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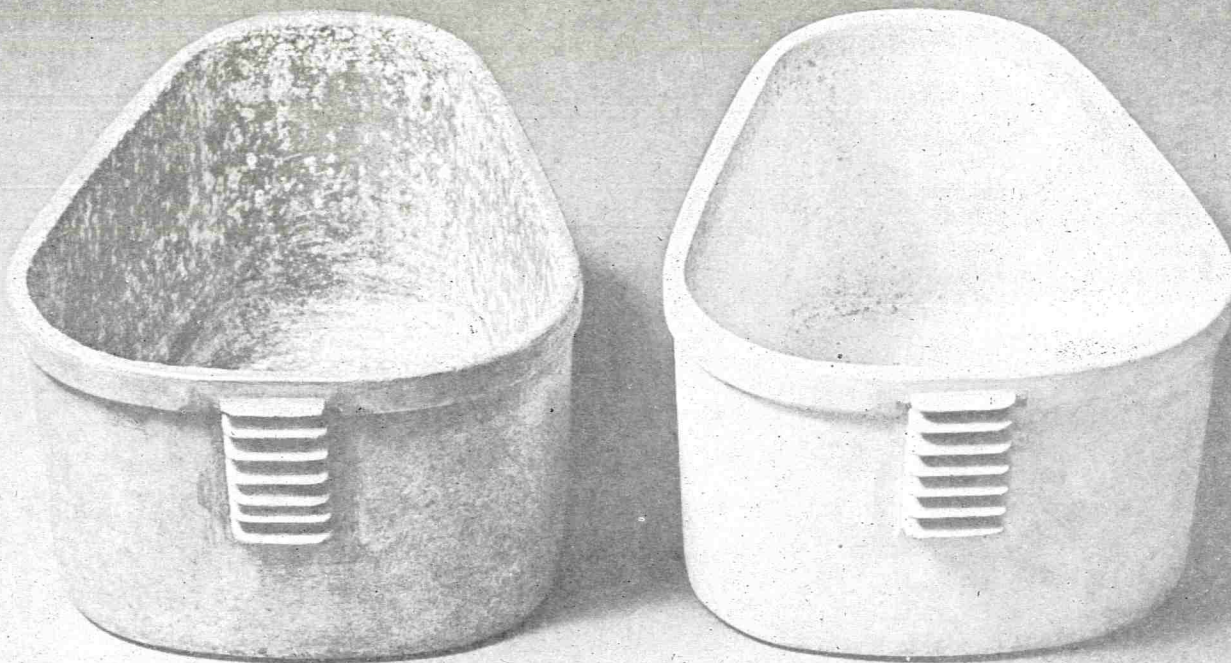
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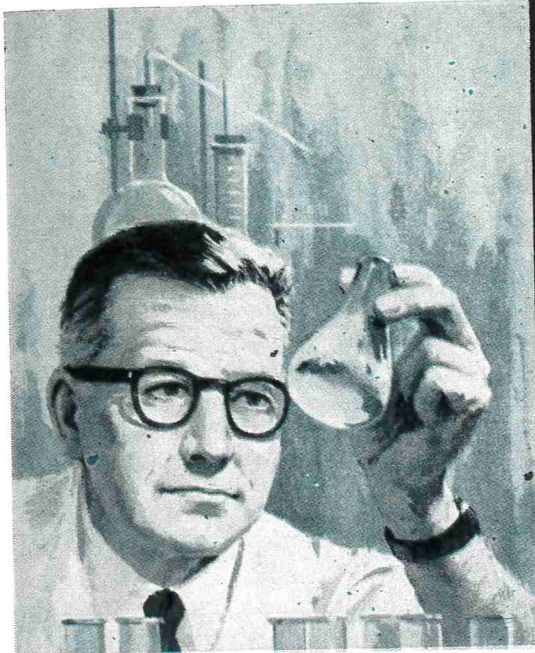
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INCLUDING MILK AND FOOD SANITATION

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Vol. 33

November, 1970

No. 11

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# THE IDENTIFICATION OF CYANIDIN-3-2<sup>G</sup> GLUCOSYL-RUTINOSIDE IN MONTMORENCY CHERRIES<sup>1</sup>

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(Received for publication July 3, 1970)

## ABSTRACT

One of the major anthocyanins in Montmorency cherries has been identified as mecocyanin (cyanidin-3-sophoroside). Evidence is presented to show that this anthocyanin is not mecocyanin but rather cyanidin-3-glucosyl-rutinoside. The identification is based on  $R_f$  values; partial and complete hydrolysis with strong acid; and infra-red, visible, and ultraviolet spectra.

The two major anthocyanin pigments of sour cherries (*Prunus cerasus* L. var. Montmorency) reportedly are antirrhinin and mecocyanin (8). Confusion exists in the literature concerning the identity of mecocyanin.

Mecocyanin was first isolated by Willstaetter and Zoellinger (14) from poppies (*Papaver rhoeas*) and identified as cyanidin diglucoside by Willstaetter and Weil (13). Li and Wagenknecht (8) identified the two major pigments of Montmorency cherries as antirrhinin (cyanidin-3-rhamnosyl- $\alpha$ 1 $\rightarrow$ 6-glucoside) and mecocyanin. Harborne (5) studied the diglucoside from *Papaver rhoeas*, concluded that it was cyanidin-3-sophoroside (glucosyl- $\beta$ 1 $\rightarrow$ 2-glucoside), and suggested this to be the structure of mecocyanin. In a study of seven varieties of cherries (excluding Montmorency) Harborne and Hall (7) found antirrhinin in all, and in six of the seven varieties they found cyanidin-3-sophoroside and a triglycoside (cyanidin-3-glucosyl-rutinoside). These authors deduced that the structure of the pigment reported as mecocyanin by Li and Wagenknecht (8) was cyanidin-sophoroside. On the basis of two-dimensional thin layer chromatography on the total pigment extracted Olden and Nybom (9) concluded that Montmorency cherries contain both cyanidin-3-sophoroside and cyanidin-3-glucosyl-rutinoside. Von Elbe et al. (12) studied the pigmentation of nine varieties of sour cherries and found that the two major pigments were cyanidin-3-rutinoside and a pigment comparable to the triglycoside observed by Olden and Nybom (9). The purpose of this report is to show that cyanidin-

3-glucosyl-rutinoside is the second major pigment in Montmorency cherries.

## MATERIALS AND METHODS

Montmorency cherries (*Prunus cerasus* L. var. Montmorency) used in this work were obtained from the University of Wisconsin Branch Experiment Station, Sturgeon Bay, Wisconsin. Cherries were pitted, individually frozen at  $-34$  C, and were stored at  $-23$  C until used.

Solvents used for chromatography were those described by Daravingas and Cain (2), and the PEW (propanol/ethyl acetate/water 7/1/2 by volume) system. The spray reagent used to visualize the sugar chromatograms was the aniline-phthalic acid reagent of Partridge (10). Isolation and purification was accomplished by the methods of Fuleki (3) and Schaller and von Elbe (11). The major bands were cut from the paper, eluted with 5% acetic acid in ethanol (v/v), the solvent was removed in vacuo at room temperature, and the pigment rechromatographed. To obtain the intermediates of acid hydrolysis the pigment was dissolved in 4N HCl and heated at 50 C (1).  $R_f$  values of the pigment were obtained in six solvent systems using descending chromatography at 20 C. The aglucone was obtained by boiling 2 mg of the pigment in 6N HCl for 30 min. The solution was cooled and the aglucone filtered, washed with 6N HCl, and dried. Identification was based on co-chromatography with synthetic cyanidin in six solvent systems, and on infrared, visible, and ultraviolet spectra.

To obtain the sugar moiety, removal of the aglucone and any unhydrolyzed pigment remaining in solution after filtration of the aglucone was accomplished by addition of a small amount of strong acid resin. A small volume of the solution was spotted onto Whatman No. 1 paper and dried under a stream of nitrogen. The sugar moiety was chromatographed against known monosaccharides in PEW. Visible and ultraviolet spectra for the pigment in 0.01% HCl/MeOH (v/v) and 0.01% HCl/EtOH were obtained with a Beckman DK-2 spectrophotometer. Infrared spectra were obtained by the KBr pellet technique on a Beckman IR-4 spectrophotometer.

## RESULTS AND DISCUSSION

Isolation of the crude pigment from the weak acid resin and separation by paper chromatography with 0.01% HCl resulted in several bands. Only the major band (Band 1; greatest  $R_f$  value) was eluted and further purified. The aglucone of this pigment, obtained by strong acid hydrolysis, was shown to be cyanidin by co-chromatography with cyanidin, and

<sup>1</sup>Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

TABLE 1. R<sub>f</sub> VALUES OF PIGMENT OF BAND I, ITS HYDROLYSIS INTERMEDIATES AND HYDROLYSIS PRODUCTS

| Pigments                                | R <sub>f</sub> values in various solvents |            |                                     |                                   |       |     | PEW<br>(R <sub>g</sub> ) <sup>1</sup> | Identification:<br>cyanidin-3- |
|---|---|------------|-------------------------------------|-----------------------------------|-------|-----|---------------------------------------|--------------------------------|
|   | 1%<br>HCl                                 | 15%<br>HAc | HAc/HCl/H <sub>2</sub> O<br>15/3/82 | HAc/HCl/H <sub>2</sub> O<br>5/1/5 | BuHCl | BAW |                                       |                                |
| Band I                                  | .61                                       | .76        | .75                                 | .88                               | .31   | .32 | —                                     | 2G-glucosyl-<br>rutinoside     |
| Hydrolyzed I                            |   |            |                                     |                                   |       |     |                                       |                                |
| Ia                                      | .36                                       | .63        | .60                                 | .83                               | .31   | .33 | —                                     | sophoroside                    |
| Ib                                      | .13                                       | .48        | .42                                 | .70                               | .35   | .40 | —                                     | rutinoside                     |
| Ic                                      | .07                                       | .33        | .25                                 | .55                               | .30   | .38 | —                                     | glucoside                      |
| Id                                      | .01                                       | .09        | .08                                 | .29                               | .69   | .64 | —                                     | cyanidin                       |
| Sugar moieties                          | —   | —          | —                                   | —                                 | —     | —   | 1.0                                   | glucose                        |
| Hydrolyzed Ia                           | —   | —          | —                                   | —                                 | —     | —   | 1.7                                   | rhamnose                       |
| Ia                                      | .07                                       | .33        | .25                                 | .55                               | —     | —   | —                                     | glucoside                      |
| Ia                                      | .01                                       | .09        | .08                                 | .29                               | —     | —   | —                                     | cyanidin                       |
| Sugar moiety                            | —   | —          | —                                   | —                                 | —     | —   | 1.0                                   | glucose                        |
| Hydrolyzed Ib                           | .07                                       | .03        | .25                                 | .55                               | —     | —   | —                                     | glucoside                      |
| Ib                                      | .01                                       | .09        | .08                                 | .29                               | —     | —   | —                                     | cyanidin                       |
| Sugar moieties                          | —   | —          | —                                   | —                                 | —     | —   | 1.0                                   | glucose                        |
| Ib                                      | —   | —          | —                                   | —                                 | —     | —   | 1.7                                   | rhamnose                       |
| Cyanidin-3-<br>sophoroside <sup>2</sup> | .34                                       | —          | .61                                 | —                                 | .22   | .33 | —                                     | —                              |
| Glucose                                 | —   | —          | —                                   | —                                 | —     | —   | 1.0                                   | —                              |
| Rhamnose                                | —   | —          | —                                   | —                                 | —     | —   | 1.7                                   | —                              |
| Cyanidin                                | .01                                       | .09        | .08                                 | .30                               | .70   | .62 | —                                     | —                              |
| Cyanidin-3-<br>glucoside                | .07                                       | .33        | .25                                 | .55                               | —     | —   | —                                     | —                              |
| Cyanidin-3-<br>rutinoside               | .14                                       | .49        | .44                                 | .71                               | .35   | .40 | —                                     | —                              |

<sup>1</sup>R<sub>g</sub> values relative to glucose<sup>2</sup>Harborne (6)

TABLE 2. SPECTRAL DATA OF PIGMENT OF BAND I AND ITS PRODUCTS OF HYDROLYSIS

| Band                      | Visible<br>max, μm | UV<br>max, μm | $\frac{E_{\text{Max}}}{E_{\text{vs}}}$<br>uv vs | Visible<br>max, μm | E <sub>440</sub> /E <sub>Max</sub> | AlCl <sub>3</sub><br>shift | Identification<br>Cyanidin-3- |
|---------------------------|--------------------|---------------|---|--------------------|------------------------------------|----------------------------|-------------------------------|
| I                         | —                  | —             | —   | 527                | 0.22                               | +                          | 2G-Glucosyl-rutinoside        |
| Ia                        | 531                | 283           | 0.66  | 527                | 0.20                               | +                          | Sophoroside                   |
| Ib                        | 538                | 284           | 0.66  | 530                | 0.22                               | +                          | Rutinoside                    |
| Ic                        | 536                | 284           | 0.61  | 529                | 0.20                               | +                          | Glucoside                     |
| Id                        | 544                | —             | —   | 536                | —                                  | +                          | Cyanidin                      |
| Cyanidin-3-<br>rutinoside | 537                | —             | —   | 529                | 0.21                               | +                          |                               |
| Cyanidin-3-<br>glucoside  | 536                | —             | —   | 528                | 0.21                               | +                          |                               |
| Cyanidin                  | 544                | —             | —   | 536                | —                                  | +                          |                               |

by infrared, visible, and ultraviolet spectra. Sugar moieties were found to be glucose and rhamnose (Table 1). This result shows that the pigment cannot be the simple diglucoside, cyanidin-3-sophoroside. The 5 position of the cyanidin moiety was shown to be open by spectral data (0.22 E<sub>440</sub>/E<sub>max</sub> ratio; 4).

Partial hydrolysis of the pigment in Band 1 revealed four degradation intermediates when chromatographed in 1% HCl (Fig. 1). The spots are labeled I (original pigment), Ia, Ib, Ic, and Id in order of decreasing rate of travel. The R<sub>f</sub> values of the intermediates and the spectral data appear in

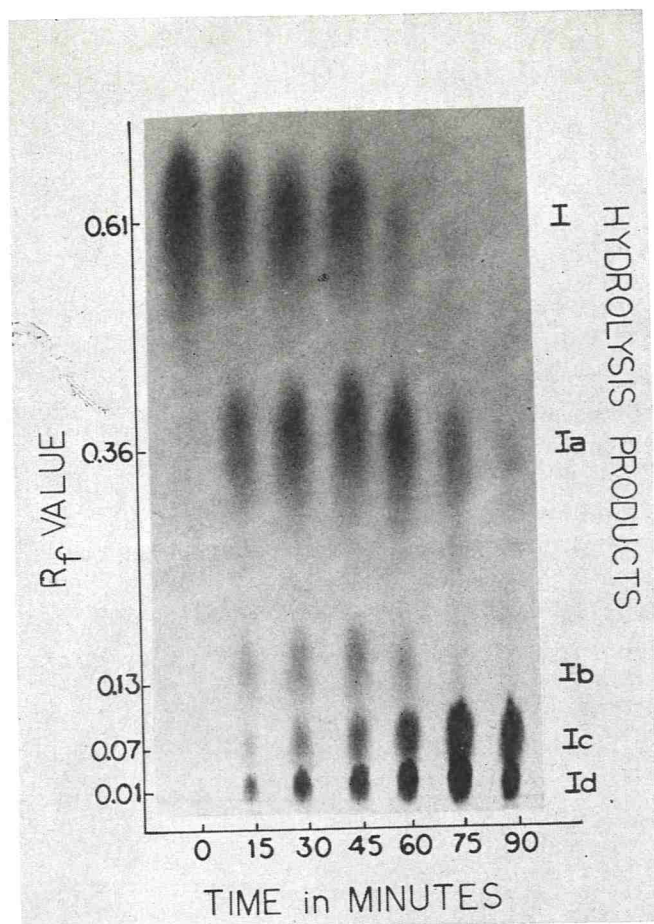


Figure 1. Partial hydrolysis of cyanidin-3-2<sup>G</sup>-glucosylrutinoside. Band I, cyanidin-3-2<sup>G</sup>-glucosyl rutinoside; Ia, cyanidin-3-sophoroside; Ib, cyanidin-3-rutinoside; Ic, cyanidin-3-glucoside; and Id, cyanidin.

Tables 1 and 2, respectively.

Presence of four intermediates rules out the possibility that this pigment (Band I) is a diglycoside, since a diglycoside would yield only two intermediates. Likewise, a linear triglycoside would yield only three intermediates. The pigment in Band I must therefore be a branched triglycoside, similar to the cyanidin-3-glucosyl-rutinoside found by Harbone and Hall (7) in six varieties of sour cherries, and reported by Olden and Nybom (9) to be in the Montmorency cherries. The work of Olden and Nybom was, however, based only on thin layer chromatography.

The  $R_f$  values of Table 1 show that controlled

partial hydrolysis of Bands Ia and Ib yielded only cyanidin-3-glucose and cyanidin, indicating that each pigment is a diglycoside containing glucose as the moiety attached directly to the ring. Furthermore, the  $R_f$  values of Band Ib are identical to those of cyanidin-3-rutinoside, and the  $R_f$  value of Ic are identical to those of cyanidin-3-glucoside. A comparison of the  $R_f$  values reported for cyanidin-3-sophoroside (6) with those of the diglycoside Ia (Table 1) suggests that these compounds are identical. These results suggest that Band I is cyanidin-3-2<sup>G</sup>-glucosyl-rutinoside.

