiment spores heated for shorter times (4, 8, 12, and 30 min) also yielded comparable counts in the different media indicating that the rate of activation as well as the

TABLE 1. EFFECT OF THE SUSPENDING MEDIUM DURING HEAT ACTIVATION (60 MIN AT 60 C) ON THE NUMBER OF ACTIVE SPORES.

| Trial | Menstruum | Viable count/ml ($\times 10^5$) |
|-------|--|--------------------------------------|
| 142 | H ₃ PO ₄ , pH 1.0 | 10 |
| | H ₂ SO ₄ , pH 1.0 | 12 |
| | HNO ₃ , pH 1.0 | 1200 |
| | HCl, pH 1.0 | 940 |
| | Distilled water | 9.0 |
| 156 | H ₃ PO ₄ , pH 1.0 | 35 |
| | H ₃ PO ₄ plus 0.1 N NaCl, pH 1.0 | 760 |
| | H ₂ SO ₄ , pH 1.0 | 34 |
| | H ₂ SO ₄ plus 0.1 N NaCl, pH 1.0 | 750 |
| | HCl, pH 1.0 | 1000 |
| 115 | 0.1 N HCl plus glutathione, 1 mg/ml | 460 |
| | 0.1 N HCl plus thioglycolate, 1 mg/ml | 340 |
| | 0.1 N HCl plus L-cysteine, 1 mg/ml | 320 |
| | 0.1 N HCl | 460 |
| | Distilled water | 4.4 |

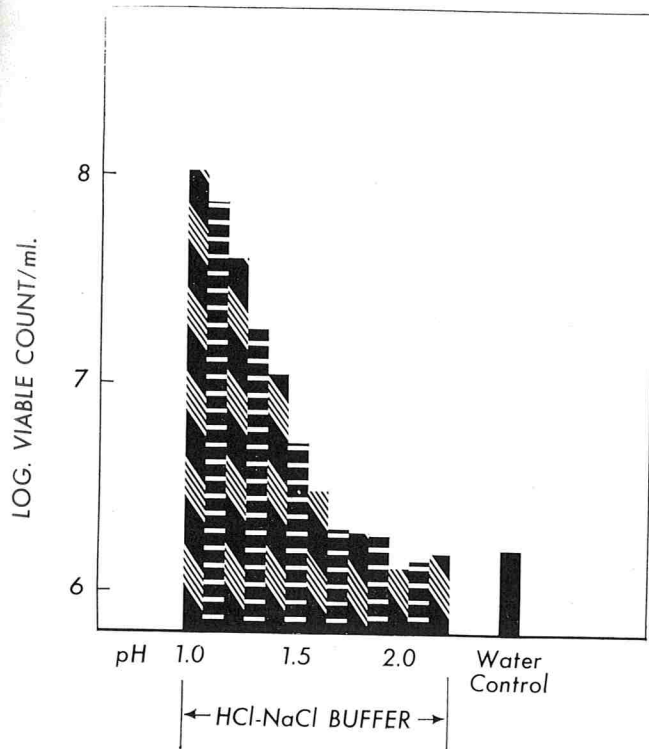


Figure 2. Effect of pH on the number of spores activated. The suspensions were heated 1 hr at 60 C.

maximal number activated was not affected.

Activated spores were stored in water for an extended time to determine whether the process was reversible as has been observed with certain bacterial spores (1, 4). In this study the spores were stored at 32 C following activation for 1 hr at 70 C in distilled water. Periodically a sample was removed for culturing; a portion was plated without additional heating while the rest was activated a second time at 70 C for 1 hr. Results showed that only a fraction of the active spores, about one-half, returned to a dormant state and that most of this reversal occurred during the first 7 days of storage (Fig. 3). Spores given a second activation exhibited an increase in viable count with time. The rupture of asci was not responsible for this increase since microscopic counts revealed no change in the number of intact asci. The data may indicate that some spores still dormant after the initial heat shock acquired the potential to be activated after ageing in the distilled water. The fact that reheating the suspensions at 70 C produced no drop in the viable count indicates that significant germination, as evidenced by a loss in heat resistance, did not occur.

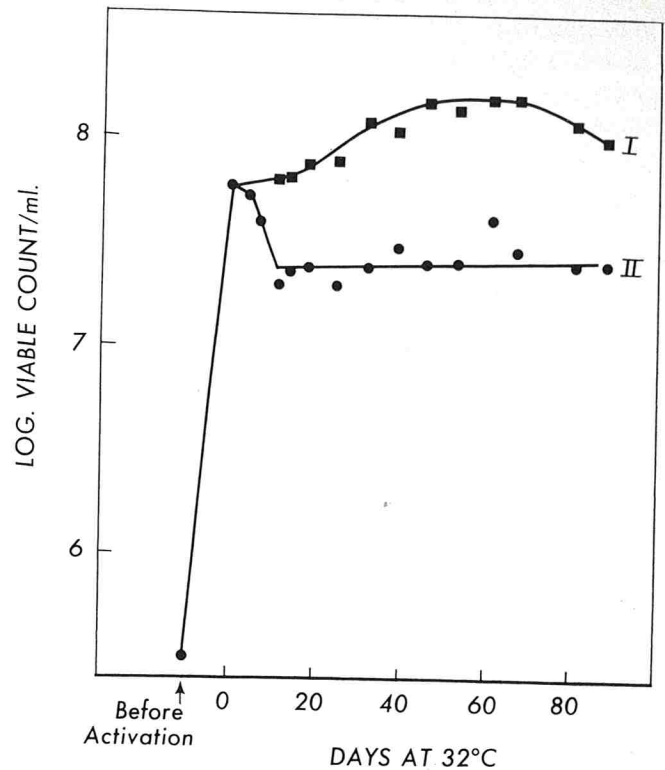


Figure 3. Effect of storage on the viable count of heat-activated spores. I. Spores reheated 60 min at 70 C prior to culturing. II. Spores not given a second heat shock.

REFERENCES

1. Church, B. D., and H. Halvorson. 1957. Intermediate metabolism of aerobic spores. I. activation of glucose oxidation in spores of *Bacillus cereus* var. *terminalis*. *J. Bacteriol.* 73:470-476.
2. Gibbs, P. A. 1967. The activation of spores of *Clostridium bifermentans*. *J. Gen. Microbiol.* 46:285-291.
3. Hull, R. 1933-34. Investigation of the control of spoilage of processed fruit by *Byssoschlamys fulva*. Report Univ. of Bristol Fruit and Veg. Preserv. Res. Sta., Campden, England.
4. Keynan, A., Z. Evenchik, H. O. Halvorson, and J. W. Hastings. 1964. Activation of bacterial endospores. *J. Bacteriol.* 88:313-318.
5. Keynan, A., G. Issahary-Brand, and Z. Evenchik. 1965. Activation of bacterial spores. p. 180-187. In L. L. Campbell and H. O. Halvorson (ed.), *Spores III*, American Society for Microbiology, Washington, D. C.
6. Maunder, D. T. 1969. Spoilage problems caused by molds of the *Byssoschlamys-Paecilomyces* group. p. 12-16. In *Byssoschlamys* seminar abstracts. Res. Circ. No. 20, N. Y. State Agric. Expt. Sta., Geneva, N. Y.
7. Put, H. M. C., and J. Th. Kruiswijk. 1964. Disintegration and organoleptic deterioration of processed strawberries caused by the mould *Byssoschlamys nivea*. *J. Appl. Bacteriol.* 27:53-58.
8. Splittstoesser, D. F., F. R. Kuss, and W. Harrison. 1970. Enumeration of *Byssoschlamys* and other heat-resistant molds. *Appl. Microbiol.* 20:393-397.

GROWTH OF SALMONELLAE IN SKIMMILK WHICH CONTAINS ANTIBIOTICS¹

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ABSTRACT

Portions of sterile skimmilk were fortified with 0.1, 0.5, and 1.0 unit of penicillin; 1.0, 10, and 100 μg streptomycin; and 0.1, 1.0, and 10 μg tetracycline per milliliter. Such skimmilks were inoculated with salmonellae, incubated at 21 or 37 C, and tested with the plate count at 3-hr intervals during an 18-hr incubation. As much as 1.0 unit of penicillin per milliliter of skimmilk had little, if any, inhibitory effect on *Salmonella typhimurium*, *Salmonella newbrunswick*, *Salmonella dublin*, or *Salmonella heidelberg* at either temperature. Streptomycin, at concentrations of 1.0 and 10 μg per milliliter failed to inhibit growth of *S. typhimurium* or *S. newbrunswick* at either temperature. Presence of 100 μg streptomycin per milliliter of skimmilk markedly inhibited *S. newbrunswick* at both temperatures and *S. typhimurium* at 21 C and completely inhibited the latter bacterium at 37 C. Neither of the salmonellae were inhibited by 0.1 μg tetracycline per milliliter of skimmilk but both bacteria were somewhat inhibited by 1.0 μg antibiotic per milliliter, particularly at 37 C, and were completely inhibited with a concentration of 10 μg tetracycline per milliliter. Concentrations of any of the three antibiotics tested that can inhibit lactic starter cultures had no apparent effect on salmonellae.

Each year approximately 2.7 million lb. of antibiotics are administered to livestock on U. S. farms either to treat diseases or as a growth stimulant (6). Although use of antibiotics as feed supplements is being reduced, the likelihood remains that these drugs can find their way into milk because they are used widely to treat mastitis and other diseases of dairy cattle.

Presence of antibiotics in milk can lead to several problems which include inhibition of lactic acid bacteria so that either an insufficient amount or no lactic acid is developed when cultured foods, such as cheese, are produced. Manufacture of rennet-type cheeses can often be completed when acid development is inadequate although the resultant cheese is likely to be of inferior quality. In addition, insufficient growth and activity of lactic acid bacteria can allow pathogenic bacteria, if present, to grow and cause the cheese to become a health hazard for the consumer. However, if the antibiotics also serve to inhibit growth of the pathogen, obviously the health hazard would be reduced. Jezeski et al. (2) demonstrated that sufficient penicillin in milk to partially inhibit lactic starter bacteria had no effect on *Staphylococcus aureus* in milk during the cheesemaking

operation nor on survival of the organism in the resulting cheese. Human infections with salmonellae often are somewhat refractory to treatment with antibiotics (3) and hence it may be reasoned that these drugs, at concentrations likely to be present in milk and affect starter cultures, will have little effect on growth of salmonellae. Data to support this conclusion were lacking. Hence experiments were conducted to determine if growth of salmonellae in milk is affected by such common antibiotics as penicillin, streptomycin, and tetracycline when they are present at concentrations needed to inhibit the commonly used lactic starter cultures. Results of the experiments are reported in this paper.

MATERIALS AND METHODS

One hundred fifty six milliliters of skimmilk were placed into each of a series of 300-ml Erlenmeyer flasks and then autoclaved for 15 min at 15 psi. One-half of the flasks of sterilized skimmilk were cooled to 21 C and the remainder to 37 C. Stock solutions of penicillin G, streptomycin, and tetracycline were prepared before addition to milk. The sterile skimmilk was fortified with sufficient of these solutions to provide the following final concentrations: penicillin, 0.1, 0.5, and 1.0 unit per milliliter; streptomycin, 1.0, 10, and 100 μg per milliliter; and tetracycline, 0.1, 1.0, and 10 μg per milliliter. Concentrations of antibiotics were selected to include those reported as able to inhibit the commonly used lactic starter cultures (4). Skimmilks thus fortified with antibiotics were inoculated separately with 24-hr old active cultures of *Salmonella typhimurium*, *Salmonella newbrunswick*, *Salmonella dublin*, and *Salmonella heidelberg*. These salmonellae were selected because they have been reported as present in raw milk or in certain dairy products (3). The inoculated skimmilks were then dispensed aseptically into a series of sterile screw-cap culture tubes (16 \times 150 mm), 10 ml per tube. Two sets of samples and controls were incubated each at 21 and 37 C. Numbers of salmonellae present in skimmilk samples initially and at 3-hr intervals during the 18-hr incubation were determined with Plate Count agar (Difco) and incubation of plates at 37 C for 48 hr.

RESULTS

Growth of salmonellae in skimmilk with added penicillin

Four species of *Salmonella* were tested for their ability to grow at 21 and 37 C in skimmilk fortified to contain 0.1, 0.5, or 1.0 unit of penicillin per milliliter. Data in Fig. 1 and 2 indicate that these concentrations of penicillin neither retarded nor enhanced growth of *S. typhimurium*, *S. newbrunswick*, *S. dublin*, or *S. heidelberg* when incubation was at 21 C.

¹Research supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison.

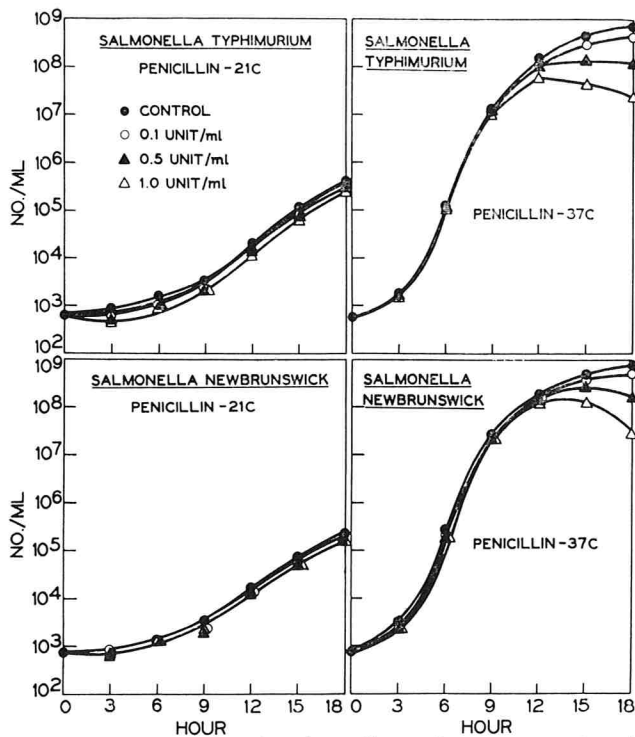


Figure 1. Growth of *Salmonella typhimurium* and *Salmonella newbrunswick* at 21 and 37 C in skim milk with and without penicillin.

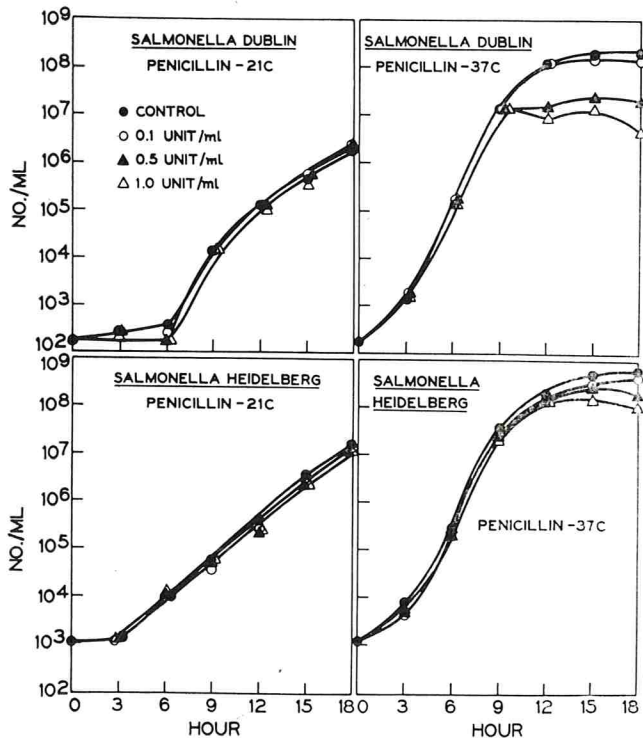


Figure 2. Growth of *Salmonella dublin* and *Salmonella heidelberg* at 21 and 37 C in skim milk with and without penicillin.

Apparently this temperature was more favorable for growth of *S. dublin* and *S. heidelberg* than it was for growth of the two other salmonellae. Both *S. dublin* and *S. heidelberg* attained populations after 18 hr

that were one or two logs greater than populations of *S. typhimurium* and *S. newbrunswick* (Fig. 1 and 2).

An increase of the incubation temperature to 37 C was accompanied by more rapid growth and hence higher populations of salmonellae than occurred at 21 C. Penicillin in the skim milk had no apparent effect on growth of salmonellae at 37 C until after 9 to 12 hr of incubation when growth was either retarded or stopped, depending on concentration of antibiotic and species of *Salmonella*. The lowest concentration of penicillin (0.1 unit/ml) was essentially without effect on all species of *Salmonella* tested. All of the species did react to the two higher concentrations (0.5 and 1.0 unit/ml) but growth of *S. heidelberg* was affected less than was growth of the other species (Fig. 2). In spite of this limited inhibition, populations of all the salmonellae approximated or exceeded 10^7 /ml after 18 hr at 37 C even when the highest concentration of penicillin was present.

Growth of salmonellae in skim milk with added streptomycin

Figure 3 provides data on the behavior of *S. typhimurium* and *S. newbrunswick* in skim milk with added streptomycin. The two lowest concentrations (1.0 and 10 $\mu\text{g}/\text{ml}$) of antibiotic had little or no effect on growth of the salmonellae at either 21 or 37 C. In contrast, the highest concentration (100 $\mu\text{g}/\text{ml}$) was markedly inhibitory to both species of *Salmonella* at both temperatures of incubation. Inhibition of *S. typhimurium* at 37 C was virtually complete, whereas limited growth occurred when this bacterium was incubated at 21 C and when *S. newbrunswick* was held at either temperature.

Growth of salmonellae in skim milk with added tetracycline

Tetracycline, when added to skim milk at a concentration of 10 $\mu\text{g}/\text{ml}$, completely inhibited, but did not inactivate *S. typhimurium* and *S. newbrunswick* at 21 and 37 C (Fig. 4). The lowest concentration (0.1 $\mu\text{g}/\text{ml}$) of tetracycline had no appreciable effect on growth of either *Salmonella* species at either incubation temperature. Growth of *S. newbrunswick* at 21 and 37 C and of *S. typhimurium* at 37 C was reduced both in terms of rate and maximum population attained when the intermediate concentration (1.0 $\mu\text{g}/\text{ml}$) was tested.

DISCUSSION

According to data summarized by Marth and Elickson (4), the lactic streptococci are partially inhibited by 0.002 to 0.1 unit and completely inhibited by 0.025 to 1.0 unit of penicillin per milliliter of milk. Whitehead and Lane (7) observed that acid development by starter cultures ceased soon after inoculation

if milk contained 0.5 unit (or more) of penicillin per milliliter. It is clearly evident from data obtained in this study that concentrations of penicillin in milk needed to inhibit the starter will have little or no effect on growth of salmonellae, thus allowing these

pathogens, if present, to grow unhindered.

Some starter cultures are partially inhibited by as little as 0.04 μg streptomycin per milliliter of milk, whereas 10 μg of the antibiotic per milliliter of milk completely inhibits *Streptococcus lactis*, *Leuconostoc citrovorum*, and *Leuconostoc dextranicum* (4). As with penicillin, concentrations of streptomycin able to partially or completely inhibit starter cultures, had no appreciable effect on growth of salmonellae.

Tetracycline, when present in milk at concentrations of 0.001 to 0.1 μg per milliliter can retard growth of starter cultures (4). Again, concentrations of this antibiotic which can inhibit starter cultures are not likely to keep salmonellae from growing.

Results from these experiments again emphasize the need for antibiotic-free milk when cultured products, and ripened cheeses in particular, are made from milk. Antibiotics commonly found in milk, if present, will partially or completely inhibit growth and acid production by the lactic acid bacteria and at the same time will allow growth of salmonellae. If rennet-type cheeses are made from milk which contains both antibiotics and salmonellae, a public health hazard could result. In earlier work, Park et al. (5) demonstrated that salmonellae grew both in milk and cheese curd when cheese was made with a starter culture that produced insufficient acid. Furthermore, salmonellae survived for a longer time in low-acid cheese (5) than they did in cheese with a normal acid content (1). Presence of antibiotics in milk to be used for cheese can create a potentially hazardous situation, if viable salmonellae also are present either because milk was inadequately heated or was inadvertently recontaminated with these bacteria after pasteurization.

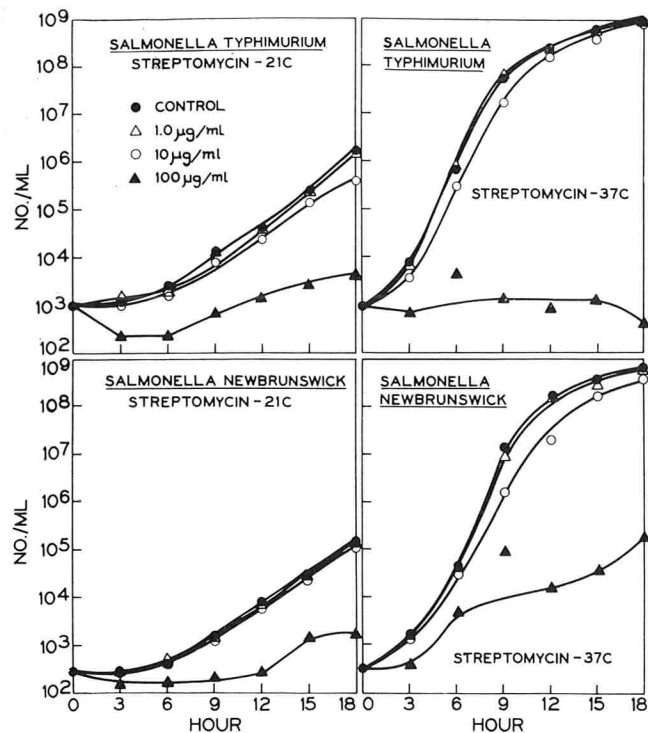


Figure 3. Growth of *Salmonella typhimurium* and *Salmonella newbrunswick* at 21 and 37 C in skim milk with and without streptomycin.

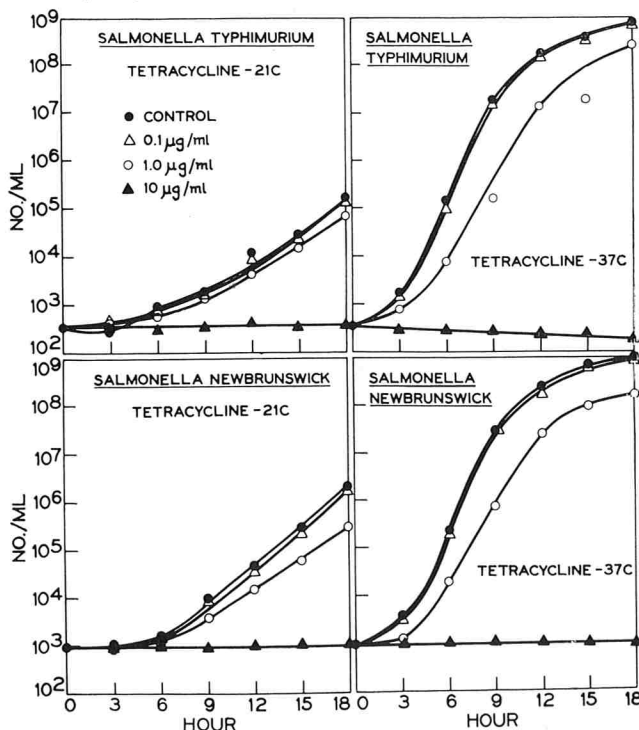


Figure 4. Growth of *Salmonella typhimurium* and *Salmonella newbrunswick* at 21 and 37 C in skim milk with and without tetracycline.

REFERENCES

- Goepfert, J. M., N. F. Olson, and E. H. Marth. 1968. Behavior of *Salmonella typhimurium* during manufacture and curing of Cheddar cheese. *Appl. Microbiol.* 16:862-866.
- Jezeski, J. J., H. A. Morris, E. A. Zottola, E. George, and F. F. Busta. 1961. The effect of penicillin in milk on the growth and survival of *Staphylococcus aureus* during the manufacture and curing of Cheddar and Colby cheese. *J. Dairy Sci.* 44:1160.
- Marth, E. H. 1969. Salmonellae and salmonellosis associated with milk and milk products. A review. *J. Dairy Sci.* 52:283-315.
- Marth, E. H., and B. E. Ellickson. 1959. Problems created by the presence of antibiotics in milk. A review. *J. Milk Food Technol.* 22:266-272.
- Park, H. S., E. H. Marth, J. M. Goepfert, and N. F. Olson. 1970. The fate of *Salmonella typhimurium* in the manufacture and ripening of low-acid Cheddar cheese. *J. Milk Food Technol.* 33:280-284.
- Van Houweling, C. D. 1967. Drugs in animal feed? *FDA Papers*, Sept.:11-15.
- Whitehead, H. R., and D. J. Lane. 1956. The influence of penicillin on the manufacture and ripening of Cheddar cheese. *J. Dairy Res.* 23:355-360.

UTILIZATION OF PHENOLPHTHALEIN MONOPHOSPHATE TO DETERMINE THE PHOSPHATASE ACTIVITY OF MILK¹

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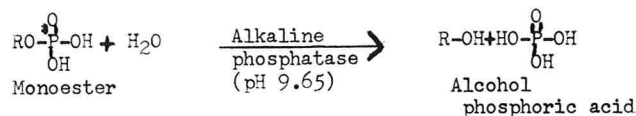
ABSTRACT

The properties of alkaline phosphatase as it exists in milk are considered. The more common methods of measuring alkaline phosphatase activity in milk are briefly reviewed, especially those employing substrates possessing "built-in" indicators which produce a chromogen directly upon hydrolysis.

The visual procedure employing phenolphthalein monophosphate as the substrate is given. The sensitivity of this method is shown to be far greater than that of the Scharer I (Rapid) method. Results of an AOAC collaborative study demonstrated that the method yields results that are as precise and either as accurate as or more accurate than those obtained by the Scharer I (Rapid) method. The quantitative spectrophotometric procedure employing the above substrate is presented. Comparison of this method with the Scharer modified spectrophotometric method on milk revealed correlation coefficients (Scharer method: 0.998 and modified new method 0.991) showing very little difference in the positive correlations of absorbance values and % raw milk. For chocolate milk, the values were 0.990 and 0.999 for the respective methods. Collaborative study of this method has demonstrated that the random error of the modified new method is almost twice that of the Scharer technique while the systematic error is only about one-fourth of the latter method.

Enzymes are organic catalysts which occur naturally in most raw foods. Indeed, milk contains many enzymes, some of the more commonly known ones are lipase, peroxidase, xanthine oxidase, and alkaline phosphatase. When milk is pasteurized, most of the enzymes are inactivated or their activity is greatly diminished. Several tests based on this observation have been developed utilizing various enzymes, e.g., amylase and catalase. However, the first reliable enzymatic test, developed by Kay and Graham in England in 1933, was based on inactivation of alkaline phosphatase.

What is phosphatase? Phosphatase is an enzyme occurring in raw milk. Actually there are three classes of phosphatases occurring in raw milk but only one of them, alkaline phosphatase, is of significance in the phosphatase test. Alkaline phosphatase is a monoesterase that catalyzes hydrolysis of monoesters:



Studies have shown that the amount of alkaline phosphatase in raw milk is variable. The activity of phosphatase per unit of milk seems to be inversely correlated to milk yield, reaching a minimum in 1 or 2 weeks after calving and rising gradually to a maximum in about 25 weeks. Breed, feed of the cow, or fat content of the milk do not appear to influence phosphatase activity. Alkaline phosphatase is associated with the fat globules of milk, i.e., it is adsorbed to the fat globule membrane surface.

ALKALINE PHOSPHATASE TESTS

Several methods have been developed for determining residual alkaline phosphatase activity. The Scharer rapid method has gained wide acceptance because of its speed and relative simplicity compared to the tedious standard laboratory methods.

While the Scharer rapid method is relatively simple and quick, it must be recognized that it does possess some inherent weaknesses. There is a constant hazard of phenol contamination from reagents, glassware, and stoppers. Reagents are unstable as is the color formed by the reaction of phenol with the dye. Visual measurement of color is sometimes difficult, particularly with borderline cases; and emulsification frequently occurs during the extraction of the phenol with butanol.

For 20 years, use of phosphate esters of indicators which produce a chromogen directly on hydrolysis has been recommended to determine phosphatase activity in milk. Esters which possess such "built-in" indicators avoid the problem of non-specific color reactions.

p-Nitrophenyl phosphate, which liberates yellow p-nitrophenol on hydrolysis, has been recommended, but it is very unstable and insensitive (requires a 2-hr incubation period for detection of contamination of pasteurized milk with 0.1% raw milk).

Phenolphthalein diphosphate is more stable and releases phenolphthalein on hydrolysis. Phenolphthalein is a lactone of a gamma hydroxy acid. Under alkaline conditions, the lactone ring is opened with

¹Presented at the 48th Annual Meeting of the New York State Association of Milk and Food Sanitarians, Rochester, New York, September 21-23, 1971.

TABLE 1. THE PROBABILITY OF CORRECT VISUAL DIFFERENTIATION BETWEEN TWO DIFFERENT LEVELS OF RAW MILK IN PASTEURIZED MILK AND CREAM

| Sample | Method | Ratio of difference ^a | No. of pairs observed | No. of correct observations | Fraction of correct observations | Confidence limits at $p = 0.95^b$ |
|--------|-----------|----------------------------------|-----------------------|-----------------------------|----------------------------------|-----------------------------------|
| Milk | New | 1:1.2 | 74 | 72 | 0.973 | 0.90-1.00 |
| Milk | Scharer I | 1:1.2 | 75 | 57 | 0.760 | 0.65-0.85 |
| Milk | New | 1:1.1 | 48 | 38 | 0.792 | 0.65-0.89 |
| Cream | New | 1:1.2 | 65 | 62 | 0.954 | 0.88-0.99 |
| Cream | Scharer I | 1:1.2 | 75 | 48 | 0.640 | 0.52-0.75 |

^a1:1.2 differences are 0.100:0.120%, 0.120:0.144%, 0.144:0.173% *for statisticians*, Vol. 1, Cambridge University Press.

are 0.100:0.110%, 0.110:0.120%, 0.120:0.132%, and 0.132:0.144, 0.173:0.208%, and 0.208-0.250% raw milk; 1:1.1 differences

^bPearson, E. S., and Hartley, H. O. 1954. *Biometrika tables* *f* raw milk.

the resulting ion losing a molecule of water to form a quinone-like structure in one ring. In addition, the other phenolic group forms a salt and the resulting ion, which is stabilized by resonance between the two benzene radicals, is highly colored red. Referring to the structure of phenolphthalein diphosphate, it will be noted that two phosphoryl radicals must be removed before free phenolphthalein is released. Hence, this substrate is not considered to be sufficiently sensitive for use as a substrate for alkaline phosphatase in milk.

A few years ago, the Warner-Lambert Research Institute succeeded in synthesizing phenolphthalein monophosphate, a substrate which is very stable and easily hydrolyzed by alkaline phosphatase to yield free phenolphthalein. A simple visual method was developed by them to determine residual alkaline phosphatase in milk (2). Our Food Science Department at Rutgers was called on to study the method and conduct an AOAC collaborative study on it. In addition, we have developed a quantitative spectrophotometric method utilizing this substrate and submitted it to collaborative study.

NEW VISUAL METHOD

Method

This method is based on release of free phenolphthalein after hydrolysis of the substrate (phenolphthalein monophosphate) and, subsequent development of a red solution which is compared visually with a standard prepared from the same milk.

1. *Reagents.* Three reagents are required to determine phosphatase activity by this method:

a. *Substrate concentrate*—This consists of 65 mM phenolphthalein monophosphate in 7.8 M 2-amino-2-methyl-1-propanol at pH 10.15 (25 C).

b. *Standard concentrate*—This is composed of 0.01% phenolphthalein and 0.04% tartrazine in the same buffer as the substrate concentrate.

c. 2.5 N NaOH

All three reagents are extremely stable provided that they are refrigerated.

2. *Procedure.* The procedure for running the test is as follows:

a. Pipette 1 ml of milk into each of two small test tubes and warm to 37 C in a water bath.

b. To one add one drop (0.04 ml) of substrate concentrate and to the other add one drop of standard concentrate.

c. Mix and incubate at 37 C for 30 min.

d. Add one drop of 2.5 N NaOH to each tube, mix and compare visually.

3. *Interpretation.* If the sample tube shows less pink color than the standard tube, milk can be considered properly pasteurized. The standard contains 4 μ g of phenolphthalein per test which is equivalent on a molar basis to 1.18 μ g of phenol. This is actually equal to the amount of phenolphthalein liberated by 1 ml of pasteurized milk contaminated with 0.1% raw milk in 30 min. This is almost identical on a molar basis to the recommended borderline level of phenol liberated in the Scharer II (Laboratory) method of 2.3 μ g phenol/ml of milk/hr. It is less than the recommended 4 μ g phenol/ml of milk/hr for the Scharer I method and the official A.O.A.C. Method II.

Tartrazine is employed in the standard to simulate the natural yellow color of phenolphthalein monophosphate. In addition, the preparation of a standard in the same milk as the sample results in colors having the same hue which is very important in any visual comparison of color densities.

Sensitivity of visual comparison

The sensitivity of the new method was compared to that of the Scharer I method for detection of slight differences in residual phosphatase activity. Pairs of samples containing different levels of raw milk in pasteurized milk and cream with a ratio of 1 to 1.2 were prepared. In each pair of prepared samples, one contained 20% higher phosphatase activity than the other as shown in Table 1. The differences in phosphatase activity were determined by both methods. The probability of correct differentiations is shown in Table 1 with 95% confidence intervals constructed. The new method achieved significantly higher probability of correct visual differentiation than the Scharer I method in detecting a 1:1.2 ratio of difference of residual phosphatase activity in either milk or cream (level of significance < 0.001). The probabilities of detecting the same ratio of differences in milk and cream by the new method were the same, while those by the Scharer I

method were slightly different (significant at $P < .25$). This observation indicates that fat content does influence the color of the butanol extract in the Scharer I method, while it has little or no effect on the new method.

A similar visual comparison was conducted to determine the ability of the new method to detect a 1 to 1.1 ratio of difference of raw milk in boiled milk. The probability of correct differentiation did decrease, but it was comparable to that of the Scharer I method in detecting a 1 to 1.2 ratio of difference of raw milk in boiled milk.

The new method is highly sensitive because of the ease of color comparison, the high rate of hydrolysis, the elimination of variations because of specific color reaction and extraction and the slight contribution of yellow color of the milk fat to the pink color of phenolphthalein.

Collaborative study (5)

Results of the collaborative study showed that the new alkaline phosphatase assay system, based on hydrolysis of phenolphthalein monophosphate, yields results that are as precise and either as accurate as or more accurate than those obtained with the Scharer I method. In addition, the new method is much simpler, has eliminated the need for positive and negative controls, and utilizes reagents that are relatively stable. It was recommended that the method as presented and studied be adopted as official, first action. This recommendation was approved by the General Referee and by Subcommittee C, and was adopted by the AOAC at the October 9-12, 1967 meetings. Use of this method and, hopefully, the subsequent endorsement of it by regulatory and industrial laboratories will assist it in achieving the status of official, final action someday.

MODIFIED NEW QUANTITATIVE SPECTROPHOTOMETRIC METHOD

Method

Dialysis through a cellulose membrane has been advocated (7) as a means of separating phenol released by action of phosphatase on disodium phenyl phosphate. This technique has been employed in our research. Concentrated buffered substrate is added dropwise to tempered milk and mixed. The mixture, having a pH of 9.6, is then transferred to a dialysis bag and dialysed against distilled water for a specified time period. After hydrolysis, the free phenolphthalein participates in solute transfer through the membrane along with the buffer and other solutes resulting in a crystal clear dialysate of pH 9.6 which can be measured directly in a spectrophotometer.

The original procedure (4) was criticized for yielding relatively low absorbance values and was subsequently modified (3) before conducting the collaborative study (6). The modification has resulted in much higher absorbance values and a decreased cost of reagent needed for the analysis.

1. Apparatus

- a. Test tubes—soft glass, large mouth 20 × 175 mm, preferably shortened to 75-100 mm and soft glass 15 × 100 mm.
- b. Pipettes—Delivery 0.1 ml, 1.0 ml, 5.0 ml, and 10.0 ml, graduated at 0.1 or 1 ml.
- c. Funnels, short stem, 35 × 75 mm.
- d. Water bath—thermostatically controlled at 37 ± 1 C.
- e. Weighing balance, analytical.
- f. Thermometer—0 to 110 C, certified against NBS.
- g. Spectrophotometer—Coleman Model 6A or equivalent.
- h. Cuvettes—Selected, round 12 × 75 mm.
- i. Rubber stoppers, No. 3.

2. Reagents and materials (only Analytical Reagent Grade Recommended)

- a. Phenolphthalein monophosphate solution (substrate concentrate) pH 10.15 at 25 C. Dissolve 3.9 g dicyclohexylamine salt of phenolphthalein monophosphate and 73.2 g 2-amino-2-methyl-1-propanol in 21.9 ml HCl. Solution is stable indefinitely under refrigeration. (Available as Phosphastrate Alkaline from General Diagnostics Div., Warner-Chilcott Laboratories, Morris Plains, N. J. 07950).
- b. Buffer—Sodium susquicarbonate dihydrate ($\text{NaHCO}_3\text{-NA}_2\text{CO}_3\cdot 2\text{H}_2\text{O}$).
- c. Phenolphthalein.
- d. Ethyl alcohol, 50% aqueous solution.
- e. Seamless, cellulose, dialyzing tubing—Flat width 1.31 inch and wall thickness 0.001 inch. Flat width 1.00 inch and wall thickness 0.0008 inch is also applicable. Available from general scientific supply houses.

Immediately before conducting test, cut tubing into 8-inch sections and place in fresh, distilled water for about 3 sec. Tie off one end, cut off excess next to knot, and store knotted bags in distilled water. Tubing may require several rinsings with distilled water to remove oily-like surface film.

3. Preparation of phenolphthalein standard curve

- a. Stock phenolphthalein solution—Dissolve 100 mg of fresh phenolphthalein in 1 liter of 50% alcohol. This solution contains 100 μg phenolphthalein per ml.
- b. Buffer solution—Dissolve 10 g of sodium sesquicarbonate dihydrate in water and make up to 100 ml.
- c. Proportion solutions for standards and final development—Pipette into test tubes (15 × 100 mm), 10 ml portion mixtures in the ratios listed in Table 2.

4. Procedure

- a. Pipette 3 ml of milk into a 15 × 100 mm. test tube and temper in a water bath at 37 C for 3-5 min.
- b. Add 3 drops of substrate concentrate to the tube and mix thoroughly, preferably with a mechanical

TABLE 2. PROPORTIONING SOLUTIONS FOR STANDARDS AND FINAL DEVELOPMENT¹

| Diluted phenolphthalein solution | Buffer solution | Final phenolphthalein concentration |
|----------------------------------|-----------------|-------------------------------------|
| (ml) | (ml) | (ug/ml) |
| 0.0 | 10.0 | 0.0 |
| 0.1 | 9.9 | 1.0 |
| 0.2 | 9.8 | 2.0 |
| 0.3 | 9.7 | 3.0 |
| 0.5 | 9.5 | 5.0 |
| 0.7 | 9.3 | 7.0 |
| 1.0 | 9.0 | 10.0 |

¹Mix above mixtures of phenolphthalein and buffer solutions, and determine absorbance values in spectrophotometer set at 550 m μ in order to produce standard curve (absorbance vs. phenolphthalein concentration).

mixer.

- c. Insert a funnel into a pre-soaked knotted, dialysis bag, transfer the above mixture and then, knot the top of the bag after expulsion of air.
 - d. Immediately suspend bag in a 20 \times 100 mm test tube containing 5 ml of distilled water and incubate in a water bath at 37 C for 3 hr. Contents of bag *must* be completely immersed in distilled water but top knot *must* remain in air.
 - e. After incubation, quickly remove bag with forceps or fingers and discard.
 - f. Read absorbance value at 550 m μ in spectrophotometer, using dialysate from a heated milk sample (80 C for 2 min) to set the instrument at 100° T. Determine phenolphthalein concentration by referring to the standard curve.
5. *Interpretation:* Tentatively, a reading of greater than 1.15 μ g phenolphthalein per 3.0 ml milk is indicative of under pasteurization or contamination of sample with raw milk.

Experimental procedure

The following procedure was employed in evaluating the modified new method. Fresh, mixed milk from the University herd was employed to prepare samples of milk con-

taining various levels of raw milk. A portion of the milk was heated to 80 C for 2 min and cooled to 15-20 C. A sample containing 1.0% raw milk was prepared by diluting 5.0 ml raw milk with heated milk to 500 ml and mixing thoroughly. Samples containing lower amounts of raw milk were prepared by diluting aliquots of above sample with appropriate amounts of heated milk and mixing thoroughly.

Immediately upon completing preparation of samples, analyses were run by the prescribed method and in some cases by the Scharer modified spectrophotometric method (1). Experiments were also conducted to determine the applicability of the modified new method to chocolate milk.

RESULTS AND DISCUSSION

The modified new method was compared with the Scharer modified spectrophotometric method on both milk and chocolate milk. Results of three trials conducted on the same series of milk and chocolate milk samples are shown in Tables 3 and 4, the phenolphthalein and phenol values being obtained by referring the average values to the standard curves.

Equations of the regression lines for milk determined from the average absorbance values (y) vs. percentages of raw milk (x) were calculated (Scharer method: $y = 0.01638 + 0.45348 x$; modified new method: $y = 0.01828 + 0.35218 x$). A slightly steeper slope was still observed for samples of milk evaluated by the Scharer method but the difference is far less than that observed in the original study (5). Correlation coefficients were calculated (Scharer method: 0.998 and modified new method: 0.991) showing very little difference in the positive correlation of absorbance values and percent raw milk as determined by the two methods.

With chocolate milk, a steeper slope was obtained for the modified new method, the slope being slightly greater (0.38513 vs. 0.35218) than that obtained on milk (Scharer method: $y = 0.00173 + 0.15156 x$;

TABLE 3. ABSORBANCE VALUES AND RESPECTIVE PHENOLPHTHALEIN (PHTHLN) AND PHENOL CONCENTRATIONS AS DETERMINED BY THE MODIFIED NEW METHOD (3 ML SAMPLE PLUS 3 DROPS SUBSTRATE VS. 5 ML WATER FOR 3 HR) AND SCHARER MODIFIED SPECTROPHOTOMETRIC METHOD ON MILK.

| Phosphatase activity, % Raw milk | Absorbance values | | | | ug phtthln/ml ¹ |
|----------------------------------|----------------------------|---------|---------|---------|----------------------------|
| | Trial A | Trial B | Trial C | Average | |
| | <i>Modified new method</i> | | | | |
| 0.05 | 0.028 | 0.027 | 0.028 | 0.028 | 0.40 |
| 0.10 | 0.042 | 0.043 | 0.048 | 0.043 | 0.63 |
| 0.25 | 0.104 | 0.110 | 0.109 | 0.109 | 1.58 |
| 0.50 | 0.213 | 0.211 | 0.224 | 0.213 | 3.10 |
| 0.75 | — | 0.305 | — | 0.305 | 4.45 |
| 1.00 | 0.345 | 0.351 | 0.330 | 0.345 | 5.00 |
| | <i>Scharer method</i> | | | | |
| 0.05 | 0.024 | 0.024 | — | 0.024 | 0.53 |
| 0.10 | 0.055 | 0.052 | — | 0.054 | 1.50 |
| 0.25 | 0.117 | 0.129 | 0.131 | 0.129 | 3.25 |
| 0.50 | 0.252 | 0.248 | — | 0.250 | 6.50 |
| 0.75 | 0.361 | 0.365 | — | 0.363 | 9.50 |
| 1.00 | 0.450 | 0.440 | 0.450 | 0.450 | 11.75 |

¹This is μ g phenol/ml for the Scharer method.

TABLE 4. ABSORBANCE VALUES AND RESPECTIVE PHENOLPHTHALEIN (PHTHLN) AND PHENOL CONCENTRATIONS AS DETERMINED BY THE MODIFIED NEW METHOD (3 ML SAMPLE PLUS 3 DROPS SUBSTRATE VS. 5 ML WATER FOR 3 HR) AND SCHARER MODIFIED SPECTROPHOTOMETRIC METHOD ON CHOCOLATE MILK.

| Phosphatase activity, % raw milk | Absorbance values | | | | ug phtlhn/ml ¹ |
|----------------------------------|----------------------------|---------|---------|---------|---------------------------|
| | Trial A | Trial B | Trial C | Average | |
| | <i>Modified new method</i> | | | | |
| 0.05 | 0.016 | 0.010 | 0.013 | 0.013 | 0.18 |
| 0.10 | 0.032 | 0.035 | 0.036 | 0.035 | 0.50 |
| 0.25 | 0.098 | 0.089 | 0.098 | 0.098 | 1.45 |
| 0.50 | 0.210 | 0.205 | 0.195 | 0.205 | 3.00 |
| 0.75 | 0.285 | 0.270 | 0.289 | 0.285 | 4.15 |
| 1.00 | 0.380 | 0.380 | 0.355 | 0.380 | 5.55 |
| | <i>Scharer method</i> | | | | |
| 0.05 | 0.005 | 0.005 | — | 0.005 | 0.14 |
| 0.10 | 0.016 | 0.012 | — | 0.014 | 0.38 |
| 0.25 | 0.043 | 0.040 | 0.036 | 0.040 | 1.04 |
| 0.50 | 0.085 | 0.085 | 0.083 | 0.085 | 2.20 |
| 0.75 | 0.125 | 0.126 | 0.131 | 0.126 | 3.26 |
| 1.00 | 0.141 | 0.142 | 0.141 | 0.142 | 3.66 |

¹This is μg phenol/ml for the Scharer method.

modified new method: $y = 0.00077 + 0.38513 x$). This is probably due to the contribution of color by the chocolate. However, interfering substances appear to have a far greater effect on results obtained by the Scharer method, the slopes for milk and chocolate milk being 0.45348 and 0.15156, respectively. Correlation coefficients were calculated (Scharer method: 0.990 and modified new method: 0.999), the differences again being very small. All four of the above correlation coefficients are significant at the 1% level.

Equations of the regression lines for (y) vs. phenolphthalein (x) values obtained on the milk and chocolate milk samples, respectively, were calculated ($y = -0.2923 + 2.2956 x$; and $y = 0.0009 + 0.6927 x$). The apparent linear correlation obtained in both instances was verified by calculating the correlation coefficients ($r = 0.996$ and 0.992) which are significant at the 1% level.

The modified new method is applicable to milk and chocolate milk and is far simpler than any of the established techniques. While a 3-hr incubation period is utilized, this should not be considered as a serious limitation as many other routine laboratory analyses can be conducted during this period.

Accuracy and precision are of utmost importance in a confirmatory test and the author believes that the modified new method assures equal or greater accuracy and precision than any of the established methods. In addition to obviating the need for positive and negative controls, the time of incubation is under far better control in the modified new method than in the Scharer modified spectrophotometric method.

Collaborative study

This study was conducted in the spring of 1971 and was reported on October 12th at the 85th Annual Meeting of the AOAC. Nine collaborators participated in the study involving six unknown milk samples. Results revealed that the random error of the modified new method was almost twice that of the Scharer modified spectrophotometric technique while the systematic error was only about one-fourth that of the latter method. A linear relationship was found between the two methods by the five collaborators who analyzed samples by both techniques, the correlation coefficients ranging from 0.993 to 0.999. The modified new method was recommended and adopted by the A.O.A.C. as official, first action

REFERENCES

1. American Public Health Association. 1967. Standard methods for the examination of dairy products. 12th ed., Amer. Public Health Ass. Inc., New York.
2. Babson, A. L., and S. J. Greeley. 1968. A new substrate for alkaline phosphatase in milk. *J. Ass. Off. Anal. Chem.* 51:555.
3. Huang, C. L., and D. H. Kleyn. 1971. Refinement of a quantitative technique for estimating alkaline phosphatase activity in milk. *J. Ass. Off. Anal. Chem.* 54:857.
4. Kleyn, D. H., and W. Yen. 1969. Spectrophotometric determination of phosphatase activity in milk utilizing a new alkaline phosphatase assay system. *J. Ass. Off. Anal. Chem.* 53:869.
5. Kleyn, D. H., and S. H. C. Lin. 1968. Collaborative study of a new alkaline phosphatase assay system for milk. *J. Ass. Off. Anal. Chem.* 51:802.
6. Kleyn, D. H., and C. L. Huang. 1971. Collaborative study of the spectrophotometric determination of phosphatase activity in milk utilizing a new alkaline phosphatase assay system. Presented at the 85th Annual Meeting of the AOAC, October 11-14, 1971.
7. Kosikowski, F. 1964. Dialysis phosphatase methods for milk and all dairy products. *J. Dairy Sci.* 47:748.

BULK MILK SAMPLING: A COMBINED DAIRY INDUSTRY AND REGULATORY FUNCTION

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ABSTRACT

Proper sampling of bulk milk is an absolutely essential routine operation. The milk hauler is the key man and for economic and other reasons must be charged with this responsibility. Coordination and pooling of manpower resources through close participation by the dairy industry and enforcement agencies is necessary. An understanding of the problems and needs by all concerned is essential. There are three basic requirements for proper bulk-milk sampling: (a) a simple, practical routine system; (b) adequate facilities; and (c) adequate training and supervision. These requirements are discussed in detail. The heart of the operation is the "Universal Sampling System." This system supplemented with proper facilities, training, and supervision will produce reliable and consistent results.

Milk occupies a unique position in the broad field of food sanitation and quality control. The perishable nature and sensitive qualities of this food make it difficult to distinguish where health protection leaves off and quality control begins. Stress conditions such as time, temperature, transportation, excessive handling, etc. caused by modern production, processing, and marketing methods and by other conditions related to the raw and processed products complicate the problems involved. Before the 1960's, protection from milk-borne diseases was the major concern and most sanitation and public health programs were directed toward this objective. A very creditable job has been done in this regard as evidenced by the almost complete absence of milk-borne diseases during the past decade.

Today the major problem is not health protection in the true sense of the word. Instead, it is the improvement and maintenance of quality so acceptable products are available to the consumer in the market-place. This quality judgment is based largely on flavor, shelf life, and appearance. The consumer demands, and has every reason to expect, that her purchase is safe, palatable, and meets nutritional standards. It then becomes the responsibility of both industry and regulatory agencies, to so conduct their affairs that this basic objective of satisfactory quality

is carried out. The perishable nature of the product and the complicated problems involved make it impossible for either the regulatory agency or the dairy industry to go it alone. The ultimate interest and objective of both parties is the same, as is the route necessary to reach the objective.

Disease protection measures are well-defined. Through a combination of animal and human health programs, pasteurization, well-designed equipment and facilities, adherence to well-established and understood routine measures, and enforcement the end result is reasonable compliance and products generally accepted as safe. These measures and legal applications are provided in Federal and State laws and regulations. The problem today, then, is provision of dairy products in the market-place which meet the esthetic and quality standards necessary for consumer confidence and acceptability. Results of reliable surveys show that entirely too much milk and too many milk products reach the public in an unsatisfactory condition (4, 5).

In today's complicated production, processing, and distribution systems there can be no clear lines of responsibility and task performance. Health authorities find it impossible to carry out the job of complete consumer protection. Nor should they be expected to, any more than for any other industry. Many production and operational conditions have little or nothing to do with health and health regulations. An example is herd feeding operations. *Health authorities should not be expected to provide the necessary surveillance for quality control! An adequate job of public health and consumer protection can only be accomplished through the coordination of the efforts of both health authorities and industry!* This includes among other things the understanding and carrying out of responsibilities, up-dating and modernizing of methods and practices, judicious and practical enforcement of laws and regulations, and public concern. It is natural that those in regulatory work should set the examples and lead the way.

It is suggested that because of its priority and basic nature, collection of farm bulk-produced milk samples be a joint endeavor of the enforcement agency and the dairy industry, and be the first to receive

¹Presented at the Sanitarians' Conference, University of Minnesota, St. Paul, Minnesota, September 17-18, 1970 and at the Dairy Fieldmen's and Sanitarians' Conference, Purdue University, November 18, 1969.

their collective attention. The task lends itself to coordination of efforts and sharing of results. Success here would set an excellent example for other operations. Students of this much-abused operation quite frankly state that bulk milk sampling cannot be successfully accomplished any other way. Many areas throughout the country using the hauler collection system over a long period of time can testify as to its practical, business-like, and effective application. May we now discuss a method, its advantages, procedures and facilities needed, and the responsibilities involved for proper bulk milk sample collection?

IMPORTANCE OF SAMPLE COLLECTION

Sample collection is a Number One priority as a means to evaluate the producer in terms of compliance with legal standards and as a basis for payment. Too much emphasis cannot be placed on the importance of sample collection and the care of samples. It is imperative that the sample be representative of the lot from which selected and that it arrive at the laboratory in the same condition it left the bulk tank, with no change in its chemical, physical, or bacteriological character.

A few examples help to substantiate the importance of sample collection. There are many uses for these samples. Analysis of a representative sample is the only way the purchaser can determine butterfat and solids contents which are so important in the purchase contract. Similarly, compliance with legal standards for chemical and bacteriological composition, quality standards, wholesomeness, and any other character needed are made possible only through sample analysis.

Examples of some adverse effects emphasize that poor sampling should not be tolerated. May we speculate for a moment on the economic and other reactions to an improperly collected or transported sample? The payment base is destroyed. The milk producer will be unjustly penalized or harassed if results are used for quality control guidance or compliance with legal standards. Laboratory work is wasted. Confusion and distrust result. Much time and effort, all of which is expensive, is expended by the fieldman, the laboratory, and others. Needless field visits, rechecks, laboratory re-runs, and investigations result. Even worse, the credibility gap becomes wider between producer, handler, laboratory, fieldman, sanitarian, and truck driver. Yes, everyone concerned is adversely affected by poor sampling. Everyone loses and no one can possibly gain. Apathy and carelessness have no place here.

Our conclusion is that proper sampling and care

of samples is equally important and necessary to all parties concerned. Our next step is to implement a method and procedure that will accomplish the necessary result. Proper sampling can only be accomplished under today's bulk-milk operation through pooling and coordinating the efforts and resources of the dairy industry and the regulatory agencies. The manner in which this may be done follows.

ESSENTIALS FOR PROPER SAMPLING

Three essentials are necessary for proper sampling. They are: (a) a simple, practical, routine system; (b) adequate facilities; and (c) adequate training and supervision.

Many systems have been tried to collect bulk milk produced samples. All except the so-called "Universal Sampling Method" or "a sample for every purpose" have serious shortcomings. It is completely out of the question for the dairy industry to routinely collect samples by sending a man to each farm. A few regulatory agencies still practice this expensive method.

Economics dictate that the routine sampling chore be assigned to the milk hauler on a regular basis. To make this practical, it is necessary that he operate under a simple routine system carried out daily in the same manner with the same facilities. He cannot be expected to do a proper job if he has to collect special samples or samples for different purposes on different days. This method confuses him, invites short-circuiting, and causes unnecessary work. The simpler the task is made, the more apt it is to be done properly.

The "Universal Sample Method"

Because of its many unique advantages, this method is rapidly becoming recognized as the best and most economical method of collecting bulk milk samples for industry and regulatory agencies. Some advocates maintain it is the only system yet developed that is satisfactory. The Farm Methods Committee of the International Association of Milk, Food, and Environmental Sanitarians has endorsed the system and recommended that it be used exclusively to sample raw milk (2). Some states have written this requirement into their regulations. Large producer organizations and dairy companies are using it exclusively in their operations. Long experience and success with its wide use in many areas are proof of its merit and value. A new use of samples collected by the system, namely sediment testing, was suggested by Roman (6) when he reported on "Testing Milk for Cleanliness of Production." He states "a Universal milk sample taken from each milk collection simplifies monitoring for cleanliness using a 4 oz

sample and 0.2 inch diameter test.”

The method is quite simply what its name implies. One sample is collected at every pick-up and it can be used for any or all of the many routine and special tests needed in modern sanitation and quality control as well as for butterfat or other tests used for payment. It does not mean that the sample can be used for all of the many uses on a given day. Obviously it is not necessary to do all of the tests needed during the month from each pick-up. It simply means that by advanced and judicious laboratory scheduling, a fresh sample of adequate size is available and can be used for any of the tests desired. The hauler simply equips himself with the facilities necessary to collect and properly transport one sample at each pick-up.

Among other advantages of the system is the fact that neither the hauler nor the milk producer knows for what purpose the sample may be used. Neither the producer nor hauler can out-guess you by preparing for sampling day and hence a day-to-day concern for quality is established. Using the samples for butterfat determinations when needed, provides an excellent check on the adequacy of agitation of the milk in the tank before the sample is taken. There are temptations connected with a system that depends on special or periodic sampling. The Universal sample removes these temptations.

How about the laboratory technician and the Universal system? Frankly, it is impossible for him to operate efficiently under the special sample system or any system other than the one described here. Since samples are available at every pick-up, he can plan his schedule to blend with his operation completely independent of the hauler. If rechecks are needed, a sample is available after the next or any subsequent pick-up.

How then can the regulatory agency obtain samples? In many areas commercial and industry laboratories do the required tests when designated officials supervise the work and certify the laboratory and the methods which are used. When it is required that an official agency make the tests, the agency need only arrange to release any samples required and collected on days previously selected. This practice is widely used. The agency provides the needed assistance in training and supervision.

Adequate facilities

Sample containers. *Standard Methods for the Examination of Dairy Products* requires that sampling containers be sterilized and of non-toxic material (1). Containers of glass or other material meeting these specifications may be used. Experience shows that washing, sterilizing, transporting, and storage of glass

and other multi-use rigid containers are subject to human error. Devised specifically to collect bulk-produced milk are sterile plastic single service bags. These bags meet the requirements of *Standard Methods* and are ideal for all kinds of sampling and especially for the “Universal Sample System” method. Their labor-saving and convenience features have gained them wide popularity.

A dipper on every farm. Many markets have obtained excellent results when a dipper was provided on every farm for sample collection. This is a 2-oz stainless steel, inexpensive dipper. This method has many advantages over that of carrying a dipper on the truck.

Refrigerated carrying cases. Sample cases able to transport milk samples on the truck at temperatures below 40 F are required by statute (1, 3) and are a necessity if accurate results are to be obtained from the samples. Prevention from freezing is also necessary. Until very recently most sample cases were poorly designed, poorly insulated, and unable to maintain the needed temperature, particularly in extreme heat and cold. There is now available a specially designed and constructed sample case which meets all of the necessary requirements of a bulk milk pick-up sample case. It is made of durable Cylolac covering with adequate blown polyurethane insulation to ensure that the samples will be kept at the proper temperature in any kind of weather.

TABLE 1. INFLUENCE OF PROPER ICING OF SAMPLES ON MILK TEMPERATURE¹. JULY 28, 1971; OUTSIDE TEMPERATURE, 84 F

| Route no. | 1st Pick up time | Time of arrival at truck terminal | Temp. of samples at arrival (°F) |
|-----------------|------------------|-----------------------------------|----------------------------------|
| 25 ² | 9:40 a.m. | 3:40 p.m. | 34 |
| 24 ² | 6:55 a.m. | 3:40 p.m. | 35 |
| 16 ² | 6:20 a.m. | 3:55 p.m. | 37 |
| 18 ² | 7:30 a.m. | 4:00 p.m. | 35 |
| 27 ² | 8:25 a.m. | 4:30 p.m. | 34 |
| 40 ³ | 6:50 a.m. | 3:30 p.m. | 42 |
| 30 ³ | 8:10 a.m. | 4:40 p.m. | 42 |

¹Properly constructed insulated sample cases used on all routes. Ice water is 20 times as penetrating and effective as air at the same temperature.

²Above samples were completely covered with ice.

³Above samples were only partly covered, not enough ice was used.

TABLE 2. WARMING OF 4 OZ MILK SAMPLES EXPOSED TO ROOM TEMPERATURE (82 F)

| Time | Temperature (°F) | Increase (°F) |
|---------|------------------|---------------|
| Start | 40 | 0 |
| 30 min | 64 | 24 |
| 60 min | 72 | 32 |
| 120 min | 78 | 38 |

This design and construction resulted from recommendations of dairy personnel and regulatory officials from all sections of the country. Nationwide testing followed with excellent reports.

Because of the case's excellent insulation and design less than one-half the amount of ice is required as compared with conventional cases. A separate bin for ice is provided. With ice conveniently available, the hauler may easily add ice to the sample compartment if there is not enough ice water to surround the samples. Research and experience have shown that ice and ice water in direct contact with the samples is the only way to properly maintain the temperature. Table 1 illustrates this fact. Temperature maintenance is a basic priority in sample collection. The necessity for a proper sample case cannot be over-emphasized.

Means of proper sample identification. The hauler must be provided with a felt point wick pencil or pen or similar means to mark or identify the sample. Accurate and legible identification is of prime importance.

Thermometer for checking temperatures. Pocket case, dial, unbreakable.

Refrigeration facilities for storage. Temperature control of samples is the most neglected of all sampling operations. It cannot exceed 40 F if changes in the bacterial and other characteristics of milk are to be prevented (1, 3). Generally, too little attention is paid to storage temperatures and to handling of samples between pick-up and analysis. The program should be geared to a minimum of handling and delay. At normal temperatures, exposed samples warm quickly. Table 2 illustrates what happens when delay is encountered in handling samples. The higher the air temperature, the faster the warming rate. Time and temperature play havoc with a perishable product. Proper facilities and careful handling

from one end of the operation to the other are a must if accurate results are to be obtained. Carelessness in one area nullifies good work elsewhere.

Training and supervision

To make the sampling program completely effective, industry and regulatory agencies must join hands in a modest and practical training and supervision program. This should be done on a routine basis.

Where training is carried out, this instruction generally consists of an illustrated lecture demonstrating good sanitation practices, elementary bacteriology, proper sampling procedures, care of samples, and examples of results of poor sampling. Proper identification, filling, and sealing of containers and the importance of temperature control are given special emphasis. Uniform guidelines are provided. A question and answer period is followed by an examination. Permits or licenses are issued to those satisfactorily passing the examination. Uniformity in methods, facilities, and interpretation of requirements and application are the backbone of the program.

REFERENCES

1. American Public Health Association. 1967. Standard methods for the examination of dairy products. 12th ed. Amer. Public Health Ass., New York.
2. Arledge, W. L. 1971. Sampling of bulk tank milk. *J. Milk Food Technol.* 34:108.
3. Grade "A" Pasteurized Milk Ordinance. 1965. Recommendation of the United States Public Health Service, Appendix B, p. 99.
4. Langlois, B. E., H. E. Randolph, and N. M. Crume. 1966. Keeping quality of dairy products obtained at retail outlets I. Low fat milk, slim milk and chocolate-flavored milk. *J. Milk Food Technol.* 29:214-217.
5. Langlois, B. E., and A. W. Rudnick, 1968. Keeping quality of dairy products obtained at retail outlets. II. Whipping cream and half and half. *J. Milk Food Technol.* 31:274-277.
6. Roman, M. H. 1971. Testing milk for cleanliness of production. *J. Milk Food Technol.* 34:438-443.

SYMPOSIUM MICROBIAL FOOD-BORNE INFECTIONS AND INTOXICATIONS

The Health Protection Branch of the Department of National Health and Welfare will sponsor a symposium entitled "Microbial Food-borne Infections and Intoxications" at the Skyline Hotel, Ottawa, Canada, October 19 and 20, 1972. The registration fee of \$40.00 provides for attendance at all symposium

functions including the technical sessions, two lunches, a reception and a banquet.

A pre-registration form may be obtained from the Symposium Secretary, Health Protection Branch, Tunney's Pasture, Ottawa 3, Canada, K1A 0L2.

PREPARATION AND UTILIZATION OF FROZEN BLOCKS OF MINCED BLACK ROCKFISH MUSCLE

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ABSTRACT

Black rockfish, which deteriorates in quality rapidly during frozen storage and thus is not fully utilized, was selected to study the concept of using the minced muscle obtained from the meat-bone separator machine to prepare modified fish blocks. The minced muscle was blended with several ingredients, which were added to protect the fish blocks against rancidity and to improve texture and flavor. These blocks had a storage life of 9 to 12 months at 0 F. Raw breaded fish sticks prepared from various lots of modified blocks that were held in frozen storage at 0 F for 6 to 12 months had an additional storage life of almost 6 months at 0 F.

In the United States, the total consumption of processed fishery products has been increasing owing to growth in population and demand for convenience foods. In 1970, production of fish sticks and portions was 116 and 233 million lb., respectively (1). The corresponding production figures for 1960 are 65 and 51 million lb. The growing world demand for food fish places constantly increasing pressure on a limited number of species. This situation demands that we make more efficient use of the food fish now landed and that we develop methods that permit the use for food of underutilized and industrial species. Among the underutilized species, appearance, size, flavor, texture, or difficulties in processing has generally precluded their acceptance as food fish. The successful use of these resources requires that the muscle be economically separated from skin and bones and that it be processed into foods that are new and/or unique in appearance, flavor, texture, or nutritional qualities to gain consumer acceptance.

Investigations at this laboratory have shown that a Japanese meatbone separator (Yanagiya¹ Model SR-s2) can be successfully used to efficiently recover the muscle from a variety of species (2). The machine removes the muscle from headed, gutted, and washed fish by pressure as the fish passes between a continuous rubber belt and a perforated stainless steel drum. The rubber belt and the drum rotate in the same direction but at differential peripheral

velocities, thus creating a shearing action. The muscle is forced through the small perforations in the drum, and the skin and bones pass to the "waste" chute. By using this machine, significantly increased yields of muscle from a variety of fish and shellfish were obtained, which should lower the cost of production (3).