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# STABILITY OF VIRUSES IN LOW MOISTURE FOODS

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(Received for publication June 16, 1970)

## ABSTRACT

Reovirus type 1, influenza virus type A, and parainfluenza virus type 3 persisted for  $\leq 3$  days in the low-moisture foods tested. Then enteroviruses tested persisted  $> 2$  weeks at room temperature and  $> 2$  months in the refrigerator. Where storage temperature was not constant, time-temperature effects were roughly cumulative. Inactivation of enteroviruses proceeded at an intermediate rate in foods stored at an intermediate temperature of 12 C. Polioviruses from feces and from tissue cultures were inactivated at comparable rates in foods. The inactivation rate of another enterovirus (ECHO-6) was similar. Neither the presence of feces nor of the fecal microflora seemed to influence the persistence of food-borne poliovirus. The moisture level in a food did not effect poliovirus inactivation under conditions of these tests. At reduced temperatures, virus was extremely stable in foods at  $\text{pH} \geq 7$ . At  $\text{pH} 5.5$ , there was a complex interaction of protein and salt content upon virus stability. Poliovirus was inactivated  $10^{-2}$  during freeze-drying of cream-style corn. The remaining virus was quite stable during storage of the product at 5 C.

Epidemiologic evidence indicates that food occasionally serves as a vehicle in virus transmission, yet virus which contaminates food is by no means certain to cause consumer infections (2). Since virus cannot propagate outside of suitable living host cells, virus in food can only persist or be inactivated. Heating, as in cooking or pasteurization, seems certain to inactivate any virus thought likely to occur in foods. If the food is not heated after contamination, virus may still be inactivated during storage or distribution, while at room temperature or below.

The persistence of viruses in milk and dairy products (4, 9), meat products (8), and a number of other foods (6, 10) has been studied by others. In the present study, we worked with foods developed for space flights, which we contaminated experimentally. Most of these foods are quite low in moisture when compared to those in general distribution. After the final processing step, which is most often freeze-drying, they are divided into serving sized portions and sealed under vacuum in laminated plastic pouches. This is the last opportunity for the food to be contaminated before it reaches the consumer. Contamination is relatively improbable; but if it occurred, it would be most likely to come from the enteric or

respiratory tract of a food handler. In an effort to mimic such accidental contamination, model enteric and respiratory viruses were added to foods, at least initially, in the body product (feces and respiratory mucus, respectively) in which they would be shed by the host.

Enteroviruses were expected to be relatively labile in this dry environment, because they are said to be inactivated rapidly by desiccation (1, 11). Myxoviruses might be stabilized by drying; this has been shown to be true of Newcastle disease virus (5). The interval between final packaging and consumption of these foods is not likely to be less than 2 weeks. During that time, the food is normally stored at approximately 5 C. Because it is physically and microbiologically stable even above room temperature, it may not be shipped under refrigeration. The objectives of the present study were to determine (a) which viruses might persist for at least 2 weeks in space foods, (b) what factors govern the persistence of viruses in these foods, and, (c) the effect of freeze-drying in food upon virus.

## MATERIALS AND METHODS

### *Tissue cultures*

Primary rhesus (*Macaca mulatta*) monkey kidney (PMK) cultures were produced, and used in virus propagation and assay, as described previously (3). Agar media for plaque assays included Earle's balanced salt solution plus 2% agamma calf serum for parainfluenza virus type 3; the same plus cysteine, protamine sulfate, and  $\text{MgCl}_2$  for enteroviruses and influenza virus type A (3); and a pancreatin overlay for reovirus type 1 (13).

### *Viruses*

Influenza virus type A (InA), strain PR 8, was the primary model respiratory virus. It was propagated in the allantoic cavities of embryonated chicken embryos. As a contaminant for foods, it was suspended in bovine respiratory mucus. Parainfluenza virus type 3 (Pf3), strain SF-4, was a secondary model respiratory virus and was propagated in PMK cultures. The poliovirus used initially as a model for the enteric viruses was obtained as feces of infants who had received the trivalent oral polio vaccine. The specimens selected were those which contained at least  $10^6$  plaque-forming units (PFU) per gram, regardless of the type of poliovirus present. Model enteric viruses in later experiments included poliovirus type 1 (Po 1), strain CHAT; poliovirus types 2 (Po 2) and 3 (Po 3) isolated from fecal specimens; echovirus type 6 (EC 6), strain D'Amori; and reovirus type 1 (Re 1), strain Lang, all propagated in PMK cultures.

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**Foods**

Foods used as model vehicles were most frequently stock items from the space food menu. Tables of average composition were supplied with these. Deliberate modifications of foods included freeze-drying of bacon squares; rehydration of spaghetti with meat sauce; and addition of acid (anhydrous propionic), salt (dry phosphate-buffered saline), and protein (lactalbumin) to banana pudding. A unit might comprise one or two bites of a bite size food, or as much as 30 g of a food intended to be rehydrated in the package, plus 0.5 g of contaminant. Each unit of food, with its contaminant, was packaged separately so that the entire contents of the package could be taken as the sample. The flexible, laminated plastic package was evacuated and flushed with nitrogen twice, then evacuated again, and heat-sealed. The time consumed in this operation was such that the virus in a "zero-time" sample had usually been in contact with the food for 1 to 2 hr before it was resuspended in diluent. Subsequent samples were taken after periods of storage at 5 or 12 C or at room temperature (22 to 24 C). The sample was generally homogenized with from 100 ml of phosphate-buffered saline (PBS) for bite size foods to 200 ml for 20 to 30 g samples and tested at further dilutions of 10<sup>-1</sup> and 10<sup>-2</sup> in two to four cultures per dilution, at 0.5 ml per culture. This was called the dilution testing technique. If low levels of poliovirus or EC 6 were expected, the entire food sample was tested by concentration techniques which have been described previously (7). No comparable methods are yet available for agents outside the enterovirus group.

In one instance, virus was added to the food before freeze-drying. The food was commercial, canned, cream-style corn and was contaminated with fecal poliovirus. One fecal specimen was dispensed in 0.5 g quantities on 2.5 cm plastic squares, frozen, and shipped to the NASA Manned Spacecraft Center, Houston. There, each preweighed contaminant was placed in a rectangular mold with 37.5 g of cream-style corn and frozen at -23 C for 24 hr. These were dried for 24 hr at a vacuum of <250 μm and a platen temperature of 51.5 C, except for a control sample. One freeze-dried serving was rehydrated immediately and frozen. The rest were sealed under vacuum, after nitrogen flushing as described above, in laminated plastic pouches. All samples were shipped on dry ice to us in Madison, Wisconsin, for virus assay.

**RESULTS**

**Viruses**

Model agents other than enteroviruses were relatively labile. InA in allantoic fluid was diluted tenfold in bovine mucus which had been neutralized (pH approximately 7) with acid, clarified by centrifugation, and boiled for 10 min before the virus was added. Mucus which had not been boiled was found to be antiviral. In a preliminary survey, InA in or on freeze-dried potato salad, beef bites, apricot cubes, gingerbread, cheese sandwiches, and cream-style corn was inactivated in 24 hr at 5 C at a rate proportional to the acidity of the food. Banana pudding (found to be at pH 7.6) was chosen for further experiments. We have no concentration method for InA as yet, so it would have been convenient to be able to test the homogenate undiluted. Unfortunately, the undiluted sample homogenate killed the tissue culture cells, so

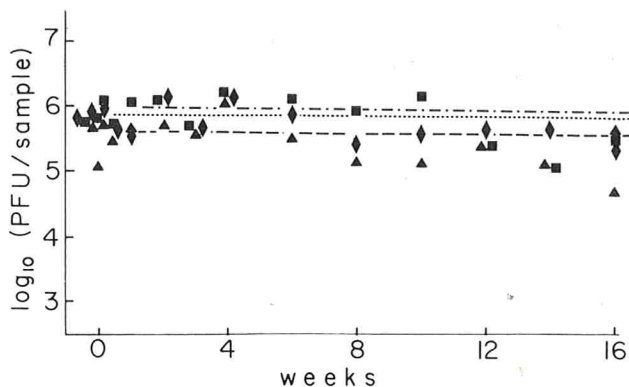


Figure 1. Inactivation of polioviruses in beef bites at 5 C (—▲ fecal poliovirus; —■ Po 1 from tissue culture, in feces; —◆ Po 1 from tissue culture, in 1% agar).

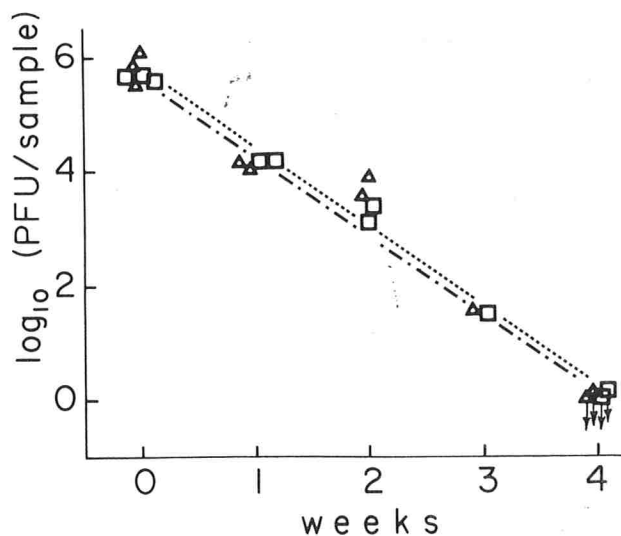


Figure 2. Inactivation of polioviruses in bacon squares at room temperature (—▲ fecal poliovirus; —□ Po 3 from tissue culture, in 1% agar; ▼ < indicated value).

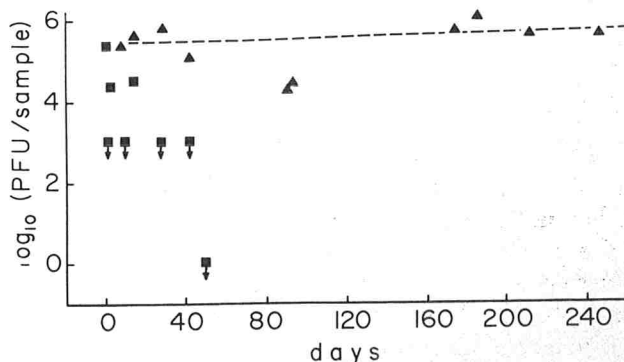


Figure 3. Inactivation of fecal poliovirus in bacon squares as a function of storage temperature (■ room temperature, —▲ 5C, ▼ < indicated value).

homogenates of banana pudding were tested at further dilutions of at least  $10^{-1}$ . Banana pudding samples were inoculated with InA and stored at 5 C and room temperature. Compared to the 0-time samples, inactivation was approximately 90% and 95% complete in 1 and 3 days at 5 C, respectively. No virus was detected at 7 days or thereafter (>99.95% inactivation) at 5 C or at room temperature. We conclude that influenza virus is not likely to persist long enough in dried foods to present a hazard to the consumer.

Pf 3 was also tested in banana pudding. No virus (i.e.,  $<1.2 \times 10^3$  PFU) was detected after 3 or 7 days' storage at 5 C in any of three experiments in which initial levels of contamination ranged from  $1.1 \times 10^4$  to  $1.4 \times 10^5$  PFU/sample. This suggests a very low stability in food for Pf 3.

A reovirus was used as an alternate model intestinal virus. Though larger and more complex than the enteroviruses, the reoviruses have shown quite similar responses to environmental factors such as salt and are relatively stable at elevated temperatures. A half-life of 2 days at 4 C in tissue culture maintenance medium and good acid stability have also been reported for reoviruses (12). Re 1 in agar was inoculated into beef bites. The zero-time samples were found to contain  $2.6 \times 10^6$  and  $1.7 \times 10^6$  PFU. After 1 and 2 weeks at room temperature and at 5 C, no virus (i.e.,  $<1.2 \times 10^3$  PFU/sample) was detected. The zero-time samples in a second experiment yielded  $3.1 \times 10^5$  and  $3.2 \times 10^5$  PFU. After 1 day at 5 C, a sample contained  $2.7 \times 10^4$  PFU. Samples after 1 and 3 days at room temperature and after 3 days at 5 C were negative. These findings indicate to us that Re 1 is not a stable virus in beef bites nor, perhaps, in other foods either. While none of these three model agents showed significant persistence in foods, the enteroviruses which were used in all the rest of these experiments proved very stable indeed.

#### *Poliovirus persistence*

Fecal poliovirus was first inoculated into bite size cheese sandwiches and stored at room temperature. The virus content declined from  $10^{5.8}$  PFU to  $<10^{3.08}$  PFU per sample in 7 days. Virus was then detectable by the concentration method through 47, but not 49, days. Fecal poliovirus in potato salad and beef bites was detectable by the dilution method after 90, but not 120, days' storage at 5 C. Malfunctions of the preparative ultracentrifuge thwarted some efforts to detect virus in these foods by the concentration method. Though some valid tests were eventually performed, no more virus was ever detected.

Fecal poliovirus was a good deal less persistent on coated gingerbread bites. The last positive tests were obtained by the dilution method after 7 days' storage at room temperature and after 28 days' storage at

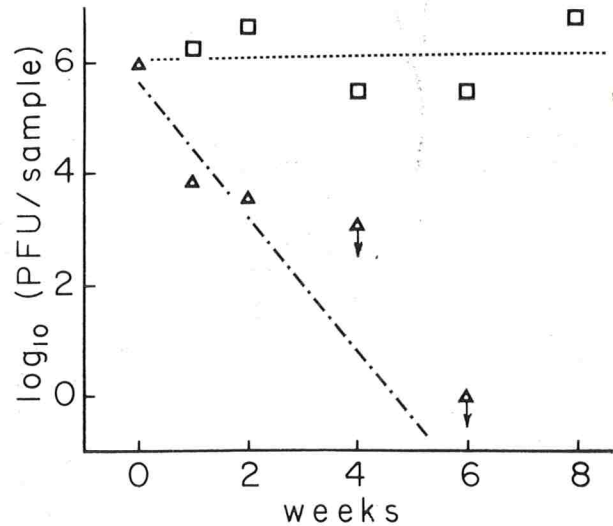


Figure 4. Inactivation of EC 6 in dry banana pudding as a function of storage temperature (—▲ room temperature, —□ 5C, ↓ < indicated value).

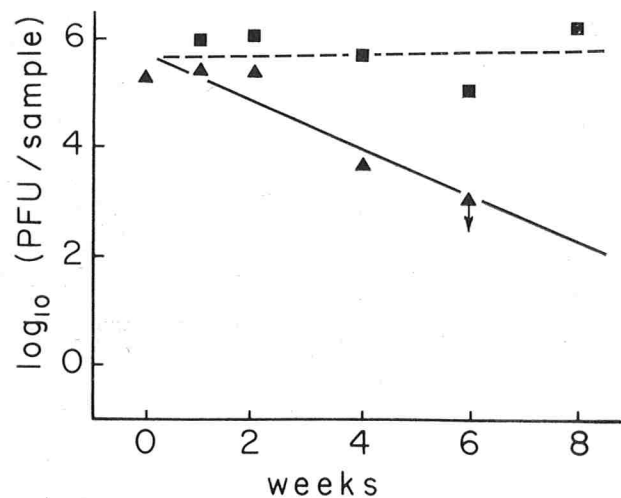


Figure 5. Inactivation of EC 6 in beef bites as a function of storage temperature (—▲ room temperature, —■ 5C, ↓ < indicated value).

5 C. The concentration method gave no positive tests. Still, it appeared that poliovirus might persist long enough even in this food, if stored at 5 C, to cause consumer infections.

#### *Mode of contamination*

Beef bites were contaminated with fecal virus, or with Po 1 of tissue culture origin mixed with feces or with 1% Noble agar (Difco), and stored at 5 C (Fig. 1). The curves shown were derived by the linear least squares method. Their slopes are  $-0.0042$  day<sup>-1</sup> for fecal virus,  $-0.0048$  day<sup>-1</sup> for tissue culture virus in feces, and  $-0.0035$  day<sup>-1</sup> for tissue culture virus in agar. These modes of contamination are approximately interchangeable at this temperature in this food. A

similar experiment was done with bacon squares stored at room temperature, except that only 1% agar was used as a carrier for the tissue culture virus (Po 3). The difference in rate of inactivation was again minimal (Fig. 2), indicating that mode of contamination was not a significant variable under these conditions, either.

#### Temperature effects

Bacon squares were contaminated with fecal poliovirus (Fig. 3). The least squares slope for virus in samples at 5 C was  $+0.0014 \text{ day}^{-1}$ , which does not differ significantly from zero. Inactivation at room temperature was significant, rapid, and perhaps not linear. Similar results were obtained with EC 6 in banana pudding (Fig. 4). The inactivation rate at room temperature was approximately  $0.17 \text{ day}^{-1}$ , whereas the slope of the curve for 5 C storage was  $+0.0014 \text{ day}^{-1}$ . EC 6 was more persistent at room temperature in beef bites (Fig. 5). The slope for the room temperature samples was  $-0.061 \text{ day}^{-1}$ , whereas that for the 5 C samples was  $+0.0016 \text{ day}^{-1}$ .