Continued investigations have revealed several potential uses for this machine-separated minced muscle. One use of major potential is the manufacture of minced fish blocks. The conventional fish block, which is the intermediate from which fish sticks and portions are prepared, is made from fillets frozen under pressure into a rectangular-shaped slab. Owing to the short supply and increased costs of the conventional fillet blocks in recent years, the potential for marketing a substitute, such as a fish block made of minced fish muscle, appears to be increasingly promising.

In our experimental studies, black rockfish (*Sebastes flavidus* and *S. brevispinis*) were used. They are an underutilized group of Northeast Pacific fish because the dark muscle, in spite of various experimental treatments, becomes rancid quickly during frozen storage. The successful stabilization of minced muscle of this species against oxidative rancidity would assure that many other species could also be used to produce minced fish blocks.

In our first experiment, we froze minced black rockfish muscle into blocks to determine its palatability and to work out processing procedures to be used in our main experiment. The minced muscle blocks were cut into sticks, batter-and-breaded, and deep-fat fried. These samples were somewhat flat in flavor and coarse-granular in texture. To improve the flavor and texture, we decided to use sugar and monosodium glutamate to enhance the flavor and salt and sodium tripolyphosphate to partially solubilize the muscle protein to serve as a binder for the particles of the coarse-minced muscle, thus permitting preparation of a cohesive minced muscle block. We refer to this type of block as a modified minced fish block.

¹Trade names referred to in this publication do not imply endorsement of commercial products.

TABLE 1. MODIFIED FISH BLOCKS, INGREDIENTS AND FUNCTIONS

| Ingredients | Percent ¹ | Function |
|-------------------------|----------------------|---|
| Minced flesh | | Nutritional base and textural properties |
| Water | 5 | Texture; solubilize water-soluble ingredients |
| Refined vegetable oil | 1 | Carrier for oil-soluble ingredients |
| Sodium chloride | 1 | Solubilize protein for textural properties; flavor |
| Sodium tripolyphosphate | 0.15 | To improve water-holding capacity |
| Sugar | 1 | Taste |
| Monosodium glutamate | 0.3 | Flavor intensifier |
| BHT ² | 0.006 | To minimize oxidation of lipids |
| BHA ² | 0.006 | To minimize oxidation of lipids |
| Polysorbate 80 | 0.075 | As emulsifier and to improve efficiency of antioxidants |
| Sorbitan monooleate | 0.075 | As emulsifier and to improve efficiency of antioxidants |

¹Percent ingredients is expressed as a percentage of the weight of minced flesh used.

²Butylated hydroxytoluene (BHT)^{*} and butylated hydroxyanisole (BHA)^{*}; in other experiments, we found that the levels of antioxidants could be further cut down as low as 0.001%.

^{*}Trade names referred to in this publication do not imply endorsement of commercial products.

The specific objectives of the study were to determine the storage characteristics at 0 F of (a) modified minced fish blocks from black rockfish and (b) raw breaded fish sticks made from the modified blocks. To simulate the storage procedure followed in the fish processing industry, the modified blocks were stored at 0 F. After various storage periods, the blocks were cut and made into raw breaded fish sticks. The fish sticks were packaged and stored at 0 F.

MATERIALS AND METHODS

Sample preparation

Black rockfish, which were held in ice aboard a commercial trawler for about 2 to 5 days and judged to be of good quality, were obtained from a local commercial processor. They were headed, gutted, washed, and passed through the meat-bone separator machine with the tension of the belt adjusted to remove as much muscle as possible. The minced muscle was then divided into 2 parts; one part was packed directly into cartons and frozen into blocks for control samples and the second part was processed into modified blocks. During a 1-year period, 5 different lots of samples were prepared.

Preparation of minced muscle blocks. Three lots of minced muscle blocks (control samples) were prepared as follows: (a) Minced muscle was packed into 13-1/2 lb., wax-coated frozen food cartons (1-1/2 inches × 11-1/2 inches × 20 inches). Cartons were then overwrapped with 2 mil polyethylene film and heat sealed. (b) The packed cartons were frozen in a plate freezer and stored at 0 F.

Preparation of modified fish blocks. Five lots of modified fish blocks were prepared by the procedure given below and 1 lot was similarly prepared except the antioxidants BHA and BHT were excluded: (a) Two percent of the minced flesh and the ingredients listed in Table 1, which were used to enhance flavor and texture and to increase the efficiency of the antioxidants, were mixed in a food blender (Waring¹ Model CB-4) for about 2 min to prepare the binder. (b) The binder was added to the rest of the minced flesh and mixed thoroughly in a Hobart¹ Model A-200 DT food mixer for 2 min at about 50 rpm. (c) The mixture was packed and frozen in the same way as the control blocks.

Preparation of raw breaded fish sticks. After storage for 6, 9, and 12 months at 0 F, the modified blocks were taken

to a commercial fish processing plant where they were cut into fish sticks with a band saw, breaded, and packed in 5-lb. institutional-size, polyethylene-coated food cartons with cellophane sheets inserted between each layer of fish sticks. The 5-lb. cartons were then packed in fiberboard master cartons and stored at 0 F to simulate commercial handling practices.

Sensory evaluation

Minced muscle blocks were evaluated initially and after 3, 4, and 6 months of storage at 0 F. The blocks were cut into 5/8-inch slices, steamed in covered aluminum containers (2 inches × 3-1/2 inches × 1-1/2 inches deep), and rated for the development of rancidity using the 10-point scale shown in Table 2. The limit of the storage life was when the mean score dropped below 6.

TABLE 2. SENSORY RATING SCALES UTILIZED IN EVALUATING THE CHANGES IN THE QUALITY OF THE MINCED FISH BLOCKS

| Quality attributes and scores | | | |
|-------------------------------|-------|--------------------------|-------|
| Rancidity | Score | Texture and flavor | Score |
| None | 10 | Like extremely | 9 |
| Trace | 8 | Like very much | 8 |
| Slight | 6 | Like moderately | 7 |
| Moderate | 4 | Like slightly | 6 |
| Excessive | 2 | Neither like nor dislike | 5 |
| | | Dislike slightly | 4 |
| | | Dislike moderately | 3 |
| | | Dislike very much | 2 |
| | | Dislike extremely | 1 |

TABLE 3. SENSORY EVALUATION OF STEAMED SAMPLES FROM 3 LOTS OF MINCED ROCKFISH (CONTROL) BLOCKS STORED AT 0 F

| Lot number | Storage time | Rancidity ¹ score (10-pt. scale) |
|------------|--------------|--|
| | (Months) | |
| 1 | 0 | 10.0 |
| | 3 | 4.0 |
| 2 | 0 | 10.0 |
| | 4 | 4.0 |
| 3 | 0 | 10.0 |
| | 4 | 10.0 |
| | 6 | 5.0 |

¹Limit of storage life was reached when score was below 6.0.

TABLE 4. SENSORY EVALUATION OF STEAMED SAMPLES OF MINCED ROCKFISH MUSCLE BLOCKS (NO ADDITIVES) AND MODIFIED MINCED ROCKFISH BLOCKS (CONTAINING ADDITIVES) STORED AT 0 F FOR 3 MONTHS

| Lot number | Additives used | Storage time | Rancidity score |
|------------|---|--------------|-----------------|
| | | (Months) | (10-pt. scale) |
| 1A | All ingredients ¹ including the antioxidants | 0 | 10.0 |
| | | 3 | 10.0 |
| | | 6 | 10.0 |
| | | 9 | 7.5 |
| 1B | All ingredients ¹ excluding antioxidants BHA and BHT | 0 | 10.0 |
| | | 3 | 4.0 |
| | | 6 | 4.0 |
| | | 9 | 4.0 |
| 1C | None | 0 | 10.0 |
| | | 3 | 4.0 |

¹Ingredients listed in Table 1.

The frozen breaded fish sticks were evaluated initially at the start of the storage test and after 4 and 6 months of storage at 0 F. The frozen fish sticks were deep-fat fried in vegetable oil heated to 350 F and presented to a panel of 5 to 10 experienced judges. Rancidity was rated with a 10-point scale; flavor and texture were judged with the 9-point hedonic scale (Table 2).

RESULTS AND DISCUSSION

Storage characteristics of minced muscle control blocks

The minced muscle frozen directly into blocks with no protective ingredients or additives became rancid in as short a time as 3 months (Table 3). This is about the time required for development of ran-

cidity in frozen, stored fillets of the same species (4).

Storage characteristics of modified fish blocks

The storage characteristics were determined³ on steamed samples of modified fish blocks, which contained no antioxidants and which contained 0.006% BHA and 0.006% BHT. The results of rancidity scores given in Table 4 show that the modified blocks containing the antioxidant mixtures (Lot 1A) showed no evidence of rancidity during the first 6 months of storage at 0 F and were not rancid after 9 months of storage except where the dark flesh particles were at the surface of the blocks. This slight rancidity of the dark flesh at the surface was masked and not detected by the sensory panel when the blocks were cut into sticks, breaded, and deep-fat fried (Table 5, Lot 1, 0 storage time). On the other hand, the modified blocks containing no antioxidants (Lot 1B) as well as the minced muscle blocks with no additives (Lot 1C) were rancid at 3 months.

The good storage characteristics of the 5 lots of modified fish blocks containing antioxidants are also shown by the sensory scores presented in Table 5 for the 5 lots of blocks when evaluated soon after being processed into breaded fish sticks.

In summary, these data indicate that the antioxidants at the levels used successfully inhibited development of rancidity in the modified blocks for at least 9 to 12 months at 0 F. This storage life compares favorably with the 6 to 12 months storage life of cod fillets (5) and with 5 to 8 months for fillets of various rockfish species at 0 F (6).

TABLE 5. SENSORY EVALUATION AFTER VARIOUS PERIODS OF STORAGE AT 0 F OF DEEP-FAT FRIED SAMPLES OF SEVERAL LOTS OF MODIFIED ROCKFISH BLOCKS AND OF BREADED FISH STICKS PREPARED FROM THE BLOCKS

| Lot number | Storage time of modified blocks | Storage time after processing into fish sticks | Rancidity ¹ | Texture ² | Flavor ² |
|------------|---------------------------------|--|------------------------|----------------------|---------------------|
| | (Months) | (Months) | (10-pt. scale) | (9-pt. scale) | |
| 1 | 12 | 0 | 9.3 | 7.3 | 7.6 |
| | | 2 | 7.8 | 7.3 | 7.0 |
| | | 6 | 5.7 | 6.5 | 4.8 |
| 2 | 9 | 0 | 10.0 | 8.0 | 6.7 |
| | | 2 | 8.2 | 7.1 | 6.7 |
| | | 6 | 7.8 | 6.6 | 5.6 |
| 3 | 9 | 0 | 9.1 | 7.2 | 7.5 |
| | | 2 | 8.6 | 7.6 | 7.1 |
| | | 6 | 8.7 | 6.6 | 6.4 |
| 4 | 9 | 0 | 10.0 | 7.0 | 7.7 |
| | | 2 | 8.8 | 6.2 | 6.4 |
| | | 6 | 8.0 | 5.3 | 5.5 |
| 5 | 6 | 0 | 9.5 | 7.6 | 7.2 |
| | | 2 | 8.2 | 7.2 | 7.1 |
| | | 6 | 7.8 | 5.9 | 5.8 |

¹Limit of storage life was reached when score for rancidity was below 6.

²Score of 5 represents neither like nor dislike.

Storage characteristics of raw breaded fish sticks

Raw breaded fish sticks that were prepared from the 5 lots of modified blocks and stored for an additional 2 months and 6 months showed a gradual increase in rancidity with storage time (Table 5). As long as the large blocks were kept intact, the development of rancidity was inhibited, but upon cutting and breading—thus exposing a large surface area—the rate of development of rancidity increased. After 6 months of frozen storage as breaded sticks, only Lot 1 (18 months total frozen storage) was judged rancid at the “slight-to-moderate” level; rancidity in all other lots was judged only “slight-to-trace.”

The texture scores for the fish sticks at the initial examination after cutting and breading were good. With storage time, the samples became slightly chewier, and the texture scores became lower. Presumably, these alterations in texture and flavor might be prevented by a small increase in the amount of sodium tripolyphosphate which would increase the water-holding capacity of the muscle protein, or by addition of antioxidants to the batter or breading material to help prevent lipid-protein interaction induced by exposure of large surface areas from cutting of blocks into sticks. Additional tests are planned to improve storage characteristics of breaded fish sticks.

CONCLUSIONS

Results of our work demonstrate promise that the underutilized black rockfish can be successfully

processed into a modified minced fish block. The modified block is prepared from machine-separated fish muscle containing additives approved for food use and has a storage life of over 9 months at 0 F. Incorporation of an antioxidant mixture of BHA/BHT in the modified block inhibited development of rancidity, which has been a major deterrent to utilization of black rockfish in processed fishery products.

Raw breaded fish sticks prepared from modified fish blocks stored at 0 F for 9 months and longer had an additional shelf life of 2 to 6 months. Shelf life was limited primarily by alteration of flavor and texture during storage.

REFERENCES

1. Anonymous. 1971. Current fishery statistics no. 5600. Fisheries of the United States, 1970. U. S. Department of Commerce, NOAA, NMFS, p. 25, March.
2. Miyauchi, D., and M. Steinberg. 1970. Machine separation of edible flesh from fish. *Fishery Ind. Res.* 6:165-171.
3. King, F. J., and J. H. Carver. 1970. How to use nearly all the ocean's food. *Commercial Fisheries Rev.* 32:12-21.
4. Stansby, M. E., and J. Dassow. 1949. Storage life of whole and split rockfish fillets. *Commercial Fisheries Rev.* 11:1-8.
5. Lane, J. P. 1964. Time-temperature tolerance of frozen seafoods. I. Review of some of the recent literature on the storage life of frozen fishery products. *Food Technol.* 18:156-162.
6. Miyauchi, D. T., and M. E. Stansby. 1952. Freezing and cold storage of Pacific Northwest fish and shellfish. Part 1—Storage life of various rockfish fillets. *Commercial Fisheries Rev.* 14:24-28.

LETTER TO THE EDITOR

Incubation temperature for Standard Plate Count

DEAR SIR:

It is good to learn (*JMFT* 35:136, 1972) that the incubation period for the SPC of raw milk can be extended to 72 hr without significantly affecting the results. (This confirms findings in collaborative studies at 3 Canadian centres in 1961-3.) This would increase the usefulness of the SPC in permitting plating on Fridays. However, it fails to improve the test as an indicator of insanitary methods, which is surely the main reason for its use. To accomplish this we must also lower the incubation temperature.

At the ADSA symposium in 1970 (*JMFT* 34:173, 1971) the writer presented data showing that psychrotroph counts might sometimes be nearly 14 times as high as SPCs. Others have reported similar findings. Thus there is little doubt that with 32 C incubation we are missing many psychrotrophs and some thermotolerant bacteria, organisms which reflect insanitary production methods.

How much lower must we go to get a more reliable reflection of the psychrotrophs present? In October 1970 Miss M. J. Orr of West of Scotland Agricultural College told me they had never found a sample where the psychrotroph count exceeded the total count at 30 C for 72 hr, the conditions recommended by the International Dairy Federation in 1958, and widely accepted outside North America. This is most

important! Collaborative studies to check on this point, as well as to compare the IDF procedure with that of the current Standard Methods, are urgently needed so that necessary changes may be made in the 14th edition of *Standard Methods*.

As in the past, opposition to a lower temperature of incubation may be expected. One objection is that many laboratories find it impossible to keep incubators at 32 C during hot weather. The validity of this objection diminishes as more laboratories are air conditioned or equipped with refrigerated incubators. But even if such facilities are lacking, surely it is better to be a few hours above the desired temperature than at too high a temperature for best results all the time.

While there is a possibility that even higher counts might be obtained by employing 28 C for 96 hr as recommended by the Iowa workers, there would be a distinct advantage if we in North America were using the same incubation conditions as are already in use in practically every other country. This applies particularly when dealing with exports of dairy products, for most importing countries employ the IDF procedure.

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INTERACTIONS OF FOOD STARTER CULTURES AND FOOD-BORNE PATHOGENS: THE ANTAGONISM BETWEEN *STREPTOCOCCUS LACTIS* AND SPOREFORMING MICROBES¹

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ABSTRACT

Lactic acid bacteria hinder development of sporeformers by production of acids, peroxides, and antibiotics. One of the best known of these antibiotics is nisin which is produced by *Streptococcus lactis* and is a permitted food additive in many countries. This paper traces the history of nisin, its effect in milk, cheese, processed cheese, canned, and other foods. Nisin primarily affects gram-positive bacteria but some of these produce nisinase, an enzyme which inactivates it. The preservative effect of nisin in heat treated foods is probably related to the retention of nisin on sporecoats. Nisin affects outgrowth of spores but not spore germination.

In natural and man-made environments the lactic acid bacteria (LAB) frequently compete successfully with sporeformers. Old cells of many of the LAB resist unfavorable physical conditions, not to the extent characteristic of spores, but sufficiently to ensure survival. For example, they resist acidity and desiccation, so that they may be readily disseminated in nature. Once they are in a suitable environment, they grow rapidly and may outgrow competitors. There are many reports that at the time milk sours the lactic flora predominates. They dominate by lowering the pH of the milk to a point inhibitory to sporeformers and by production of peroxides and antibiotics. It is worth pointing out that the number of antibiotics reported to be produced by sporeforming organisms far exceeds those formed by LAB (55). The lack of inhibition of LAB by sporeformers may result from antibiotics not being formed in milk or cheese or to the antibiotic remaining bound to the producer organism. However, Martin et al. (73) found that sporeformers could inhibit lactic starter cultures.

Peroxides may contribute significantly to the inhibition of sporeformers by LAB. Clostridia, which are anaerobic sporeformers, have no catalase or peroxidase and may be virtually defenceless against peroxides produced by LAB. The lack of development of clostridia in Swiss-type cheese was shown to result from production of inhibitory substance by LAB

in the cheese (50). Later it was shown that this inhibitor was probably peroxide (100). With aerobic organisms producing catalase, the sensitivity of the cells to peroxides seems to depend on the size of the inoculum, i.e. the amount of preformed enzyme which protects the cells. If one considers the situation at the beginning of a growth cycle with only few cells present and adequate amounts of preformed catalase absent, then the intrinsic sensitivity of the cells determines the effect of peroxides. Under such testing conditions a strain of *Lactobacillus lactis* (catalase-negative) was sensitive to hydrogen peroxide at 135 $\mu\text{g}/\text{ml}$ and a catalase positive strain of *Staphylococcus aureus* was inhibited by 5 $\mu\text{g}/\text{ml}$ (100).

In soured milk drinks, *Streptococcus lactis* appears to have an important effect on bovine tubercle organisms. The tubercle organism could not be recovered from soured milk. This effect may be caused by acidity (93) or by antibiotic production (75) or by a combination of these factors.

An interesting aspect of the antagonism between *S. lactis* and sporeformers is the antibiotics produced by the lactic acid bacteria. The best known antibiotic was called nisin. Streptococci belonging to serological group N are the principal producers of this substance and the name was derived from the letters Group N Inhibitory Substance (76).

HISTORY OF NISIN

Lactic streptococci producing an inhibitor against other microbes were first observed by L. A. Rogers in 1928 (88), and they were further studied by Whitehead and collaborators in New Zealand in 1933 (101, 102). Whitehead and collaborators were seeking the cause of slow acid development in cheesemaking when they discovered "inhibitory streptococci." Further practical dairying aspects were studied in Britain in 1943 by Meanwell (78) and Hoyle and Nichols (53). They were concerned with the effect of lactic streptococci on each other and wished to avoid strain dominance in multiple strain starters.

In the meantime, the physiology of nisin production, its assay, conditions for pilot plant production,

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its purification, and chemical composition were all reported (3, 9, 10, 12, 13, 41, 42, 43, 49, 74, 76, 80, 94). In the U.S.A. in 1951 Baribo & Foster (7, 8) essentially confirmed the results of the European workers though they felt uncertain that they were dealing with nisin. This was partially because it was only slowly realized that nisin was not a single substance but a family of antibiotics. It became known that one strain produced five different substances, that the same strain could produce mixtures which differed according to growth conditions (44), and that different strains of *S. lactis* produced different antibiotics (46). *Streptococcus cremoris* was also known to produce an antibiotic termed diplococcin, which differed from nisin in many respects (84).

The question was also asked but not resolved, whether streptococci other than the lactics are able to produce antibiotics. Murray and Loeb (82) reported that three strains of hemolytic streptococci grown on blood agar produced antibiotics and using cellophane sacs obtained cell-free antibiotic preparations. Hirsch and Wheater, in a large survey involving about 10,000 isolates of streptococci, only obtained antibiotic effects with lactic streptococci (51).

This is a brief and incomplete account of the early work on nisin. Since 1966 interest in nisin has been revived along modern lines of molecular biology. The work of Gross and co-workers is leading to a much better understanding of the composition and structure of nisin (2, 31, 32). The biosynthetic mechanisms (54, 57, 58) and the significance of the antibiotic to the producer organism are also being studied (56).

STREPTOCOCCUS LACTIS VERSUS SPOREFORMERS IN MILK

There are several reports on the effect of *S. lactis* on aerobic sporeformers, especially *Bacillus cereus*. The latter is of some importance as a possible food-poisoning organism. The effect mostly investigated was the development of a fault of milk called "bitty cream" or cream rising. This results from the *B. cereus* lecithinase acting on milk fat-globule membranes (92); such milk, when only mildly heated, goes "bitty," i.e. the fat-globules coalesce thus spoiling the appearance of coffee or tea. Garvie and Stone (23) investigated this defect but found little or no effect of nisin or non-nisin producing cultures when the milk was kept at 15 C or higher. At 5 C or 10 C the sporeformer did not develop. A nisin producing strain did not retard development of the sporeformer but at the lower temperature of storage, after an initial increase, numbers of *B. cereus*, declined (23). These findings appear to apply also to *B. subtilis* and were confirmed and extended to

V.H.T. treated milk (40, 79, 81, 90).

More recently "cream rising" has also been associated with a specific milk globulin fraction which helps to attach bacteria (*S. cremoris* and *B. cereus*) to the fat globule membrane (91).

STREPTOCOCCUS LACTIS VERSUS SPOREFORMERS IN CHEESE AND PROCESSED CHEESE

In 1951 Hirsch and collaborators used nisin producing starters in Swiss type cheese to control "blowing" faults caused by *Clostridium butyricum* or *Clostridium tyrobutyricum* (48, 77). Later nisin became available in a skim-milk carrier and both the addition of nisin and the use of the cultures were extensively investigated (33); in many western countries nisin became an accepted and legal food additive (34, 66). Much of the early work was reviewed by Berridge in 1953 (11) and the effectiveness of nisin appears to have been established. Nevertheless, some of the results remain contradictory to this day. For example Pulay and collaborators (18, 85), working in Hungary, found that nisin had side effects in cheese manufacture; it retarded other lactic acid bacteria so that the cheese became more liable to faults caused by development of resistant gram-negative flora. On the other hand Kooy and Pette in the Netherlands (69, 70) used nisin producing starters successfully in the manufacture of Edam cheese. Kooy (68) was the first to show that the decrease of nisin content observed in some cheeses was linked with the growth of *Lactobacillus plantarum* and suggested that this organism made a nisinase (see below). More recently, Goudkov and Sharpe (26) studied the inter-relationship of milk lactic acid flora with *C. tyrobutyricum*, *Clostridium beijerinckii*, *Clostridium sporogenes*, and *Clostridium perfringens* in aseptically and non-aseptically made cheese. The latter clostridia occurred only as spores and did not develop in cheese, whereas the first was able to develop and caused rancidity if the pH was above 5.3. The starter flora, which was not examined for nisin formation, was inhibitory to the growth of *C. tyrobutyricum*.

STREPTOCOCCUS LACTIS VERSUS SPOREFORMERS IN CANNED AND OTHER FOODS

Streptococcus lactis cells, do not survive the heat treatment involved in preparation of canned foods. However, nisin used as an adjunct to canning, inhibits development of spores and has been extensively tested as a food additive (83).

Much of this work has been excellently reviewed, earlier by Hawley (33-36) and more recently by Jarvis and Morisetti (66). The use of antibiotics in thermally processed foods is a large subject which can-

not be dealt with adequately in this review. The acceptance of nisin as an additive in canned foods is based on several criteria, principal among these is that it is non-toxic (21, 89), it is degraded by intestinal bacteria and enzymes (4, 17, 39, 65, 96), it is of little therapeutic value because of its insolubility at physiological pH (30), and it does not form cross resistant mutants (43, 45, 86).

Among the clostridia, *Clostridium botulinum* is one of the more nisin resistant species (36, 59) so that in canned foods a minimum "botulinum cook" is always necessary. If so used, or used in products which are subject to spoilage but present no health hazard, nisin has been reported to be effective. Thus it was reported effective in beans in tomato sauce and peas (25, 67), tomatoes (98), chocolate flavoured milk (37, 98), vegetable purees (99), soups (5, 6, 36) and other foods (20, 35). For a more comprehensive list of foods see ref. 34. The writers' experience has been that nisin was effective in cheese and processed cheese and in soups, inhibiting flat sour thermophilic spoilage. Nisin was useful in soups when this was made from high quality starting materials but it was ineffective when heavily contaminated lentils were used as the starting materials (24). Similarly, we found that nisin was unable to prevent spoilage of prepacked bacon from which we isolated a nisin-resistant flora.

RESISTANCE TO NISIN

Nisin resistant strains of *Escherichia coli* and *Streptococcus faecalis* did not inactivate nisin (43), a finding confirmed by Ramseier (86). However, Kooy (68) reported an extracellular nisinase in *Lactobacillus plantarum* and nisinase has been reported in other lactic streptococci (22, 72), in *Staphylococcus aureus* (16), and in *Streptococcus thermophilus* (1). The latter was the first serious study in which the "enzyme" was shown to be inducible and specific in its substrate requirements to nisin. The enzyme was extensively studied in *B. cereus* by Jarvis (60-64). He showed that the enzyme was active against both subtilin and nisin [polypeptide antibiotics known to contain a similar amino-acid residue (32)]. Nisinase is specific to these antibiotics and acts by reducing an unsaturated bond; this inactivates the antibiotics without causing detectable change in their molecular weights (64).

Gould (27) and Gould and Hurst (29) suggested that the resistance of bacilli to nisin could be correlated with the method of spore-coat opening. Small celled species (e.g. *Bacillus subtilis*) appear to open their coats by mechanical pressure (type M) and on agar culture slides, the spore-coats remain visible

long after vegetative outgrowth has taken place. M-Type spores are sensitive to 2-10 units of nisin/ml. Large spored species (e.g. *B. cereus*) open their coats by lysis and in agar slide cultures the spore-coats tend to disappear after vegetative growth commences. These lytic spores (L type) are much more nisin-resistant and generally they are insensitive to even 100 units/ml.

Nisinase has recently been suggested as a means of identifying nisin in foods (66) and, although preliminary results are encouraging, the enzyme is not yet available in a purified and isolated form.

IN VITRO EFFECT OF NISIN ON SPOREFORMERS

Ramseier (87) carried out extensive investigations on the *in vitro* effect of nisin on *C. butyricum*. He confirmed earlier findings that nisin was bactericidal (45, 47). Nisin was absorbed from solution by spores, especially germinated spores (28), and acted on them by releasing 260 m μ absorbing substances. In this respect, as well as many others, nisin acted similarly to cationic detergents. However, nisin was also effective on vegetative cells, young cells being equally sensitive to old cells. Old cells did not release "pool substances" so that this facet of the mode of action remains obscure. Early reports suggested that nisin decreased the heat resistance of bacterial spores (71, 83). This effect was more apparent than real (14, 15, 19, 83) and resulted from carryover of antibiotic into the culturing medium. Spores damaged by heat were claimed to have increased nisin sensitivity (38) but this was not confirmed (98). The carryover resulted from adsorption of nisin to the spore-coat and spores incubated with trypsin could be germinated and grown normally (95). Tramer (97) reported that *Bacillus stearothermophilus* spores did not bind nisin firmly and that these spores did not germinate in the presence of nisin but Hitchins et al. (52) and Gould (28) observed phase darkening and loss of heat resistance with 56 strains of bacilli studied. They concluded that nisin prevented outgrowth rather than germination.

REFERENCES

1. Alifax R., and R. Chevalier. 1962. Study of the nisinase produced by *Streptococcus thermophilus*. J. Dairy Res. 29:233-240.
2. Bailey, F. J., and A. Hurst. 1971. Preparation of a highly active form of nisin. Can. J. Microbiol. 17:61-67.
3. Baranova, L. P., and N. S. Egorov. 1971. Effect of aminoacids on the growth of *Streptococcus lactis* and the biosynthesis of nisin (Transl.). Antibiotiki 15:686-688.
4. Barber, R. S., R. Braude, and A. Hirsch. 1952. Growth of pigs given skim milk soured with nisin producing strep-

- tococci. *Nature* 169:200-201.
5. Bardsley, A. 1962. Antibiotics in food canning. *Food Technol., Aust.* 14:532-537.
6. Bardsley, A. 1962. Antibiotics in food canning. *Food Technol. Aust.* 14:606-611.
7. Baribo, L. E., and E. M. Foster. 1951. Some characteristics of the growth inhibitor produced by a lactic streptococcus. *J. Dairy Sci.* 34:1128-1135.
8. Baribo, L. E., and E. M. Foster. 1951. The production of growth inhibitor by lactic streptococci. *J. Dairy Sci.* 34:1136-1144.
9. Bavin, E. M., A. S. Beach, R. Falconer, and R. Friedmann. 1952. Nisin in experimental tuberculosis. *Lancet* 1:127-129.
10. Berridge, N. J. 1949. Preparation of the antibiotic nisin. *Biochem. J.* 45:486-493.
11. Berridge, N. J. 1953. The antibiotic nisin and its use in the making and processing of cheese. *Chem. Ind.* 1953:1158-1161.
12. Berridge, N. J., and J. Barrett. 1952. A rapid method for the turbidimetric assay of antibiotics. *J. Gen. Microbiol.* 6:14-20.
13. Berridge, N. J., G. G. F. Newton and E. P. Abraham. 1952. Purification and nature of the antibiotic nisin. *Biochem. J.* 52:529-535.
14. Campbell, L. L., and E. E. Sniff. 1959. Effect of subtilin and nisin on the spores of *Bacillus coagulans*. *J. Bacteriol.* 77:766-770.
15. Campbell, L. L., E. E. Sniff, and R. T. O'Brien. 1959. Subtilin and nisin as additives that lower the heat-process requirements of canned foods. *Food Technol.* 13:462-464.
16. Carlson, S., and H. M. Bauer. 1957. A study of problems associated with resistance to nisin, an antibiotic produced by *Streptococcus lactis*. *Arch. Hyg. Bakteriol.* 141:445-459.
17. Claypool, L., B. Heinemann, L. Voris, and C. R. Stumbo. 1966. Residence time of nisin in the oral cavity following consumption of chocolate milk containing nisin. *J. Dairy Sci.* 49:314-316.
18. Csiszar, J., and G. Pulay. 1956. Research on *Streptococcus lactis* producing antibiotics against clostridia. I. The activity spectrum of nisin producing strains and raising the production of antibiotics. *Int. Dairy. Cong. Proc.* 2 Pt. II:423-431.
19. Denny, C. B., L. E. Sharpe, and C. W. Bohrer. 1961. Effects of tylosin and nisin on canned-food spoilage bacteria. *Appl. Microbiol.* 9:108-110.
20. Eastoe, J. E., and J. E. Long. 1959. The effect of nisin on the growth of cells and spores of *Clostridium welchii* in gelatin. *J. Appl. Bacteriol.* 22:1-9.
21. Frazer, A. C., M. Sharratt, and J. R. Hickman. 1962. The biological effects of food additives. I. Nisin. *J. Sci. Food Agr.* 13:32-42.
22. Galeslot, Th. E. 1956. Lactic acid bacteria which destroy the antibioticum (nisin) of *S. lactis*. *Ned. Melk. Zdschr.* 10:143-155.
23. Garvie, E. I., and M. J. Stone. 1953. The associative growth of *Bacillus cereus* and *Streptococcus lactis* in milk. *J. Dairy Res.* 20:29-35.
24. Gibbs, B. M., and A. Hurst. 1964. Limitations of nisin as a preservative in non-dairy foods. p. 151-165. *In* N. Molin (ed) *Microbial inhibitors in foods*. Almqvist and Wiksell, Stockholm.
25. Gillespy, T. G. 1957. Nisin trials. *Fruit and veg. Cann. Quick Freez. Res. Assoc. Leaflet No. 3*, Chipping Campden, England.
26. Goudkov, A. V., and M. E. Sharpe. 1966. A preliminary investigation of the importance of clostridia in the production of rancid flavour in cheddar cheese. *J. Dairy Res.* 33:139-149.
27. Gould, G. W. 1962. Microscopical observations on the emergence of cells of *Bacillus* from spores under different cultural conditions. *J. Appl. Bacteriol.* 25:35-41.
28. Gould, G. W. 1964. Effect of food preservatives on the growth of bacteria from spores. p. 17-24. *In* N. Molin (ed) *Microbial inhibitors in foods*. Almqvist and Wiksell, Stockholm.
29. Gould, G. W., and A. Hurst. 1962. Inhibition of *Bacillus* spore development by nisin and subtilin. 8th Int. Cong. Microbiol. Abstr. A2-11.
30. Gowans, J. L., N. Smith, and H. W. Florey. 1952. Some properties of nisin. *Brit. J. Pharmacol. Chemother.* 7:438-449.
31. Gross, E., and J. L. Morell. 1970. The assignment of sulfide bridges of β -methyllanthionine to a novel bicyclic structure of identical ring size. *J. Amer. Chem. Soc.* 92:2919-2920.
32. Gross, E., J. L. Morell, and L. C. Craig. 1969. Dehydroalanilylsine: Identical COOH-terminal structures in the peptide antibiotics nisin and subtilin. *Proc. Natl. Acad. Sci.* 62:952-956.
33. Hawley, H. B. 1955. The development and use of nisin. *J. Appl. Bacteriol.* 18:388-395.
34. Hawley, H. B. 1957. Nisin in food technology. *Food Mfr.* 32:370-376, 430-434.
35. Hawley, H. B. 1961. "Nisin" thermal adjunct for processing meats to be packaged in plastic film. *Packaging* 32 (no. 381):93-96.
36. Hawley, H. B. 1962. The uses of antibiotics in canning. p. 272-288. *In* M. Woodbine (ed) *Antibiotics in agriculture*. Butterworths, London.
37. Heinemann, B., C. R. Stumbo, and A. Scurlock. 1964. Use of nisin in preparing beverage-quality sterile chocolate-flavoured milk. *J. Dairy Sci.* 47:8-12.
38. Heinemann, B., L. Voris, and C. R. Stumbo. 1965. Use of nisin in processing food products. *Food Technol.* 19:592-596.
39. Heinemann, B., and R. Williams. 1966. Inactivation of nisin by pancreatin. *J. Dairy Sci.* 49:312-313.
40. Hermeir, J., A. Tobalay, and G. Mocquot. 1962. The effect of inoculation with milk streptococci on the growth of *Bacillus cereus* in U.H.T. milk. *Int. Dairy Cong. A:*785-792.
41. Hirsch, A. 1946. Some general aspects of the inhibitory streptococci. *Proc. Soc. Appl. Bacteriol.* 1-4.
42. Hirsch, A. 1950. The assay of the antibiotic nisin. *J. Gen. Microbiol.* 4:70-83.
43. Hirsch, A. 1951. Growth and nisin production of a strain of *Streptococcus lactis*. *J. Gen. Microbiol.* 5:208-221.
44. Hirsch, A. 1951. Various antibiotics from one strain of *Streptococcus lactis*. *Nature* 167:1031-1032.
45. Hirsch, A. 1954. Some polypeptide antibiotics. *J. Appl. Bacteriol.* 17:108-115.
46. Hirsch, A., and E. Grinsted. 1951. The differentiation of lactic streptococci and their antibiotics. *J. Dairy Res.* 18:198-204.
47. Hirsch, A., and E. Grinsted. 1954. Methods for the enumeration of anaerobic sporeformers from cheese, with observations on the effect of nisin. *J. Dairy Res.* 21:101-110.
48. Hirsch, A., E. Grinsted, H. R. Chapman, and A.T.R. Mattick. 1951. A note on the inhibition of an anaerobic sporeformer in swiss-type cheese by a nisin producing streptococcus. *J. Dairy Res.* 18:205-206.
49. Hirsch, A., and A. T. R. Mattick. 1949. Some recent applications of nisin. *Lancet* 1:190-197.

50. Hirsch, A., M. McClintock, and G. Mocquot. 1952. Observations on the influence of inhibitory substances produced by the lactobacilli of Gruyère cheese on the development of anaerobic spore-formers. *J. Dairy Res.* 19:179-186.
51. Hirsch, A., and D. M. Wheeler. 1951. The production of antibiotics by streptococci. *J. Dairy Res.* 18:193-197.
52. Hitchins, A. D., G. W. Gould, and A. Hurst. 1963. The swelling of bacterial spores during germination and outgrowth. *J. Gen. Microbiol.* 30:445-453.
53. Hoyle, M. and A. A. Nichols. 1948. Inhibitory strains of lactic streptococci and their significance in the selection of cultures for starter. *J. Dairy Res.* 15:398-408.
54. Hurst, A. 1966. Biosynthesis of the antibiotic nisin by whole *Streptococcus lactis* organisms. *J. Gen. Microbiol.* 44:209-220.
55. Hurst, A. 1969. Polypeptide antibiotics. p. 167-182. In G. W. Gould and A. Hurst (ed) *The bacterial spore*. Academic Press, New York.
56. Hurst, A., and H. Kruse. 1970. The correlation between change in absorbcency, calcium uptake and cell bound nisin activity in *Streptococcus lactis*. *Can. J. Microbiol.* 16:1205-1211.
57. Ingram, L. C. 1969. Synthesis of the antibiotic nisin: Formation of lanthionine and β -methyl-lanthionine. *Biochim. Biophys. Acta* 184:216-219.
58. Ingram, L. C. 1970. A ribosomal mechanism for synthesis of peptides related to nisin. *Biochim. Biophys. Acta* 224:263-265.
59. Ingram, M., E. M. Barnes, and J. M. Shewan. 1956. Problems in the use of antibiotics for preserving meat and fish. *Food Sci. Abstr.* 28:121-136.
60. Jarvis, B. 1966. Observations of the inactivation of nisin by enzymes from *Bacillus cereus* and *Bacillus polymyxa*. *Chem. Ind.* 1967:882.
61. Jarvis, B. 1967. Inactivation of nisin by enzymes from *Bacillus cereus* and *B. polymyxa*. 4th meeting Federation Europ. Biochem. Soc. Abstracts #502, p. 126.
62. Jarvis, B. 1967. Resistance to nisin and production of nisin-inactivating enzymes by several *Bacillus* species. *J. Gen. Microbiol.* 47:33-48.
63. Jarvis, B. 1970. Enzymic reduction of the C-terminal dehydroalanillysine sequence in nisin. *Proc. Biochem. Soc.* 119:56P.
64. Jarvis, B., and J. Farr. 1971. Partial purification, specificity and mechanism of action of the nisin-inactivating enzyme from *Bacillus cereus*. *Biochem. Biophys. Acta* 227:232-240.
65. Jarvis, B., and R. R. Mahoney. 1969. Inactivation of nisin by α -chymotrypsin. *J. Dairy Sci.* 52:1448-1450.
66. Jarvis, B., and M. D. Morisetti. 1969. The use of antibiotics in food preservation. *Int. Biodeterior. Bull.* 5:39-61.
67. Kiss, I., N. Kiss-Kutz, J. Farkas, and K. Vas. 1968. Further data on the application of nisin in pea preservation. *Elelmiszertudomány* 2:51-58.
68. Kooy, J. S. 1952. Strains of *Lactobacillus plantarum* which destroy the antibiotic made by *Streptococcus lactis*. *Neth. Milk Dairy J.* 6:223-230.
69. Kooy, J. S., and J. W. Pette. 1952. The inhibition of butyric acid fermentation in cheese by using antibiotic producing streptococci as starter. *Neth. Milk Dairy J.* 6:317-322.
70. Kooy, J. S., and J. W. Pette. 1952. Inhibition of the growth of lactate fermenting butyric acid bacteria by antibiotics from lactic acid streptococci. *Neth. Milk Dairy J.* 6:302-316.
71. Lewis, J. C., H. D. Michener, C. R., Stumbo, and D. S. Titus. 1954. Antibiotics in food processing: Additives accelerating death of spores by moist heat. *J. Agr. Food Chem.* 2:298-302.
72. Lipinska, E., and M. Strazalkowska. 1959. Experiments on training pure cheese cultures to resist antibiotics produced by *Streptococcus lactis*. *Int. Dairy Congr. Proc.* 2:572-579.
73. Martin, J. H., and D. B. Kenkare, and W. J. Harper. 1962. Inhibition of lactic starter cultures by selected spore-forming organisms. *J. Dairy Sci.* 45:654.
74. Mattick, A. T. R., and A. Hirsch. 1944. A powerful inhibitory substance produced by group N streptococci. *Nature* 154:551-552.
75. Mattick, A. T. R., and A. Hirsch. 1946. Sour milk and the tubercle bacillus. *Lancet* 1:417-418.
76. Mattick, A. T. R., and A. Hirsch. 1947. Further observation on an inhibitory substance (nisin) from lactic streptococci. *Lancet* 2:5-12.
77. McClintock, M., L. Serres, J. J. Marzolf, A. Hirsch, and A. T. R. Mattick, 1952. Action inhibitrice des streptocoques producteurs de nisine sur le developpement des sporules anaerobies dans le fromage de Gruyere fondu. *J. Dairy Res.* 19:187-193.
78. Meanwell, L. J. 1943. The influence of raw milk quality on "slowness" in cheesemaking. *Proc. Soc. Agr. Bacteriol.* 19:(abstr.).
79. Mikolajcik, E. M., and J. W. Kearney. 1969. Behaviour of *Bacillus cereus* in cultured and acidified skim milk. *J. Dairy Sci.* 52:897.
80. Mocquot, G., and E. Lefebvre. 1956. A simple procedure to detect nisin in cheese. *J. Appl. Bacteriol.* 19:322-323.
81. Moycho, W., and W. Gromska. 1956. Antagonistic action of *Streptococcus lactis* against *Bacillus subtilis* and *Pseudomonas fluorescens*, observed in milk. *Acta Microbiol. Pol.* 5:267-270.
82. Murray, R. G. E., and L. J. Loeb. 1950. Antibiotics produced by micrococci and streptococci that show selective inhibition within the genus streptococcus. *Can. J. Res.* 28E:177-185.
83. O'Brien, R. T., D. S. Titus, K. A. Devlin, C. R. Stumbo, and J. C. Lewis. 1956. Antibiotics in food preservation. II. Studies on the influence of subtilin and nisin on the thermal resistance of food spoilage bacteria. *Food Technol.* 10:352-355.
84. Oxford, A. E. 1944. Diplococci, an anti-bacterial protein elaborated by certain milk streptococci. *Biochem. J.* 38:178-182.
85. Pulay, G. 1956. II. Unfavourable effects of nisin type antibiotics on the quality of cheese. *Int. Dairy Cong. Proc.* 2 Pt. II:432-443.
86. Ramseier, H. R. 1959. The action of coliform bacteria on the stability of nisin with special regard to the influence of the test medium. *Int. Dairy Congr. Proc.* 2:566-571.
87. Ramseier, H. R. 1960. The action of nisin on *Clostridium butyricum*. *Arch. Mikrobiol.* 37:57-94.
88. Rogers, L. A. 1928. The inhibiting effect of *Streptococcus lactis* on *Lactobacillus bulgaricus*. *J. Bacteriol.* 16:321-325.
89. Shtenberg, A. J., and A. D. Ignat'ev. 1970. Toxicological evaluation of some combinations of food preservatives. *Food Cosmet. Toxicol.* 8, 369-380.
90. Simonart, P., R. Poffe, and M. Weckx. 1962. *Bacillus cereus* and its detection in milk. *Int. Dairy Cong. Proc. C:* 403-409.
91. Stadholders, J., and G. Hup. 1970. Complexity and specificity of euglobulin in relation to inhibition of bacteria and to cream rising. *Neth. Milk Dairy, J.* 24:79-95.
92. Stone, J. M. 1952. The action of lecithinase of *Bacil-*

lus cereus on the globule membrane of milk fat. *J. Dairy Res.* 19:311-315.

93. Tacquet, A., F. Tison, and B. Devulder. 1961. Action bactericide du yaourt sur les mycobacteries. *Ann. Inst. Pasteur. Paris* 100:581-587.

94. Taylor, J. I., A. Hirsch, and A. T. R. Mattick. 1948. The treatment of bovine streptococcal and staphylococcal mastitis with nisin. *Veterinary Record* 61:197-198.

95. Thorpe, R. H. 1960. The action of nisin on spoilage bacteria. I. The effect of nisin on the heat resistance of *Bacillus stearothermophilus* spores. *J. Appl. Bacteriol.* 23: 136-143.

96. Tiemann, U., and H. D. Belitz. 1969. About nisin: I. Effect of proteases on nisin activity. *Z. Lebensmittel-Unters. Forsch.* 141(5):260-262.

97. Tramer J. 1964. The inhibitory action of nisin on

B. stearothermophilus. p. 25-33. In N. Molin (ed) *Microbial inhibitors in foods*. Almquist and Wiksell, Stockholm.

98. Tramer J. 1966. Nisin in food preservation. *Chem. Ind.* 1966:446-450.

99. Vas, K. 1964. Nisin in the food industry. *Deut. Lebensm. Rundsch.* 60:63-67.

100. Wheater, D. M., A. Hirsch, and A. T. R. Mattick. 1952. Possible identity of "lactobacillin" with hydrogen-peroxide produced by lactobacilli. *Nature* 170:623-626.

101. Whitehead, H. R. 1933. A substance inhibiting bacterial growth produced by certain strains of lactic streptococci. *Biochem. J.* 27:1793-1800.

102. Whitehead, H. R., and W. Riddet. 1938. Slow development of acidity in cheese manufacture. *New Zeal. J. Agr.* 46:225-229.

THE LEADERSHIP ROLE IN FUTURE ENVIRONMENTAL MANAGEMENT¹

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ABSTRACT

Current public health organization will undergo a dramatic change during the decade of the 70's. Two reasons for this anticipated change are: (a) growth of ecological awareness by the general public, and (b) greater public acceptance of the principle that good medical care is a right, rather than a privilege. These forces have placed the agency-employed environmentalist in a position of unaccustomed visibility and with the popular support he has long sought. Leadership in environmental management can be his but only if he can move quickly and meet certain criteria, among these the realization that actions designed to correct or prevent environmental degradation must be problem-oriented, rather than program-oriented.

The organizational structure of the traditional public health agency will, I believe, undergo profound changes during the decade of the 70's. So profound, in fact, that it may well cease to exist as we know it day. This will be caused by two movements on the current social scene which will play a commanding role in determining the shape and structure of public health in the future. These are:

CONDITION OF ENVIRONMENT

First, the snowballing popular recognition of the

condition of the environment in which we all must live. Unlike other contemporary trends this one has the active support of a wide array of social and economic groups. Rallying around the banner of ecology is the largest assortment of ill-matched allies since the Crusades. The young and the old, the left and the right, the religious and the non-religious, the "Silent Majority" and the activist, are all increasingly concerned with the quality of the environment in which they live. Although signs of a fad exist in this movement there is no doubt that a strong element of very deep concern exists and can be expected to persist as long as the quality of life continues to decline.

MEDICAL CARE

Second, the growing general acceptance of the principle that good medical care is a right, rather than a privilege. Translating this premise into programs, the Federal government has developed two financing mechanisms, Medicare and Medicaid, which have enabled many people, formerly dependent upon public health agencies for medical services, to purchase such services in the private market. In addition to the inflationary effect these programs have had upon medical costs, they have also in effect reduced the need for the "clinic" approach to

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QUALITY CONTROL IN THE CONFECTIONERY INDUSTRY¹

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ABSTRACT

The following topics were highlighted in this paper: (a) Candy manufacture is basically the physical chemistry of super-saturated solutions. (b) Quality control is necessary to produce a clean, uniform, and legal product. (c) Quality control is necessary to control costs. (d) The high solids content in finished confections requires special packaging. (e) Effects of small quantities of foreign material such as ash and protein may affect quality at the temperature used to manufacture most candy. (f) Methods of producing grain within the confection or by adding seed to produce grain. (g) Candy manufacturers should develop purchasing specifications in the absence of government or industry standards. (h) Specifications should meet Federal Food and Drug Administration requirements. (i) It is important to know the microbiology of certain ingredients, particularly products of animal origin. These sensitive ingredients should be quarantined on receipt and not used until found safe. (j) The candy manufacturer is legally responsible for the products offered for sale.

The title of this paper is rather broad, so broad in fact that much of the material is applicable to production of many foods, other than confectionery. It is customary and desirable to define the parameters of a discussion, and logically in this discussion, I should define "Quality Control."

These two words generate pleasing thoughts in the minds of everyone, but what does this phrase actually mean? If you ask any food manufacturer, whether it be a one-man operation or a firm employing several thousand individuals, he will say he has a quality control program. A quality control program may consist of, in the simplest form, the cook tasting a soup to see if there is enough seasoning, or it may consist of the more detailed procedures using sophisticated laboratory equipment and taste panels.

I know of one candy manufacturer who disliked pineapple flavor and refused for years to include this flavor as one of the many flavored candies manufactured by his firm. He did not care whether the item would sell or not—he didn't like it and that was it. You might call this a biased organoleptic control program. This is one of the advantages of a taste panel.

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WHY QUALITY CONTROL?²

- (a) To produce a uniform product so as to insure continued consumer acceptance.
 - (b) To control cost so as to obtain a return on the initial investment.
 - (c) To comply with government regulations.
- All of the above are of equal importance.

Most candy manufacturers started out as small manufacturers, usually in the kitchen of their homes. There are built-in safety factors because of the temperature required and the relatively low moisture content of the final product. I have had visits from a number of individuals throughout the years, wanting information as to how to expand their kitchen operations.

A product made in the kitchen that has gained local acceptance may have potential in an expanded market. However, limited production for quick sale and with a minimum of problems of shelf life and distribution is one thing. But expanded production entailing greater overhead as to personnel, capital investment in building and equipment, labor, and many other factors, plus the ability to compete in the open market is something else.

The more I see of the food field, the more I realize that when considering the manufacture of a food item, the first thought should be given to employing a lawyer knowledgeable in food and drug law, and a food technologist. Another requirement is that the candy manufacturer should be well aware of the sanitation requirements of the particular item he plans to manufacture.

COST CONTROL

There is no point in manufacturing a candy item and selling it at a loss. Everyone is well aware that basic costs are increasing. You meet them by increased production through automation, sales, and marketing techniques. I have mentioned the kitchen operation where the candy manufacture, packaging, and distribution was performed by the family.

There are a number of interesting stories of how several candy manufacturers established a complete chemical and microbiological laboratory. One manufacturer was producing a chocolate-coated bar. His

production sheets showed a constant rate of production for his market. All at once he noticed they were using more chocolate for the same number of bars produced per unit time. Chocolate is not an inexpensive ingredient. He called me and I inquired if he had checked the viscosity of each chocolate shipment on receipt. He said "No," but he had obtained the same coating from the same supplier for years. On test, the coating viscosity was too high, and he was coating the bar centers with too much chocolate.

I suggested that he buy a viscosimeter and check all incoming shipments. In addition, I suggested that he set up a laboratory and check production runs of bar center versus coating. This is a simple technique of weighing the finished bar and then removing the coating by a fat solvent. The difference in weight is the coating. This simple procedure demonstrated to this manufacturer the importance of laboratory control.

Once this firm saw what a laboratory could do, they set up a complete statistical quality control program including a well equipped laboratory.

Another firm encountered production difficulties. Indications were that the sweetened condensed skim milk was out of control. Analysis of the milk revealed a high percentage of water. I was informed that based on calculations, the processor had been spending over \$300 a month for water. More will be said about ingredients later in this paper.

GOVERNMENT REGULATIONS

Why government regulations? Regulations are not on the books because some regulatory official had nothing else to do. Basically, the purpose of government regulations is to protect the consumer.

The following statement is an excerpt from the annual report of a regulatory agency.

"It is encouraging to record organized industry efforts to effect basic corrections of conditions and practices that have formerly caused tremendous losses of food through failure to protect it from contamination by rodents and insects. Under the leadership of their trade associations, several large industries have set up organizations to deal with sanitation on an industry-wide basis. The dairy industry in years past had blazed a path by setting up improvement groups with a view toward better plant sanitation and protection of raw materials. During the year, other industries, such as the milling, baking, canning, and confectionery groups have employed specialists in sanitation to assist the member firms, both in establishing general concepts of sanitation and in solving particular problems that confront them. Other industries are contemplating similar steps toward an informed industry compliance with requirements essential to the proper handling of food. In addition to industry-wide groups, many companies have employed technically trained people to deal with

sanitary controls within their own organizations."

"Regulatory attention to filth and decomposition cannot be relaxed, however. The availability of more labor, which it was hoped would relieve some phases of the problem, has been partially offset by higher costs of production, an excuse given by some manufacturers for curtailing attention to sanitation. Unfit raw materials are still being used by too many manufacturers who consider their responsibility discharged when the factory itself is operated under sanitary conditions. It is equally important to the consumer to have his food produced in sanitary factories and to have it protected during every stage of its handling from the farm to the table."

The source of the above statements is the Annual Report of the Food and Drug Administration of 1946. The food industry has come a long way since 1946 with particular regard to environmental sanitation. Since then, in addition to environmental problems, more attention is being given to bacteriological hazards, pesticides, residues, food additives, genetic mutagenesis, etc. and you might say more attention is being given to many other things in the food supply that you do not ordinarily see.

The confectionery industry uses many agricultural ingredients, both domestic and foreign. The philosophy of regulatory enforcement being what it is, the candy manufacturer is responsible for the quality of the ingredients used, the processing, packaging, and distribution through many channels up to and including the consumer. His name is on the package and to the consumer and to the regulatory official he is responsible, regardless of who may have abused his product.

INGREDIENT QUALITY CONTROL

Ingredients used in candy manufacture are subjected to much higher temperatures than in many other foods. For details of the physical and chemical methods used to test ingredients and finished goods, you should consult the *Official and Tentative Methods of the Association of Analytical Chemists*, tenth edition, 1970. There are a number of other tests the candy manufacturer may use to screen ingredients prior to using more detailed tests contained in the A.O.A.C.

Sugar

Sucrose, either cane or beet, may be suitable for table use or in other foods, but the elevated temperatures used in candy manufacture will emphasize differences in sugar composition usually considered of minor importance.

Sugar is manufactured by the general process of seeding a super-saturated sugar solution. The crystals are removed by centrifuging and are then washed. The first crystallization or "strike" is the purest crystal and is referred to as "strong sugar". This refers to the resistance of this sugar to inversion.

Then, the mother liquor is again concentrated and seeded. The ash, protein, and other materials in the mother liquor are increased in concentration with each strike. The opportunity for these components of the mother liquor to become entrapped in the lattice work of the crystals is therefore increased with each strike. Last strike sugar is easier to invert than first strike sugar. I am informed that the crystals resulting from each strike are blended to produce a uniform product. Comparison of first strike molasses with last strike molasses, as with black strap molasses, illustrates the point.

At this point brief mention should be made as to how texture control is obtained in various confections. Sugar is the main ingredient and a sugar solution will illustrate the point. Sufficient water is added to sugar to prepare a sirup. Heat is added to dissolve the sugar. The water must then be removed. Slow removal of the water may result in over inversion of the sugar. Therefore, fast water removal is desirable in most types of hard candies. This fast removal of water is usually done under vacuum.

As water is removed, viscosity of the solution is increased. The increased viscosity inhibits crystal formation. It is therefore possible, by removing a great deal of the water, as with hard candy, to about 1.5%, to produce a liquid that, on cooling, is supercooled. This produces a clear glassy like material without obvious grain.

Fondant is a mixture of sugar and corn sirup. A hot solution of these sugars is agitated at a constant rate. The sugar crystallizes into fine crystal. Each crystal is surrounded by a thin layer of sirup. Finished fondant may be used to seed other supersaturated solutions of sugar. In the industry this is called a "bob" sirup. A solution of sugars is prepared and, while hot, a quantity of fondant is added. It is important not to completely melt the fondant, as the seed will be destroyed. The seeded sirup is run into moulds. As the seeded sirup cools, the fondant generates crystals throughout the entire solution.

There is another example that is also interesting. Fudge, as made in the home, is prepared by heating sugar, milk, and cocoa, or chocolate to a definite temperature. It is known that the lid should be kept off the pot for the first few minutes. After cooking, the pot is permitted to cool until the hand can be placed comfortably on the bottom of the pot. Then the sirup is beaten until it changes color and it is then poured into a suitable container.

What has actually happened? The lid on the pot caused water of condensation to form and water ran down the inside of the pot, dissolved the sugar crystals on the side of the pot which, in turn, prevent-

ed the seeding of the sirup during cooking. The cooling after the final cooking increased the supersaturation of the sirup solution until there were more crystals in solution than would ordinarily occur at this temperature. Beating of the sirup caused grain to form. The agitation produced small crystals and prevented agglomerates from forming that would give a rough-textured fudge. Fudge preparation in the home or in the factory obeys the laws of the physical chemistry of supersaturated solutions.

The total amount of ash derived from sugar, water, and other ingredients can influence the characteristics of candy, particularly hard candy and fondant.

Municipal water supplies are usually constant with respect to odor and taste. Also they maintain the chemical components within limits. The candy manufacturer using these water supplies adjusts his manufacturing techniques to these conditions. The following two case histories indicate the necessity of knowing water quality in more detail. An eastern manufacturer of hard candy decided to build a branch plant in Louisiana. One of the reasons was that he would be near a sugar supply. The plant was put in operation. The candy produced was sticky and not what he expected. What he had failed to check was the hardness of the water. In this particular locality the water hardness was 149. This plant did not remain in production.

The second example involved a candy manufacturer in the state of Ohio. The water supply was obtained from the city. The source of the water was from deep wells. This manufacturer was experiencing difficulty in the manufacture of hard candy in the spring of the year; during the fall his fondant was discolored. What happened was that in the spring, the water table was high and water contained dissolved carbon dioxide. This seemed to buffer the alkaline salts in the water. During the fall the water table was low and water contained considerable alkaline ash. As this was city water, there was little he could do about it. He considered ion exchange treatment of processing water, distillation, or the purchase of liquid sugar. He finally resolved the problem by purchasing liquid sugar. I know of a number of firms that have established branch manufacturing facilities. The first thing the chemist does is to take water samples and prepare candy according to the standard formulas. Water quality, for years, had not been generally considered important in candy manufacturing. The National Confectioners Educational and Scientific Foundation decided to sponsor a study to quantitate the effect of dissolved mineral in water on sugar inversion. A grant was made by the Foundation to the Food Science Department, University of Wisconsin, Madison. The concentrations of

the dissolved salts used in the experiments were within the limits recommended by the Public Health Service. Standard concentrations of sugar solutions were prepared. Tests were conducted at 250 F.

Some of the results are as follows. "Under the conditions of the experiment, presence of Na_2CO_3 and NaHCO_3 caused a browning reaction. City of Madison water gave similar results. The presence of MgCl_2 resulted in an increase of reducing sugar. After heating for 30 min, at 250 F, a browning reaction developed apparently caused by degradation of the sugars. The presence of CaCl_2 in the solution also resulted in an increase of concentration of reducing sugars but not accompanied by the browning reaction."

The details of this study are contained in *Research Report 71, College of Agriculture and Life Sciences, Research Division, University of Wisconsin, Madison, October 1970*. Results of this study illustrate that a food manufacturer should know his water supply as he would know any other ingredient.

There may be a difference between cane and beet sugar. Cane sugar is usually more highly refined. The candy maker can observe a pan of boiling solution of sugar and may be able to determine if it is cane or beet sugar. I hasten to add that the quality of beet sugar has been improving and beet sugar can be refined equally as well as cane sugar. The general quality of beet sugar is acceptable in a number of classes of candy, while cane sugar is preferred for white goods. Most candy manufacturers also specify crystal size.

In any quality control program it must be recognized that the supplier has production problems. Many candy plants, on receiving a shipment of sugar, do a fast quality check by using the Hooker Method, developed by the U. S. Bureau of Standards and published in circular C-440. This circular, incidentally, is out of print. In substance, the test is as follows:

One-half a pound (227 g) of sugar is placed in a copper casserole of the following dimensions, 4 9/16 inches diameter at the top, 2 1/4 inches at the bottom, and (a) height (of) 2 9/16 inches (inside measurements). After the addition of 3 oz (89 ml) of distilled water, the casserole is placed over the naked flame of a burner. Flame should be regulated previously to such a size that the total time of heating required to bring the temperature to 350 F (177 C) is 21-25 min. (It has been found that this condition will be fulfilled if 200 ml of water at room temperature is brought to a point of vigorous boiling in 4 1/2 to 5 min.)

The contents of the casserole are continuously stirred until the sugar has dissolved, and the stirring rod is then removed. If the size of the flame has been properly adjusted, the solution should start to boil in about 5 min after being first put over the flame. At this point, an inverted watch glass is placed over the casserole; otherwise the sugar will tend to crystallize as the evaporation proceeds.

After the heating has continued precisely 15 min from the time when the casserole was first placed over the flame, the watch glass is removed, and the solution is then thoroughly and constantly stirred without a moment's interruption until the boiling has reached exactly 350 F (177 C,) the thermometer being used as a stirring rod. The casserole is then instantly removed from the flame and its contents are as rapidly as possible emptied upon a polished copper slab 14 × 14 1/4 inches in size. In a few minutes, the mass becomes brittle and can be broken up for further tests.

The Hooker procedure is deficient chiefly in reproducibility. The test has been modified in a number of laboratories such as using porcelain casseroles instead of copper. The end result of this procedure is the characteristic color produced. The test is relative since it does not give quantitative measurements. Often a sugar sample subjected to this test gives a tan color. If the sum total of the color-producing materials does not affect the candy in which it is to be used, then the sugar is acceptable for the intended purpose.

It takes very little protein and ash to produce dark colored sugar. The acceptable amount of color is an individual company decision. The merit of this test is that it is quick and gives a general rule of thumb for any needed further tests. Heavily discolored samples are rejected regardless of the reason.

Today, there are more and more custom blends of liquid sugars being offered to the food processor. Delivery is usually in tank cars or trucks. It saves the cost of handling, dissolving the sugar, etc. The economics of liquid sugar depend a great deal on the distance from the supplier. This is reflected in shipping costs, plus the cost of water removal, the storage facilities, etc.

Corn syrup

Checks are made as to dextrose equivalents, color, total solids, and response to heating. The wide variety of corn syrups offered and the quantities used make it imperative that the candy manufacturer make sure the right syrup goes into the right storage tank. Color is important in the manufacture of white goods. Solids are also important in the formulation of the candy.

Whipping agents

Whipping agents used in confectionery are animal or vegetable in origin. Gelatin, soy protein, egg white solids are commonly used. In addition to providing light texture, they serve as protective colloids to slow down formulation of sugar agglomerates. Development of these agglomerates gives a rough texture in candy, particularly in fondant. Gelatin is usually purchased on the basis of the Bloom Test.

The test was named after the man who developed the method. Basically, it is a measure of the amount

of force necessary to penetrate a solution of gelatin of known concentration. Measurements on foaming characteristics are also done.

Manufactured milk

The confectionery industry uses negligible quantities of fluid milk. Most of the milk used in candy formulas is usually some variety of condensed or dried milk. The constituents of some of these manufactured milks are generally specified in U.S.D.A. Guidelines. However, in automatic processing, these milks should be uniform within specified limits because automatic equipment may not compensate for variations in ingredients.

Fats and oils

Butter and hard butters are used to impart flavor, lubrication, and textural qualities. Tests for quality include taste, obvious rancidity, and free fatty acids for borderline rancidity. Fats are also well known for their ability to absorb odors. The heat used in processing candy will accelerate oxidative changes and a product of low consumer acceptability results. The melting point of the hard butters used is important in controlling texture and volume.

Nuts

Some of the nuts used in confections are peanuts, pecans, almonds, coconut, filberts, Brazil nuts, and cashews. There are U.S.D.A. grading specifications for these nuts.

Chocolate and chocolate products

Chocolate by definition is the ground portion of the cacao bean. It is what is commonly referred to as chocolate liquor or baking chocolate. The Federal Food and Drug Administration has Standards of Identity for Cacao and Cacao Products.

I might mention that technically, there is no such thing as "white chocolate." To be called chocolate, the ingredient must contain chocolate liquor. Quality control consists primarily of viscosity tests to make sure the proper coverage of products is obtained. Flavor is equally important. Analysis of cacao butter content and milk protein is also routine. Tasting for proper flavor is highly desirable.

There is considerable similarity between the blending of the various cacao beans and the blending of coffee beans to obtain specific flavors.

Cocoa powder quality is evaluated by suspending the powder in boiling water, with or without sugar, for tasting compared to a standard. Color can also be compared to a standard. Fineness can be determined by a screen test using a petroleum solvent or by use of a calibrated microscope. The pH is also important in some uses.