Inactivation at 12 C was intermediate to those at 5 C and at room temperature (Fig. 6). Cheese sandwiches and Po 1 in agar comprised the model system. The least squares curves have been plotted with misgivings, for the entire inactivation curve at 5 C or at 12 C is not straight, though a straight segment appears to begin at 1 week at each temperature. If each of these linear segments is extrapolated to the Y-axis, it appears that only 1% of the initial virus takes part in the process then being observed.

Fecal poliovirus in bacon squares served to determine the effects of fluctuating storage temperatures. The results of two experiments are reported together (Fig. 7). Inactivation in sample sets 1, held continuously at room temperature, gave a least squares slope of  $-0.20 \text{ day}^{-1}$ , and periods spent at room temperature by sample sets 2 and 3 resulted in inactivation at roughly comparable rates. Infectivity levels were approximately stable during storage at 5 C, except for the fourth week of sets 2. Each of the 4 week samples was tested by the concentration technique, which is not as quantitative as the dilution technique.

#### Food composition

Poliovirus stability in the earliest studies had been greatest in bacon squares at 5 C. The table of composition indicated that they were relatively high in protein (54.9% by weight) and ash (10%) and intermediate in acidity (pH 5.2). Their only unique feature was their moisture content, which at 13.4% was the highest on the space food menu.

A portion of a batch of bacon squares was freeze dried (approximately 4.5% moisture), and these and

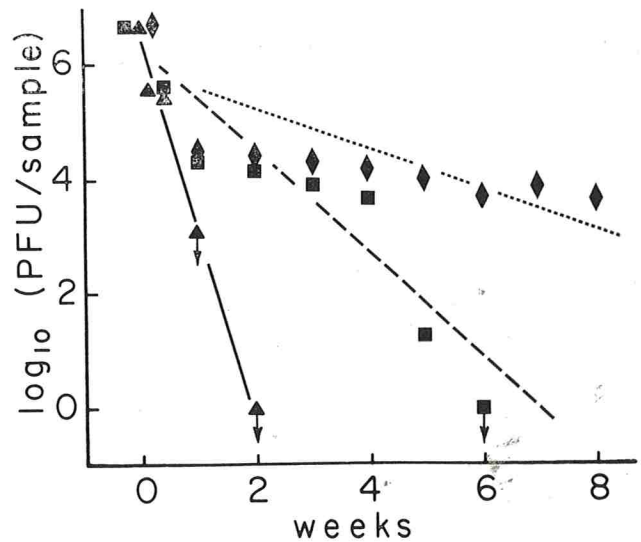


Figure 6. Inactivation of Po 1 in cheese sandwiches as a function of storage temperature (—▲ room temperature, - - -■ 12C, ·····◆ 5C, ↓ < indicated value).

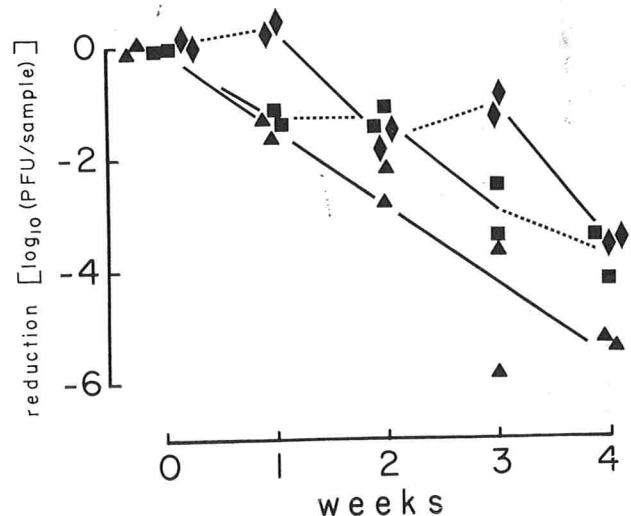


Figure 7. Inactivation of fecal poliovirus in bacon squares as a function of storage temperature (▲ sample set 1, ■ 2, and ◆ 3; storage at — room temperature and ····· 5C).

some standard bacon squares of the same batch (approximately 12.9% moisture) were inoculated with fecal poliovirus. Computed linear least squares slopes were  $-0.0022 \text{ day}^{-1}$  for the standard and  $-0.0029 \text{ day}^{-1}$  for the freeze dried bacon squares at 5 C (Fig. 8). Another experiment was done at room temperature. The contaminant was Po 3 in agar, and at least two samples were taken from each set each time (one of the 3-week samples was lost in titration). The results indicated that moisture differences, over this range, had little influence upon poliovirus stability in bacon squares at room temperature (Fig. 9).

Freeze-dried spaghetti with meat sauce was rehydrated according to directions; the calculated

moisture content was 81%. The contaminant was fecal poliovirus, so that both the microflora of the food and of the feces were present. Half of the samples received 2% tetracycline. This had a great influence upon the decomposition of the food, but little upon the inactivation of the virus (Fig. 10). The packages without tetracycline were gassy and foul-smelling within a week, and liquefaction of the food solids was far advanced by the third week. Only mild gas production and a slight odor of putrefaction were observed within 3 weeks in the presence of the antibiotic. The rates of virus inactivation were  $-0.12$  and  $-0.10 \text{ day}^{-1}$  with and without tetracycline, respectively, or somewhat slower than most of those observed with low moisture foods at these temperatures. This still did not explain the relative stability of poliovirus in bacon squares, which has been unaffected by reducing moisture.

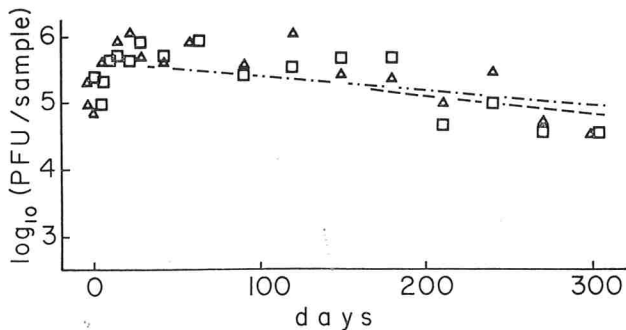


Figure 8. Inactivation of fecal poliovirus during storage at 5°C as a function of moisture level in bacon squares (—▲ standard, ~ 12.9% moisture; - - -□ freeze-dried, ~ 4.5% moisture).

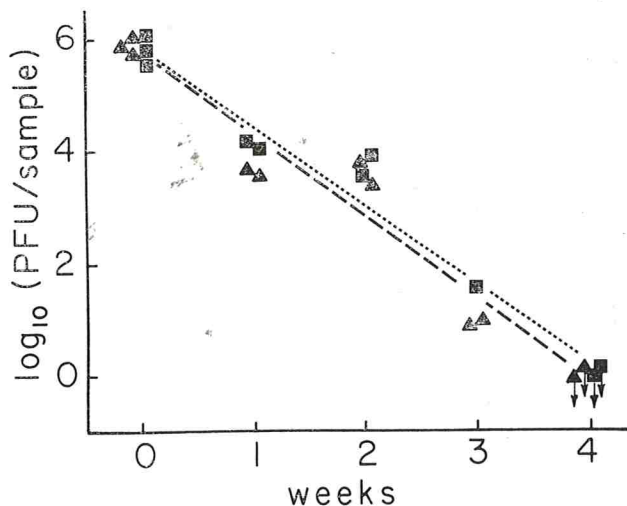


Figure 9. Inactivation of Po 3 during storage at room temperature as a function of moisture level in bacon squares (—▲ standard, ~ 12.9% moisture; ·····■ freeze-dried, ~ 4.5% moisture; ↓ < indicated value).

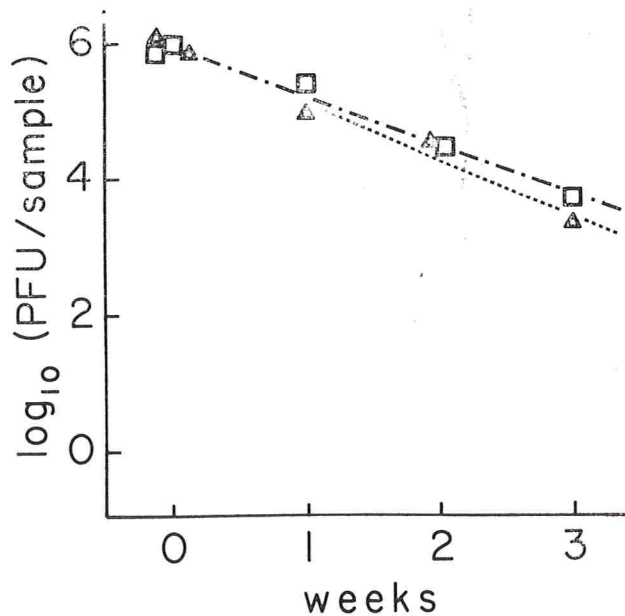


Figure 10. Inactivation of fecal poliovirus at room temperature in rehydrated spaghetti with meat sauce (—□ 0% and ·····▲ 2% tetracycline added).

We decided to select a bland food and add components to it to determine which influenced enterovirus stability. Banana pudding, with tabulated values of 1.1% moisture, pH 7.3, 2% protein, and 3.5% ash, was chosen. The pH of the batch we received was approximately 7.6 when water was added. Anhydrous propionic acid was chosen for pH adjustment: it is a liquid over the entire range of temperatures being studied so it can be measured and mixed without adding water; it is neither hygroscopic nor extremely volatile; and it is strong enough that a small volume will produce the desired pH, but not so strong that the food is charred or decomposed at the first point of contact. This does not mean that propionic acid is typical of all the substances which make foods acid, but it made a logical beginning. In 10 g of banana pudding, 0.42 ml of propionic acid would reduce the pH (on rehydration) to 5.5, while 1.0 ml yielded pH 4.0. Slightly more acid was sometimes needed to achieve the same pH when other additives were present. In a preliminary comparison, inactivation at 5°C of Po 3 in agar in banana pudding was increased slightly at pH 5.5 and a great deal more at pH 4.0 (Fig. 11). The slopes of the least squares curves shown were  $-0.047$ ,  $-0.079$ , and  $-0.15 \text{ day}^{-1}$ , in decreasing order of pH.

Protein was the next food component examined. Lactalbumin was added to banana pudding to levels of 0, 15, 30, and 60%. One set of samples was at pH 7.6, and another was adjusted to pH 5.5 with propionic acid. We contaminated the samples with Po 2 in agar and stored them at 12°C (Fig. 12). Inacti-

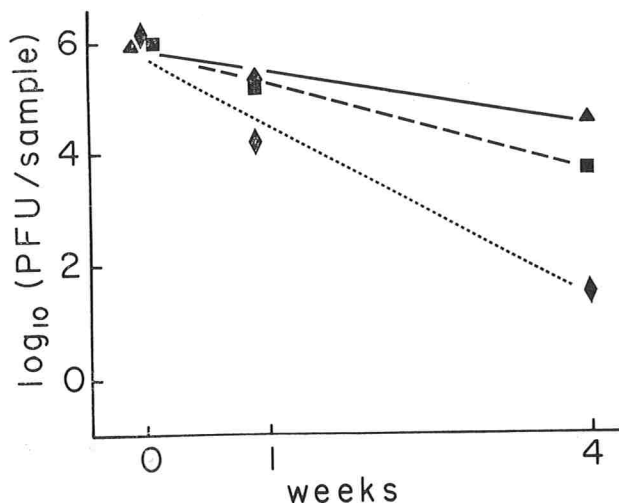


Figure 11. Inactivation of Po 3 at 5C in dry banana pudding as a function of acidity (—▲ pH 7.6, - - -■ pH 5.5, .....◇ pH 4.0).

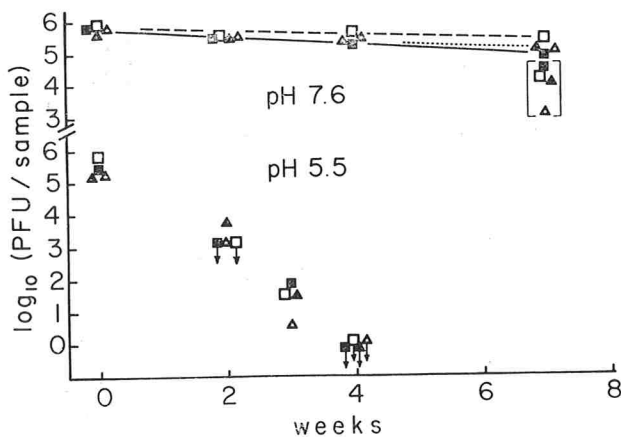


Figure 12. Inactivation of Po 2 at 12C in dry banana pudding as a function of added protein (—▲ 0%, - - -▲ 15%, .....□ 30%, and —■ 60% lactalbumin; < indicated value; bracketed points, samples stored last 2 weeks at room temperature).

vation at pH 7.6 was consistent and fairly slow, even during 2 weeks at room temperature. The data were not consistent enough or numerous enough to warrant estimating curves for the samples at pH 5.5. We surmise that protein level was not a very important factor in food-borne virus stability under the conditions of this experiment.

The effect of salt appeared to be pH dependent. One cannot account for all of the salts present in a food on the basis of the various ions reported in the composition tables. The ash content of banana pudding is said to be 3.5%, of which about 70% can be identified in the tables, whereas bacon squares have 10% ash. We added 6.5% dry phosphate-buffered saline (PBS) mix to a portion of banana pudding and dispensed this and some of the original product

into individual samples. The samples were contaminated with Po 2 in agar and stored at 5 C. As is shown in Fig. 13 (upper points), there was essentially no difference in inactivation rate with and without added salt. Only the former is shown as a curve; its slope was calculated to be  $-0.0061 \text{ day}^{-1}$ . Another set of samples was prepared in the same manner, except that the pH of the food was adjusted to 5.5. The lower curve is for pH 5.5 without added salt; its slope is  $-0.0058 \text{ day}^{-1}$ , or essentially the same as that shown above. Inactivation with high salt at low pH obviously was much more rapid (though non-linear).

We also examined the interaction of salt and protein content. These components had had little influence upon virus stability at reduced temperatures at pH 7.6. Since this experiment was to be conducted at 12 C, the pH selected was 5.5. The food was banana pudding, with and without 50% lactalbumin and 6.5% dry PBS mix; and the contaminant was Po 1 in agar. Inactivation was fairly rapid in the high protein-low salt and low protein-high salt combinations (Fig. 14). The third sample from each of these two sets had to be tested by the concentration method and somewhat earlier than we had originally planned. The slopes of these inactivation curves were very nearly the same ( $-0.25$  and  $-0.24 \text{ day}^{-1}$ , respectively). The high protein-high salt combination is near in gross composition to the freeze-dried bacon squares; but inactivation is perhaps more rapid in the former ( $-0.025 \text{ day}^{-1}$ ) than would be predicted from the latter ( $0.0029^{-1}$ ), even in view of the increase in storage temperature from 5 C to 12 C. The control samples (low protein-low salt) showed a rate of  $-0.033 \text{ day}^{-1}$ .

#### Freeze-drying virus-contaminated food

All but one of the experiments described were carried out in low moisture foods, most of which had been freeze-dried. We wanted to know whether the virus could withstand freeze-drying in food and what effect this had upon the stability of the virus in subsequent storage. Cream-style corn was chosen as the model vehicle because the original, moist form of this food is a grocer's shelf item (canned cream-style corn). The greatest loss of virus evidently occurred during the freeze-drying step (Fig. 15). A further apparent loss of infectivity in transit from Houston to Madison seems not to have been real; the titer of the sample after freeze-drying is identical to the least squares estimate for the stored samples at zero time. Contaminated food samples stored at 5 C since arrival in Madison have shown slow loss of infectivity; the slope of the least squares curve shown in Fig. 15 is  $-0.0032 \text{ day}^{-1}$ . One sample was divided into small volumes and frozen after rehydration.

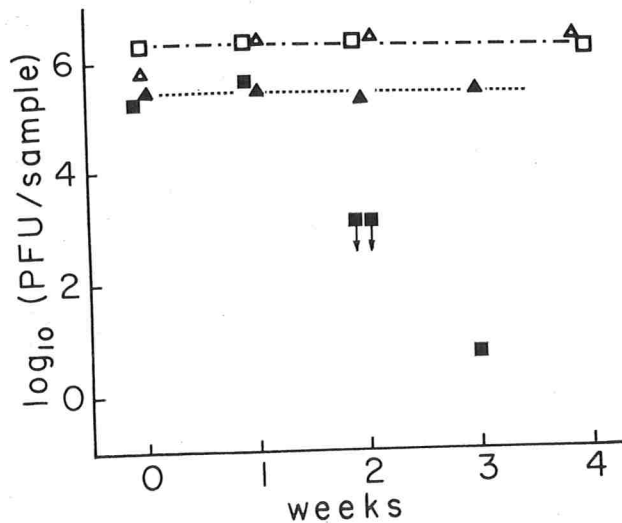


Figure 13. Inactivation of Po 2 at 5C in dry banana pudding as a function of added salt (pH 7.6,  $\Delta$  0% and  $\square$  6.5% added dry PBS; pH 5.5,  $\Delta$  0% and  $\blacksquare$  6.5% added dry PBS;  $\downarrow$  < indicated value).

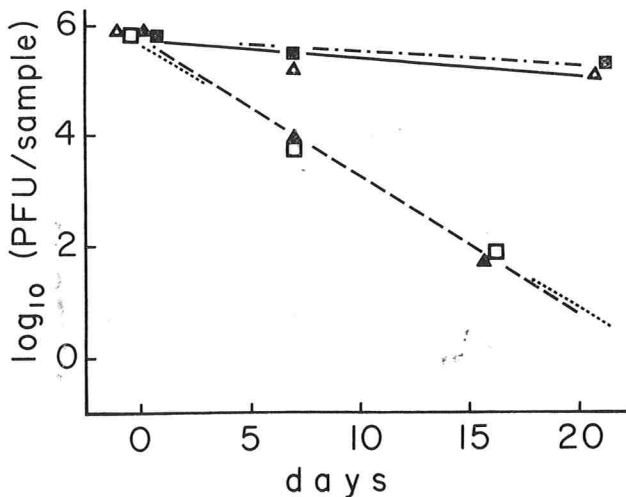


Figure 14. Inactivation of Po 1 at 12C in dry banana pudding adjusted to pH 5.5, as a function of added salt and protein (added dry PBS and lactalbumin:  $\Delta$  0% and 0%;  $\square$  0% and 50%;  $\blacksquare$  6.5% and 0%; and  $\blacksquare$  6.5% and 50%, respectively).

Portions of this, tested after periods of up to 12 weeks at  $-20^{\circ}\text{C}$ , have shown an apparent inactivation rate of  $-0.0008\text{ day}^{-1}$ . This does not differ significantly from zero; more than 3 years would be required for 90% inactivation at this rate.

#### DISCUSSION

The results of many of these experiments have been expressed as the slope of a curve derived by the linear least squares method. This may not be valid, for two reasons. First, there is some doubt that virus

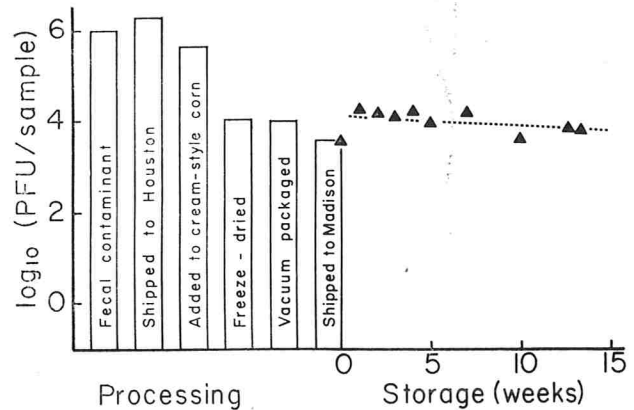


Figure 15. Inactivation of fecal poliovirus in processing and during subsequent storage at  $5^{\circ}\text{C}$  in cream style corn.

inactivation is a truly exponential process under all of these conditions; if activation is not linear, one cannot summarize it with a line. Second, the points plotted along many of these curves were determined by two different methods and are not strictly comparable. Though the concentration method has been remarkably successful with a variety of foods in this study, it is not really a quantitative technique.

While the least squares slope may not summarize the inactivation process adequately, it is the only basis we have for comparing results among experiments. It also permits comparison with the results of others for food categories such as meat products. In our study in beef bites, Po 1 was inactivated at approximately  $-0.004\text{ day}^{-1}$  at  $5^{\circ}\text{C}$ , and EC 6 at  $-0.061\text{ day}^{-1}$  at room temperature. Inactivation of coxsackievirus types B3 and B5 in mince meat, reported by Kalitina (8), was approximately  $-0.03\text{ day}^{-1}$  at  $2^{\circ}\text{C}$  and  $-0.05$  to  $-0.07\text{ day}^{-1}$  at  $20^{\circ}\text{C}$ . Po 1 in pork "junior food" was inactivated at  $-0.07\text{ day}^{-1}$  at  $4^{\circ}\text{C}$  and  $-0.03\text{ day}^{-1}$  at  $20^{\circ}\text{C}$  in experiments by Heidelbaugh and Giron (6). Inactivation of Po 1 was quite rapid in extremely acid foods; we have derived rate estimates of  $-0.25\text{ day}^{-1}$  in cranberry sauce (pH 2.7) and  $-0.21\text{ day}^{-1}$  in orange juice (pH 2.9) at  $4^{\circ}\text{C}$  from their data. Banana pudding, adjusted to pH 4.0 with propionic acid, was the most acid food in our studies. The inactivation rate for Po 3 in this was estimated at  $-0.16\text{ day}^{-1}$  at  $5^{\circ}\text{C}$ . Though inactivation rates may not be entirely valid as we have expressed them, they seem to agree rather well with those derived from others' results with high-moisture foods. The loss of infectivity in freeze-drying was less by a factor of 10 to 100 than those experienced with other foods by Heidelbaugh and Giron (6).

We have identified several factors which seem to influence the stability of viruses in low-moisture foods. Preeminent among these is the kind of virus; reovirus type 1 and two myxoviruses (influenza A and parain-

fluenza virus type 3) were rapidly inactivated in foods at 5 C. Enteroviruses, including all three types of polioviruses and EC 6, were invariably more persistent than the other model agents. There was no apparent difference in stability among enteroviruses, nor did source (tissue cultures or the human intestines) nor carrier (feces or 1% agar) influence the rate at which the enteroviruses were inactivated. The emphasis here has been upon freeze-dried foods, but there has been no indication that enterovirus stability is directly dependent upon moisture content.

Temperature proved a very significant factor in virus inactivation. Enteroviruses were inactivated more rapidly at room temperature than at 12 C or at 5 C, and may not have been inactivated at all at -20 C. Time-temperature effects appeared to be roughly cumulative in one experiment with fluctuating temperature. Though inactivation in bacon squares at 5 C has been negligible in short-time experiments, poliovirus has been less stable in bacon squares than in some other foods at room temperature. Therefore, the effect of temperature and of food composition upon inactivation rate appear to be interdependent.

Acidity appears to be a prime factor influencing the stability of enteroviruses in foods at reduced temperatures. Inactivation in foods near pH 7 has generally been slower than in acid foods. Acidity also appears to have influenced the way in which other food components affect virus stability. Neither high protein nor salt was significant in neutral food, but the two together stabilized virus at pH 5.5. These statements should be hedged by pointing out that they are based upon data from one food (dry banana pudding) adjusted with one acid (propionic) to one pH (5.5) and supplemented with one protein (lactalbumin) and one form of salt (dry PBS). The data obtained from this experimental system have not been at odds with those from whole foods of varying composition, but they obviously have not covered all of the possibilities. A real knowledge of the influence of these factors upon virus inactivation requires that a much broader set of combinations of these factors be tested. We have already detected an anomalous interaction between salt and protein in acid food. If additional work is done, there are likely to be several more surprises.

## ACKNOWLEDGEMENTS

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# GROWTH AND ACTIVITY OF *STREPTOCOCCUS CREMORIS* IN SKIMMILK FORTIFIED WITH SOME VOLATILE COMPOUNDS<sup>1</sup>

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## ABSTRACT

Skim milks were fortified with 1, 10, 100, 1000, 10,000, and 100,000 ppm of the following milk-associated volatile compounds: acetaldehyde, isobutyraldehyde, propionaldehyde, acetone, 2-butanone, 2-pentanone, ethanethiol, 1-propanethiol, 2-propanethiol, chloroform, ether, acetonitrile, ethylene dichloride, and methylsulfide. They were then inoculated with *Streptococcus cremoris*, incubated at 22 C, and tested after 2, 5, 8, 11, and 16 hr of incubation for total numbers and titratable acid.

The aldehydes, at concentrations of 10 - 100 ppm and higher, markedly reduced both growth and acid production with the effects most pronounced at and beyond 11 hr of incubation. Ketones tested were less inhibitory than the aldehydes and, at the higher concentration, caused a more pronounced reduction in acid development than in growth. The thiols, especially at concentrations of 100 and 1000 ppm, markedly reduced growth but this was not reflected in a similar reduction of acid production. Chloroform and ether were more detrimental to acid formation than to growth, whereas ethylene dichloride caused marked inhibition when measured by either parameter. A slight reduction in growth but stimulation of acid production were caused by 10, 100, and 1000 ppm of acetonitrile and methylsulfide.

Raw and heated milks contain a large number of volatile compounds, some of which have been listed in a previous report (3). Kulshrestha and Marth (3) tested 27 such compounds associated with milk for their ability to inhibit *Streptococcus lactis*, *Streptococcus cremoris*, *Streptococcus diacetilactis*, *Leuconostoc citrovorum*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhimurium*. These tests involved the disc assay method and with it the most volatile of the compounds studied failed to inhibit the bacteria. It was believed by Kulshrestha and Marth (3) that the chemicals evaporated from discs so rapidly that they failed to diffuse into the agar. Consequently, a series of experiments were conducted to determine if the compounds, when added to sterile skim milk, would affect growth of and acid production by *S. cremoris*. Results of the trials are given in this paper. A preliminary report of this work has been presented (2).

## MATERIALS AND METHODS

### Chemicals

The 14 chemicals used in this study included those which exhibited no inhibitory activity against bacteria when tested by the disc assay method (3). Chemicals and their sources are: acetaldehyde, chloroform, and ether from the Mallinckrodt Chemical Works, St. Louis; acetone from University of Wisconsin stores; acetonitrile and ethylene dichloride from J. T. Baker Chemical Co., Phillipsburg, N. J.; 2-butanone, methylsulfide, 2-pentanone, and propionaldehyde from Eastman Organic Chemicals, Distillation Products Industries, Rochester, N. Y.; and ethanethiol, isobutyraldehyde, 1-propanethiol, and 2-propanethiol from Aldrich Chemical Co., Inc., Milwaukee, Wisconsin.

### Procedure

Decimal dilutions (1 to 100,000 ppm) of the chemical being studied were prepared in screw cap culture tubes containing autoclaved skim milk. Ten sets of 6 tubes per set were prepared with one tube in each set containing 1, 10, 100, 1000, 10,000, or 100,000 ppm of chemical. Five sets of 6 tubes per set were prepared in a similar fashion except that sterile distilled water was added in lieu of the chemical. Five sets of tubes containing the chemical and five sets of tubes with added sterile distilled water were inoculated by adding to each tube 0.1 ml of a 1:10 dilution of an active 18-24 hr old *S. cremoris* culture. The remaining five sets of tubes were used as uninoculated controls. All tubes were incubated at 22 C.

One set of tubes containing skim milk plus all concentrations of chemical, and inoculated with *S. cremoris*, one set of tubes containing skim milk plus added distilled water (in lieu of the chemical) and inoculated with *S. cremoris*, and one set of tubes containing skim milk plus all concentrations of chemical but not inoculated were incubated for 2, 5, 8, 11, and 16 hr. Inoculated tubes of skim milk were tested for plate counts using Elliker's agar (1) and skim milks were checked for titratable acid and pH. The titratable acid was increased slightly by 100,000 ppm isobutyraldehyde and by 10,000 and 100,000 ppm of propionaldehyde. Other concentrations of these chemicals and all concentrations of the other chemicals used had no appreciable effect on the initial titratable acid of the milk. The increase in acidity attributable to the chemical was taken into account where necessary when calculations were made. Results of pH determinations are not reported since they reflect trends shown by data on titratable acid.

## RESULTS

Chemicals at concentrations of 1, 10, 100, 1000, 10,000 and 100,000 ppm were tested to determine

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TABLE 1. DIFFERENCES IN POPULATION OF AND TITRATABLE ACID PRODUCED BY *Streptococcus cremoris* IN SKIMMILK FORTIFIED WITH ACETALDEHYDE, ISOBUTYRALDEHYDE, AND PROPIONALDEHYDE.

| Time (hr) | Acetaldehyde (ppm) <sup>1</sup>                  |       |        | Isobutyraldehyde (ppm) <sup>2</sup> |       |        | Propionaldehyde (ppm) <sup>3</sup> |       |        |
|-----------|--|-------|--------|-------------------------------------|-------|--------|------------------------------------|-------|--------|
|           | 100  | 1000  | 10,000 | 10                                  | 100   | 10,000 | 100                                | 1000  | 10,000 |
|           | Difference from control in log of population (%) |       |        |                                     |       |        |                                    |       |        |
| 2         | -5.3   | -5.7  | -7.0   | -0.8                                | -0.7  | -2.0   | -0.2                               | +0.2  | -2.7   |
| 5         | -3.8   | -10.0 | -12.9  | -1.5                                | -3.8  | -5.5   | -0.4                               | -1.9  | -14.5  |
| 8         | +2.6   | -9.3  | -19.4  | -0.3                                | -1.9  | -5.0   | -2.3                               | -1.3  | -21.8  |
| 11        | +0.4   | -12.6 | -28.9  | -5.7                                | -6.5  | -6.4   | -5.9                               | -9.4  | -30.1  |
| 16        | +1.0   | -13.9 | -23.3  | -7.6                                | -7.7  | -10.5  | -1.1                               | -11.1 | -36.7  |
|           | Difference from control in titratable acid (%)   |       |        |                                     |       |        |                                    |       |        |
| 2         | — <sup>4</sup>                                   | —     | —      | +4.3                                | 0.0   | 0.0    | 0.0                                | 0.0   | +13.0  |
| 5         | —  | —     | —      | -13.0                               | -8.7  | -8.7   | 0.0                                | 0.0   | +8.3   |
| 8         | —  | —     | —      | 0.0                                 | +4.3  | -8.7   | -3.7                               | -3.7  | 0.0    |
| 11        | —  | —     | —      | -10.0                               | -20.0 | -24.3  | -6.3                               | -15.6 | -15.6  |
| 16        | —  | —     | —      | -23.7                               | -37.3 | -54.2  | -17.2                              | -62.5 | -51.6  |

<sup>1</sup>Control: Log no./ml 6.61, 7.07, 7.42, 7.83, and 8.11 at 2, 5, 8, 11, and 16 hr respectively. Data on titratable acid not available for control or treated samples.

<sup>2</sup>Control: Log no./ml 6.16, 6.86, 7.45, 8.29, and 8.69 and per cent titratable acid 0.23, 0.23, 0.23, 0.30, and 0.59 at 2, 5, 8, 11, and 16 hr, respectively.

<sup>3</sup>Control: Log no./ml 6.27, 7.24, 7.76, 8.62, and 9.20 and per cent titratable acid 0.23, 0.24, 0.28, 0.32, and 0.64 at 2, 5, 8, 11, and 16 hr, respectively.

<sup>4</sup>— = No result.

TABLE 2. DIFFERENCES IN POPULATION OF AND TITRATABLE ACID PRODUCED BY *Streptococcus cremoris* IN SKIMMILK FORTIFIED WITH ACETONE, 2-BUTANONE, AND 2-PENTANONE.

| Time (hr) | Acetone (ppm) <sup>1</sup>                       |      |        | 2-Butanone (ppm) <sup>2</sup> |      |        | 2-Pentanone (ppm) <sup>3</sup> |      |        |
|-----------|--|------|--------|-------------------------------|------|--------|--------------------------------|------|--------|
|           | 100  | 1000 | 10,000 | 100                           | 1000 | 10,000 | 100                            | 1000 | 10,000 |
|           | Difference from control in log of population (%) |      |        |                               |      |        |                                |      |        |
| 2         | -4.8   | -1.3 | -3.3   | -5.0                          | -1.5 | -5.3   | -2.3                           | -1.6 | -5.6   |
| 5         | -3.1   | -1.4 | -2.7   | -3.3                          | -0.9 | -6.5   | -0.4                           | -1.0 | -15.4  |
| 8         | -2.6   | -5.0 | -5.3   | +7.3                          | +0.8 | -6.2   | 0.0                            | -1.3 | 0.0    |
| 11        | 0.0  | +0.1 | -4.4   | -0.9                          | -0.4 | -1.6   | -0.2                           | -3.8 | 0.0    |
| 16        | -9.6   | -2.0 | -4.8   | -2.4                          | -0.2 | -11.6  | -0.2                           | -1.3 | +0.1   |
|           | Difference from control in titratable acid (%)   |      |        |                               |      |        |                                |      |        |
| 2         | +4.8   | +4.8 | 0.0    | 0.0                           | 0.0  | -5.0   | 0.0                            | 0.0  | 0.0    |
| 5         | 0.0  | 0.0  | 0.0    | -4.8                          | -4.8 | -4.8   | +4.2                           | 0.0  | -4.2   |
| 8         | -4.3   | -4.3 | -4.3   | -12.5                         | -8.3 | -16.7  | 0.0                            | 0.0  | -7.7   |
| 11        | -4.0   | -4.0 | -8.0   | +6.5                          | +6.5 | -22.6  | +6.7                           | +3.3 | -20.0  |
| 16        | -2.3   | -4.7 | -16.3  | 0.0                           | -3.0 | -47.1  | +3.2                           | -6.5 | -62.9  |

<sup>1</sup>Control: Log no./ml 6.31, 7.12, 7.41, 8.01, and 8.99 and per cent titratable acid 0.21, 0.22, 0.23, 0.25, and 0.43 at 2, 5, 8, 11, and 16 hr, respectively.

<sup>2</sup>Control: Log no./ml 6.46, 7.03, 7.13, 8.02, and 9.21 and per cent titratable acid 0.20, 0.21, 0.24, 0.31, and 0.70 at 2, 5, 8, 11, and 16 hr, respectively.

<sup>3</sup>Control: Log no./ml 6.41, 7.22, 7.85, 8.16, and 9.13 and per cent titratable acid 0.23, 0.24, 0.26, 0.30, and 0.62, and at 2, 5, 8, 11, and 16 hr, respectively.

their effects on growth and activity of *S. cremoris*. Data in tables are limited to those obtained when the 10, 100, and 1000 ppm or 100, 1000, and 10,000 ppm concentrations were used. The lowest concentration listed is generally the one at which a minimal but detectable difference was observed.