The quality of chocolate coatings is judged by taste, gloss, snap, or brittleness. To obtain these characteristics, the coatings must be tempered. Cacao butter in chocolate is a mixture of triglycerides and like all fats, exhibits polymorphism. Each triglyceride has its own physical characteristics. There are several classifications of these triglycerides proposed by various authors. For purposes of this paper the terminology of Dr. S. V. Vaeck will be used. He calls the triglycerides alpha, beta, and beta-prime. The stability of each crystal varies as to melting point, latent heat of fusion, shelf life, and contraction from the liquid phase. The following table illustrates the variations in each type.

| Type | Melting point °C | Melting point °F | Latent heat of fusion | Approx. life | Contraction from liquid |
|----------|------------------|------------------|-----------------------|--------------|-------------------------|
| β | 34-35 | 93.2-95 | 36 cal/g | stable | 0.097 ml/g |
| β' | 27-29 | 80.6-84.2 | 28 cal/g | 1 month | 0.080 ml/g |
| α | 21-24 | 69.8-75.2 | 19 cal/g | 1 hr | 0.060 ml/g |

Of these forms, only the β form is stable. All others are temporary and will in time change over to the stable form.

To obtain the stable form in a general sense, raise the temperature to about 140 F for sweet chocolate, and to 120 F for milk-containing coatings. These temperatures will melt all of the triglycerides. The temperature is then gradually lowered as constant agitation is applied. As the temperature is lowered, the three types of crystals are produced. The temperature is lowered to about 86 F and then raised to 89-90 F. The rise in temperature destroys all but the beta-crystal. The coating is still liquid and by controlled application to the centers the beta-crystals will cause only beta-crystals to form as the coating is cooled.

Chocolate-coated candy must be protected against adverse conditions of temperature and humidity. This requires controlled temperature and humidity. Packaging is also important. To illustrate, there is a defect that appears on chocolate called, "bloom." It presents a gray appearance and it may appear to be mold. The cause of the bloom is heat and/or moisture. Heat melts the fat and on recrystallization it presents a gray appearance on the surface of the candy. Moisture melts the sugar and it, in turn, on recrystallization, shows a gray appearance on the surface of the candy. Examination with a good hand lens will reveal the facts. Latent heat in the centers, when they are coated, will migrate to the surface and dissolve the fat and also cause fat bloom.

MICROBIOLOGICAL CONSIDERATIONS

Up to this point, I have mentioned briefly some of the physical and chemical quality control tests used

by the confectionery manufacturer. In recent years, food production has changed from local production and consumption to a complex system of distribution made possible by improved packaging and preservation techniques. Any error of sanitation in this complex system may be rapidly disseminated nationally or world wide. This is illustrated by the activities of the regulatory officials to eradicate *Salmonella* from the food supply. The remainder of my comments will deal with microbiological control.

Candy manufacture for purposes of microbiological control can be separated into two categories. (a) cold processed confections, and (b) hot processed confections. In the first category would fall mainly moulded chocolate, chocolate coatings for cream centers, and certain panned items. The temperatures used in processing rarely reach what is generally termed pasteurization, or better yet, sterilization. The moisture content of these candies is quite low. Hot processed confections, on the other hand, would include hard candy, fudges, toffees, caramels, gums, jellies, marshmallow and nougats, etc.

Most finished candies have a high solids content. Acidulants used as flavor enhancers and the flavors themselves may be bacteriostatic or bacteriocidal.

It has been estimated there are between two and three thousand varieties of confections. It would be an impossible task to evaluate each of these confections as to a possible bacteriological hazard. For this reason the effect of the sum total of ingredients on *Salmonella* survival in the finished candy is being evaluated by a study under the sponsorship of the National Confectioners Educational and Scientific Foundation at the Food Research Institute, University of Wisconsin, Madison.

The role of bacteria in diseases of man and animals has been known since the time of Louis Pasteur. Investigators of illness during the early years were busy searching for a specific microorganism as a causative agent of disease. During the past few years, heavy emphasis has been placed on *Salmonella*. Outbreaks of bacterial food-borne illness have been reported from time to time. The reports of the Center for Disease Control have done much to highlight the necessity of trying to eliminate *Salmonella* from the food supply.

About 1300 serotypes of *Salmonella* are known to cause illness in man or animals. Rather than becoming involved in the details of methods of laboratory examinations, which are available, let us confine the remainder of this discussion as it applies to the subject of this paper.

Sugar products

The staphylococci, *Shigella*, streptococci, certain clostridia, the aerobic bacilli such as *Bacillus cereus*,

and certain strains of *Escherichia coli* have been incriminated in food borne illnesses. To my knowledge there have not been microbial problems with dry sugar, liquid sugar, and corn syrups. The viscosity of liquid sugars is high enough to inhibit microbial growth. In the storage of liquid sugars, care must be exercised to prevent water of condensation from forming inside the tanks. This condensate may run down the sides and layer on the surface of the sugar solution.

Microorganisms present in the air will seed this diluted solution and then will grow. Vertical storage tanks offering a minimum of exposed liquid surface (as contrasted to horizontal tanks), filtered air intake vents, temperature controls in the tank storage area, and ultra-violet lights installed inside the tanks will do much to prevent microbial growth from developing in any condensate that may form on the surface of these ingredients.

Animal and vegetable proteins and fats

The most common agents of animal origin used in candy manufacture are gelatin, egg white solids, and certain milk proteins. Vegetable proteins, mainly those derived from soy beans, are also used. All of these agents are processed ingredients and those, particularly of animal origin, should be quarantined on receipt and released for use only after they have been tested for the presence of undesirable microorganisms.

To my knowledge there has been little microbial difficulty with the fats and oils used in confectionery.

Nuts and coconut

The manufacturing confectioner uses large quantities of peanuts, pecans, filberts, almonds, Brazil nuts, cashews, and coconut. Some of these nuts such as peanuts, almonds, filberts, and pecans are domestic in origin. Filberts and almonds are also imported. Coconut and Brazil nuts and cashews are all imported. Nut meats, with the exception of cashews, peanuts, and almonds, may receive little or no heat treatment. We have unpublished data that the dry-roasting (325 F for 20 min) of peanuts containing 5.0 - 6.0% moisture and, experimentally inoculated with 10^8 *E. coli* per gram, destroyed the bacterium. Laboratory studies at the Food Research Institute, University of Wisconsin, using methods similar to commercial oil roasting of peanuts at 270 F for 5 min or longer resulted in the destruction of *Salmonella*.

Coconut is pasteurized after being processed. One major coconut supplier has the following published specifications for desiccated coconut:

| | |
|----------------------|-------------|
| Standard Plate Count | 5000/g max. |
| Yeast | 100/g max. |
| Mold | 50/g max. |
| Coliform | 10/g max. |

Salmonella
Staphylococcus
 (Coagulate-positive)

Negative
 Negative

Pecans may or may not be given a light dry or oil roast. Usually they are used raw. The basis for heating most nuts is for blanching to improve the texture, and in some instances as a preventive technique to control insect infestation. To my knowledge there is little published data on the effect of roasting on the bacterial population of nut meats.

Aflatoxin reared its head a number of years ago after a commercial poultryman in England fed what was later determined to be aflatoxin-containing peanut meal to young turkeys.

Mycotoxins as a causative agent for disease have been known to the scientific community for years. Probably one of the oldest mycotoxins is ergot caused by the fungus *Claviceps purpurea*. It is found on grains and most often on rye.

Following the aflatoxin incident in England about 10 years ago, all peanut products were surveyed. Peanut meal is not used for food in the U.S. However, whole peanuts and peanut products are used and have been found to contain aflatoxin. Grading of various kinds of peanuts by the U.S.D.A. requires that peanuts contain less than 20 ppb of aflatoxin to move in interstate commerce. Most large users of peanuts have certified laboratories that do analysis for aflatoxin. Control procedures consist of harvesting and storage of peanuts, in-shell and shelled, under conditions that will not be conducive to mold growth.

The U.S.D.A., the FDA, and the shellers and peanut buyers have an active research program directed toward minimizing or hopefully eliminating aflatoxin from animal and human foods. Some of the phases of this program study conditions affecting aflatoxin development during the growing of the peanuts, harvesting, storage, shelling, and transportation. Sampling procedures and analytical methods are being continually evaluated. Mold resistant varieties of peanuts are also being investigated.

The following is a list of nuts for which the U.S. Department of Agriculture has established grades: shelled almonds, 1960; almonds in shell, 1964; Brazil nuts in shell, 1966; filberts in shell, 1961; shelled pecans, 1956; pecans in shell, 1962; English walnuts in shell, 1964; peanuts (white Spanish), farmers stock, 1958; peanuts (Virginia type), farmers stock, 1955; peanuts (runner type), farmers stock, 1955; peanuts (shelled Spanish type), 1959, peanuts (shelled Virginia type), 1959; peanuts (shelled runner type), 1956; and peanuts (cleaned Virginia type), 1955.

The grades are based on size, shape, limits as to defects, foreign material etc. Although nuts are

graded according to U.S. Department of Agriculture regulations, in addition, they are required to meet the standards of the Federal Food and Drug Act.

According to the Import Detention Lists, thousands of pounds of Brazil nuts have been denied entry to the U.S. because of aflatoxin content, domestically produced pecans have been seized by the FDA for contamination with *E. coli*. There have also been sporadic problems of *Salmonella* in coconut, chiefly *Salmonella senftenberg*.

The Salmonella problem

Most of the difficulties from the standpoint of *Salmonella* in the candy industry have arisen from chocolate coatings containing milk. Chocolate and chocolate coatings are purchased by most candy manufacturers. The manufacture of chocolate starts with roasting the cacao beans, removing the shells, and then grinding the beans into chocolate liquor. From this point on the amount of heat used is just enough to keep the chocolate liquor fluid. Dry materials such as milk are blended into the mass. There is not sufficient heat used to pasteurize chocolate. Also the moisture is below 1.5%.

Once *Salmonella* or other microorganisms gain entry into the chocolate, the usual manufacturing processes will not destroy them. Some research has been published in which water was added to experimentally inoculated chocolate and the temperature raised to pasteurize the chocolate. The added water must be removed and this requires additional processing. To my knowledge, pasteurization is not generally practiced by the chocolate-coating manufacturer.

Most of the studies on *Salmonella* in the past few years have been done on the relationship of water, nutrients, and temperature to the encouragement of *Salmonella* growth. Little research until recently has been published on what happens when you limit the amount of available water. Our industry encountered a problem with *Salmonella* in chocolate coatings. The Research Committee of the National Confectioners Association arranged a conference with Dr. E. M. Foster, Director, Food Research Institute, University of Wisconsin, Madison. Following this conference, a program of specific studies on confectionery was instituted. The first paper which resulted from these studies was "Heat Resistance of *Salmonella typhimurium* and *Salmonella senftenberg* 775 W in Milk Chocolate." The latter microorganism is a laboratory curiosity. However, it has been found in other foods. It survives in environments usually destructive to other *Salmonella*. The thinking was that if you could eliminate *S. senftenberg* 775 W, you should be able to eliminate other *Salmonella*.

This study brought out a very important fact, namely that other *Salmonella* sero-types become resistant when the available water is reduced. Since this first study was undertaken, we have continued to support several programs on the environmental

factors affecting the death or dormancy of *Salmonella* under conditions of candy manufacture. The results of these studies have been and will continue to be published in scientific journals.

THE LEADERSHIP ROLE

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public health which has in the past consumed such a large share of public health's budget. With the increasing probability that some form of "national health insurance" will be instituted during this decade the need for traditional public health services will decrease.

A NEW TEAM

These two forces operating simultaneously have propelled the environmentalist into an unaccustomed place at center stage, whether or not the involved individual and his employing agency presently realize it. The facade has been stripped away and it is now increasingly obvious that the environmentalist is not a member of the so-called "health team" so popular in some public health quarters. He stands alone and performance now expected from him is that of a soloist, as far as public health professionals are concerned. To perform to his capabilities he must instead turn to a "team" made up of people who may never have stepped inside a public health department, indeed, may have little interest in or knowledge of the narrow medical view of health.

In addition to this divestiture of the concept of the "health team," other notions, long principles of faith in public health, are being questioned and discarded. Among these is the use of the term "environmental health" with its implication of program based solely on disease prevention, and the growing use of designations such as "environmental management" which more nearly describe the broader base upon which current ecological programming has its foundations. This does not mean that health is eliminated as a reason for environmental programs; quite the contrary, health is more fully recognized as having components other than absence of physical illness. It recognizes that the "healthy"

man enjoys social and economic well-being, and an absence of undesirable stress, as well as freedom from physical pain. It further recognizes that correction of conditions that lead to a state of disease are more appropriately handled through the use of techniques and tools alien to the science of medicine. It further implies, in fact boldly states, that management of man's environment is independent from the purview of the specialty of preventive medicine. This again results from the very broad foundations upon which programs designed to manage the environment are based.

A BROADER BASE

As proof that this broader base is becoming more widely recognized we have only to turn to the recent Federal reorganization of agencies aimed at restoring the quality of our environment. Once the almost exclusive domain of the Public Health Service, the legislative and executive branches of our government saw fit to fragment such programs over many agencies. The recent reversal of this trend of dissipation and the beginning of a policy of concentration of environmental programs into a single agency far removed from the Public Health Service only reinforces the fact that, at a Federal level, it has been recognized for some time that environmental problems are multi-faceted in nature, and that "health," as it is narrowly defined by the medical profession, is but one of its many components, and often a minor one.

This is not to say that environmental programming can remain visible and viable only in an agency separate from the traditional health department, but to say that it cannot remain viable if limited by the traditional health department concepts of

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WASTES GENERATED IN THE MANUFACTURE OF SAUERKRAUT¹

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ABSTRACT

Forty three surveys were made of two sauerkraut factories to obtain quantitative data on the wastes generated. Trim losses (solid wastes) were over 35 tons per 100 tons of raw cabbage processed and the discarded brine represented about 29% of the salted, shredded cabbage. Other sources of waste effluents were vat soak water, vat wash water, and can cooling water. From 294 to 395 gal of waste effluents were discharged per ton of shredded cabbage processed; the BOD ranged from about 11 to 13 lb. per ton. Brine had enough nutrients (BOD, nitrogen, and phosphorus) for optimal biological stabilization.

Sauerkraut is a clean, sound product, of characteristic acid flavor, obtained by the lactic acid fermentation of properly prepared and shredded cabbage in the presence of 2 to 3% salt. It contains, on completion of the fermentation, not less than 1.5% titratable acidity, expressed as lactic acid (4). Over 10 million cases of sauerkraut are packed annually in the United States with New York State, the leader, producing over 3.6 million (5).

Sauerkraut waste water is unfavorable for conventional waste treatment because of its extremely high BOD, low pH, and high sodium chloride content (11). There is thus a need for improved methods to treat this waste water. Before treatment facilities can be designed, however, data must be available regarding quantities and properties of the wastes. Securing this information was a major objective of this study.

A second objective was to obtain information that might point to changes in processing methods that could be made to reduce waste generation. Other in-plant studies (3, 9, 10) have shown that economies in water usage and consequent effluent reduction often can be effected in food processing.

MATERIALS AND METHODS

The research was carried out during the 1971 season in the factories of two of our larger sauerkraut producers. The analyses and flow data are the results of 43 surveys performed on different days.

Waste water flow rates were measured by the calibrated

container-stop watch procedure (2). Samples of waste water were sealed in one-pint polyethylene bags and transported to the laboratory over ice in an insulated chest. The analyses for BOD, COD, pH, total solids, lactic acid, and NaCl were done as soon as the samples reached the laboratory. Samples to be analyzed for Kjeldahl nitrogen, and phosphorus were frozen immediately and stored at -23 C until the tests could be made. Standard methods were used for determining 5-day BOD, COD, and total solids (1). In the BOD test, the modified Winkler method was used to determine dissolved oxygen (1). Lactic acid and NaCl were determined by micro titrations (12). Kjeldahl nitrogen and phosphorus were analyzed by AOAC methods (6).

RESULTS AND DISCUSSION

Sauerkraut process

The cabbage is delivered to the factory by truck. It then is transported via conveyor belt to the coring machine. Following this, the cored head is conveyed to the trimming table where outer leaves and bad spots are removed. This latter operation represents a source of solid waste.

Next the cabbage is shredded and transported to the fermentation vat. Salt, 2.25 to 2.5 lb. per 100 lb. of cabbage, is applied evenly as the shreds are distributed in the vat.

Juice is released from the cabbage almost immediately after addition of the salt. To assure a maximum fill of cabbage into a vat, much of this "early brine" may be withdrawn from the vat and discarded during or shortly after the filling. As the data will show, this early brine can be a significant source of liquid waste in the sauerkraut process.

After the vat is filled, it is covered with a plastic sheet that is weighted with water. The fermentation is considered complete when the titratable acidity, expressed as lactic acid, has reached 1.5%, and the shreds are fully cured. This process requires four or more weeks.

The final step is the filling of the fermented cabbage and brine into retail packages (cans, jars, or flexible pouches). Only a portion of this "late brine" is added to the packages, the remainder which must be discarded represents a second important source of liquid waste.

Volumes and properties of waste effluents

¹Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 1930.

TABLE 1. SOURCES AND VOLUMES OF SAUERKRAUT WASTE EFFLUENTS

| Vat no. | Shredded cabbage in vat | Early brine | Late brine | Vat soak water | Vat wash water |
|-----------|-------------------------|-------------|------------|----------------|----------------|
| | (tons) | (gal) | (gal) | (gal) | (gal) |
| Factory A | | | | | |
| 21 | 69 | 3,120 | 2,290 | 16,000 | 132 |
| 24 | 65 | 2,280 | 2,019 | 15,000 | 180 |
| 25 | 69 | 3,120 | 3,362 | 14,500 | 150 |
| 26 | 65 | 1,900 | 2,139 | 13,700 | 135 |
| 29 | 66 | 1,920 | 1,506 | 14,000 | 165 |
| 49 | 65 | 3,120 | 750 | 15,000 | 150 |
| 53 | 65 | 3,000 | 480 | 14,000 | 195 |
| 51 | 65 | 1,980 | 3,744 | — | 180 |
| 52 | 65 | 2,475 | 1,250 | — | — |
| | Mean | 66 | 2,546 | 14,600 | 161 |
| Factory B | | | | | |
| 9 | 33 | 162 | 1,872 | 9,000 | 85 |
| 10 | 32 | 24 | 2,145 | 10,000 | 120 |
| 11 | 34 | 144 | 1,963 | 9,500 | — |
| 12 | 34 | 615 | 2,041 | 10,000 | — |
| 13 | 34 | 372 | 2,184 | 9,500 | — |
| | Mean | 33.4 | 263 | 9,600 | 103 |

In addition to brines, other sources of waste effluent were the vat soak water, vat wash water, and can cooling water. The volume and composition of these different wastes are presented in Tables 1 and 2.

Although the soak water required to maintain vat integrity during the storage season, was very high in volume (Table 1), its strength was relatively low (Table 2). Its low BOD suggests that it should be readily biologically treatable although it could present a problem in hydraulic loading. The trend to the fiberglass lining of wooden vats and the use of other materials for vat construction would indicate that soak water will be even less of a consideration in the future.

The water used to clean vats before filling was of greater strength but the volume was low. Vat wash water, therefore, was not a significant waste source. Use of alkaline cleaning compounds undoubtedly accounted for its somewhat higher pH.

At the time of filling the vats with cabbage, Factory A released considerably more juice, the early brine, than did Factory B (Table 1). This difference in practice is best illustrated in Table 3 where the data are expressed as waste per ton of cabbage and, therefore, compensate for differences in vat capacities. It can be seen that Factory A discharged an average of five times greater volume of early brine than did Factory B. Sooner or later, of course, the excess cabbage juice must be discarded and Factory B which had released the modest amount of early brine had a greater volume of late brine to discharge at the end of the fermentation (Tables 1, 3).

Inspection of data in Table 3 affords a comparison between the two practices. We conclude from the data that the volume of brine generated by the two

methods is comparable. Although the total pounds of BOD appear to be higher in the Factory B brines, the difference may not be statistically significant; it is noted that, conversely, Factory A brines seemed to contain the higher total solids. The greater quantity of lactic acid discarded by Factory B resulted from the larger volume of cabbage juice that was retained in the vats during the sauerkraut fermentation. It is likely that the increment in lactic acid generated by Factory B has economic significance since acid effluents generally require neutralization before biological treatment.

Data on cooling water are not presented in the tables because it was not possible to relate water discharge to sauerkraut production. Studies at Factory B showed this water to have a low BOD, 27-40 mg/l, and a discharge flow rate of 590 to 750 gal per hour.

Data on material balance (Table 4) show the quantity of sauerkraut produced from 100 tons of cabbage. It can be seen that about 29% of the salted shredded cabbage is discarded as brine. The data also show that there is a problem of solid as well as liquid waste in the manufacture of sauerkraut. The trim loss of 35.3 tons is an average for 16 different loads of cabbage; individual values ranged from 28.7 to 41 tons. Trim losses were high in 1971, probably because of a wet growing season. According to some sauerkraut packers, a loss of 26 to 30% is a more common figure. The solid wastes are generally returned to the growing field.

Because of their high strength, the surplus brines present the greatest problem with respect to treatment. The average BOD of the brines discarded by the two factories was 11.75 lb. per ton of shredded cabbage (Table 3). The total BOD from a 65-ton

vat would, therefore, equal 764 lb. which, when expressed on a population equivalent basis, means that it is equal to that contributed in one day by a population of about 4500 persons.

Since sauerkraut brine has only a limited sales volume and no simple or inexpensive means for reduction of brine is apparent, a solution to the problem of excessive brine formation remains to be resolved. Although the recent report of breeding new high dry matter cabbage for potential reduction of hydraulic loads in sauerkraut fermentation appears promising (7), the relative chemical composition of such newly developed varieties and their effects upon BOD load remains to be established.

It is likely that the extremely high BOD of waste brines may require their segregation for separate treatment. Our analyses of brine nitrogen and phosphorous contents (Table 2) indicate that neither compound would have to be added as a nutrient supplement to achieve optimal biological stabilization. Thus the average BOD:N:P ratio was found to be very close to the recommended 100:5:1 (8).

One possible problem associated with the brines is their high salt concentration; levels as high as 4.5% were found in this study (Table 2). Little of this salt probably would be removed from the waste water by conventional treatment systems. Furthermore, certain concentrations of sodium chloride might interfere with the activity of system's biomass. The ideal solution would be to reduce the amount of salt discharged; the 18 lb. per ton of shredded cabbage (Table 3) represents a loss of almost 40%. Unfortunately, alterations in the sauerkraut process that would waste less salt are presently not known. Unpublished studies by one of us (JRS) have shown the adverse affects of diminished salt content upon the textural properties of the finished product.

REFERENCES

1. Anon. 1965. Standard methods for the examination of water and waste water. 12th ed. pp. 244, 393, 400, 406. Amer. Public Health Ass., New York, N. Y.
2. Anon. 1969. A guide for waste management in the food processing industries. p 3.3. National Canners Ass., Berkeley, Calif.
3. Anon. 1968. Cannery waste treatment, utilization and disposal. Publ. No. 39. State Water Resources Control Board, Sacramento, Calif. 274 p.
4. Anon. 1967. United States standards for grades of bulk sauerkraut. Federal Register, May 24 (32 F.R. 7568). 3 p.
5. Anon. 1970. The almanac of the canning, freezing, preserving industries. p. 391. E. E. Judge & Sons, Westminster, Md.
6. Anon. 1960. Methods of analysis. 9th ed. p 9-12. Ass. Off. Agr. Chem., Washington, D. C.
7. Dickson, M. H., and J. R. Stamer. 1970. Breeding cabbage for high dry matter and soluble solids. J. Amer.

TABLE 2. CHARACTERISTICS OF SAUERKRAUT EFFLUENT

| Effluent source | Factory | pH | Total solids (mg/l) | COD (mg/l) | BOD (mg/l) | Lactic acid (mg/l) | NaCl (mg/l) | Kjeldahl Nitrogen (mg/l) | Total phosphorus (mg/l) |
|-----------------|---------|----------------------|---------------------|---------------|---------------|--------------------|---------------|--------------------------|-------------------------|
| Vat soak water | A | 6.5-8.5 ^a | 168-708 | 24-140 | 12-104 | | | | |
| | B | 7.5 ^b | 478 | 64 | 60 | | | | |
| Vat wash water | A | 8.4-8.6 | 378-1,528 | 22-220 | 10-85 | | | | |
| | B | 8.5 | 776 | 83 | 43 | | | | |
| Early brine | A | 9.5-11.0 | 1,325-9,935 | 176-520 | 141-416 | | | | |
| | B | 10.4 | 2,448 | 303 | 236 | | | | |
| Late brine | A | 9.4-9.5 | 1,440-1,270 | 180-260 | 105-160 | | | | |
| | B | 9.5 | 1,355 | 220 | 132 | | | | |
| Range of data | A | 3.7-6.0 | 46,740-75,040 | 14,630-25,350 | 8,250-9,550 | 450-4,770 | 32,600-42,300 | 420-600 | 70-97 |
| | B | 5.2 | 56,630 | 16,620 | 9,070 | 1,510 | 34,000 | 540 | 85 |
| Mean of data | A | 3.9-5.9 | 42,350-64,620 | 15,000-21,700 | 9,750-13,000 | 540-1,710 | 22,700-45,000 | 470-600 | 99-116 |
| | B | 5.2 | 55,120 | 17,730 | 11,140 | 926 | 36,800 | 555 | 106 |
| Range of data | A | 3.3-3.7 | 53,320-61,700 | 24,200-32,500 | 20,500-28,150 | 14,800-21,900 | 22,400-28,400 | 910-1,250 | 159-220 |
| | B | 3.5 | 57,730 | 28,960 | 24,300 | 18,600 | 26,800 | 1,090 | 189 |
| Mean of data | A | 3.4-3.8 | 50,420-61,760 | 24,000-30,000 | 21,000-26,000 | 14,000-16,800 | 24,000-29,000 | 870-970 | 185-264 |
| | B | 3.6 | 55,450 | 27,130 | 23,200 | 15,850 | 26,800 | 927 | 207 |

TABLE 3. WASTE LOAD PER TON OF SHREDDED CABBAGE FERMENTED

| Factory | Effluent | Volume (gal) | Total solids (lb.) | BOD (lb.) | Lactic acid (lb.) | NaCl (lb.) |
|---------|-------------|-----------------|-----------------------|--------------|----------------------|---------------|
| A | Soak water | 221 | 0.9 | 0.11 | 0 | 0 |
| | Wash water | 2 | 0.04 | 0.004 | 0 | 0 |
| | Early brine | 40 | 20.5 | 3.10 | 0.52 | 11.7 |
| | Late brine | 31 | 15.5 | 7.60 | 4.9 | 7.1 |
| | Total | 394 | 36.94 | 10.81 | 5.42 | 18.8 |
| B | Soak water | 287 | 1.8 | 0.10 | 0 | 0 |
| | Wash water | 3 | 0.03 | 0.003 | 0 | 0 |
| | Early brine | 8 | 3.7 | 0.75 | 0.06 | 2.5 |
| | Late brine | 61 | 29.0 | 12.10 | 8.3 | 14.0 |
| | Total | 359 | 34.53 | 12.95 | 8.36 | 16.5 |

TABLE 4. MATERIAL BALANCE OF SAUERKRAUT PRODUCTION (FACTORY A)

| | (Tons) |
|---------------------------|--------|
| Raw cabbage | 100.00 |
| Solid wastes (trim loss) | 35.30 |
| Shredded cabbage in vat | 64.70 |
| Salt added | 1.70 |
| Liquid wastes | |
| Early brine | 11.00 |
| Late brine | 8.50 |
| Total | 19.50 |
| Yield of finished product | 46.90 |

Soc. Hort. Sci. 95:702-722.

8. Eckenfelder, W. W., Jr., and D. J. O'Connor. 1961.

Biological waste treatment. Pergamon Press, New York. 299 p.

9. Hang, Y. D., D. L. Downing, and D. F. Splittstoesser. 1971. Sanitation and water usage in the processing of sour cherries. *J. Milk Food Technol.* 34:428-430.

10. Rambo, R. S. 1968. Vegetable canning processing wastes. Ph.D. thesis. University of Wisconsin, Madison. 157 p.

11. Splittstoesser, D. F., and Y. D. Hang. 1970. Effect of processing conditions on fruit and vegetable waste effluents. p. 24-28. Proceedings of the Eastern Exp. Sta. Collaborators Conference on Agricultural and Processing Wastes in the Eastern Region: A perspective. Publ. No. ARS73-70, U. S. Department of Agriculture, Philadelphia, Pa.

12. Stamer, J. R., M. H. Dickson, J. B. Bourke, and B. O. Stoyla. 1969. Fermentation patterns of poorly fermenting cabbage hybrids. *Appl. Microbiol.* 18:323-327.

THE LEADERSHIP ROLE

(Continued from Page 431)

"health," "environmental health," "sanitation," and "preventive medicine." Such programming can and will function within any organization structure providing that structure does not limit ecological programming to the restrictive boundaries determined by tradition. Programs aimed at correcting ecological problems will continue to grow in importance and it makes little difference to the public, to the general governmental organization involved, or even to the eventual solution of the problems, whether or not these activities are performed by a public health agency. The current demand is for results, not internecine combat over organizational structure and responsibility based on misdirected pride.

FUTURE ROLE OF ENVIRONMENTALIST

The present corps of paid professional environmentalists (sanitarians, engineers, wildlife managers, etc.) can play a key role in future ecological programs, but only if they:

- (a) Understand that ecological problems demand corrective programs which are as multi-faceted

as the problems. A limited concern with health, with social problems, with economic concerns, with preservation, or with other restricted goals means an equally limited impact on the problems; and an equally limited degree of support.

- (b) Are willing to accept leadership. As stated above, it makes no difference to the suffering public who takes this role; and there is no shortage of competitors.
- (c) Realize that they are currently in a unique position to assume leadership. The local environmentalist is, or should be, the person to whom the locality now looks to for aid and advice in solving ecological problems.
- (d) Understand and utilize the political, social, and economic factors in their locality in developing solutions.
- (e) Make use of the current popular interest in, and concern over, environmental degradation. Never before, and possibly never again, will the environmentalist have the public support he now has. Never before has there been the multitude of organizations and individuals concerned with a common goal. Some need leadership, some

(Continued on Page 447)

THE PROPIONIC-ACID BACTERIA—A REVIEW

III. MISCELLANEOUS METABOLIC ACTIVITIES¹

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ABSTRACT

This is the third and final section of a review of the propionic-acid bacteria. This section discusses some of their miscellaneous metabolic activities such as: erythritol metabolism, formation of diacetyl-acetoin and vitamin B₁₂, production of volatile compounds not previously mentioned, propionin (an antiviral agent), lipids and phospholipids, and slime formation. Concluding comments provide a brief summary of particular points of interest.

The first two papers in this series have been primarily concerned with the growth and metabolism of propionibacteria. Up to this point, the discussion has dealt in detail with reactions of the propionic-acid fermentation and the formation of acetate, CO₂, and propionate. This paper will consider several other more or less unrelated metabolic processes. The anaerobic fermentation of erythritol is included because this is a relatively unique process. Production of diacetyl may be related to flavor development in Swiss cheese and also may provide additional insight into the formation of this commercially important compound. Other metabolically related and economically important compounds to be discussed include volatile compounds not previously mentioned and vitamin B₁₂. The demonstration of antiviral activity by propionibacteria would not be considered commonplace, whereas slime production by this genus is not altogether unexpected; however, both of these functions may have as yet unappreciated value.

ERYTHRITOL METABOLISM

Although few microorganisms are able to grow anaerobically with erythritol, a 4-carbon polyalcohol, *Propionibacterium pentosaceum* uses this four carbon alcohol as a carbon source producing propionic, acetic, formic, and succinic acids (266). Barker and Lipmann (14) found that, in the presence of sodium pyruvate and sodium fluoride, cell suspensions of *P. pentosaceum* metabolizing erythritol seemed to produce acid not accounted for as glyceric acid-

3-P. Since no appreciable carbon dioxide is released from the fermentation of erythritol by these bacteria, Barker and Lipmann postulated that their cell suspensions were converting erythritol to a 4-phosphate, oxidizing the 4-phosphate to erythrose-4-phosphate, and then oxidizing the erythrose-4-phosphate, via a diphosphate intermediate, to 4-phosphoerythronic acid.

Shetter (191), in 1951, was able to demonstrate that extracts of *P. pentosaceum* would catalyze the ATP-mediated phosphorylation of erythritol to D-erythritol-4-phosphate (L-erythritol-1-phosphate), but could not establish the role of this compound in the metabolism of erythritol. Later, Holten and Fromm (81) purified and determined the properties of erythritol kinase to further elucidate the metabolic pathway. The studies presented in their paper suggest the first step to be as follows:

$$\text{Erythritol} + \text{ATP} \xrightleftharpoons{\text{erythritol kinase}} \text{L-erythritol-1-phosphate} + \text{ADP}$$

They found that *P. pentosaceum* grown in the absence of erythritol shows little erythritol kinase activity, suggesting that the enzyme was adaptive.