#### Aldehydes

Data obtained when three aldehydes, acetaldehyde, isobutyraldehyde, and propionaldehyde, were tested are given in Table 1. Acetaldehyde, at concentra-

tions of 1,000 and 10,000 ppm, markedly reduced growth of *S. cremoris*, particularly during the later stages of incubation. Unfortunately, data on acid production are unavailable and hence it cannot be said for certain that this characteristic of *S. cremoris* was affected by acetaldehyde.

Isobutyraldehyde, even at a concentration of 10 ppm reduced both growth and acid production of *S. cremoris*. Inhibition, particularly of acid production, was even more pronounced when higher

concentrations (100 and 1000 ppm) were tested. It should be mentioned that a reduction in acid production after 11 and 16 hr of incubation is most meaningful since the organism, under conditions used in these experiments, was most actively producing acid at these times. Proliferation of the organism occurred earlier and hence a reduction in growth may be noted before 8 hr of incubation have elapsed. Generally, however, inhibition of growth also was most evident after 11 and 16 hr of incubation.

Propionaldehyde was somewhat less inhibitory than isobutyraldehyde in that 100 ppm of the chemical was needed before *S. cremoris* exhibited a marked

reduction in acid production. The two higher concentrations (1000 and 10,000 ppm) substantially reduced both growth and acid production, particularly after 11 hr of incubation.

#### Ketones

Table 2 summarizes data obtained when acetone, 2-butanone, and 2-pentanone served as test chemicals. Although acetone appeared to have a minimal effect on growth, the high level (10,000 ppm) did reduce acid production by approximately 16%. Similar results were obtained with 2-butanone and 2-pentanone. In general, the ketones tested were less

TABLE 3. DIFFERENCES IN POPULATION OF AND TITRABLE ACID PRODUCED BY *Streptococcus cremoris* IN SKIMM LK FORTIFIED WITH ETHANETHIOL, 1-PROPANETHIOL, AND 2-PROPANETHIOL.

| Time (hr)  | Ethanethiol (ppm) <sup>1</sup> |       |       | 1-Propanethiol (ppm) <sup>2</sup> |       |       | 2-Propanethiol (ppm) <sup>3</sup> |       |       |
|--|--------------------------------|-------|-------|-----------------------------------|-------|-------|-----------------------------------|-------|-------|
|  | 10                             | 100   | 1000  | 10                                | 100   | 1000  | 10                                | 100   | 1000  |
| Difference from control in log of population (%) |                                |       |       |                                   |       |       |                                   |       |       |
| 2  | -1.4                           | -2.5  | -3.4  | -4.6                              | -7.3  | -8.6  | -2.9                              | -5.9  | -6.2  |
| 5  | -2.6                           | -4.9  | -14.3 | -3.0                              | -5.0  | -18.2 | -0.9                              | -5.7  | -10.8 |
| 8  | -2.1                           | -4.8  | -18.8 | -5.4                              | -7.2  | -35.8 | -0.9                              | -2.0  | -12.9 |
| 11   | -3.2                           | -7.3  | -27.6 | -6.7                              | -14.3 | -43.5 | -4.2                              | -5.2  | -19.1 |
| 16   | 0.0                            | -11.6 | -37.6 | -6.7                              | -24.9 | -48.2 | -3.1                              | -15.3 | -24.6 |
| Difference from control in titratable acid (%)   |                                |       |       |                                   |       |       |                                   |       |       |
| 2  | +8.7                           | +8.7  | 0.0   | +5.3                              | +5.3  | +5.3  | +10.0                             | +10.0 | +5.0  |
| 5  | +4.3                           | +4.3  | +4.3  | +4.8                              | 0.0   | 0.0   | 0.0                               | 0.0   | 0.0   |
| 8  | -8.7                           | -8.7  | +4.0  | +4.5                              | 0.0   | 0.0   | -4.2                              | -4.2  | -4.2  |
| 11   | +3.1                           | 0.0   | -9.4  | 0.0                               | 0.0   | -25.8 | +6.5                              | +6.5  | -3.3  |
| 16   | +4.1                           | +4.1  | -21.9 | -5.9                              | -5.9  | -17.6 | +4.3                              | +4.3  | -2.9  |

<sup>1</sup>Control: Log no./ml 6.43, 7.20, 7.52, 8.20, and 8.97 and per cent titratable acid 0.23, 0.23, 0.25, 0.32, and 0.73 at 2, 5, 8, 11, and 16 hr, respectively.

<sup>2</sup>Control: Log no./ml 6.97, 7.59, 8.19, 8.51, and 8.77 and per cent titratable acid 0.19, 0.21, 0.22, 0.31, and 0.51 at 2, 5, 8, 11, and 16 hr, respectively.

<sup>3</sup>Control: Log no./ml 6.46, 7.03, 7.38, 8.02, and 9.21 and per cent titratable acid 0.20, 0.21, 0.24, 0.31, and 0.70 at 2, 5, 8, 11, and 16 hr, respectively.

TABLE 4. DIFFERENCES IN POPULATION OF AND TITRABLE ACID PRODUCED BY *Streptococcus cremoris* IN SKIMMILK FORTIFIED WITH CHLOROFORM AND ETHER.

| Time (hr)  | Chloroform (ppm) <sup>1</sup> |       |       | Ether (ppm) <sup>2</sup> |       |       |
|--|-------------------------------|-------|-------|--------------------------|-------|-------|
|  | 10                            | 100   | 1000  | 10                       | 100   | 1000  |
| Difference from control in log of population (%) |                               |       |       |                          |       |       |
| 2  | +0.9                          | 0.0   | -4.3  | +0.4                     | 0.0   | -0.7  |
| 5  | -3.0                          | -3.0  | -14.2 | -3.1                     | -3.1  | -2.4  |
| 8  | +0.5                          | 0.0   | -19.8 | -1.1                     | -2.4  | -0.1  |
| 11   | -0.1                          | -0.8  | -2.9  | +0.4                     | -4.2  | -0.6  |
| 16   | +0.1                          | -9.1  | -11.1 | -1.6                     | -1.7  | -3.6  |
| Difference from control in titratable acid (%)   |                               |       |       |                          |       |       |
| 2  | 0.0                           | 0.0   | +5.0  | +4.5                     | +4.5  | +4.5  |
| 5  | -9.1                          | -9.1  | -9.1  | -8.7                     | -8.7  | -4.3  |
| 8  | +4.3                          | +4.3  | -4.3  | -4.3                     | 0.0   | -4.3  |
| 11   | -3.8                          | -3.8  | -3.8  | -4.2                     | -4.2  | -4.2  |
| 16   | -8.9                          | -48.9 | -48.9 | -17.9                    | -17.9 | -17.9 |

<sup>1</sup>Control: Log no./ml 6.48, 7.30, 7.89, 8.26, and 8.89 and per cent titratable acid 0.20, 0.22, 0.23, 0.27, and 0.45 at 2, 5, 8, 11, and 16 hr, respectively.

<sup>2</sup>Control: Log no./ml 5.52, 6.21, 7.18, 7.85, and 8.89 and per cent titratable acid 0.22, 0.23, 0.23, 0.24, and 0.39 at 2, 5, 8, 11, and 16 hr, respectively.

TABLE 5. DIFFERENCES IN POPULATION OF AND TITRATABLE ACID PRODUCED BY *Streptococcus cremoris* IN SKIMMILK FORTIFIED WITH ACETONITRILE, ETHYLENE DICHLORIDE, AND METHYLSULFIDE.

| Time (hr) | Acetonitrile (ppm) <sup>1</sup>                  |       |       | Ethylene dichloride (ppm) <sup>2</sup> |       |        | Methylsulfide (ppm) <sup>3</sup> |      |       |
|-----------|--|-------|-------|--|-------|--------|----------------------------------|------|-------|
|           | 10   | 100   | 1000  | 100                                    | 1000  | 10,000 | 10                               | 100  | 1000  |
|           | Difference from control in log of population (%) |       |       |  |       |        |                                  |      |       |
| 2         | -3.8   | -9.8  | -3.8  | -1.8                                   | -3.9  | -24.5  | +0.7                             | +1.2 | +1.3  |
| 5         | -11.6  | -18.2 | -11.8 | -7.0                                   | -7.0  | -34.4  | -1.8                             | -2.4 | -3.0  |
| 8         | -0.8   | -1.2  | -10.1 | -4.8                                   | -2.6  | -28.1  | -0.8                             | -0.1 | +0.1  |
| 11        | -0.4   | -1.3  | -3.1  | -3.9                                   | -11.5 | -55.7  | 0.0                              | -2.3 | -10.2 |
| 16        | -0.1   | -1.6  | -3.9  | -21.7                                  | -13.5 | -62.8  | +0.3                             | -4.4 | -5.7  |
|           | Difference from control in titratable acid (%)   |       |       |  |       |        |                                  |      |       |
| 2         | +4.8   | 0.0   | 0.0   | 0.0                                    | 0.0   | -4.5   | 0.0                              | 0.0  | 0.0   |
| 5         | +4.8   | +4.8  | +4.8  | 0.0                                    | 0.0   | -9.1   | +4.0                             | +4.0 | 0.0   |
| 8         | +4.3   | +4.3  | +21.8 | -4.3                                   | -13.0 | -13.0  | +3.7                             | +3.7 | 0.0   |
| 11        | 0.0  | 0.0   | 0.0   | -10.0                                  | -23.3 | -36.7  | +7.1                             | +7.1 | +3.6  |
| 16        | +3.7   | +1.9  | +1.9  | -39.0                                  | -47.5 | -64.4  | +2.3                             | +2.3 | +2.3  |

<sup>1</sup>Control: Log no./ml 6.31, 7.05, 7.70, 7.80, and 7.93 and per cent titratable acid 0.21, 0.21, 0.23, 0.26, and 0.54 at 2, 5, 8, 11, and 16 hr, respectively.

<sup>2</sup>Control: Log no./ml 6.16, 6.86, 7.45, 8.29, and 8.69 and per cent titratable acid 0.22, 0.22, 0.23, 0.30, and 0.59 at 2, 5, 8, 11, and 16 hr, respectively.

<sup>3</sup>Control: Log no./ml 6.03, 7.09, 7.75, 8.11, and 8.96 and per cent titratable acid 0.25, 0.25, 0.27, 0.28, and 0.44 at 2, 5, 8, 11, and 16 hr, respectively.

detrimental to growth and activity of *S. cremoris* than were the aldehydes.

#### Thiols

The effects of ethanethiol, 1-propanethiol, and 2-propanethiol on *S. cremoris* are recorded in Table 3. Ethanethiol markedly reduced growth when present at concentrations of 100 and 1,000 ppm and acid production only at the highest level. In fact, acid production was slightly enhanced when the low concentration (10 ppm) was tested. Although stimulation of acid production is indicated, more work is needed before this can be claimed as a certainty. The low (10 ppm) level of 1-propanethiol and 2-propanethiol acted much as ethanethiol at the same concentration. High concentrations of 1-propanethiol and 2-propanethiol markedly reduced growth but this was not accompanied by the reduction in acid development which might be expected. In general, the thiols tested were more inhibitory to growth than the ketones and nearly equal to the aldehydes, but the reduction in growth was not always accompanied by a corresponding reduction in acid production.

#### Other compounds

Chloroform and ether (Table 4) at all concentrations tested caused a marked reduction in acid production but this was not accompanied by a corresponding reduction in growth. This suggests that the quantity of acid formed per cell was reduced through the action of the chemicals. Chloroform and ether were among the more effective of the compounds tested in terms of inhibiting acid production.

Ethylene dichloride at the three levels reported (Table 5) was particularly inhibitory both to growth and acid production. It was clearly the most effective of all compounds tested when both parameters are considered. In contrast to this, acetonitrile (Table 5) caused a minimal reduction in growth and a slight but clearly evident increase in acid production. The effects of methylsulfide (Table 5) were similar to those observed with acetonitrile. Again, stimulation of acid production is indicated but additional work is needed to verify this assumption.

#### DISCUSSION

Data reported previously by Kulshrestha and Marth (3) together with the information in this paper suggest that some of the lactic streptococci may be sensitive to some of the volatile compounds which have been identified as present in milk. Although the work in both of these reports has been concerned with the effect of single compounds, it must be remembered that in milk they exist as mixtures. The possibility of synergistic or antagonistic effects among some of the compounds cannot be ruled out until experimental evidence to the contrary has been collected.

Although the compounds used in this and the previous study (3) are representative of those which have been recovered from milk, all volatile substances identified as present in milk have not been tested and hence their effect on starter cultures is unknown. At this point it is not known whether the volatile compounds listed previously (3) are present in all

milks at all seasons of the year. It is common knowledge that variations in the major milk constituents occur during a lactation, among cows within a breed, and among cows of different breeds. Certainly the level and types of volatile compounds present also may be expected to vary in a similar fashion. Such variation could serve to influence the behavior of starter cultures even if other conditions which govern their growth and activity are carefully controlled.

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## THE FUTURE OF EDIBLE OILS IN THE DAIRY INDUSTRY<sup>1</sup>

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Canada*

Today in the dairy industry profit levels expressed on any base one prefers—return on capital, cents per quart of milk, per cent of sales, etc. are at an all-time low. Too low to attract the capital that will be needed if the industry is to keep pace with the technology of the seventies. In order to adequately assess the future of edible oils in the dairy industry, it is essential to know where we are and where we have come from.

If any industry in Canada was ever at the crossroads it is the processing segment of the Canadian Dairy Industry. Harrassed at the federal level by the Canadian Dairy Commission, who appears resigned to a substantial reduction in the industry—at the provincial level by the Ontario Milk Marketing Board, who appears resolutely dedicated to increasing the producer's share of the sales dollar and by regulations confining the method of coloring margarine and the prohibition of blending vegetable oils with milk solids. One should not overlook the restrictions imposed by Health and Welfare, Department of Consumer and Corporate Affairs, or even the municipal restrictions imposed by iron curtains—Montreal and Quebec.

For more than twenty years following World War II there was minimum action undertaken by any government—federal or provincial to solve the long term supply problems confronting the dairy industry. Surpluses of butter, cheese, and powder, the pivotal

products of the industry, grew larger and were solved only by fire sale pricing on the world market. Suddenly in the dying years of the soaring sixties, the pot has started to boil. Within the past two years the Federal Minister of Agriculture convened two sessions of a study group of top level personnel interested in the future of the dairy industry and this was followed by the Agriculture Congress to study in depth the recommendations of the Federal Task Force on Agriculture.

The statement issued by the delegation from the University of Saskatchewan on the last day of this important Congress held at Ottawa in March of 1969, points up the existing aura of gloom.

"It is becoming clear to delegates that after every tree has been shaken in a search for markets and after every avenue has been explored in the transfer of resources to alternate crops and after every stone has been turned looking for price raising schemes, there still will not be enough revenue to yield comparable returns to the resources presently in Canadian agriculture.

What may be needed is to reduce not just the number of farms but land and capital as well—to shrink the whole agricultural plant down to the point where resources yield comparable returns."

Personally, I felt that the Federal Task Force authors who prepared the various position papers examined the situation primarily from an economic viewpoint and gave scant attention to the socio-economic aspects. The savings realized by the elim-

<sup>1</sup>Presented at the annual meeting of the Ontario Milk and Food Sanitarians Association, Toronto, Ontario, January 28, 1970.

# AN AGRICULTURAL ENGINEER'S OPINION ABOUT LAGOONS

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## ABSTRACT

The lagoon waste disposal method will work adequately for the dairy producer if it is properly designed for a specific herd size and it is operated correctly. This paper describes the two lagoon systems -aerobic and anaerobic- and gives the design specifications for a 50 cow herd. The operations specifications and requirements are also given.

The problem of waste handling from the present dairy producing herd is sheer immensity. If manure from cattle is to be used to advantage as fertilizer on the land it should be applied at specific times. This means that the manure must be stored for various lengths of time. Since storage facilities require considerable investment, the dairy producer hesitates to make the investment but will purchase commercial fertilizer. He is agreeable to a disposal method that simply lets the waste disappear. The lagoon is a method that if properly designed should let the natural biological process make the waste disappear.

There are several methods of waste disposal possible for the dairy producer. There are combinations of these different methods, or portions of them. The producer therefore, is faced by a complex problem out of which he must form a decision as to what total system he will utilize.

The lagoon method of waste disposal is certainly not new. Events recorded in history and archaeological discoveries tell us that the lagoon system was used thousands of years ago. The thing that is probably different today from then is the immense quantity of waste compared to the volume then.

The problem of animal waste disposal in the United States is immense. There are approximately 200,000,000 people in the U. S. today. According to Hansen (1) each of these persons is supported by: 2 laying hens, 4 broilers, 1/2 turkey, 1/2 cow, 1/3 pig, 1/10 sheep, and several other small animals too numerous to mention. C. A. Johnson (2) reported a daily manure production from all poultry and livestock of 6,000,000 tons in 1959. The daily total quantity today must be much larger than that.

If an attempt was made to use all this manure for fertilizer, and assuming 25 tons applied to an acre each year, it would require over 86,000,000 acres per year. By comparison, the state of Colorado encompasses approximately 62,000,000 acres. In addition to this present day quantity problem, it is predicted that there will be 400,000,000 people in the U. S. by the year 2000. For this reason it appears that the animal waste disposal problem is sheer immensity.