Wawszkiewicz and Barker (248) determined the nature of the major products accumulating in fluoride-poisoned, pyruvate-enriched cell suspensions incubated with randomly labeled erythritol ¹⁴C and studied the effects of some unlabeled suspected intermediates on the system. They found that erythritol metabolism proceeds via a series of phosphorylated intermediates, of which L-erythritol-1-P is the primary member. L-erythritol-1-P can serve as a hydrogen donor for the reduction of 2,6-dichloroindophenol in cell-free extracts. Although the oxidation product was not isolated, indirect evidence suggested that it was L-erythrulose-1-P and that this compound is probably the next intermediate in the erythritol fermentation (247). Labeling dilution experiments indicated that D-erythrose-4-P is not a direct intermediate in the sequence. The formation of labeled formaldehyde in the reaction mixtures lends support to the view that L-erythrulose-1-P, formed from L-erythritol-1-P, undergoes cleavage between the C₃ and C₄ positions to yield formaldehyde and dihydroxyacetone-P. This cleavage, combined with

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other reactions known to occur in the propionic-acid bacteria, provides a source of the labeled C₃, C₅, C₆, and C₇ sugar phosphates accumulating in the cell suspensions incubated with labelled erythritol ¹⁴C. This also provides an explanation for the substantial quantities of formic acid found during the fermentation of erythritol. The pathway of erythritol metabolism in *P. pentosaceum*, as conceived on the basis of the results of their experiments, is outlined in Fig. 1.

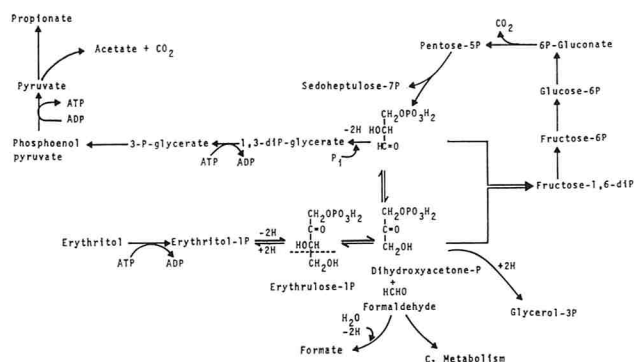


Figure 1. The pathway for erythritol metabolism in *P. pentosaceum* (Modification of that presented by Wawszkiewicz and Barker (248).

FORMATION OF DIACETYL-ACETOIN

Production of diacetyl and acetoin by propionic-bacteria has been observed by several investigators (7, 122, 123, 133, 229, 267), but the biosynthetic pathway is still unclear. According to Antila (7) diacetyl synthesis in propionibacteria involves the intermediate formation of α -acetolactic acid. Factors affecting the production of diacetyl by propionibacteria has been investigated extensively by Lee et al. (122, 123). They found that greater accumulation of diacetyl occurred at 21 C than at 32 or 37 C. The optimum pH for production was between 4.0 and 4.5. They also found that diacetyl production in milk cultures of propionibacteria is followed by reduction to acetoin and 2, 3-butylenediol. Fermentation of pyruvate, glucose, or citrate yielded detectable amounts of diacetyl, but glucose yielded the highest level of the dicarbonyl. Addition of citrate to the milk increased the diacetyl concentration and delayed the diacetyl-destructive phase, substantiating that the tricarboxylic acid could be one of the precursors of the dicarbonyl. Lee et al. (123) also examined cell-free extracts of *Propionibacterium shermanii* for diacetyl reductase to explain the diacetyl-destructive phase encountered with this culture. Under their experimental conditions, diacetyl reductase activity in cell-free extracts was very low or absent.

Acetaldehyde has been found to be produced in small amounts by propionibacteria (101), even though it was considered not to be involved in the propionic-acid fermentation. Since active acetaldehyde is thought to be an intermediate of acetoin formation, it is possible that acetaldehyde or a derivative may participate in the fermentation. If propionibacteria are able to synthesize their own leucine, valine, and isoleucine, they should have the α -acetolactate synthetase enzyme. This enzyme also serves an important role in the biosynthetic pathway for acetoin and diacetyl. Formation of diacetyl is interesting because the pathway of biosynthesis would serve as an excellent carbon-dioxide-generating system, since relatively large amounts of CO₂ are produced.

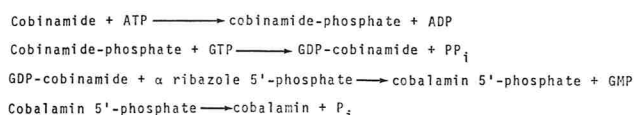
PRODUCTION OF VITAMIN B₁₂

Although there are numerous processes that have been described for production of vitamin B₁₂, only a few have clearly been used on a commercial scale. The processes using the *Propionibacterium* species have been considered the most productive and were used commercially. Both batch and continuous processes have been described. A number of media have been used for growth of the bacteria and accumulation of this vitamin in these organisms. Some of the formulations have included a variety of materials of natural origin including cheese whey (25, 130), distiller's waste (94), spent potato wash (132), casein hydrolysates (143, 287), corn steep liquor (169), and corn starch (11, 187). In most fermentation processes, the vitamin B₁₂ yields have been correlated with cellular yields, and inclusion in the media of additional growth factors have increased cell production (97). Since cobalt is part of the cobamide molecule, inclusion of this element in the media resulted in marked increases in production of vitamin B₁₂ (24, 76). Sathyanarayana and Washington (185) found that a concentration of 3 mg/l of cobalt stimulated a three-fold increase in vitamin B₁₂ production by *Propionibacterium freudenreichii*.

Practically all the cobamides formed in the fermentation are retained in the cells, and the first step is the separation of the cells from the fermentation medium. This is usually accomplished by centrifugation, which concentrates the propionibacteria into a cream-like material that can then be dried to yield a product containing approximately 175 mg of B₁₂ activity per pound (160). The vitamin B₁₂ activity is released from the cells by acid, heat, cyanide, or other treatments (160). Barker et al. (16) found that addition of cyanide solutions decomposes the coenzyme form of the vitamin and results in formation of the cyanocobalamin. A num-

ber of investigators (16, 89, 96, 98, 161, 222) have developed methods of isolation of the cyanocobalamin thus formed.

The synthesis of vitamin B₁₂ by propionibacteria has been extensively investigated. A number of researchers have studied the regulation of synthesis (17, 27), the intermediates formed (22, 28, 39, 65, 66, 68, 96, 183, 203), the coenzymes involved in biosynthesis (16, 84, 88, 89, 90, 98, 222, 223), the biosynthesis of analogs (23, 73, 108, 139, 161), the precursors involved (67, 95, 155, 156), and the conditions affecting synthesis (24, 26, 91, 109, 142, 186, 204, 245, 246, 286). Recently, Renz (178) proposed a reaction sequence for the enzymatic synthesis of vitamin B₁₂ from cobinamide in *P. shermanii* as follows:



This scheme substantiates investigations of Friedmann (67), where he isolated several of the precursors involved in this pathway.

PRODUCTION OF OTHER VOLATILE COMPOUNDS

Production of volatile acids by propionibacteria has been subjected to several investigations. Shaw and Sherman (188) were among the first to observe that these organisms produce acetic and propionic acids from lactose and lactates. Quantitative analysis of the major free fatty acids by Langler and Day (117) and identification of a number of volatile compounds by Langler et al. (118) in Swiss cheese led to formulation of a synthetic flavor mixture that approximated the flavor of the natural product. Although propionibacteria have often been implicated in some of the changes during Swiss-cheese ripening, there is little information available in the literature concerning volatile compounds other than fatty acids produced by these organisms. Recently, Keenan and Bills (101) conducted a study to determine the volatile compounds produced by *P. shermanii*. Gas-chromatographic investigation of milk cultures of three strains of *P. shermanii* showed that acetaldehyde, propionaldehyde, ethanol, and propanol were produced by all strains. One strain produced dimethyl sulfide in quantities significant from the standpoint of flavor threshold in milk systems.

PROPIONIN, AN ANTIVIRAL AGENT

Extracts of *P. freudenreichii* exhibit antiviral activity against Columbia SK virus *in vivo* and *in vitro* and against vaccinia virus *in vitro* (40). The active agent(s) is referred to as propionin. Ramanathan et al. (174) succeeded in partial purification of propion-

in A by organic-solvent extraction, followed by Sephadex, and paper chromatography. Preliminary identification indicated propionin to be peptide in nature. The name propionin was applied to what was at first assumed to be a single antiviral substance, but it is now clear that several propionins are present. Later, Ramanathan et al. (176) described the complete chemical purification of propionin into B and C types. Ramanathan et al. (175) investigated a high-molecular-weight substance with lethal (not antiviral) activity against lymphocytic choriomeningitis virus infection in mice. They described its method of isolation and presented preliminary data on its chemical nature.

LIPIDS AND PHOSPHOLIPIDS

Certain morphological and physiological similarities exist between various genera of microorganisms. Mannose-containing phospholipids are well characterized components of mycobacteria. Recently a phosphatidyl-myoinositol monomannoside has been identified in *P. shermanii* by Brennen and Ballou (19), which was considered as an additional factor indicating a close taxonomic relationship between the genera *Propionibacterium* and *Mycobacterium*. Prottey and Ballou (170) studied the lipids of *P. shermanii* in detail and found that the most abundant mannose-containing lipid is diacyl myoinositol monomannoside. Small amounts of decanoic, lauric, palmitic, and stearic acids are present, but the major fatty acids have not as yet been identified.

SLIME PRODUCTION

Extracellular material of gelatinous consistency is formed by many genera of bacteria, particularly those showing mucoid growth phases. The material may be a firmly adherent, discrete cover layer on each cell. This form is commonly called a capsule. Capsule-forming strains of bacteria often produce much loose slime in addition to capsules. Propionibacteria have been reported to produce slime in growth media, although there is no mention in the literature of capsule formation. In 1921, Sherman and Shaw (189) noted that, in a suitable nutrient broth, propionibacteria exhibit a heavy slimy growth. Skogen (198) studied the various factors that influence the production of slime by these bacteria. He found that, as with other genera, pH of the medium, temperature of incubation, the carbon source, and variation within strains were the primary influencing factors. Skogen's results from paper and thin-layer chromatographic analyses of the capsular material indicated that mannose and lesser amounts of glu-

cose and galactose were present in the loose slime and capsules produced by a *Propionibacterium zeae* strain.

The function slime serves in these microbes is unknown. It can only be hypothesized that it serves to protect the cell against bacteriophage attack or other deleterious conditions. Its immunological properties also should not be ignored. Attempts to isolate phage from propionibacteria have not been successful in our laboratories (*unpublished data*, ISU). Formation of slime by these microorganisms may be a partial reason for these failures.

COMMENTS

Nutrition of propionibacteria has been extensively investigated and much of the early literature on this bacterial genus is concerned with this subject. The absolute requirements for their nutrition is known, and additional research specifically in this area has not seemed necessary. Since these organisms play an important role in the manufacture of Emmental cheeses, development of a completely selective medium for differentiation and enumeration of propionibacteria would be an important aid in investigation of the many problems associated with growth or lack of growth of these organisms in cheese. Since Emmental cheeses are manufactured using a combination of different bacterial starters, and because of the constant presence of adventitious flora in the product at all stages of curing, selectivity in differentiating the propionibacteria from the over-all population is necessary. Present procedures require verification of colony identity by laborious isolation and identification. Further research on selective enumeration of propionibacteria would aid investigators trying to determine the exact role of propionibacteria in the cheese-curing process.

With regard to propionic-acid fermentation, we have seen that CO_2 exchange with a carboxyl group does not provide a reliable indication of a primary fixation reaction in the propionate biosynthesis, since exchange could occur in reactions that will not yield a net fixation of CO_2 . The CO_2 involved in carbon fixation is the active species rather than HCO_3^- , as speculated, in reactions catalyzed by phosphoenolpyruvate carboxytransphosphorylase. In regard to the requirement for metal cations, thus far it has not been possible to isolate the apoenzyme from carboxytransphosphorylase. It seems that an apoenzyme is formed, but it also has been shown that this apoenzyme has an unusually high affinity for both EDTA and Co^{++} . These results lend considerable support to the conclusion that the apoenzyme is a metalloprotein. Conversion or exchange of P_i into PP_i requires both type I and type II metals and

phosphoenolpyruvate. Furthermore, conversion of PP_i into P_i and phosphoenolpyruvate requires both type I and type II metals and CO_2 . The CO_2 evidently is required to form oxaloacetate via the phosphoenolpyruvate carboxytransphosphorylase reaction, and then the PP_i is converted to phosphoenolpyruvate by reversing the same reaction. Evidence has been presented that the pyruvate reaction may involve a heavy metal complexed by thiol and, thus, inhibited. Therefore, in the presence of thiols, the pyruvate reaction is practically eliminated when the concentration of CO_2 is high. The unequivocal demonstration that carboxytransphosphorylase is a metalloprotein must await the results of metal analysis. Proof has thus far not been obtained because only a limited amount of enzyme has been available.

One of the breakthroughs relative to the mechanism of action of biotin in fixation reactions is the discovery by Lynen's group (131) that biotin can be carboxylated enzymatically. We have seen that the reaction catalyzed by the methylmalonyl-oxaloacetic transcarboxylase is a new type of biochemical reaction that permits movement of carboxyl groups from compounds involved in one metabolic pathway to compounds involved in another pathway without the intervention of free CO_2 . Transcarboxylase is a biotin-containing enzyme that catalyzes the reversible carboxylation of pyruvate by methylmalonyl-CoA with the formation of oxaloacetate and propionyl-CoA. Within this enzyme, several subunits have been isolated, and it is certain that there is a 6 S subunit that contains cobalt and zinc and a 1.3 S subunit that contains biotin. This finding is particularly important in light of the results of kinetic studies, which indicate that separate reaction sites exist for the two half-reactions of transcarboxylase and that the biotin ring may migrate between the sites. Structural and catalytic similarities among all the biotin enzymes lead one to speculate that a system involving half-reactions that occur on separate subunits may be a common feature of the biotin enzymes. All the evidence with transcarboxylase, kinetic data, location of the metal and biotin on different subunits, and the results of oxalate inhibition, are in accord with the view that there is migration of the biotin between regions of the enzyme. It seems that this may be a general property of all biotin enzymes. One wonders if the transcarboxylase of the propionibacteria is but one example of a group of transcarboxylases. For economy and control of reactions, it would seem advantageous for the cell to be able to transfer carboxyl groups just as phosphates are transferred. Carboxyl groups, like phosphates, may be utilized in coupled reactions

instead of being generated each time they are needed.

Much of the preliminary work on acetoin-diacetyl production by propionibacteria has been concerned with determination of optimum conditions of production. Discovery of the pathway by which these compounds are synthesized may provide additional insight into the metabolic diversity of these organisms.

Finally, a comment should be made on an area of research which has largely been neglected. This is the investigation of amino-acid biosynthesis in propionibacteria, and contrary to earlier opinion, these organisms are able to synthesize a large number of amino acids. There are reports in the literature concerning the ability of propionibacteria to synthesize intermediates involved in amino-acid metabolism and their ability to transaminate a variety of compounds from an amino acid donor, but these reports are few and are not of a comprehensive nature (29, 56, 85, 104, 146).

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REFERENCES

- Allen, S. H. G., R. Kellermeyer, R. Stjernholm, B. Jacobson, and H. G. Wood. 1963. The isolation, purification and properties of methylmalonyl racemase. *J. Biol. Chem.* 238: 1637-1642.
- Allen, S. H. G., R. W. Kellermeyer, R. Stjernholm, and H. G. Wood. 1963. Partial reconstruction of the propionic acid fermentation with purified enzymes. *Fed. Proc.* 22:355.
- Allen, S. H. G., R. W. Kellermeyer, R. Stjernholm, and H. G. Wood. 1964. Purification and properties of enzymes involved in the propionic acid fermentation. *J. Bacteriol.* 87:171-187.
- Allen, S. H. G., H. G. Wood, R. L. Stjernholm, and R. W. Kellermeyer. 1962. Separation and purification of methylmalonyl-CoA racemase and isomerase from propionic acid bacteria. *Fed. Proc.* 21:244.
- Antila, M. 1954. Über die Propionsäurebakterien im Emmentaler Käse Meijerit. *Aikakausk.* 16:1-132.
- Antila, M. 1956/57. Der Aminosäurebau durch Propionsäurebakterien. *Meijerit. Aikakausk.* 18/19:1-6.
- Antila, M. 1956/57. Die Bildung von Azetoin und Diazetyl bei Propionsäurebakterien. *Meijerit. Aikakausk.* 18/19:7-14.
- Antila, M., and M. Hietaranta. 1953. Growth inhibition of propionic acid bacteria by propionate. *Meijerit. Aikakausk.* 15:3-10.
- Antila, M., and M. Hietaranta. 1953. The inhibitory effect of lactates on growth and fermentation of propionic acid bacteria. *Meijerit. Aikakausk.* 15:11-19.
- Antila, V., and M. Antila. 1968. The content of free amino acids in Finnish cheese. *Milchwissenschaft* 23: 597-602.
- Arkad'eva, Z. A., and L. I. Vorob'eva. 1966. Sran-vietel' roe izuchenie 7 shtammov propionokislykh bakterii v svyazis obrazovaniem vitamina B₁₂. (Comparative study of 7 strains of propionic acid bacteria with respect to their production of vitamin B₁₂). *Vestn. Mosk. Univ.* 21:84-87.
- Ayres, G. C. deMello, and F. J. S. Lara. 1962. Fumarate hydratase from *Propionibacterium pentosaceum*. *Biochim. et Biophys. Acta* 62:435-437.
- Barban, S., and S. J. Ajl. 1951. Interconversion of propionate and succinate by *Propionibacterium pentosaceum*. *J. Biol. Chem.* 192:63-72.
- Barker, H. A., and F. Lipmann. 1944. On lactic acid metabolism in propionic acid bacteria and the problem of oxido-reduction in the system fatty-hydroxyketo acid. *Arch. Biochem.* 4:361-370.
- Barker, H. A., and F. Lipmann. 1949. The role of phosphate in the metabolism of *Propionibacterium pentosaceum*. *J. Biol. Chem.* 179:247-257.
- Barker, H. A., R. D. Smith, H. Wissbach, J. I. Toohey, J. N. Ladd, and B. E. Volcani. 1960. Isolation and properties of crystalline cobamide coenzymes containing benzimidazole or 5, 6-dimethylbenzimidazole. *J. Biol. Chem.* 235:480-488.
- Barosinski, B. 1966. Some aspects of the mechanism of biosynthesis of vitamin B₁₂ in cells of *Propionibacterium shermanii*. *Bull. Acad. Polon. Sci. Ser. Sci. Biol.* 14:143-147.
- Bauchop, T., and S. R. Elsdén. 1960. The growth of microorganisms in relation to their energy supply. *J. Gen. Microbiol.* 23:457-469.
- Brennen, P., and C. E. Ballou. 1968. Phosphatidylmyoinositol monomannoside in *Propionibacterium shermanii*. *Biochem. Biophys. Res. Commun.* 30:69-75.
- Berger, J. M., M. J. Johnson, and W. H. Peterson. 1938. The proteolytic enzymes of bacteria. II. The peptidases of some common bacteria. *J. Bacteriol.* 36:521-545.
- Berge's manual of determinative bacteriology. 1957. 7th ed. The Williams and Wilkins Co., Baltimore, Md.
- Bernhauer, K., R. Wagner, and W. Zahn. 1965. Synthesen auf dem Vitamin B₁₂-Gebiet. XIX. Darstellung und biosynthetische Verwertbarkeit von N-(Cobryl)-1-aminoäthan-2-phosphonsäure und N-(Cobryl-1-aminoäthylthiophosphate. *Biochem. Z.* 342:207-216.
- Borisova, T. G., Z. L. Loginova, T. A. Palagina, and N. A. Rabinovich. 1968. Conditions affecting the biosynthesis of true vitamin B₁₂ from its analogs in an aqueous suspension of *Propionibacterium shermanii*. (Engl. Sum.) *Prikl. Biokhim. Mikrobiol.* 4:153-156.
- Bukin, V. N., and G. V. Pronyakova. 1960. The biosynthesis of vitamin B₁₂ and porphyrins by *Propionibacterium*. *J. Biochem. (Tokyo)* 47:781-786.
- Bullerman, L. B., and E. C. Berry. 1966. Use of cheese whey for vitamin B₁₂ production. I. Whey solids and yeast extract levels. *Appl. Microbiol.* 14:353-355.
- Bykhovskii, V. Ya., G. V. Mantrova, and N. I. Zaitseva. 1969. Effect of the age of the culture and cultivation conditions on the synthesis of vitamin B₁₂ and porphyrins by *Propionibacterium shermanii*. (Engl. Sum.) *Priklad. Biokhim. Mikrobiol.* 5:32-36.
- Bykhovskii, V. Ya., N. I. Zaitseva, and V. N. Bukin. 1969. Some aspects of the regulation of the biosynthesis of vitamin B₁₂ and porphyrins in *Propionibacterium shermanii*. *Dokl. Akad. Nauk. SSSR* 185:459-461.
- Bykhovskii, V. Ya., N. I. Zaitseva, and G. V. Mantrova. 1964. On the use of delta-aminolevulinic acid for the biosynthesis of vitamin B₁₂ by resting cells of *Propionibacterium shermanii*. (Engl. Sum.) *Dokl. Akad. Nauk. SSSR* 157:692-695.

29. Cagen, L. M., and H. C. Friedmann. 1968. Enzymatic phosphorylation of serine by inorganic pyrophosphate. *Biochem. Biophys. Res. Commun.* 33:528-533.
30. Carson, S. F. 1953. Significant advances in the study of carbon metabolism in microorganisms. *Proc. VIth Intern. Congr. Microbiol.* (Rome).
31. Carson, S. F., D. S. Anthony, B. Kuna, B. J. Bachmann, and M. Long. 1948. Studies on the mechanism of the propionic acid fermentation. *Bacteriol. Proc.* 12:G24.
32. Carson, S. F., J. W. Foster, S. Ruben, and H. A. Barker. 1941. Radioactive carbon as an indicator of carbon dioxide utilization. V. Studies on the propionic acid bacteria. *Proc. Natl. Acad. Sci. U.S.A.* 27:229-235.
33. Carson, S. F., J. W. Foster, S. Ruben, and M. D. Kamen. 1940. Radioactive carbon as a tracer in the synthesis of propionic acid from CO₂ by the propionic acid bacteria. *Science* 92:433-434.
34. Carson, S. F., and S. Ruben. 1940. CO₂ assimilation by propionic acid bacteria studied by the use of radioactive carbon. *Proc. Natl. Acad. Sci. U.S.A.* 26:422-426.
35. Chaix, P., and C. Fromageot. 1937. Sur la nécessité de substances sulfurées pour la réalisation de la glycolyse bactérienne. *Enzymologia*, 1:321-327.
36. Chaix, P., and C. Fromageot. 1942. Influence of Co on the respiration of *Propionibacterium pentosaceum*. *Trav. Membres. Bull. Soc. Chim. Biol.* 24:1128-1131.
37. Chaix-Audemard, P. 1940. Sur les relations existant entre respiration et fermentation chez *Propionibacterium pentosaceum*. Thesis: Fac. Sci., Lyon, 104 p. (Engl. Sum.)
38. Cooper, T. G., T. T. Tchen, H. G. Wood, and C. R. Benedict. 1968. The carboxylation of phosphoenolpyruvate and pyruvate. I. The active species of "CO₂" utilized by phosphoenolpyruvate carboxykinase, carboxytransphosphorylase, and pyruvic carboxylase. *J. Biol. Chem.* 243:3857-3863.
39. Coulter, C. L., S. W. Havkinson, and H. C. Friedmann. 1969. Isolation and molecular structure of vitamin B₁₂ 5'-phosphate. *Biochim. Biophys. Acta.* 177:293-302.
40. Cutting, W. C., A. Furst, D. Read, D. Grant, H. Cords, J. Megna, and E. Butterworth. 1960. Antiviral extracts from propionibacteria. *Antibiot. and Chemother.* 10:623-625.
41. Davis, J. J., J. M. Willard, and H. G. Wood. 1969. Phosphoenolpyruvate carboxytransphosphorylase. III. Comparison of the fixation of carbon dioxide and the conversion of phosphoenolpyruvate and phosphate into pyruvate and pyrophosphate. *Biochemistry* 8:3127-3136.
42. Delwiche, E. A. 1948. Mechanism of propionic acid formation by *Propionibacterium pentosaceum*. *J. Bacteriol.* 56:811-820.
43. Delwiche, E. A. 1949. Vitamin requirements of the genus *Propionibacterium*. *J. Bacteriol.* 58:395-398.
44. Delwiche, E. A. 1950. A biotin function in succinic acid decarboxylation by *Propionibacterium pentosaceum*. *J. Bacteriol.* 59:439-442.
45. Delwiche, E. A., and S. F. Carson. 1953. A citric acid cycle in *Propionibacterium pentosaceum*. *J. Bacteriol.* 65:318-321.
46. Delwiche, E. A., E. F. Phares, and S. F. Carson. 1956. Succinic acid decarboxylation system in *Propionibacterium pentosaceum* and *Veillonella gazogenes*. I. Activation, decarboxylation and related reactions. *J. Bacteriol.* 71:598-603.
47. Demeter, K. J., and A. Janoschek. 1941. Vorkommen und Entwicklung der Propionsäurebakterien in verschiedenen Käsearten. *Zentralbl. Bakt. II. Abt.* 103:257-271.
48. Difco Manual. 1953. 9th ed. Difco Laboratories, Detroit, Mich. p. 125.
49. Dorner, W. 1943. Allgemeine und milchwirtschaftliche Mikrobiologie. Frauenfeld und Leipzig. P. 200.
50. Eggerer, H., P. Overath, F. Lynen, and E. R. Stadtman. 1960. On the mechanism of the cobamide coenzyme dependent isomerization of methylmalonyl-CoA to succinyl-CoA. *J. Amer. Chem. Soc.* 82:2643-2644.
51. El-Hagarawy, I. S. 1960. Factors affecting the organic acid production by propionibacteria used in the manufacture of Swiss cheese. *Diss. Abstr.* 20:2459.
52. El-Hagarawy, I. S., W. J. Harper, and W. L. Slatter. 1957. Organic acid production of propionibacteria. II. Effect of growth accessory substances on propionic and acetic acid production in milk. *J. Dairy Sci.* 40:707-712.
53. El-Hagarawy, I. S., W. L. Slatter, and W. J. Harper. 1957. Organic acid production by propionibacteria. I. Effect of strains, pH, carbon source, and intermediate fermentation products. *J. Dairy Sci.* 40:579-587.
54. El-Hagarawy, I. S., W. L. Slatter, W. J. Harper, and I. A. Gould. 1954. Factors affecting the organic acid production of propionibacteria used in manufacture of Swiss cheese. *J. Dairy Sci.* 37:638.
55. Eldredge, E. E., and L. A. Rogers. 1914. The bacteriology of cheese of the Emmental type. *Centr. Bacteriol. Parasitenk. (Abt. II).* 40:5.
56. Ellfolk, N. 1953. Studies on aspartase. I. Quantitative separation of aspartase from bacterial cells and its partial purification. *Acta Chem. Scand.* 7:824-830.
57. Emilsson, E., and G. Sjöström. 1947. The preparation of sterile experimental cheese on the laboratory scale, and some experiments on the ability of some bacteria to breakdown citric acid in cheese. *Svenska Mejeritidn.* 39:467-472.
58. Evans, H. J., and H. G. Wood. 1968. The mechanism of the pyruvate, phosphate dikinase reaction. *Proc. Natl. Acad. Sci. U.S.A.* 61:1448-1453.
59. Field, M. F. 1958. A study of the nature of the stimulatory effect of autoclaved glucose on the growth of propionibacteria. *Diss. Abstr.* 18:1217.
60. Field, M. F., and H. C. Lichstein. 1955. Conditions affecting the growth of propionic bacteria in synthetic media. *Bacteriol. Proc.* p. 120-121.
61. Field, M. F., and H. C. Lichstein. 1957. Factors affecting the growth of propionibacteria. *J. Bacteriol.* 73:96-99.
62. Field, M. F., and H. C. Lichstein. 1958. Growth stimulating effect of autoclaved glucose media and its relationship to the carbon dioxide requirement of propionibacteria. *J. Bacteriol.* 76:485-490.
63. Field, M. F., and H. C. Lichstein. 1958. Influence of casein hydrolysates and amino acids on glucose fermentation by *Propionibacterium freudenreichii*. *J. Bacteriol.* 76:491-494.
64. Fitz, A. 1878. Über Spaltpilzgährungen. *Ber.* 11: 1890; 1879, 12:474; 1880, 13:1309; 1882, 15:867; 1883, 16: 844; 1884, 17:1188.
65. Friedmann, H. 1965. Partial purification and properties of a single displacement trans-N-glycosidase. *J. Biol. Chem.* 240:413-418.
66. Friedmann, H. 1965. The formation of α -glycosidic 5'-nucleotides by a single displacement trans-N-glycosidase. *J. Biol. Chem.* 240:406-412.
67. Friedman, H. C. 1968. Vitamin B₁₂ biosynthesis. Evidence for a new precursor, vitamin B₁₂ 5'-phosphate. *J. Biol. Chem.* 243:2065-2075.
68. Friedmann, H. C., and D. L. Harris. 1964. On the enzymatic origin of a likely intermediate in cobalmin biosynthesis. *Fed. Proc.* 23(2 pt. 1):528.
69. Fromageot, C., and G. Bost. 1938. La fermentation du glucose par quelques bacteries propioniques croissant en "milieux definis". *Enzymologia* 2:225-240.

70. Fromageot, C., and P. Chaix. 1937. Respiration et fermentation chez *Propionibacterium pentosaceum*. *Enzymologia* 3:288.
71. Fukui, G. M. 1952. Studies on growth and respiration mechanisms of the propionic acid bacteria. Thesis, Cornell University, Ithaca, New York.
72. Gerwin, B. I., B. E. Jacobson, and H. G. Wood. 1969. Transcarboxylase. VIII. Isolation and properties of a biotin-carboxyl carrier protein. *Proc. Natl. Acad. Sci. U.S.A.* 64:1315-1322.
73. Guest, J. R., S. Friedman, D. D. Woods, and E. L. Smith. 1962. A methyl analog of cobamide coenzyme in relation to methionine synthesis in bacteria. *Nature* 195:340-342.
74. Gunsalus, I. C., B. L. Horecker, W. A. Wood. 1955. Pathways of carbohydrate metabolism in microorganisms. *Bacteriol. Rev.* 19:79-128.
75. Gurmani, S., S. P. Mistry, and B. C. Johnson. 1960. Function of vitamin B₁₂ in methylmalonate metabolism. I. Effect of a cofactor form of B₁₂ on the activity of methylmalonyl-CoA isomerase. *Biochim. Biophys. Acta* 38:187-188.
76. Hendlin, D., and M. L. Rugar. 1950. The effect of cobalt on the microbial synthesis of LLD-active substances. *Science* 111:541-542.
77. Hettinga, D. H., E. R. Vedamuthu, and G. W. Reinbold. 1968. Pouch method for isolating and enumerating propionibacteria. *J. Dairy Sci.* 51:1707-1709.
78. Hietaranta, M., and M. Antila. 1953. The influence of biological nutrients on the growth of propionic acid bacteria in milk. XIII Int. Dairy Congr. 3:1428-1431.
79. Hietaranta, M., and M. Antila. 1954. Some aspects of citric acid breakdown in Emmental cheese. *Mejerit. Finl. Svensk.* 16:91-94.
80. Hitchner, E. R. 1934. Some physiological characteristics of the propionic acid bacteria. *J. Bacteriol.* 28:473-479.
81. Holten, D., and H. J. Fromm. 1961. Purification and properties of erythritol kinase from *Propionibacterium pentosaceum*. *J. Biol. Chem.* 236:2581-2584.
82. Hunter, J. E., and W. C. Frazier. 1961. Gas production by associated Swiss cheese bacteria. *J. Dairy Sci.* 44:2176-2186.
83. Ierusalimskii, N. D. 1967. Biochemical mechanisms of the regulation of the growth rate of microorganisms. (Engl. Sum.) *Izv. Akad. Nauk. SSSR Ser. Biol.* 3:339-350.
84. Janicki, J., F. Peđziwilk, J. Skupin, J. Kowalczyk, K. Nowakowska, and K. Trojanowska. 1967. The role of corrinoid protein complexes in the synthesis of the methionine methyl group. (Engl. Sum.) *Bull. Acad. Pol. Sci. Ser. Sci. Biol.* 15:191-195.
85. Janicki, J., J. Skupin, and K. Nowakowska. 1963. Effect of S-adenosyl-L-methionine ("active methionine") on the biosynthesis of corrinoids by *Propionibacterium shermanii*. (Engl. Sum.) *Bull. Acad. Polon. Sci.* 11:19-21.
86. Johns, A. T. 1951. Mechanisms of the propionic acid formation by propionibacteria. *J. Gen. Microbiol.* 5:337-345.
87. Kamar, C. S., G. W. Reinbold, and R. V. Hussong. 1952. A plating method for the isolation and enumeration of propionibacteria. *J. Dairy Sci.* 35:915-919.
88. Kamikubo, T., and H. Narahara. 1967. Some observations on the pathways of biosynthesis of corrinoids and their coenzyme-forms in *Propionibacterium arabinosum*. *Vitamins (Kyoto)* 36:137-141. (Engl. Sum.)
89. Kamikubo, T., and H. Narahara. 1968. Purification of corrinoids and their coenzyme forms with Amberlite XAD-1 and XAD-2. *Vitamins (Kyoto)* 37:225-229. (Engl. Sum.)
90. Kamikubo, T., H. Narahara, K. Murai, and N. Kumamoto. 1966. Die Biosynthese von 2-Chloradenin-Cobamid und seiner Coenzym-Form sowie Versuche über die biosynthetische Verwertbarkeit von 2, 8-Dichloradenin. *Biochem. Z.* 346:159-166.
91. Kanopkaite, S. I., and A. S. Gibavichyute. 1965. Effect of some factors on the biosynthesis of vitamin B₁₂, thiamine, riboflavin, and folic acid. (Engl. Sum.) *Tr. Akad. Nauk. Lit. SSSR B(1.36)*:185-194.
92. Kanopkaite, S. I., A. I. Olyakene, and A. V. Yaroshyus. 1965. Phosphatase and phosphorylase activity of *Propionibacterium shermanii* during impaired cellular respiration and phosphorylation. (Engl. Sum.) *Tr. Akad. Nauk. Lit. SSSR B(3.38)*:279-295.
93. Katagiri, H., and Y. Ichikawa. 1953. *Propionibacterium*. *Chem. Abstr.* 47:3923.
94. Kato, T., and S. Shimizu. 1963. Chemical studies on vitamin B₁₂ and related compounds. IV. Isolation of cobinamide-like compound from fermentation products. *J. Vitaminol.* 9:94-100.
95. Kato, T., and S. Shimizu. 1963. Chemical studies on vitamin B₁₂ and its related compounds. VI. Paper-electrophoretic examination on cobamides in *Propionibacterium shermanii* cells cultivated in the presence of selected precursors. *J. Vitaminol.* 9:305-308.
96. Kato, T., and S. Shimizu. 1963. Chemical studies on vitamin B₁₂ and its related compounds. VII. Paper-electrophoretic examination on cobamides in *Propionibacterium shermanii* cells cultivated in the presence of hydroxocobalamin or cyanocobalamin. *J. Vitaminol.* 9:309-312.
97. Kato, T., and S. Shimizu. 1963. Chemical studies on vitamin B₁₂ and its related compounds. VIII. Selection of culture media for production of 5,6-dimethylbenzimidazolylcobamide coenzyme by *Propionibacterium shermanii*. *J. Vitaminol.* 9:313-316.
98. Kato, T., S. Shimizu, and S. Fukui. 1964. Chemical studies on vitamin B₁₂ and its related compounds. IX. Formation of cobinamide coenzyme by cultivation of *Propionibacterium shermanii* in the presence of cobinamide. *J. Vitaminol.* 10:89-98.
99. Kaziro, Y., L. F. Hass, P. D. Boyer, and S. Ochoa. 1962. Mechanism of the propionyl carboxylase reaction. II. Isotopic exchange and tracer experiments. *J. Biol. Chem.* 237:1460-1468.
100. Kaziro, Y., E. Leone, and S. Ochoa. 1960. Biotin and propionyl carboxylase. *Proc. Natl. Acad. Sci. U.S.A.* 46:1319-1327.
101. Keenan, T. W., and D. D. Bills. 1968. Volatile compounds produced by *Propionibacterium shermanii*. *J. Dairy Sci.* 51:797-799.
102. Kellermeyer, R. W., S. H. G. Allen, R. Stjernholm, and H. G. Wood. 1964. Methylmalonyl isomerase. IV. Purification and properties of the enzyme from propionibacteria. *J. Biol. Chem.* 239:2562-2569.
103. Kellermeyer, R. W., and H. G. Wood. 1962. Methylmalonyl isomerase. A study of the mechanism of isomerization. *Biochemistry.* 1:1124-1131.
104. Kinney, R. W., and C. H. Werkman. 1958. Transamination in *Propionibacterium jensenii*. *Iowa State Coll. J. Sci.* 32:455-461.
105. Kiuru, V. J. T. 1948. The effect of oxidation-reduction potential on the rate of development of propionic acid fermentation. *Meijerit. Aikakausk.* 10:67-74.
106. Kiuru, V. J. T. 1949. Factors affecting the velocity of propionic acid fermentation. XII. Int. Dairy Congr. 2:499-528.
107. Knappe, J. 1970. Mechanism of biotin action. *Ann. Rev. Biochem.* 39:757-776.
108. Konigk, E. 1964. Biosynthese einiger im Alkanola-

min-Anteil fluorierter B₁₂-Analogo mit Hilfe von *Propionibacterium shermanii*. Biochem. Z. 341:123-125.

109. Kramli, A., and L. I. Vorob'eva. 1964. Effect of oxidation-reduction conditions on the formation of vitamin B₁₂ by propionic acid bacteria. Mikrobiologiya 33:408-414.

110. Krane, W. 1961. Die chemisch-physikalischen Bedingungen für das Wachstum und das Gärvermögen von Propionsäurebakterien und Buttersäurebazillen. Milchwissenschaft 16:184-187, 355-359, 620-629.

111. Krebs, H. A., and L. V. Eggleston. 1941. Biological synthesis of oxaloacetic acid from pyruvic acid and carbon dioxide. II. The mechanism of carbon dioxide fixation in propionic acid bacteria. Biochem. J. 35:676-687.

112. Kurmann, J. 1960. Ein vollsynthetischer Nährboden für Propionsäurebakterien. Pathol. et Microbiol. 23:700-711.

113. Kurmann, J. 1960. Neue Beobachtungen über die Aufbewahrung und Behandlung der Propionsäurebakterienkultur. Schweiz. Milchztg. 43:1.

114. Kurmann, J. 1962. Eine methodische Verbesserung zur Bestimmung des Propionsäurebakteriengehaltes der Ementhalerkerseimilch. XVI Int. Dairy Congr. C:377-382.

115. Kurtz, F. E., J. A. Hupfer, E. A. Corbin, R. E. Hargrove, and H. E. Walter. 1958. Interrelationships between pH, populations of *Propionibacterium shermanii*, levels of free fatty acids, and the flavor ratings of Swiss cheese. J. Dairy Sci. 41:719.

116. Kurtz, F. E., J. A. Hupfer, E. A. Corbin, R. E. Hargrove, and H. E. Walter. 1959. Interrelationships between pH, populations of *Propionibacterium shermanii*, levels of free fatty acids, and the flavor ratings of Swiss cheese. J. Dairy Sci. 42:1008.

117. Langler, J. E., and E. A. Day. 1966. Quantitative analysis of the major free fatty acids in Swiss cheese. J. Dairy Sci. 49:91-93.

118. Langler, J. E., L. M. Libbey, and E. A. Day. 1967. Identification and evaluation of selected compounds in Swiss cheese flavor. J. Agr. Food Chem. 15:386.

119. Lara, F. J. S. 1959. The succinic dehydrogenase of *Propionibacterium pentosaceum*. Biochim. et Biophys. Acta 33:565-567.

120. Leaver, F. W. 1953. Formation of propionic acid from lactic acid by *Clostridium propionicum* and by *Propionibacterium arabinosum*. Fed. Proc. 12:471.

121. Leaver, F. W., H. G. Wood, and R. Stjernholm. 1955. The fermentation of three carbon substrates by *C. propionicum* and *Propionibacterium*. J. Bacteriol. 70:521-530.

122. Lee, S. Y., E. R. Vedamuthu, C. J. Washam, and G. W. Reinbold. 1969. Diacetyl production by propionibacteria. J. Dairy Sci. 52:893.

123. Lee, S. Y., E. R. Vedamuthu, C. J. Washam, and G. W. Reinbold. 1970. Diacetyl production by *Propionibacterium shermanii* in milk cultures. Can. J. Microbiol. 16:1231-1242.

124. Lengyel, P., R. Mazunder, and S. Ochoa. 1960. Mammalian methylmalonyl isomerase and vitamin B₁₂ coenzymes. Proc. Natl. Acad. Sci. U.S.A. 46:1312-1318.

125. Lichstein, H. C. 1955. The biotin requirements of the genus *Propionibacterium*. Arch. Biochem. Biophys. 58:423-430.

126. Lichstein, H. C. 1955. The presence of bound biotin in purified preparations of oxaloacetic carboxylase. J. Biol. Chem. 212:217-222.

127. Lichstein, H. C. 1957. Bound biotin in oxaloacetic carboxylase preparations. Arch. Biochem. Biophys. 71:276-277.

128. Lichstein, H. C. 1958. On the specificity of biotin in the metabolism of propionibacteria. Arch. Biochem. Biophys. 77:378-386.

129. Lochmüller, H., H. G. Wood, and J. J. Davis. 1966. Phosphoenolpyruvate carboxytransphosphorylase. II. Crystallization and properties. J. Biol. Chem. 241:5678-5691.

130. Lutskova, M. 1966. Production of vitamin B₁₂ in form of biomass by means of microbiological synthesis on whey nutrient medium. XVII Int. Dairy Congr. E/F:75-77.

131. Lynen, F., J. Knappe, E. Lorch, G. Jütting, and E. Ringelmann. 1959. Die Biochemische Funktion des Biotins. Agnew. Chem. 71:481-486.

132. Majchrzak, R. S. Nawrocki, and B. Czarnocka-Rocznikowa. 1966. Optimal medium for synthesis of coenzymes by propionibacteria. Acta Microbiol. Pol. 15:173-186.

133. Malik, A. C., G. W. Reinbold, and E. R. Vedamuthu. 1968. An evaluation of the taxonomy of *Propionibacterium*. Can. J. Microbiol. 14:1185-1191.

134. Mazunder, R., T. Sasakawa, Y. Kaziro, and S. Ochoa. 1961. A new enzyme in the conversion of propionyl coenzyme A to succinyl coenzyme A. J. Biol. Chem. 236:PC53.

135. Menon, I., Aravindakshan, and D. Shemin. 1967. Concurrent decrease of enzymic activities concerned with the synthesis of coenzyme B₁₂ and of propionic acid in propionibacteria. Arch. Biochem. Biophys. 121:304-310.

136. Minkevich, I. G., N. V. Stepanova, and N. D. Ierusalimskii. 1968. Optimization of the working conditions of a device for continuous cultivation of propionic bacteria. Mikrobiologiya 37:298-307.

137. Moat, A. G., and E. A. Delwiche. 1950. Utilization of coenzyme A by *Propionibacterium freudenreichii*. J. Bacteriol. 60:757-762.

138. Molinari, R., and F. J. S. Lara. 1960. The lactic dehydrogenase of *Propionibacterium pentosaceum*. Biochem. J. 75:57-65.

139. Muller, O., and G. Muller. 1963. Zur chemie und Biochemie der "Cobalamine." XXIV. Weitere Synthesen von Corrinoid-Coenzym-Analogen und Umwandlung einiger dieser Derivate durch *Propionibacterium shermanii*. Biochem. Z. 337:179-194.

140. Neronova, N. M., and S. I. Ibragimova. 1969. Fermentation of volatile acids and vitamin B₁₂ by *Propionibacterium shermanii*, growing in continuous culture with an excess of lactate. Mikrobiologiya 38:420-424.

141. Neronova, N. M., S. I. Ibragimova, and N. D. Ierusalimskii. 1967. Effect of propionate concentration on specific growth rate of *Propionibacterium shermanii*. Mikrobiologiya 36:404-409.

142. Neronova, N. M., N. D. Ierusalimskii, and A. I. Anchurova. 1962. Formation of vitamin B₁₂ by propionic acid bacteria with respect to conditions of cultivation. Mikrobiologiya 31:165-169.

143. Neujahr, H. Y., W. G. Kurz, and G. Rossi-Ricci. 1960. Studies on vitamin B₁₂ production by *Propionibacterium shermanii* grown in sewage. Arkiv. Kemi. 15:363-374.

144. Niethammer, A., and M. Hitzler. 1960. Synthetic culture of *Propionibacterium* (Orla-Jensen) van Niel. Zbl. Bakt., Abt. II 113:478-479.

145. Nieuwenhof, F. F. J., J. Stadhouders, and G. Hup. 1969. Stimulating effect of lactobacilli on the growth of propionibacteria in cheese. Neth. Milk Dairy J. 23:287-289.

146. Nollstadt, K., L. Klein, and I. Ukstins. 1962. Stimulation of methionine methyl synthesis in an enzyme system from pig liver by *Propionibacterium shermanii* boiled extract. Fed. Proc. 21:4.

147. Northrop, D. B. 1969. Transcarboxylase: VI. Kinetic analysis of the reaction mechanism. J. Biol. Chem. 244:5808-5819.

148. Northrop, D. B., and H. G. Wood. 1969. Transcarboxylase: V. The presence of bound zinc and cobalt. J.

Biol. Chem. 244:5801-5807.

149. Northrop, D. B., and H. G. Wood. 1969. Transcarboxylase: VII. Exchange reactions and kinetics of oxalate inhibition. *J. Biol. Chem.* 244:5820-5827.

150. Orla-Jensen, S. 1898. Studien über die Lochbildung in den Emmentaler Käsen. *Zentr. Bakteriolog. Parasitenk., Abt. II* 4:217, 265, 325.

151. Orla-Jensen, S. 1931. *Dairy bacteriology*. 2nd ed. P. Blakiston's Son and Co., Inc., Philadelphia, Penn.

152. Overath, P., G. M. Kellerman, and F. Lynen. 1962. Zum Mechanismus der Umlagerung von Methylmalonyl-CoA in Succinyl-CoA. II. Versuche zur Wirkungsweise von Methylmalonyl-CoA Isomerase und Methylmalonyl-CoA Racemase. *Biochem. Z.* 335:500-518.

153. Overath, P., E. R. Stadtman, G. M. Kellerman, and F. Lynen. 1962. Zum Mechanismus der Umlagerung von Methylmalonyl-CoA in Succinyl-CoA. III. Reinigung und Eigenschaften der Methylmalonyl-CoA Isomerase. *Biochem. Z.* 336:77-98.

154. Park, H. S., G. W. Reinbold, E. G. Hammond, and W. S. Clark, Jr. 1967. Growth of propionibacteria at low temperatures. *J. Dairy Sci.* 50:589-591.

155. Pawelkiewicz, J. 1955. Forerunners in the biosynthesis of nucleotide cyanocobal amine. III. The influence of aureomycin on the synthesis of the vitamin group B₁₂ with propionic acid bacteria. *Acta Biochim. Pol.* 2:321-327.

156. Pawelkiewicz, J. 1955. Precursors of the biosynthesis of vitamins of the B₁₂ group. *Bull. Acad. Pol. Sci. Cl. II* 3:3-4.

157. Pawelkiewicz, J., and A. B. Legocki. 1963. Propionate (acetate) kinase of *Propionibacterium shermanii*. *Bull. Acad. Pol. Sci.* 11:569-572.

158. Peltola, E. 1940. Effect of salt on bacteria of importance in Emmental cheese-making. *Meijerit. Aikakausk.* 2:11-21.

159. Peltola, E., and M. Antila. 1953. The effect of oxidizing salts on the oxidation-reduction potential and ripening of Emmental cheese. *XIII Int. Dairy Congr.* 2:729-731.

160. Peppler, H. J. 1967. *Microbial technology*. Reinhold Publishing Corp., New York, N. Y. p. 287.

161. Perlman, D., and J. M. Barrett. 1958. Biosynthesis of cobalamin analogs by *Propionibacterium arabinosum*. *Can. J. Microbiol.* 4:9-15.

162. Pett, L. B., and A. M. Wynne. 1933. The metabolism of propionic acid bacteria. I. The degradation of phosphoric acid esters by *Propionibacterium jensenii* (van Niel). *Trans. Roy. Soc. Can., Sec. V*, 27:119.

163. Phares, E. F., and S. F. Carson. 1960. Role of methylmalonyl-CoA and biotin in bacterial succinate decarboxylation. *Bact. Proc.* p. 154.

164. Phares, E. F., E. A. Delwiche, and S. F. Carson. 1956. Succinic acid decarboxylation systems in *Propionibacterium pentosaceum* and *Veillonella gazogenes*. II. Evidence for an active "C₁" complex. *J. Bacteriol.* 71:604-610.

165. Phares, E. F., M. V. Long, and S. F. Carson. 1960. Properties of methylmalonyl isomerase of *Propionibacterium pentosaceum*. *Proc. Amer. Chem. Soc.* 138:21c.

166. Phelps, A. S., M. J. Johnson, and W. H. Peterson. 1939. Carbon dioxide utilization during the dissimilation of glycerol by the propionic acid bacteria. *Biochem. J.* 33:726-728.

167. Pomerantz, S. H. 1958. A mass analysis study of the metabolism of propionate by *Propionibacterium arabinosum*. *J. Biol. Chem.* 231:505-517.

168. Pomerantz, S. H., and H. G. Wood. 1958. A mass analysis study of formaldehyde fixation and cleavage of lactate by *Propionibacterium arabinosum*. *J. Biol. Chem.* 231:

519-531.

169. Pronyakova, G. V. 1960. Biosynthesis of vitamin B₁₂ and porphyrins in *Propionibacterium* cultures. *Biokhimiia (Engl. Transl.)* 25:223-229.

170. Prottey, C., and C. E. Ballou. 1968. Diocyl myoinositol monomannoside from *Propionibacterium shermanii*. *J. Biol. Chem.* 243:6196-6201.

171. Pulay, G., S. N. Toth, and A. R. Bakos. 1959. Importance of iron in the metabolism of some microorganisms of importance to the dairy industry. *XV Int. Dairy Congr.* 2:775-781.

172. Quastel, J. H., and D. M. Webley. 1941. Vitamin B₁ and bacterial oxidations. I. Dependence of acetic acid oxidation on vitamin B₁. *Biochem. J.* 35:192-206.

173. Quastel, J. H., and D. M. Webley. 1942. Vitamin B₁ and bacterial oxidations. II. The effects of magnesium, potassium and hexosediphosphate ions. *Biochem. J.* 36:8-33.

174. Ramanathan, S., G. Read, and W. C. Cutting. 1966. Purification of propionin, an antiviral agent from propionibacteria. *Proc. Soc. Exp. Biol. Med.* 123:271-273.

175. Ramanathan, S., E. Furusawa, and W. C. Cutting. 1968. An anti-ICM agent from propionibacteria. *Chemotherapy* 13:271-275.

176. Ramanathan, S., C. Walyne, and W. C. Cutting. 1968. Antiviral principles of propionibacteria. Isolation and activity of propionics B and C. *Proc. Soc. Exp. Biol. Med.* 129:73-77.

177. Rappoport, D. A., and H. A. Barker. 1954. Fermentation of arabinose-1-C¹⁴ by propionic acid bacteria. *Arch. Biochem. Biophys.* 49:249-251.

178. Renz, R. 1968. Reaction sequence in the enzymatic synthesis of vitamin B₁₂ from cobinamide in *Propionibacterium shermanii*. *Hoppe Seyler's Z. Physiol. Chem.* 349:979-981.

179. Ritter, P., and H. Schwab. 1968. Cheesemaking experiments concerning the effect of micrococci on the activity of propionic acid bacteria in Emmental cheese. *Schweiz. Milchztg.* 94(14) *Wiss. Beil. Nr.* 117:977-988.

180. Ritter, P., H. Schwab, and H. Holzer. 1967. Testing the stimulatory or inhibitory effect of micrococci on propionic bacteria. *Schweiz. Milchztg.* 93(34) *Wiss. Beil. Nr.* 113:929-930.

181. Rodzevich, V. I., T. G. Borisova, A. A. Shilova, N. G. Misyureva, G. M. Dobrolinskaya, and O. P. Kozhemyakina. 1967. Use of the starch enzymic hydrolysate in nutrient media for *Propionibacterium shermanii*. (Engl. Sum.) *Priklad. Biokhim. Mikrobiol.* 3:259-263.

182. Rollman, N., and G. Sjöström. 1946. The behavior of some propionic acid bacteria strains against NaCl, NaNO₃, and heating. *Svenska Mejeritidningen (Engl. Transl.)* 38(19):199-201; (20):209-212.

183. Ronzio, R. A., and H. A. Barker. 1967. Enzymic synthesis of guanosine diphosphate cobinamide by extracts of propionic acid bacteria. *Biochemistry* 6:2344-2354.

184. Rose, I. A., E. L. O'Connell, P. Noce, M. F. Utter, H. G. Wood, J. M. Willard, T. G. Cooper, and M. Benziman. 1969. Stereochemistry of the enzymatic carboxylation of phosphoenolpyruvate. *J. Biol. Chem.* 244:6130-6133.

185. Sathyanarayana Rao, S., and D. R. Washington. 1964. Influence of cobalt on *Propionibacterium freudenreichii*. *Nature* 202:212-213.

186. Shaposhnikov, V. N., S. A. Arkad'eva, L. I. Vorob'eva, and L. G. Kolobova. 1969. Effect of organic acids on vitamin B₁₂ synthesis by *Propionibacterium shermanii*. *Priklad. Biokhim. Mikrobiol. (Engl. Transl.)* 5:105-107.

187. Shaposhnikov, V. H., and L. I. Vorob'eva. 1963. Development of propionic acid bacteria and synthesis of vita-

- min B₁₂ in synthetic and natural media. *Mikrobiologiya*. 32:204-208.
188. Shaw, R. H., and J. M. Sherman. 1923. The production of volatile fatty acids and carbon dioxide by propionic acid bacteria with special reference to their action in cheese. *J. Dairy Sci.* 6:303-309.
189. Sherman, J. M., and R. H. Shaw. 1921. Associative bacterial action in the propionic acid fermentation. *J. Gen. Physiol.* p. 657.
190. Sherman, J. M., and R. H. Shaw. 1923. The propionic acid fermentation of lactose. *J. Biol. Chem.* 56:695-700.
191. Shetter, J. K. 1956. Formation of D-erythritol 4-phosphate by *Propionibacterium pentosaceum*. *J. Amer. Chem. Soc.* 78:3722-3723.
192. Shtikkel', E. I., R. K. Seidalina, and R. M. Dzyubanova. 1967. Changes in the properties of propionic acid bacteria in nutritive media with ascorbic acid. (Engl. Sum.) *Izv. Akad. Nauk. Kaz. SSSR Ser. Biol.* 2:36-39.
193. Siegel, L., and S. Englard. 1961. Beef-heart malic dehydrogenases. I. Properties of the enzyme purified from extracts of acetone-dried powders. *Biochim. Biophys. Acta* 54:67-76.
194. Silverman, M., and C. H. Werkman. 1938. Vitamin B₁ in bacterial metabolism. *Proc. Soc. Exp. Biol. Med.* 38:823-827.
195. Silverman, M., and C. H. Werkman. 1939. Adaptation of the propionic acid bacteria to vitamin B₁ synthesis including method of assay. *J. Bacteriol.* 38:25-32.
196. Siu, P. M. L., H. G. Wood, and R. L. Stjernholm. 1961. Fixation of CO₂ by phosphoenolpyruvic carboxytransferase. *J. Biol. Chem.* 236:PC 21-22.
197. Siu, P. M. L., and H. G. Wood. 1962. Phosphoenolpyruvic carboxytransferase, a carbon-dioxide fixation enzyme from propionic acid bacteria. *J. Biol. Chem.* 237:30-44-3051.
198. Skogen, L. O. 1970. Capsulation of *Propionibacterium*. Unpublished M. S. Thesis. Iowa State University Library, Ames, Iowa.
199. Smith, R. M., and K. J. Monty. 1959. Vitamin B₁₂ and propionate metabolism. *Biochem. Biophys. Res. Commun.* 1:105-110.
200. Snell, E. E., F. M. Strong, and W. H. Peterson. 1939. Growth factors for bacteria. VIII. Panthothenic and nicotinic acids as essential growth factors for lactic and propionic acid bacteria. *J. Bacteriol.* 38:293-308.
201. Stadtman, E. R. 1952. The purification and properties of phosphotransacetylase. *J. Biol. Chem.* 196:527-534.
202. Stadtman, E. R., P. Overath, H. Eggerer, and F. Lynen. 1960. The role of biotin and vitamin B₁₂ coenzyme in propionate metabolism. *Biochem. Biophys. Res. Commun.* 2:1-7.
203. Stefaniak, O., B. Lubienka, and M. Janicka. 1968. Isolation and purification of corrinoids synthesized by *Propionibacterium shermanii*. *Acta Microbiol. Pol.* 17:83-90.
204. Stefaniak, O., and M. Muszynska. 1969. Effect of pH and temperature on the content of corrinoids released from *Propionibacterium shermanii*. *Acta Microbiol. Pol.* 18:25-29.
205. Stepanova, N. V., Y. M. Romanovshii, and N. D. Ierusalimskii. 1965. A mathematical model for the growth of microorganisms in a continuous culture. (Engl. Sum.) *Dokl. Akad. Nauk. SSSR* 163:1266-1269.
206. Stjernholm, R. 1958. Formation of trehalose during dissimilation of glucose by *Propionibacterium*. *Acta Chem. Scand.* 12:646-649.
207. Stjernholm, R. 1960. Fermentation of D-ribose-1-C¹⁴ or glucose-1-or 2-C¹⁴ with extracts of *Propionibacterium shermanii*. *Bacteriol. Proc.* 60:181.
208. Stjernholm, R., and F. Flanders. 1962. Metabolism of D-ribose-1-C¹⁴ and C¹⁴-labeled D-gluconate in an enzyme system of the genus *Propionibacterium*. *J. Bacteriol.* 84:563-568.
209. Stjernholm, R., and H. G. Wood. 1960. Trehalose and fructose as indicators of metabolism of labeled glucose by the propionic acid bacteria. *J. Biol. Chem.* 235:2753-2756.
210. Stjernholm, R., and H. G. Wood. 1960. Glycerol dissimilation and occurrence of a symmetrical three-carbon intermediate in propionic acid fermentation. *J. Biol. Chem.* 235:2757-2761.
211. Stjernholm, R., and H. G. Wood. 1961. Methylmalonyl isomerase: II. Purification and properties of the enzyme from propionibacteria. *Proc. Natl. Acad. Sci. U.S.A.* 47:303-312.
212. Stjernholm, R., and H. G. Wood. 1963. The symmetrical C₃ in the propionic acid fermentation and the effect of avidin on propionate formation. *Iowa State J. Sci.* 38:123-140.
213. Stolp, H. 1955. Ernährungsphysiologische Untersuchungen an anaeroben Bakterien. I. Zur Ernährungsphysiologie der Clostridien. *Arch. Mikrobiol.* 21:273-293.
214. Stone, R. W., and C. H. Werkman. 1936. Role of phosphoglyceric acid in the dissimilation of glucose by the propionic acid bacteria. *Iowa State Coll. J. Sci.* 10:341-343.
215. Stone, R. W., and C. H. Werkman. 1937. The occurrence of phosphoglyceric acid in the bacterial dissimilation of glucose. *Biochem. J.* 31:1516-1523.
216. Stone, R. W., H. G. Wood, and C. H. Werkman. 1937. Phosphorylation and first stages in glucose breakdown by propionic acid bacteria. *J. Bacteriol.* 33:101.
217. Swick, R. W. 1959. Evidence for a transcarboxylation reaction in propionibacteria. *Proc. Am. Chem. Soc.* 136:70c.
218. Swick, R. W. 1961. The conversion of acrylate to succinate in propionibacteria. *Fed. Proc.* 20(1 pt 1):80.
219. Swick, R. W. 1962. Propionic acid metabolism: Mechanism of the methylmalonyl isomerase reaction and the reduction of acrylyl coenzyme A to propionyl coenzyme A in propionibacteria. *Proc. Natl. Acad. Sci. U.S.A.* 48:288-293.
220. Swick, R. W., and G. G. Moss. 1961. Propionic acid metabolism. II. The isomerization of methylmalonyl coenzyme A. U. S. Atomic Energy Comm. Res. and Develop. Rept. ANL-6464. 110-113.
221. Swick, R. W., and H. G. Wood. 1960. The role of transcarboxylation in propionic acid fermentation. *Proc. Natl. Acad. Sci. U.S.A.* 46:28-41.
222. Tamao, Y., T. Kato, S. Shimizu, and S. Fukui. 1964. Chemical and biochemical studies of vitamin B₁₂ and its related compounds. XIII. Comparison of availabilities of cyanocobalamin and hydroxocobalamin in 5,6-dimethylbenzimidazolyl cobamide coenzyme formation by *Propionibacterium shermanii*. *J. Vitaminol.* 10:211-217.
223. Tamao, Y., Y. Morikawa, S. Shimizu, and S. Fukui. 1968. Coenzyme activity of 5'-deoxyadenosyl-10-chlorocobalamin in propanediol dehydratase system. *Biochim. Biophys. Acta* 151:260-266.
224. Tatum, E. L., E. B. Fred, H. G. Wood, and W. H. Peterson. 1936. Essential growth factors for propionic acid bacteria. I. Sources and fractionation. II. Nature of the Neuberg precipitate fraction of potato: replacement by ammonium sulphate or by certain amino acids. *J. Bacteriol.* 32:157-166, 167-174.
225. Thompson, R. C., 1943. The B-vitamin requirements of the propionibacteria. *J. Bacteriol.* 46:99-104.
226. Tittsler, R. P. 1940. The influence of hydrogen ion concentration upon the growth of *Propionibacterium*. *J.*

- Bacteriol. 39:95-96.
227. Tittler, R. P., and G. P. Sanders. 1953. Effects of pH on the growth of *Propionibacterium shermanii* and its relation to the quality of Swiss cheese. *J. Dairy Sci.* 36:574.
228. Trotman-Dickenson, A. F. 1959. Free radicals. John Wiley and Sons, New York, N. Y. p. 10.
229. Tomka, G. 1949. Acetoin and diacetyl production of the rod shaped propionic acid bacteria. XII Intern. Dairy Congr., Proc. 2:619-622.
230. Vandemark, P. J., and G. M. Fukui. 1956. An enzymatic study of the utilization of gluconic acid by *Propionibacterium pentosaceum*. *J. Bacteriol.* 72:610-614.
231. Van Niel, C. B. 1928. The propionic acid bacteria. J. W. Boissevain and Co., Haarlem. (thesis: Delft).
232. Vedamuthu, E. R., and G. W. Reinbold. 1967. The use of candle oats jar incubation for the enumeration, characterization and taxonomic study of propionibacteria. *Milch-wissenschaft* 22:428-431.
233. Virtanen, A. I. 1923. Über die Propionsäuregärung. *Soc. Sci. Fennica, Comment. Physic.-Math.* 1:1-23. 1925, 2:1-13.
234. Virtanen, A. I. 1934. On the enzymes of bacteria and bacterial metabolism. *J. Bacteriol.* 28:447-460.
235. Volk, W. A. 1954. The effect of fluoride on the permeability and phosphatase activity of *Propionibacterium pentosaceum*. *J. Biol. Chem.* 208:777-784.
236. Volk, W. A. 1956. Formation of L-arabinose phosphate by extracts from *Propionibacterium pentosaceum*. *Fed. Proc.* 15:376.
237. Volk, W. A. 1959. The enzymatic formation of D-arabinose 5-phosphate from L-arabinose and adenosine triphosphate (ATP) by *Propionibacterium pentosaceum*. *J. Biol. Chem.* 234:1931-1936.
238. Volk, W. A. 1960. Purification and properties of phosphoarabinoisomerase from *Propionibacterium pentosaceum*. *J. Biol. Chem.* 235:1550-1553.
239. Von Freudenreich, E., and S. Orla-Jensen. 1906. Über die Emmentalerkäse stattfindende Propionsäuregärung. *Zentr. Bakteriol. Parasitenk. Abt. II* 17:529.
240. Vorob'eva, L. I. 1958. Carbon balance in fermentation of lactic and pyruvic acids by propionic acid bacteria. *Mikrobiologiya* 27:286-291.
241. Vorob'eva, L. I. 1958. Conditions of glycerol fermentation by propionic acid bacteria. *Dokl. Akad. Nauk. SSSR* 121:641-644.
242. Vorob'eva, L. I. 1959. Effect of aeration on propionic acid fermentation. *Mikrobiologiya* 28:209-214.
243. Vorob'eva, L. I., G. A. Golozubova, and V. I. Safonov. 1968. Catalase of propionic acid bacteria. *Priklad. Biokhim. Mikrobiol.* 4:654-658.
244. Vorob'eva, L. I., S. F. Kozyreva, and L. L. Lisenkova. 1967. Effect of light on metabolism of propionic acid bacteria. *Mikrobiologiya* 36:410-415.
245. Vorob'eva, L. I., and V. S. Kuznetsova. 1964. Effect of $MnSO_4$ on vitamin B₁₂ production by propionic acid bacteria. *Mikrobiologiya* 33:26-30.
246. Vorob'eva, L. I., A. A. Lukin, R. M. Muslekh, and N. A. Baranova. 1967. Effect of ethylenimine and UV-rays on vitamin B₁₂ biosynthesis and other specific physiological-biochemical features of *Propionibacterium shermanii*. (Engl. Sum.) *Vestn. Mosk. Univ. Ser. 6 Biol. Pochvoved* 22:41-44.
247. Wawzkiewicz, E. J. 1968. Erythritol metabolism by *Propionibacterium pentosaceum*. The role of L-erythrulose 1-phosphate. *Biochemistry* 7:683-687.
248. Wawzkiewicz, E. J., and H. A. Barker. 1968. Erythritol metabolism by *Propionibacterium pentosaceum*. The over-all reaction sequence. *J. Biol. Chem.* 243:1948-1956.
249. Werkman, C. H., R. W. Stone, and H. G. Wood. 1937. The dissimilation of phosphate esters by the propionic acid bacteria. *Enzymologia* 4:24-30.
250. Werkman, C. H., and H. G. Wood. 1942. Heterotrophic assimilation of carbon dioxide. *Advances in Enzymology* 2:135-182.
251. Whiteley, H. R. 1953. The mechanism of propionic acid formation by succinate decarboxylation. I. The activation of succinate. *Proc. Natl. Acad. Sci. U.S.A.* 39:772-779.
252. Whiteley, H. R. 1953. The mechanism of propionic acid formation by succinate decarboxylation. II. The formation and decarboxylation of succinyl CoA. *Proc. Natl. Acad. Sci. U.S.A.* 39:779-785.
253. Whittier, E. O., and J. M. Sherman. 1923. Propionic acid and ketones from whey. *Ind. Eng. Chem.* 15:229-231.
254. Wierzbicka, H., A. B. Legocki, and J. Pawelkiewicz. 1967. Determination of acetate and propionate using propionate (acetate) kinase from *Propionibacterium shermanii*. *Acta Biochem. Pol.* 14:1-6.
255. Wiggert, W. P., and C. H. Werkman. 1939. Fluoride sensitivity of *Propionibacterium pentosaceum* as a function of growth conditions. *Biochem. J.* 33:1061-1069.
256. Willard, J. M., J. J. Davis, and H. G. Wood. 1969. Phosphoenolpyruvate carboxytransferase. IV. Requirement for metal cations. *Biochemistry* 8:3137-3144.
257. Winkler, S. 1953. The inhibitory action of propionic acid bacteria. XIII Int. Dairy Congr. 3:1164-1167.
258. Wood, H. G. 1934. The physiology of the propionic acid bacteria. Thesis, Iowa State (College) University, Ames, Iowa.
259. Wood, H. G. 1968. Mechanism of formation of oxaloacetate and phosphoenolpyruvate from pyruvate. *J. Vitaminol.* 14:59-67.
260. Wood, H. G., S. H. G. Allen, R. Stjernholm, and B. Jacobson. 1963. Transcarboxylase. III. Purification and properties of methylmalonyloxaloacetic transcarboxylase containing tritiated biotin. *J. Biol. Chem.* 238:547-556.
261. Wood, H. G., A. A. Andersen, and C. H. Werkman. 1938. Nutrition of the propionic acid bacteria. *J. Bacteriol.* 36:201-214.
262. Wood, H. G., J. J. Davis, and H. Lochmüller. 1966. The equilibria of reactions catalyzed by carboxytransferase, carboxykinase, and pyruvate carboxylase and the synthesis of phosphoenolpyruvate. *J. Biol. Chem.* 241:5692-5704.
263. Wood, H. G., E. Erb, and C. H. Werkman. 1937. Dissimilation of pyruvic acid by the propionic acid bacteria. *Iowa State Coll. J. Sci.* 11:287-292.
264. Wood, H. G., J. J. Davis, and J. M. Willard. 1969. Phosphoenolpyruvate carboxytransferase. V. Mechanism of the reaction and the role of metal ions. *Biochemistry* 8:3145-3155.
265. Wood, H. G., and F. W. Leaver. 1952. Fermentation of glucose-3, 4-C¹⁴ and glucose-1-C¹⁴ by *Propionibacterium arabinosum*. *Fed. Proc.* 11:313-314.
266. Wood, H. G., and F. W. Leaver. 1953. CO₂ turnover in the fermentation of 3, 4, 5 and 6 carbon compounds by the propionic acid bacteria. *Biochim. Biophys. Acta* 12:207-222.
267. Wood, H. G., R. G. Kulka, and N. L. Edson. 1956. The metabolism of glucose-1-C¹⁴ in an enzyme system from *Propionibacterium*. *Biochem. J.* 63:177-182.
268. Wood, H. G., H. Lochmüller, C. Riepertinger, and F. Lynen. 1963. Transcarboxylase. IV. Function of biotin and the structure and properties of the carboxylated enzyme. *Biochem. Z.* 337:247-266.
269. Wood, H. G., and R. Stjernholm. 1961. Transcar-

boxylase. II. Purification and properties of methylmalonyl-oxaloacetic transcarboxylase. Proc. Natl. Acad. Sci. U.S.A. 47:289-303.