It can be seen from the foregoing data that it is impossible to attempt to utilize all animal manure on the land as fertilizer. In addition some storage facilities must be available so that the manure can be stored and spread when it will do the most good, such as is done for commercial fertilizer. Since storage facilities for manure are quite costly, and commercial fertilizer at the present time is economical, the dairy producer hesitates to make the investment in manure storage facilities, but will invest in commercial fertilizer. He thus is agreeable to a waste disposal system that lets the waste disappear.

The lagoon is a method that if properly designed should theoretically let natural biological processes make the waste disappear. It should be pointed out that there will be no financial return from this method, and whatever cost is put into construction and operation must be paid for from milk production.

## TYPES OF LAGOONS

Basically, there are two types of lagoons, aerobic and anaerobic. Aerobic decomposition results in complete breakdown of organic compounds into water and carbon dioxide. Neither of these is harmful or disagreeable. Anaerobic decomposition does not result in complete break down. Some organic material is left as sludge. Disagreeable and dangerous gases - ammonia, methane, and hydrogen sulfide as well as an acidic effluent are by-products. The aerobic method is much more desirable, but much harder to achieve, because of physical sizing usually a ratio of 20 to 1 of surface area. Therefore, the anaerobic method is employed in most instances. Actually, a combination of the two is desired for complete safe disposal.

Available data (4) indicates that 1 ft<sup>3</sup> of anaerobic lagoon volume for each pound of body weight of the animal is needed. Therefore, a 1200 pound dairy cow requires 1200 ft<sup>3</sup>. The depth can, and possibly should be, as great as possible since the anaerobic bacteria can work at these greater depths and the physical sizing is made much easier. In addition, there will be less surface area exposed for the volume contained. Usually a depth from 10 to 12 ft can be accomplished by a bull-dozer in a practical way.

A 50 cow dairy herd would thus require a volume of 60,000 ft<sup>3</sup>. At a 10 ft depth, this would be a surface area of 6,000 ft<sup>2</sup>. If the surface were square, each side would be approximately 78 ft in length, providing the walls are vertical. This is impractical unless the walls are hard surfaced, which increases construction costs. The practical way is to slope the wall approximately 1 to 3.

To have a lagoon of the volume needed for the 50 cow dairy herd, and maintaining an average depth of 10 ft, a lagoon with a square surface should be approximately 100 ft on each side, with the sides sloping at the rate of 1 to 3, to where the flat bottom would be approximately 45 ft on a side.

The manure from the lot should be deposited near the center of the lagoon. The physical design requirements for getting this job done is too lengthy to describe here, but having the raw material deposited near the center is highly desirable for proper decomposition. The waste from the milking parlor also can be deposited in the lagoon, and should be discharged near the center. This will be an anaerobic lagoon, and the products of decomposition will cause disagreeable odors. The odors come mainly from two gases, ammonia and hydrogen sulfide.

#### STARTING A LAGOON

A new lagoon should be started by filling it to the operating level with water. Manure and waste should be added gradually to allow time for bacterial growth. Summer months are much preferred for starting because the warmer temperature can start the bacterial growth at a faster rate (5). As waste is deposited in the lagoon, there will be some overflow. At times when atmospheric evaporation rate is high there may be little or no overflow. During these times provision must be made to add water to keep the lagoon at the operating level. The overflow, or effluent, from an anaerobic lagoon can still cause contamination. It may be clear, but it is far from pure. The effluent must not enter a running stream or flow onto another person's property. It may be further treated in several ways, therefore some planning is required.

#### HANDLING EFFLUENT

An acceptable method is to use an aerobic lagoon, usually called the second stage. Since a large percentage of the solid waste has been previously decomposed, there is very little volume to process, and the aerobic lagoon, or second stage, can be much smaller than the anaerobic lagoon. It should be approximately one-half the size of the first lagoon (3) and not over 5 ft deep. The shallow depth aids sun-

light penetration of the water to encourage algal growth. Wind action also aids in remixing of air into the volume of water to support aerobic bacteria.

The only sacrifices in this dual system are that the anaerobic lagoon produces disagreeable gases and decomposition is not as complete as aerobic action, but it does keep the physical requirements to manageable limits. A fact that may present tremendous management problems is adding or restricting water to the lagoon to maintain the liquid at the operating level.

Another way to handle the effluent would be to install an irrigation pump and maintain the level of the anaerobic lagoon by pumping the top part of the liquid out through pipes into fields. The distribution in the field could be by open pipe, or an irrigation nozzle. The main thing to watch is that no runoff takes place. A small amount of labor may be required if irrigation nozzles are moved to distribute the liquid evenly for irrigation purposes. The irrigation pump will work well with this system since the material is mostly liquid.

A hauling tank could be utilized for distribution onto fields rather than the irrigation pipe, but more labor and time is usually involved. Treatment of the effluent may be by a tile disposal field (6). This would be similar to the well known human septic tank disposal field. The design limitation is the percolation rate of the soil.

It is estimated from the sparse data available, that in the anaerobic lagoon sludge buildup will accumulate at the rate of 50 to 60 ft<sup>3</sup> per year per cow. Due to the sloping sides, there will be approximately 1.5 ft depth of sludge accumulation during the first year, 1 ft accumulation during the second year, 0.75 ft during the third, and 0.6 ft during the fourth year. At this rate, and with no increase in herd size, the lagoon should operate approximately 5 years before cleaning out of the sludge is necessary. It must be noted that as the sludge accumulates in the bottom, the volume of the lagoon is lessened. Since the design volume includes this fact, the decomposing action should continue to take place adequately for the time specified.

The anaerobic sewage lagoon appears to offer the dairy producer an economical and low labor requirement waste disposal system. It must be designed correctly, and managed according to the design. It should be designed for the conditions where it will be located and not on data for locations farther north or south where the average temperature is different. Large quantities of disagreeable odors quite likely will be generated, and future complaints from them may be anticipated. There will be a sludge buildup that must be taken care of at some future date.

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## THE FUTURE OF EDIBLE OILS

(Continued from Page 496)

ination of federal subsidies should be compared to alternate costs required to deal with this hard core problem of inefficient farms. Surely it is a national problem.

### CONTRACT OR EXPAND

Do you realize that there are only approximately 150,000 farmers in Canada who divide the federal subsidy of around \$110,000,000. That is a simple arithmetic average of \$700.00 per farmer. I don't know what the median subsidy payment would be but there are quite a few shippers who receive more than \$10,000 each from this subsidy. The direct subsidies have increased from 20c per cwt. in 1965 to \$1.31. Granted, the Dairy Commission policy of charging back to the fund a share of the costs of disposing of surpluses has reduced the per cwt. somewhat. The point that I would like to make as an ordinary tax payer, surely some policy such as creating annuities for certain farmers in exchange for their withdrawal from producing milk should be initiated. Over a period of time a substantial cut back could be achieved in an orderly manner that would cause no great hardship to anyone, yet have a terminal aspect to the hundreds of millions being spent on subsidies as the recipients of these annuities passed from the scene.

The National Dairy Council, who represented the processing segment of the Canadian Dairy Industry, made one of the few submissions to the Congress that took a positive approach. Let's strive to expand rather than contract our industry. While most papers pleaded for continued subsidies, increased tariffs and grants, The National Dairy Council proposed a policy of expansion of the domestic market; more market research and promotion of dairy foods; review of standards; improved quality of milk; and consolidation program for processors to increase efficiency.

During the past year The National Dairy Council

has been striving to stimulate interest in a major national advertising campaign and in a substantial market research campaign. Discussions were held with the Canadian Dairy Commission; the Dairy Farmers of Canada; and The Department of Industry, Trade, and Commerce. Frankly, the results have been most encouraging. The Dairy Farmers of Canada were co-sponsors of our proposal of advertising and market research before the Canadian Dairy Commission and the Agriculture Congress convened by the federal government.

### ADVERTISING AND MARKET RESEARCH

For the record, it should be noted that a great deal of the advertising for dairy products in 1970 underwritten by the Dairy Farmers of Canada and the Ontario Milk Marketing Board. The processors without exception commend the producer for their efforts in this area and the processor only regrets that his inadequate profit levels forces his advertising budgets to be one of the lowest in the food industry.

And I am most reluctant to comment on the expenditures for market research. Canada ranks right next to the bottom in this category. We can only trust that an awareness is being created that will alter this poor showing. The Dairy Farmers realize the importance of market research and are giving serious consideration to recommending the withdrawal of the federal sales tax, even on margarine, to aid in unblocking the path to new dairy food development.

The Canadian Dairy Commission convened a top level seminar late in 1969 to discuss with the National Dairy Council and Dairy Farmers of Canada, the merits of market research and new product development. A unanimous concensus at that session agreed that a select Committee representing the Commission, Dairy Farmers and National Dairy Council with a full-time qualified staff, should be formed immediately to explore the potential of new dairy foods. Again, the Department of Industry, Trade & Commerce has been most constructive. The De-

(Continued on Page 505)

# PREDOMINANT MICROORGANISMS ON RAW PLANT FOODS<sup>1, 2</sup>

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## ABSTRACT

Considerable variation was observed in the microbial populations present on raw plant foods. Equipment contamination and microbial growth on the product following harvest often were responsible for high microbial counts. Because of these factors, vegetables protected by a pod or husk frequently were more heavily contaminated than those exposed to soil and air throughout their growth history.

Similar types of microorganisms were isolated from raw and post-blanch samples of peas and green beans. The samples recontaminated after the blanch generally contained a higher proportion of catalase-negative cocci.

Some of the properties of the more numerous groups are presented. Many of the isolates were sufficiently different from "type" cultures that they could not be readily placed into described species.

The term "raw plant food" covers a variety of commodities—from the cereals to the pulses to the leafy vegetables. Furthermore, it includes products to be sold as fresh produce as well as those destined to be canned, frozen, dried, or fermented. Much of this report will be devoted to one type of raw plant food, vegetables harvested for canning or freezing.

The literature contains only a limited amount of information regarding the microflora of vegetables as received from the growing field. This is understandable since a common early processing step is a blanch that destroys a very high percentage of the contaminants. The microflora of the raw vegetable, therefore, may have little bearing on that of the processed food.

## NUMBERS OF MICROORGANISMS

The counts reported in the literature (Table 1) illustrate several points: (a) Samples of a given vegetable may differ widely with respect to microbial populations. The counts on potatoes, for example, ranged from  $75 \times 10^3$  to  $28 \times 10^6$  per gram. (b) There appears to be little relation between the degree of intrinsic exposure or protection and the total count. The numbers of organisms on peas protected by a pod, and on corn protected by a husk, were as high

as those on potatoes, carrots, and other vegetables that were grown in contact with the soil.

Our own data, the results of many surveys (10, 11), illustrate the same thing (Fig. 1). The histogram shows that peas, which had been essentially sterile up until their removal from the pod, yielded counts considerably higher than green beans which had been exposed to contamination from soil and air throughout their growth history.

## FACTORS AFFECTING COUNTS

Viners were responsible for the high counts on our peas. We know this because our samples were collected directly from this equipment immediately after shelling. No opportunity was presented, therefore, for subsequent contamination or microbial growth. The problem with the viners was that because they were located outside the factory door, too often their sanitation was completely overlooked.

Another example of "outside" equipment is the mechanical harvesters that are ever gaining in use. Our studies with Concord grapes (5) showed that because of contaminated harvester surfaces, the machine-picked fruit gave counts that were ten-fold or more higher than those on grapes that we personally had harvested by hand. However, when commercial hand-picked fruit as received at the factory was com-

TABLE 1. "TOTAL" COUNTS REPORTED FOR RAW VEGETABLES.

| Vegetable | Count per g x 10 <sup>3</sup> | Reference                  |
|-----------|-------------------------------|----------------------------|
| Beet      | 3,200                         | Vaughn (17)                |
| Carrot    | 440                           | Vaughn (17)                |
| Potato    | 75 - 28,000                   | Vaughn (17)                |
| Cabbage   | 4 - 2,000                     | Keipper and Fred (4)       |
| Corn      | 100 - 10,000                  | Smart (6)                  |
| Lima bean | 1 - 150                       | Smart (6)                  |
| Cucumber  | 16,000                        | Etchells et al. (1)        |
| Kale      | 1,200 - 10,000                | Smart and Brunstetter (7)  |
| Spinach   | 2,000 - 23,000                | Smart and Brunstetter (7)  |
| Peas      | 220 - 30,000                  | Splittstoesser et al. (11) |
| Snap bean | 600 - 3,000                   | Splittstoesser et al. (10) |

<sup>1</sup>Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 1809.

<sup>2</sup>Presented as part of a seminar on Spoilage Bacteria, Indicator Organisms, and Pathogens in Raw Plant Foods at the 70th Annual Meeting of the American Society for Microbiology, Boston, Massachusetts, April 26-May 1, 1970.



pared with the mechanically-harvested product, little difference in counts was evident (Fig. 2). Contaminated lug boxes along with actual microbial growth on the berries were thought to be responsible for the higher counts on the commercially hand-picked fruit. It was concluded that by paying attention to harvester sanitation, the mechanically picked grapes should yield the lower microbial counts.

Growth on the vegetable between the time of harvest and processing appears to be one of the factors responsible for high counts on green beans. In one survey it was observed that the microbial counts on beans that had just arrived from the growing field were 80 thousand per gram compared with a count of 5 million per gram on similar product that had been held in the factory yard for 8 hr. The beans had been held in large crates and the day was warm. Apparently sufficient moisture was released from the vegetable to provide an excellent growth medium for microorganisms.

PREDOMINANT MICROBIAL GROUPS

In addition to numbers of microorganisms, we have been interested in the kinds that make up the predominant groups. One phase of the work was to compare the microflora of raw vegetables with that on vegetables collected from various processing stages following the blanch (14). The original objective was to learn whether a characteristic microflora developed on processing lines that were handling a given vegetable.

Our findings with peas and green beans suggested that the kinds of microorganisms on the raw and blanched vegetable were quite similar. The most obvious difference was that recontamination following the blanch resulted in a higher proportion of

TABLE 2. THE INCIDENCE OF GENERAL MICROBIAL TYPES ON RAW AND BLANCHED PEAS.

| Microbial type                  | Per cent distribution <sup>1</sup> |             |
|---------------------------------|------------------------------------|-------------|
|                                 | Raw peas                           | Post-blanch |
| <i>Catalase-positive</i>        |                                    |             |
| Rods, Gram-negative             | 14                                 | 21          |
| Rods, Gram-positive             | 37                                 | 21          |
| Cocci, Gram-positive            | 17                                 | 13          |
| <i>Catalase-negative</i>        |                                    |             |
| Cocci, Gram-positive            | 26                                 | 42          |
| <i>Other</i>                    |                                    |             |
| Yeasts, "actinos", lactobacilli | 6                                  | 3           |

<sup>1</sup>Number of isolates: 380 from raw peas, 1100 from the post-blanch samples.

TABLE 3. THE INCIDENCE OF GENERAL MICROBIAL TYPES OF RAW AND BLANCHED BEANS

| Microbial type                  | Per cent distribution <sup>1</sup> |             |
|---------------------------------|------------------------------------|-------------|
|                                 | Raw beans                          | Post-blanch |
| <i>Catalase-positive</i>        |                                    |             |
| Rods, Gram-negative             | 38                                 | 14          |
| Rods, Gram-positive             | 29                                 | 19          |
| Cocci, Gram-positive            | 7                                  | 17          |
| <i>Catalase-negative</i>        |                                    |             |
| Cocci, Gram-positive            | 17                                 | 41          |
| <i>Other</i>                    |                                    |             |
| Yeasts, "actinos", lactobacilli | 9                                  | 9           |

<sup>1</sup>Number of isolates: 430 from raw green beans, 1300 from the post-blanch samples.

catalase-negative cocci. With peas, the incidence of these organisms increased from 26% on the raw vegetable to 42% on the blanched product (Table 2). The fact that the data represent a relatively large number of isolates, over 1400, suggests that some of these changes in distribution probably were real.

An even greater increase in the incidence of catalase-negative cocci was observed with green beans, from 17% on the raw vegetable to 41% on the post-blanch samples (Table 3). This is understandable when one considers that equipment contamination was primarily responsible for the microbial populations on peas both before and after the blanch. With beans, on the other hand, the microflora of the raw vegetable reflected contamination from soil and air along with actual growth on the vegetable while contaminated equipment was the source of organisms on the post-blanch samples. We suspect that a study of the microflora of green beans prior to harvest, that is in the growing field, might reveal even greater differences in the predominant groups. By the time the vegetable is delivered to the processing plant, the predominant microflora already may have been altered significantly.

GROUP PROPERTIES

Some of the isolates within the different morphological groups have been subjected to additional study in order to learn more about their identity and to obtain additional information regarding some of their properties.

Almost all of the gram-positive, catalase-positive cocci on raw vegetables were micrococci rather than staphylococci. The basis for this identification was that the isolates metabolized sugars oxidatively rather than fermentatively, and were coagulase-negative.