270. Wood, H. G., and R. Stjernholm. 1962. Assimilation of carbon dioxide by heterotrophic organisms. The bacteria. Vol. III p. 41-118, Academic Press, New York, N. Y.

271. Wood, H. G., R. Stjernholm, and F. W. Leaver. 1955. The metabolism of labeled glucose by the propionic acid bacteria. J. Bacteriol. 70:510-520.

272. Wood, H. G., R. Stjernholm, and F. W. Leaver. 1956. The role of succinate as a precursor of propionate in the propionic acid fermentation. J. Bacteriol. 72:142-152.

273. Wood, H. G., R. W. Stone, and C. H. Werkman. 1937. The intermediate metabolism of the propionic acid bacteria. Biochem. J. 31:349-359.

274. Wood, H. G., E. L. Tatum, and W. H. Peterson. 1937. Growth factors for bacteria. IV. An acidic ether-soluble factor essential for growth of propionic acid bacteria. J. Bacteriol. 33:227-242.

275. Wood, H. G., and C. H. Werkman. 1934. Pyruvic acid in the dissimilation of glucose by the propionic acid bacteria. Biochem. J. 28:745-747.

276. Wood, H. G., and C. H. Werkman. 1934. The propionic acid bacteria: On the mechanism of glucose dissimilation. J. Biol. Chem. 105:63-72.

277. Wood, H. G., and C. H. Werkman. 1936. Mechanism of glucose dissimilation by the propionic acid bacteria. Biochem. J. 30:618-623.

278. Wood, H. G., and C. H. Werkman. 1936. The utilization of CO₂ in the dissimilation of glycerol by the propionic acid bacteria. Biochem. J. 30:48-53.

279. Wood, H. G., and C. H. Werkman. 1938. The utilization of CO₂ by the propionic acid bacteria. Biochem. J. 32:1262-1271.

280. Wood, H. G., and C. H. Werkman. 1940. The fixation of CO₂ by cell suspensions of *Propionibacterium pentosaceum*. Biochem. J. 34:7-14.

281. Wood, H. G., and C. H. Werkman. 1940. The relationship of bacterial utilization of CO₂ to succinic acid formation. Biochem. J. 34:129-137.

282. Wood, H. G., C. H. Werkman, A. Hemingway, and A. O. Nier. 1941. Heavy carbon as a tracer in heterotrophic carbon dioxide assimilation. J. Biol. Chem. 139:365-376.

283. Wood, H. G., C. H. Werkman, A. Hemingway, and A. O. Nier. 1941. Note on the degradation of propionic acid synthesized by *Propionibacterium*. Iowa State Coll. J. Sci. 15:213-214.

284. Wood, H. G., C. H. Werkman, A. Hemingway, and A. O. Nier. 1941. The position of carbon dioxide carbon in succinic acid synthesized by heterotrophic bacteria. J. Biol. Chem. 139:377-381.

285. Yousten, A. A., and E. A. Delwiche. 1961. Biotin and vitamin B₁₂ coenzymes in succinate decarboxylation by *Propionibacterium pentosaceum* and *Veillonella alcalescens*. Bact. Proc. 61:175.

286. Zodrow, K. 1963. Studies on the effect of 5,6-dimethyl benzimidazole on the biosynthesis of corrinoids by propionibacteria. Acta Microbiol. Pol. 12:61-68.

287. Zodrow, K., J. Chelkowski, O. Stefaniak, and D. Czarnecka. 1963. The effect of different casein hydrolysates on the growth and biosynthesis of corrinoids by propionibacteria. Acta Microbiol. Pol. 12:259-262.

288. Zodrow, K., O. Stefaniak, J. Chelkowski, and K. Szcsepska. 1963. Influence of Ca-pantothenate and biotin on the growth and biosynthesis of corrinoids by propionibacteria. Acta Microbiol. Pol. 12:263-266.

289. Zodrow, K., and O. Stefaniak. 1963. Effect of temperature on the growth and the production of corrinoids by *Propionibacterium shermanii*. Acta Microbiol. Pol. 12:271-280.

THE LEADERSHIP ROLE (Continued from Page 435)

need resources, some need active support. The environmentalist to various degrees can offer help in each of these situations.

- (f) Realize that pressures for relevant programming are now coming from citizens up through the bureaucratic chain, and are problem-centered; instead of the traditional system in which programs come down from the Federal level, and are program-centered.

The time remaining, during which the environmentalist can take a leadership role, is rapidly dwindling, and in some areas it may already be too late. The ecological movement is demanding action at a faster rate than bureaucratic organizations are accustomed to respond; indeed faster than they may be able to respond. The demand for more rapid

action may well lead to changes within organizations as well as changes in structure. Because of the time loss involved in communicating up and down the several layers of authority, a condition seemingly intrinsic to bureaucracy, we may well find it necessary to delegate greater responsibility for decision-making to the lowest levels. There is a built-in danger of error in doing this, but there is an even greater danger in continuing the bureaucratic game of "buck-passing" with its attendant delay, spreading of responsibility, and flaccid decisions. We must accept the fact that mistakes will be made, that better training programs are a must, and that criticism will become a way of life. The alternative is to remain with customary programs and approaches in a traditional structure, insulated from the heat (and from the problems), self-confident in our increasingly superfluous knowledge and skills, as contemporary society with its plethora of problems passes us by.

E-3-A SANITARY STANDARDS FOR STORAGE TANKS FOR EGGS AND EGG PRODUCTS

Serial #E-0100

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

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

A.

SCOPE

A.1

These standards cover the sanitary aspects of storage tanks for eggs and egg products.

A.2

In order to conform with these E-3-A Sanitary Standards, storage tanks shall comply with the following design, material, fabrication, and cleaning criteria.

B.

DEFINITIONS

B.1

Product: Shall mean the eggs and egg product stored in the tank.

B.2

Storage Tank: Shall mean a cylindrical, rectangular, oval or other equally satisfactory shape tank except a vertical tank whose inside height is in excess of 10 feet¹ and the tank is used for the storage or storage and cooling of a product.

B.3

Product Contact Surfaces: Shall mean all surfaces which are exposed to the product and surfaces from which liquids may drain, drop, or be drawn into the product.

B.4

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

B.5

Mechanical Cleaning or Mechanically Cleaning: shall denote cleaning, solely by circulation and/or

¹Vertical tanks in excess of 10 feet inside height are defined as silo-type tanks.

flowing chemical detergent solutions and water rinses onto and over the surfaces to be cleaned, by mechanical means.

C.

MATERIALS

C.1

All product contact surfaces shall be of stainless steel of the AISI 300 series² or corresponding ACI³ types (See Appendix, Section E.), or stainless steel that is non-toxic and non-absorbent and which under conditions of intended use is equally corrosion resistant to stainless steel of the AISI 300 series² or corresponding ACI³ types, except that:

C.1.1

Rubber and rubber-like materials may be used for umbrellas for vertical agitator assemblies, gaskets, seals and parts used in similar applications. These materials shall comply with the applicable provisions of the "E-3-A Sanitary Standards for Multiple-Use Rubber and Rubber-Like Materials Used as Product Contact Surfaces in Egg Processing Equipment, Serial #E-1800".

C.1.2

Plastic Materials may be used in sight and/or light openings and for umbrellas for vertical agitator assemblies, bearings, gaskets, seals, and parts used in similar applications. These materials shall comply with the applicable provisions of the "3-A San-

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

³Alloy Casting Institute Division, Steel Founders' Society of America, 21010 Center Ridge Road, Rocky River, Ohio 44116.

itary Standards for Multiple-Use Plastic Materials Used as Product Contact Surfaces for Dairy Equipment, Serial #2000", as amended.

C.1.3

Where functional properties are required for specific applications, such as agitator bearing surfaces and rotary seals, where dissimilar materials are necessary, carbon and/or ceramics may be used. Ceramic materials shall be inert, non-porous, non-toxic, non-absorbent, insoluble, resistant to scratching, scoring, and distortion by the temperature, chemicals, and methods to which they are normally subjected in operation or cleaning and bactericidal treatment.

C.1.4

Glass may be used in sight and/or light openings and when used shall be of a clear heat resistant type.

C.2

The materials used for the lining shall not be less than No. 14 U. S. standard gauge.

C.3

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

D.**FABRICATION****D.1**

All product contact surfaces shall be at least as smooth as a No. 4 mill finish on stainless steel sheets. (See Appendix, Section F.)

D.2

All permanent joints in product contact surfaces shall be welded. All welded areas of product contact surfaces shall be at least as smooth as the adjoining surfaces.

D.3

All product contact surfaces shall be easily accessible for cleaning, either when in an assembled position or when removed. Removable parts shall be readily demountable.

D.4

All product contact surfaces shall be self draining except for normal clingage. The bottom pitch of a vertical tank designed for mechanical cleaning shall be at least 3/4 inch per foot toward the outlet. Horizontal rectangular tanks designed for

mechanical cleaning which have a built-in bottom pitch, shall have a pitch of at least 1/4 inch per foot toward the outlet.

Horizontal tanks shall be so constructed that they will not sag, buckle, or prevent complete drainage of water when the tank has a pitch of not more than 1 inch in 100 inches. (See D.22).

D.5

If it is necessary to enter the tank to clean any or all of the product contact surfaces, the tank shall have the following minimum dimensions:

- (1) 36-inches in height by 48-inches in diameter, or 48-inches square.
- (2) 36-inches in height, 36-inches in width, by 48-inches in length, if oval or rectangular.

D.6

The inside radii of all welded or permanent attachments shall be not less than 1/4 inch. Where the head(s) joins the lining of the tank the radius shall not be less than 3/4 inch.

D.7

There shall be no threads on product contact surfaces.

D.8

Sanitary pipe and fittings shall conform with "E-3-A Sanitary Standards for Fittings Used on Egg and Egg Products Equipment and Used on Sanitary Lines Conducting Egg and Egg Products, Serial #E-0800".

D.9

One or more fittings to accommodate indicating and/or recording temperature sensing devices shall be provided.

D.9.1

They shall conform to one of the following types:

D.9.1.1

The applicable fittings found in the "E-3-A Sanitary Standards for Instrument Fittings and Connections Used on Egg Products Equipment, Serial #E-0900", as amended and supplements thereto.

D.9.1.2

Fittings for temperature sensing devices which do not pierce the tank lining, but which have temperature sensing element receptacles securely attached to exterior of the lining.

D.9.2

The fittings for temperature sensing devices shall be located to permit the registering of the temperature of the product when the tank contains no more than 20% of its capacity.

D.10

The outlet shall be located where readily accessible and in a position to provide complete drainage of the tank. The top of the terminal end of the outlet passage shall be lower than the low point of the bottom of the lining at the outlet. The outside diameter of the outlet opening shall be at least as large as that of 1-1/2 inch E-3-A sanitary tubing.

D.11

Inlet and Outlet connections in the tank shall be provided with welding stub ends, bolted or clamp type flanges or E-3-A sanitary threaded connections. The face of a bolted or clamp type flange or a E-3-A sanitary threaded connection below the maximum normal product level shall be as close as practical to the outer shell of the tank. (See Appendix, Section G and Section H.)

D.12

The manhole shall be located at the outlet end or side of the tank or the top of the tank. The inside dimensions of the manhole opening shall not be less than 15" × 20" oval, or 18" diameter.

A top manhole opening shall be not less than 3/8 inch higher than the surrounding area and if the exterior flange is incorporated in it, it shall slope and drain away from the opening. The sleeve or collar of a manhole opening for an inside swing type manhole cover shall be pitched so that liquids cannot accumulate.

D.13

The cover for a manhole in the end or side wall shall be either of the inside or outside swing type. If the cover swings inside, it shall also swing outside, away from the opening. Threads or ball joints employed to attach the manhole cover(s) shall not be located within the lining. The cover for a manhole in the top shall be of the outside swing type.

D.14

Gaskets shall be removable. Any gasket groove or gasket retaining groove shall not exceed 1/4 inch in depth or be less than 1/4 inch wide. The minimum radius of any internal angle in a gasket groove or gasket retaining groove shall be not less than 1/8 inch.

D.15

Unless otherwise specified, means for mechanical agitation of product shall be provided, that when operated intermittently or continuously, will be sufficient to maintain uniformity of product throughout the tank. The agitator, if not designed for mechanical cleaning, shall be located in such a manner that it shall be readily accessible and re-

movable for manual cleaning. The opening for a vertical agitator shall have a minimum diameter of 1 inch on tanks which require removal of the agitator shaft for cleaning or be of a diameter that will provide a 1 inch minimum annular space between the agitator shaft and the inside surface of the opening on a tank which does not require removal of the agitator for cleaning. An umbrella or drip shield of sanitary design that can be raised or dismantled, to permit cleaning of all of its surfaces, shall be provided to protect against the entrance of dust, oil, insects and other contaminants into the tank through the annular space around the agitator shaft. The agitator shaft, if removable, shall be provided with an easily accessible, readily demountable coupling of either a sanitary type located within the lining or a coupling located outside the lining provided that it is above the umbrella provided to protect the annular space around the shaft. A bottom support or guide, if used, shall be welded to the lining and shall not interfere with drainage of the tank and the inside angles shall have minimum radii of 1/8 inch. When the agitator shaft has a bearing cavity, the diameter of the cavity shall be greater than the depth. A seal for the agitator shaft, if provided, shall be of a packless type, sanitary in design with all parts readily accessible for cleaning. A sanitary seal for the agitator shaft shall be provided for (1) a horizontal agitator, (2) a vertical agitator when it is specified that the tank is to be located so that the portion of the shaft outside the tank is not in a processing area (See D.26.) and (3) an agitator in a tank having means for mechanically cleaning the tank.

D.16

A sample cock shall be provided. It shall be of a type that has its sealing surface relatively flush with the product contact surface of the tank and have an inside diameter no less than that of one inch E-3-A sanitary tubing.

D.17

Sight and light openings, when provided, shall be of such design and construction that the inner surfaces drain inwardly, and if the tank is designed for mechanical cleaning, the inner surface of the glass (or plastic) shall be relatively flush with the inner surface of the lining. The inside diameter of the opening shall be at least 3-3/4 inches. The external flare of the opening shall be pitched so that liquid cannot accumulate.

D.18

An opening for a pressure transmitter, if provided, shall be in a portion of the tank that is in the

processing area, and if the tank is designed for mechanical cleaning, the transmitter shall be relatively flush with the inner surface of the lining.

D.19

An opening for a gauge, if provided, shall be in the portion of the tank in the processing area. The inside diameter of the opening shall be not less than 1.75 inches.

D.20

A hooded air vent of sufficient free opening area to prevent back pressure during filling and to prevent vacuum during emptying of the tank shall be provided in the front head near the top of the tank or in the top of the tank. (See Appendix, Section K.) The vent shall terminate in a processing area and shall drain into the tank. It shall be provided with a perforated cover having openings not greater than 1/16 inch diameter, or slots not more than 1/32 inch wide. Woven wire mesh shall not be used for this purpose. It shall be so designed that parts are readily accessible and readily removable for cleaning.

D.21

Storage tanks having an inside height of more than 96 inches shall be provided with means (see suggestions Appendix, Section I) that will facilitate manual cleaning and inspection of all product contact surfaces or means shall be provided for mechanically cleaning the product contact surfaces of the tank and all non-removable appurtenances thereto (See suggestions Appendix, Section J).

D.22*Means of supporting tanks***D.22.1**

The means of supporting tanks designed to be installed wholly within a processing area shall be one of the following:

D.22.1.1

With legs. Adjustable legs shall be provided of sufficient number and strength and so spaced that the filled tank will be adequately supported. Legs shall have sealed bases. Exterior of legs and leg sockets shall be readily cleanable. Legs shall be such that the product outlet is sufficiently high to allow for adequate cleaning and will provide an 8-inch minimum clearance between the floor and the tank outlet valve or bracing whichever is lower. The legs of cylindrical horizontal tanks shall be installed so that the leg will be vertical when the tank lining is pitched 1/4 inch per foot toward the outlet.

D.22.1.2

Mounted on a slab or island. The base of the tank

shall be such that it may be sealed to the mounting surface. (See Appendix, Section L.)

D.23

The tank shall be insulated with insulating material of a nature and amount sufficient to prevent, in 18 hours, an average temperature change of greater than 2° F in the tank full of water when the average difference between the temperature of the atmosphere surrounding the tank is 30° F above or below that of the water in the tank, provided that the insulating material shall have an insulating value equivalent of not less than 2 inches of cork. Tanks specified for installation partially outside of a building shall be insulated with insulation material having an insulating value of not less than 3 inches of cork over non-refrigerated areas. (See D.26). Insulation material shall be installed in such a manner as to prevent shifting or settling.

D.24

Non-product contact surfaces shall be free of pockets and crevices and be readily cleanable and those to be coated shall be effectively prepared for coating.

D.25

The outer shell shall be smooth and effectively sealed except for a vent or weep hole in the outer shell of the tank. The vent or weep hole shall be located in a position that will provide drainage from the outer shell and shall be vermin proof. Outside welds need not be ground.

D.26

Storage tanks shall have an information plate in juxtaposition to the name plate giving the following information or the information shall appear on the name plate:

- (a) The insulating value of the insulation as expressed in the following or a similar statement:
The insulation of this tank is or is equivalent to ----- inches of cork.
- (b) If the tank has a vertical agitator and a sanitary seal is not provided for the agitator shaft, the following or a similar statement shall be expressed:
This tank is designed to be located wholly within a processing area.

*APPENDIX***E.***STAINLESS STEEL MATERIALS*

Stainless steel conforming to the applicable composition ranges established by AISI² for wrought products, or by ACI³ for cast products, should be considered in compliance with the requirements

of Section C.1 herein. Where welding is involved the carbon content of the stainless steel should not exceed 0.08%. The first reference cited in C.1 sets forth the chemical ranges and limits of acceptable stainless steels of the 300 series.

Cast grades of stainless steel corresponding to types 303, 304, and 316, are designated CF-16F, CF-8, and CF-8M, respectively. These cast grades are covered by ASTM⁴ specifications A296-68 and A351-70.

F.

PRODUCT CONTACT SURFACE FINISH

Surface finish equivalent to 150 grit or better as obtained with silicon carbide, is considered in compliance with the requirements of Section D.1 herein.

G.

INLET AND OUTLET CONNECTIONS

The distance between the nearest point on the outer shell of the tank to (1) the face of a bolted or clamp type flange or (2) the face of a E-3-A sanitary threaded connection on an inlet or outlet connection below the normal product level should not exceed the smaller of (1) twice the nominal diameter of the connection or (2) five inches.

H.

VALVES

Valves on inlet and outlet connections in the tank below the maximum normal product level should be of the close coupled plug-type or of the close coupled compression-type.

I.

MANUAL CLEANING

If the inside height of a tank exceeds 96 inches, one means for manual cleaning is to weld a stainless steel rung on each end of the tank to support a removable platform at a height which will facilitate cleaning and inspection.

J.

MECHANICAL CLEANING

One cleaning method found to be satisfactory is to pump the cleaning solution to the dome of the tank or the upper portion of the tank surface, as the case may be, through stainless steel lines with C-I-P fittings or welded joints and distribute it

in such a manner as to provide flooding over all interior surfaces. The tank should be installed with sufficient pitch to accomplish draining and to have a fast flushing action across the bottom. The pitch should be at least 1/4 inch per foot. Means should be provided for manual cleaning of all surfaces not cleaned satisfactorily by mechanical cleaning procedures. NOTE: Cleaning and/or sanitizing solutions should be made up in a separate tank—not the storage tank.

K.

AIR VENTING

To insure adequate venting of the tank which will protect it from internal pressure or vacuum damage during normal operation, the critical relationship between minimum vent-size and maximum filling or emptying rates should be observed. The size of the free vent opening of a tank should be at least as large as those shown in the table below:

| Minimum Free Vent Opening Size (inches, I.D.) | Maximum Filling or Emptying Rate (gallons per minute) |
|---|---|
| 1-3/4 | 175 |
| 2-1/4 | 300 |
| 2-3/4 | 400 |

The above sizes are based on normal operation and are sized to accommodate air only and not liquid. A perforated vent cover, if used, should have a free opening area equal to at least 1-1/2 times the area of the vent opening in the tank. The venting system covered in the preceding paragraphs is intended to provide for venting during filling and emptying; however, it is not adequate during cleaning. During the cleaning cycle, tanks when cleaned mechanically should be vented adequately by opening the manhole door to prevent vacuum or pressure build up due to sudden changes in temperature of very large volumes of air⁵. Means should be provided to prevent excess loss of cleaning solution through the manhole opening. The use of tempered water of about 95° F for both pre-rinsing and post-rinsing is recommended

⁵For example, when a 6,000 gallon tank (with 800 cu. ft. of 135° F hot air after cleaning) is suddenly flash cooled by 50° F water sprayed at 100 gpm the following takes place: Within one second, the 800 cu. ft. of hot air shrinks approximately 51 cu. ft. in volume. This is the equivalent in occupied space of approximately 382 gallons of product. The shrinkage creates a vacuum sufficient to collapse the tank unless the vent, manhole, or other openings allow the air to enter the tank at approximately the same rate as it shrinks. It is obvious, therefore, that a very large vent such as the manhole opening is required to accommodate this air flow.

⁴Available from American Society for Testing and Materials, 1916 Race Street, Philadelphia, Pa. 19103.

to reduce the effect of flash heating and cooling. Provisions should be made to prevent overflowing with resultant vacuum or pressure damage to the tank.

L.

SLABS OR ISLANDS

When a tank is designed to be installed on a slab or an island, the dimensions of the slab or island should be such that the tank will extend beyond the slab or island at least one inch in all horizontal

directions. The slab or island should be of sufficient height so that the bottom of the outlet connection is not less than 8 inches above the floor. The surface of the slab or island should be coated with a thick layer of waterproof mastic material which will harden without cracking. The junction of the outer shell of the tank and the slab or island should be sealed.

These standards are effective June 28, 1972.

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NEW MANUAL KEEPS FOOD SERVICE OPERATIONS OUT OF TROUBLE

More stringent enforcement of sanitary codes and the advent of OSHA (Occupational Safety and Health Act) is making life a bit tedious for many food service operations. Governmental concern for sanitation and cleanliness in restaurants, bakeries, supermarkets, school lunch kitchens, cafeterias, hospital kitchens, fast food outlets and drive-ins have been an area of mounting problems for food service people.

A new and unique manual for easy compliance to sanitary regulations has been developed in non-commercial form by the Sparta Brush Company Inc. of Sparta, Wisconsin. The hard cover, loose leaf format includes a synopsis of the federal sanitation code, sanitizing chemicals, water temperatures, selection and use of specialized brushes and "how to" sections on the proper cleaning of equipment and utensils.

The manual was introduced at the National Restaurant Show on May 21, 1972 and according to Joe Larson, Sparta Brush president, "Food service operators and restaurant supply dealers alike welcomed this nuts and bolts manual with a sigh of relief. It helps them to keep inside the law as far as sanitary clean-up is concerned and eliminates questionable areas in the OSHA rulings." The manual covers all types of equipment and food handling utensils in all areas of food preparation and service.

Sparta Brush subsidized this non-commercial manual and is offering copies for sale at a price of \$1. Price includes a ring binder and provides for periodic revisions and updating of information on newly printed pages. Copies can be obtained by remitting \$1 to Sparta Brush Company Inc., Sparta, Wisconsin 54656. Specify manual SM-003.

FUNGI AND FOODS

"Fungi and Foods" is the title of the 7th Annual Symposium sponsored by the Western New York Section of the IFT and the New York State Agricultural Experiment Station, Geneva to be held October 19, 1972 at the Sheraton Gatehouse, Rochester, New York. The program will include seven speakers. For more information, contact D. L. Downing, New York State Agricultural Experiment Station, Geneva, New York 14456.

PROCESSORS BEGIN PRE-REGISTERING FOR FOOD & DAIRY EXPO

Advance registrations for Food & Dairy Expo '72 are flowing rapidly into the office of the sponsoring Dairy and Food Industries Supply Assn., as shown by staffers Julie McMahon and Cecil Cassidy.

Food plant personnel in a wide variety of capacities — owners, engineers, marketing directors, researchers, operations managers, purchasing agents,

management — are registering now for the October 1-5 exposition.

Expo attendees who register in advance do themselves a favor. They avoid long lines and unnecessary delays at Show-time; their Expo kits are awaiting them when they arrive at Convention Hall.

Pre-registrants receive a handy confirmation card, hotel reservation form, notice on group flight arrangements and announcement of social activities. Hotel forms include room rates and a map specifying hotel locations. Advance registrants have priority for choice hotel space.

Response so far indicates a record number of representatives from the bakery, beverage, canning, confectionery, dairy and meat industries will be in attendance. The Atlantic City Convention Hall Show will feature nearly 300 exhibits of equipment and supplies for the processing industries.

Write to DFISA, 5530 Wisconsin Ave., Washington, D. C. 20015 for registration and hotel forms.

DAIRY QUALITY CONTROL CONFERENCE

A Dairy Quality Control Conference will be held at Penn State University on October 10, and 11, 1972. This Conference is aimed at dairy plant processors and plant management personnel. Topics will include discussion of pasteurization processes and quality control measures. Other topics will include talks on soft serve and shake mix problems, quality control of Yoghurt, and acidulated dairy products.

Consumer-oriented panels will discuss attitudes of consumers regarding the dairy industry, labeling problems and nutritive value of dairy products.

Other topics will include needed change in regulatory control, and plant control of volume and fat losses.

All meetings will be held in the J. O. Keller Building on the campus of The Pennsylvania State University, University Park, Pa. 16802. Registration fee is \$25.00. For information contact the Agricultural Conference Coordinator, 410 J. O. Keller Building, University Park, Pa. 16802.

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METHODS FOR PRODUCTION OF HIGH QUALITY RAW MILK

(A Summary of Annual Reports Prepared From 1955 to 1970 by
the IAMFES Dairy Farm Methods Committee)

COMPILED AND EDITED BY

J. C. FLAKE, A. E. PARKER, J. B. SMATHERS, A. K. SAUNDERS AND E. H. MARTH

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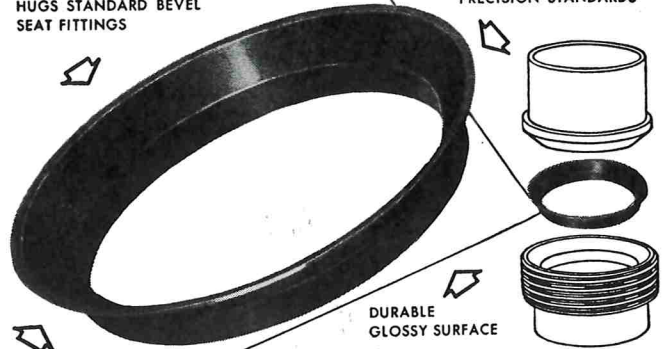
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Dairy authorities speak out
on better cow milking.



R. R. Hacker / University of Guelph / Canada

Prime the udder for maximum milk let-down

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

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

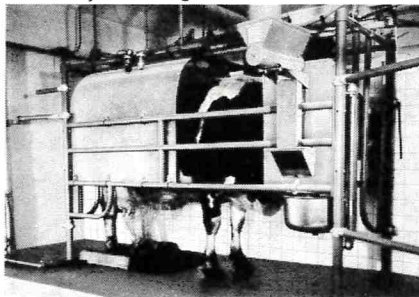
Washing or massaging the udder causes a stimulation of the nerve endings, releasing a hormone called oxytocin from the pituitary gland. In the udder, oxytocin causes a contraction of the muscle tissue thus expelling milk into larger ducts and cisterns. A recent experiment demonstrated that the sounds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

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

A milking machine should not be placed on a cow until her teats are

plump and fully distended from the pressure of the milk. Teat cups will draw in flabby teats and loose udder tissue when milk pressure is low. If this happens, the teat can be injured or partially blocked and milking time will be longer.

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



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

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

PERCENTAGES OF COWS THAT MILKED OUT IN VARIOUS INTERVALS

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

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

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

Surge... the accent is on YOU!

This is one of a series of topics developed by noted Dairy authorities. For a complete set write for a free booklet.

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