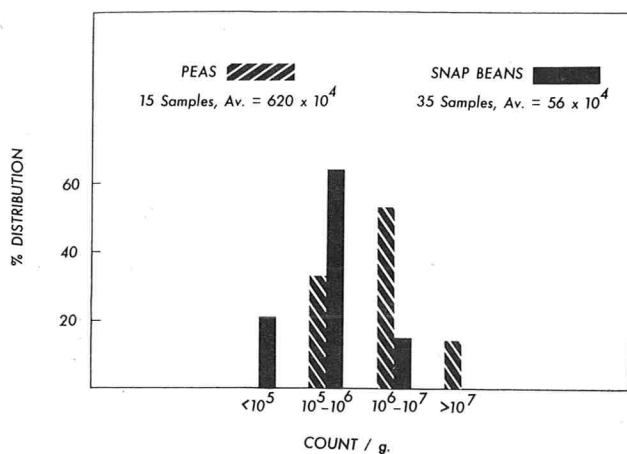


Figure 1. Frequency distribution histogram comparing "total" counts on raw peas and green (snap) beans.

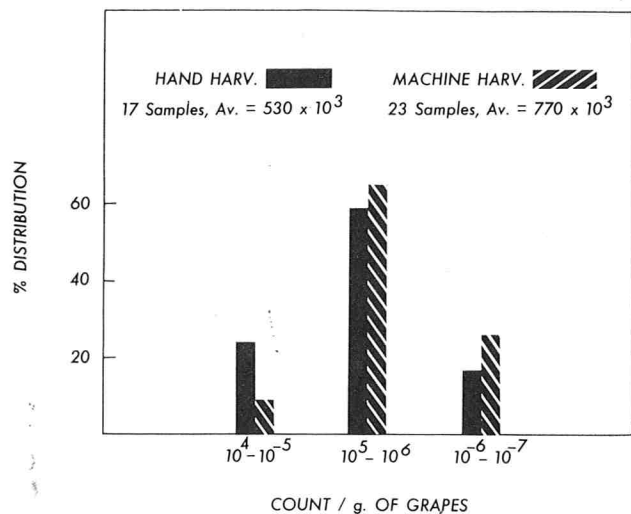


Figure 2. Frequency distribution histogram comparing the yeast and mold counts on hand-picked and mechanically harvested Concord grapes.

Similar results have been obtained with post-blanch vegetables in that many samples were negative for staphylococci and those that were contaminated with the organism invariably contained less than 10 per gram (13).

Studies on 71 cultures of gram-negative, catalase-positive rods indicated that a number of genera were represented. The most numerous types, 28 cultures, were non-motile and produced non-pigmented colonies. About 50% were oxidative (3) and were thought to be either achromobacters or non-motile species of *Pseudomonas*. The others resembled members of the genus *Alcaligenes* in that they did not produce acid from carbohydrates.

The second largest subgroup, 22 cultures, consisted of polar flagellates. It was concluded that most were species of *Pseudomonas* although a few yellow-pigmented isolates were thought to have been species of *Xanthomonas* (14).

Only nine of the gram-negative rod isolates were found to be coliforms. This agrees with previous findings which showed that although coliforms are a common contaminant of raw vegetables, they usually are not sufficiently numerous to be considered part of the predominant microflora (12).

A great majority of the gram-positive, catalase-positive rods, over 98%, were nonsporeforming species. The isolates were studied in greater detail than the previously discussed groups because it appeared that their identification, even as to genus, might prove to be very difficult. We were unwilling to merely lump the organisms under the title "coryneforms" which is one method of handling this poorly described group.

Our approach to the problem was to compare the isolates with known species using numerical taxonomic procedures (15). One hundred isolates from raw and post-blanch vegetables were studied along with five corynebacteria (that are pathogenic to plants,) three arthrobacters, and two species of microbacteria. A group of tests and observations that yielded 114 codable characters were made on the cultures. Similarity coefficients that took into account only positive matches were calculated using the formula of Sneath (8). Groups then were formed on the basis of single linkage, that is, a culture was added to a given group at the highest per cent similarity shared by it and any existing member of the group.

The results (Fig. 3) were that a majority of the vegetable isolates clustered into six groups. Each point in the figure represents an individual culture, thus, the data illustrate the level at which the isolates joined and the size of the different groups. Seventy five per cent of the vegetable isolates and none of the named cultures were included at these similarity levels. At 60% S, the six groups had merged and at 50% one large group encompassed all 100 vegetable isolates and 9 out of the 11 known cultures.

TABLE 4. SOME OF THE PROPERTIES POSSESSED BY 75% OR MORE OF THE CATALASE-POSITIVE, GRAM-POSITIVE RODS WITHIN THE INDIVIDUAL GROUPS

| Group           | I    | II   | III   | IV    | V     | VI    |
|-----------------|------|------|-------|-------|-------|-------|
| No. Isolates    | 13   | 5    | 13    | 21    | 18    | 5     |
| <i>Property</i> |      |      |       |       |       |       |
| Pleomorphic     | +    | -    | +     | -     | +     | +Br.  |
| Pigment         | yel. | -    | yel.  | yel.  | yel.  | -     |
| Hugh & Leifson  | var. | var. | oxid. | oxid. | oxid. | oxid. |
| Glucose-salts   | -    | -    | +     | +     | +     | +     |
| Gelatin         | -    | +    | +     | +     | +     | -     |

TABLE 5. SOME OF THE PROPERTIES SHARED BY 75% OR MORE OF THE HOMO FERMENTATIVE COCCI WITHIN EACH GROUP.

| Property   | Group C | Group D | Group E |
|--|---------|---------|---------|
| pH 9.6 broth, growth                                   | -       | -       | +       |
| 6.5% NaCl broth, growth                                | -       | -       | +       |
| 60 C 60 min, survive                                   | -       | -       | +       |
| 37 C, growth   | 50%     | +       | +       |
| 45 C, growth   | -       | +       | +       |
| NH <sub>3</sub> from arginine                          | +       | -       | +       |
| H <sub>2</sub> O <sub>2</sub> on MnO <sub>2</sub> agar | +       | +       | -       |
| Lactose, ferment                                       | -       | +       | +       |
| Malate, utilize  | +       | +       | +       |

TABLE 6. SOME OF THE PROPERTIES SHARED BY 75% OR MORE OF THE HETERO FERMENTATIVE COCCI WITHIN EACH GROUP.

| Property   | Group A | Group B |
|--|---------|---------|
| Dextran from sucrose                                   | -       | +       |
| Ferment arabinose and xylose                           | +       | +       |
| Ferment lactose  | +       | -       |
| Utilize malate   | -       | +       |
| H <sub>2</sub> O <sub>2</sub> on MnO <sub>2</sub> agar | +       | -       |
| Esculin  | -       | +       |
| α-hemolysis  | +       | -       |

The fact that the named cultures joined at such low similarity levels indicated that the vegetable isolates were not closely related to the corynebacteria, arthrobacters, and microbacteria with which they had been compared.

Table 4 shows some of the properties shared by at least 75% of the cultures within the individual groups. It can be seen that many of the organisms were similar to each other with respect to some of the more readily discernible properties such as pigmentation and pleomorphism. The morphology of the Group VI isolates plus a few in Group V included branched cells. Most of the cultures metabolized carbohydrates oxidatively although about 50% in Groups I and II were fermentative. Cultures in four groups grew well in a synthetic glucose ammonium salts medium indicating relatively simple nutritional requirements. Only the Group VI organisms grew after four serial transfers in the medium, however, suggesting a growth factor requirement by the others. Flagella were found only on the Group V organisms. A variety of types were observed, from polar monotrichous to peritrichous. This lack of homogeneity in Group V

is supported by the fact that its formation began at the low similarity of 71% S.

It is felt that the organisms in Groups I through IV were quite similar to the coryneforms that have been isolated from a variety of foods. The Group VI, and perhaps Group V, isolates may be closer to the arthrobacters which in turn may mean that their presence reflects soil contamination. Their relationship to the arthrobacters is suggested by their branched morphology and simpler nutritional requirements. One notable difference was that while most arthrobacters are strongly proteolytic, the Group VI cultures failed to hydrolyze gelatin.

Early studies on the catalase-negative, gram-positive cocci indicated that similar types were present on raw and blanched vegetables. They also revealed that many of the cultures, particularly the homofermentative strains, did not closely resemble described species. As a result, numerical taxonomy was again used as a means of grouping the cultures

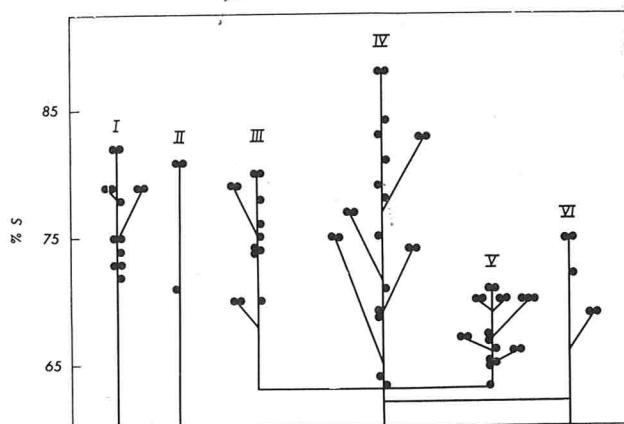


Figure 3. Dendrogram showing the per cent similarity at which each of the Gram-positive, catalase-positive rod cultures clustered. Each point represents an individual culture.

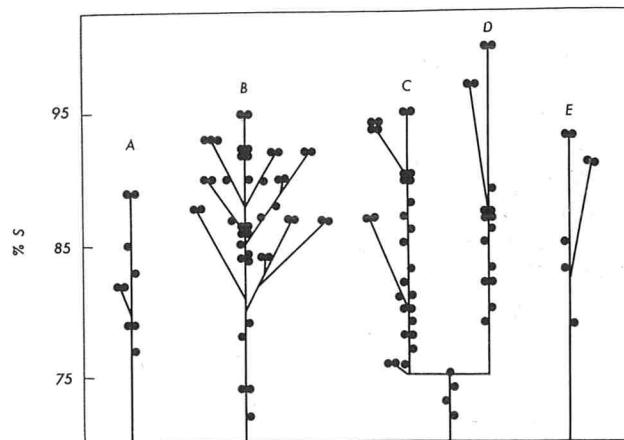


Figure 4. Dendrogram showing the per cent similarity at which each of the Gram-positive, catalase-negative coccus cultures clustered. Each point represents an individual culture.

and comparing them with established species. (16).

In this study, 135 isolates from post-blanch samples of green beans and corn were compared on the basis of 88 characters. Also tested were four strains of *Streptococcus faecalis* and single cultures of *Streptococcus faecium*, *Streptococcus lactis*, groups G, K, and N streptococci, and *Pediococcus cerevisiae*. The results (Fig. 4) were that a majority of the vegetable isolates, but none of the known or "type" cultures, clustered into five groups.

Table 5 presents some of the properties of the homofermentative cocci, Groups C, D and E. The Group E isolates were typical enterococci in that they grew at 10 and 45 C, in a pH 9.6 medium, tolerated 6.5% NaCl, and survived heating for 30 min at 60 C. They were not beta hemolytic, did not liquefy gelatin, and appeared to share traits with both *S. faecalis* and *S. faecium*. They resembled *S. faecalis* in their ability to reduce 0.04% potassium tellurite and to utilize malate but were similar to *S. faecium* in that they fermented arabinose, produced only a weak reaction in litmus milk, and did not utilize sorbitol, pyruvate, or gluconate.

The Group C cultures resembled the lactic streptococci in that they grew at 10 C but not at 45 C and produced ammonia from arginine. Only 50% of the cultures grew at 37 C suggesting that the group tended to be psychrophilic. All of the cultures were lactose-negative.

The Group D organisms grew at 10 and 45 C but shared few other properties with the enterococci and did not merge with them until the low similarity level of 69%. On the basis of growth temperature they resembled *Streptococcus uberis* but differed from this species in that they did not ferment sorbitol, utilize glycerol aerobically, or produce ammonia from arginine.

Groups A and B were heterofermentative cocci that produced significant quantities of CO<sub>2</sub> in glucose broth (Table 6). The Group B organisms were typical of *Leuconostoc mesenteroides* in that they produced dextran from sucrose and fermented pentoses. On the basis of these tests as well as their negative reaction for hydrogen peroxide, they appeared to be very similar to cultures isolated by Whittenbury (18) from grass silage.

The Group A organisms differed in many respects from those in Group B and the two groups did not merge until the low similarity level of 70%. This indicated that the Group A isolates were not merely dextran-negative strains of *L. mesenteroides*. Garvie (2) has proposed a new species, *Leuconostoc paramesenteroides*, for the non-dextran producing, pentose fermenting leuconostocs. These data support her proposal.

Little is known regarding the extent that different raw plant foods differ with respect to their microflora. Fruits, of course, with their low pH and high sugar harbor a flora considerably different from peas and beans. It is possible that because of inhibitors, the presence or absence of specific nutrients, etc., certain low acid vegetables also may possess a significantly different microflora. Our studies on post-blanch samples of whole kernel corn lend support to this in that on this vegetable catalase-negative cocci generally made up over 90% of the "total" count population.

#### ACKNOWLEDGEMENTS

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## THE FUTURE OF EDIBLE OILS

(Continued from Page 499)

partment has issued several studies that point out the impact of economies of scale in the butter industry and another in the substitute dairy products problem.

### NEW DAIRY FOODS FROM ABROAD

Last fall the Department of Industry, at the request of the National Dairy Council, arranged with Canadian embassies overseas to bring into Canada live samples of new dairy foods that might possess market potential in Canada. The results were most interesting.

The Department of Industry had flown in over 100 different foods from half a dozen countries—dry, frozen, chilled, liquid—you name it! Gelled milks, quarg cheese, a butter-margarine combination from Holland. (Isn't it ironical that some European countries stipulate that a per cent of butter must be added to margarine in order to increase the per capita consumption of butter, while in Ontario this is strictly taboo.) Kefir from Germany, a rice milk pudding from Greece (really excellent); butter and meat spreads from Holland; mousse from England; clarified butter; custards from Netherlands; garlic butter; flavoured milks; and many more. I recall how Mr. Ron Clark of Ottawa remarked that every one of the over 100 products possessed interesting market potential in Canada. Several of the food items were combinations of dairy and vegetable oil.

Arising from this project has come a more major one. The Department of Industry, Trade and Commerce is organizing a European trip for 15 processors to conduct an on-the-site inspection tour. The trip is scheduled for February 20 through to March 8, 1970 and will cover processing and research establishments in England, Holland, France, Switzerland, Germany, and Sweden, in a search to seek out new foods that the dairy processor can introduce to the Canadian consumer.

It is interesting to note in the Department of In-

dustry report on substitutes, the statement "Dairy processors, if they are to remain viable and maintain and expand their sales volume, will have to be in a position not only to manufacture these new products but also to be capable of competing with other food processors in marketing substitute dairy products."

### CHANGES IN THE INDUSTRY

There have been many public statements that the small farmer is inefficient and that a large number must leave agriculture. In 1963 there were 235,000 farms shipping milk—in 1969 this figure will be around 150,000.

Likewise, there are a group of processors who are unlikely, because of size limitations, to be able to survive and will be phased out. This natural attrition is happening at a rather startling rate. In 1961 there were 1,710 dairy processing plants in Canada. By 1969 it is estimated the number will be 1,100. It is forecast that by 1980 the number of producers and processors will be reduced by a further 30%. This trend to agri-business on the producer side and to an oligarchy-type of processing industry appears irreversible. I am reluctant to comment on the sociological repercussion resulting from the disappearance of the family farm and the independent processing entrepreneur from the scene except to say that I am deeply concerned about the long term adverse sociological "side-effects."

If the dairy processing industry wants to go anywhere in the seventies then we must use the methodology of the seventies. This will mean a consolidation of plants far beyond anything any of us has contemplated up to now. This consolidation will be necessary to collate the volume that will be required to sustain the sophisticated processes of the seventies.

Let me give you an example: The British Milk Marketing Board has recently built a new butter and powder plant. This plant built at a cost of several millions of dollars can handle over 1,000,000

(Continued on Page 515)

# DIVERSE ORIGINS OF UBIQUITOUS ENVIRONMENTAL CARCINOGENIC HAZARDS AND THE IMPORTANCE OF SAFETY TESTING<sup>1</sup>

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## ABSTRACT

A vast array of chemical carcinogens are present in the environment, many of natural origins and many as a result of man's use and abuse of technology. The background occurrence of such chemical hazards must be taken into account when evaluating the safety of proposed or existing chemical commodities, particularly those which, intentionally or unintentionally, become incorporated into the food supply. This report briefly describes hazardous chemicals present in the environment, how they are detected, how this information relates to potential human problems, and the importance of testing chemicals prior to their use by humans. Our past mistakes with biologically foreign chemical commodities serve as sufficient warning that the wholesale adulteration of the environment is to be avoided.

Recently, the American public has been confronted with many reports concerning the possible hazardous nature of certain food additives, drugs, insecticides, and herbicides. These reports have often been accompanied by conflicting views on the wisdom of restricting the use of commodities representing a potential threat to the members of the community that use them. On the premise that such controversy, in part, results from inadequate dissemination of important information regarding this area of public health, the present review is prepared.

## THE CANCER PROBLEM

Most Americans are aware of the magnitude of the cancer problem in the United States and throughout the world. Cancer ranks second behind cardiovascular disease as a cause of illness and death in the United States. An estimated 960,000 Americans are presently under medical care for cancer, with an estimated 625,000 new cases of cancer each year in this country (1). Nearly 330,000 Americans will

die of cancer in 1970 which represents a rate of about 900 people per day. An estimated 52 million Americans now alive, one of every four individuals, will eventually have cancer unless preventative measures are found.

The direct costs for diagnosis, treatment, and care of cancer patients amounted to \$1.2 billion in 1962 (44). A total of 54 million work days lost in 1962 as a result of illness and disability caused by cancer represents a loss of 221,000 man years of productivity or \$1 billion in terms of 1962 dollars. Thus, the dimensions of the cancer problem are painfully obvious.

Despite significant and steady improvements in early diagnosis and treatment of cancer, only one in three people afflicted with cancer will survive at least 5 years after the first diagnosis of their disease. The old cliché, "an ounce of prevention is worth a pound of cure" would appear most appropriate when dealing with the cancer problem. The determination of methods of cancer prevention necessarily requires the identification of the causes of cancer. The incidence of cancer has gradually increased over the last few decades, and it is becoming more and more obvious that environmental factors are playing causal roles in this problem. In fact, it has been estimated that about one-half of all human cancer is due to environmental factors (7).

Agents that produce cancer in humans and other animals are called carcinogens and include radiations, viruses, and chemicals. It has been repeatedly estimated, however, that probably 80 to 90% of human cancers are caused by chemicals (7, 13), most of which are present in our environment—being placed there by Nature and man. Practical preventative measures require first the identification of these carcinogens, and second the elimination of human exposure to these hazardous chemicals if at all possible.

Although this review is concerned only with chemical carcinogenesis, human exposure to chemicals poses other potential health hazards. The production of permanent heritable defects in mammalian germ cells by chemical mutagens in the environment is an area only beginning to receive systematic study (19, 24).

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<sup>2</sup>Career Development Awardee of the National Cancer Institute, United States Public Health Service (1 K4 CA-08245).

<sup>3</sup>Predoctoral Fellow of the National Institutes of Health, General Medical Sciences (1 FO1 GM 44402).

Similarly, the production of congenital birth defects by chemical teratogens represents a very real hazard as witnessed by the approximately 10,000 infants throughout the world that were deformed by the tranquilizer thalidomide (25, 70). In fact, one-third of the beds in children's hospitals are occupied by congenitally defective children. Indeed, congenital disease represents the third most common cause of death in the newborn (23).

#### DETECTION OF CHEMICAL CARCINOGENS

Epidemiology is the study of observing and recording the results of Nature's experiments on man, and man's experiments on himself (58). The discovery of a relationship between smoking and lung cancer is a classical example of epidemiological investigations of a situation in which man has been and continues to experiment on himself (58, 65). As we shall see later, such studies have been most important in detecting chemical causes of cancer in similar situations. This being true, it is obviously more desirable to detect chemical carcinogens, whenever possible, before we allow human exposure.

Laboratory investigations with experimental animals provide a second means of detecting chemical carcinogens (60, 69). This method requires the production of cancer in animals by exposing them to the chemical being tested, and is utilized to test potential carcinogens found in the environment, and potentially marketable chemicals as required by the Food and Drug Administration and other regulatory agencies.

In order to understand important characteristics of these animal tests, representative experiments with a group of chemicals known as nitrofurans will be considered. Some chemicals of this type, used in various parts of the world as drugs and also employed as food additives in Japan, have recently been shown to produce a variety of cancers in different experimental animals (21, 22, 43). For example, feeding rats a diet containing *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]acetamide has been shown to produce a very high incidence of breast cancer (Fig. 1) (20).

In such studies, results are reported in terms of the percentage of treated animals developing cancer, and are compared with the percentage of animals developing cancer in a control group of animals that did not receive exposure to the chemical being tested. Nearly every organ system is capable of yielding a tumor in response to an appropriate chemical carcinogen. If the incidence of cancer of a particular organ in treated animals is significantly greater than that observed in control animals, the chemical is judged to be a carcinogen. In the above test (Fig. 1), the incidence of breast cancer in rats fed the nitro-furan derivative was 91% after 66 weeks, whereas the

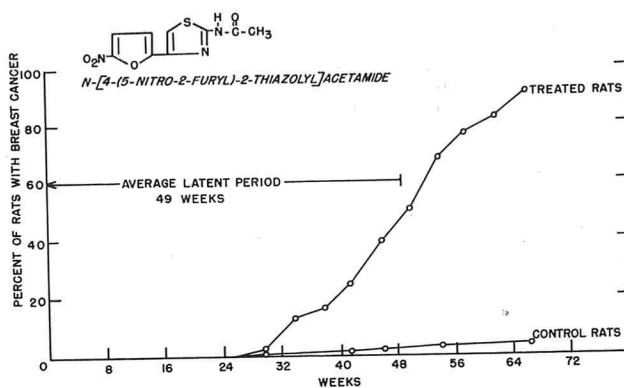
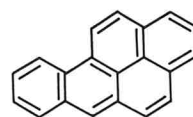
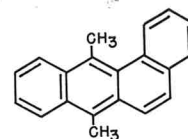


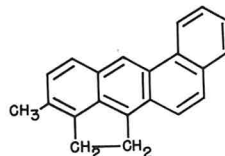
Figure 1. Production of breast cancer in rats by feeding *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]acetamide (20).



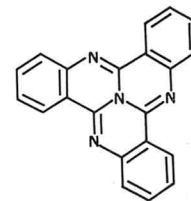
3,4-BENZPYRENE



9,10-DIMETHYL-1,2-BENZANTHRACENE



3-METHYL CHOLANTHRENE



TRICYCLOQUINAZOLINE

Skin, Subcutaneous tissue, and Mammary Gland Cancer in Rodents

Figure 2. Examples of carcinogenic polycyclic aromatic hydrocarbons.

incidence was only 5% in control animals after this period of time. The detection of such a carcinogen is relatively easy because of its strong, obvious activity. The detection of weak carcinogens or weakly carcinogenic mixtures of substances (12) is often considerably more difficult (18). However, it is doubtful that an affected individual feels much consolation with the knowledge that his cancer was produced by a weak carcinogen rather than a strong carcinogen.

A very important characteristic of all tests of chemical carcinogens is the latent period, which is that period between the time of onset of exposure to a carcinogen and the time of appearance of the resulting cancer. In the example in Fig. 1, the average latent period for development of breast cancer in rats was about 49 weeks. It is important to remember, however, that some carcinogens have latent periods many years in length and often the latent period is much longer in duration than the exposure period necessary to produce cancer. In other words, cancer produced by a chemical often does not appear for

a very long time after exposure of an animal to that chemical.

### HAZARDOUS CHEMICALS IN THE ENVIRONMENT

The list of chemical carcinogens present in the environment is rapidly increasing in length as further epidemiological and animal studies are conducted. Consequently, no attempt can be made here to list every carcinogen known, or thought, to pose a hazard for man.

In 1775, a London surgeon, Percivall Pott, reported what was the first strong indication that chemicals cause cancer in man (49), although reports suggesting chemicals as a cause of human cancer date back to at least 1761 (53). Dr. Pott recorded that cancer of the scrotum was commonly observed in chimney sweeps who at the early age of six or seven began their occupation of creeping up and down the sooty chimneys of London to clean them. This contact with soot along with poor hygiene was regarded as the cause of the cancerous "soot wart" seen in these pitiful children (49).

It took science over 100 years to begin to understand this problem. The answer was finally realized by the isolation from coal tars and soot of pure chemical carcinogens that were capable of producing many types of cancer in a variety of animal species (8). The hazardous chemicals present were members of what is now a large class of chemical carcinogens known as polycyclic aromatic hydrocarbons (Fig. 2). Of all the chemical carcinogens, this type may be the most prevalent in the human environment. Carcinogenic polycyclic aromatic hydrocarbons have been isolated in tobacco smoke (65), automobile exhaust and polluted urban atmospheres (56), wood smoke (54), smoked foods (40), and mineral oils (47).

Epidemiological studies suggest direct relationships between the prevalence of certain types of cancer in humans and exposure to polycyclic aromatic hydrocarbons. Over 1000 cases of cancer of the scrotum in workmen exposed to certain mineral oils containing 3,4-benzpyrene have been recorded (47). A relatively high incidence of cancer of the stomach in farm families living in Iceland appears to be related to the consumption of many homesmoked foods by these rural people and consequently a greater exposure to polycyclic aromatic hydrocarbons present in foods of this type (62). Similarly, a higher incidence of lung cancer in non-smokers living in large cities than in non-smokers living in rural areas may be related to the degree that the air is polluted by exhaust fumes containing such carcinogens (54), although the precise role of atmospheric pollution in the human lung cancer problem remains uncertain (72).

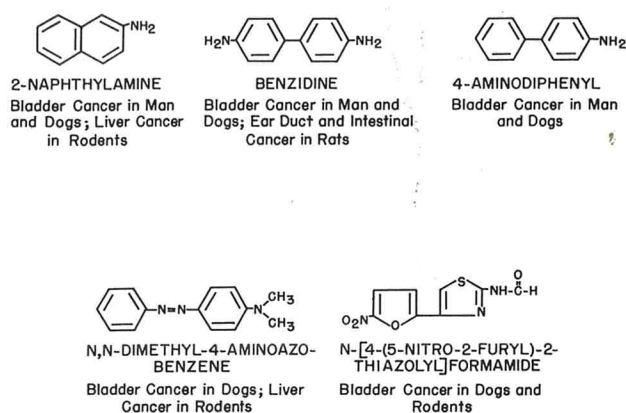


Figure 3. Examples of carcinogenic aromatic amine derivatives.

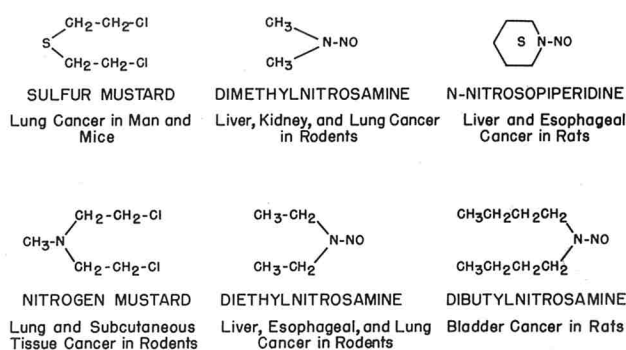


Figure 4. Examples of chemical carcinogens which exist as or give rise to alkylating agents *in vivo*.

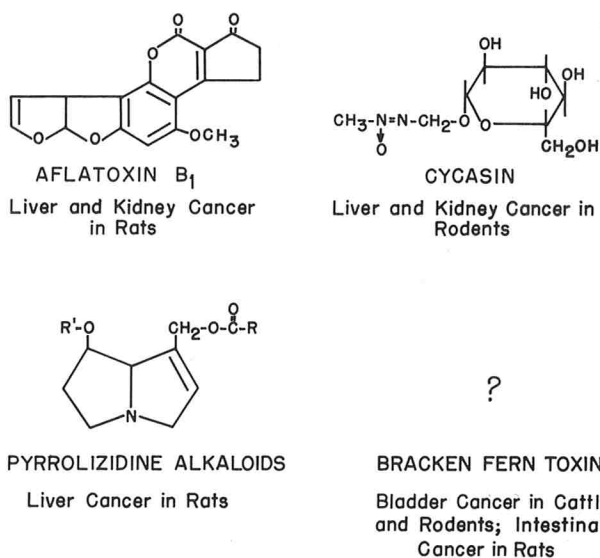


Figure 5. Examples of naturally-occurring chemical carcinogens.

Another indication of chemical carcinogenesis in man was reported in 1895 by Dr. L. Rehn in Germany who observed an association between the occurrence of cancer of the urinary bladder and exposure of workmen to chemicals in the German dye industry.



Since that time, scientific studies leave little doubt that many of these chemicals known as aromatic amines, are responsible for the production of certain human cancers (Fig. 3) (30).

As an example, as many as 76% of workers exposed to 2-naphthylamine or benzidine in a single dye factory have developed cancer of the bladder (14). Similar unfortunate situations also have occurred in the industries that utilized aromatic amines in the production of rubber and cables. An estimated 2500 to 3000 cases of bladder cancer related to the occupational exposure to aromatic amines have been reported throughout the world, and more than 600 cases have been reported in the United States (30). Industry and government have cooperated in the institution of safety measures to minimize human exposure to these chemicals. However, it might be considered unfortunate by the detached observer that in England, for instance, 15 years passed between the convincing demonstration that certain aromatic amines cause bladder cancer in man and the time at which these chemicals were placed under governmental control (13). It is very likely that a number of workmen received a sufficient exposure to carcinogenic aromatic amines in this time period to bring about their untimely demise. At that time, as in the present, the problem of legal control was complicated by the unwillingness of the community to give up the economic advantages which the use of such hazardous chemicals presents.

Many aromatic amine derivatives are now known to be carcinogenic (Fig. 3) and many are present or have been proposed for use in the human environment. Of interest is the recent isolation of a known human bladder carcinogen, 2-naphthylamine, from tobacco smoke (27). Indeed, there is evidence of a relationship between smoking and cancer of the bladder (65).

Another important class of chemical carcinogens is known as alkylating agents, many of which are very strong carcinogens and many of which are receiving considerable attention as potential environmental contaminants (Fig. 4). One instance of human exposure to alkylating agents dates back to World War I when soldiers were exposed to the potent war gas, sulfur mustard, and again in World War II when soldiers were exposed to the "improved" nitrogen mustard. American veterans so exposed are said to show an increased risk of lung cancer (59). In addition, a high incidence of cancer of the lung and upper respiratory tract in workmen engaged in the manufacture of mustard gases has been observed (67).

Carcinogenic alkylating agents can also be produced *in vivo* from certain nitroso compounds such

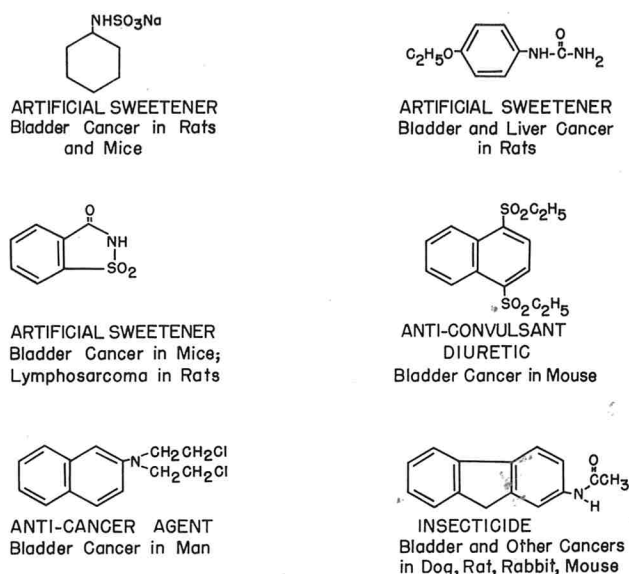


Figure 6. Examples of chemical carcinogens used or proposed for use by man.

as dimethylnitrosamine and *N*-nitrosopiperidine (Fig. 4) (39). Dialkylnitrosamines produce cancer of the lung, liver, kidney, bladder, or esophagus in a diverse group of animal species and have been isolated from certain foods such as mushrooms and smoked fish and meat products (17, 39). It is very likely that the occurrence of these hazardous chemicals in foodstuffs is related to the use of nitrite in their preparation. The ability of nitrite to react with secondary amines to form carcinogenic dialkylnitrosamines certainly presents potential hazards to the human population (38). Alkylating agents as a class of chemical carcinogens are also beginning to receive attention as atmospheric contaminants (55).

Numerous other classes of chemical carcinogens exist including aliphatic chlorocarbons, certain hormones, inorganic chemicals, and miscellaneous liver toxins. Many of these also occur in the environment and the reader is referred to other sources for detailed information in these areas (13, 29-31, 59). The chemical carcinogens discussed thus far are primarily produced by man and are either accidentally or intentionally introduced into the environment by man's use and abuse of technology. It is difficult at this time to assess the exact extent to which these chemicals are related to the human cancer problem. However, the fact remains that our environment is becoming increasingly contaminated with a variety of products of industrial and individual use.

Recent attention has been drawn to certain insecticides as possible carcinogens. A few years ago, an epidemic of liver cancer afflicted the domestic trout population in the United States (35). Attempts to determine possible causal factors resulted in the provocative finding that the insecticide, DDT, when

fed to trout in low doses, produced liver cancer. Similar results were observed in mice by feeding DDT or a number of other insecticides (33). These findings are of considerable importance in view of the widespread use of insecticides in agriculture. The reproductive failure and population declines of predatory birds serve as sufficient warning (48) that "the number of hazards to animal and human populations inhabiting areas which our rivers drain have reached dangerous proportions (59)." The delta area of the Mississippi River appears to act as a septic tank for much of the country and it has been often suggested that contaminated water runoff may be related to the high incidence of both lung cancer and bladder cancer for which the inhabitants of the area are noted (30, 59).

Aside from the chemical hazards described above, many of which we are all exposed to in varying degrees, recent studies have demonstrated the presence of naturally-occurring chemical carcinogens of plant origin. Exposure to these carcinogens would usually be realized by the utilization of such plant materials as food stuffs. The possibility that humans are being exposed to hazardous chemicals present in the food supply has caused considerable concern among many members of the general public probably because this problem is, so to speak, closer to home. For this reason, the remainder of this review will consider the sources of hazardous chemicals in foodstuffs, with particular attention to food additives and the relevance and importance of safety testing.

#### POTENTIAL CHEMICAL HAZARDS IN THE FOOD SUPPLY

The food supply represents a major route of exposure of human and animal populations to non-nutrient chemicals of potentially hazardous nature. There are three principle means by which these chemicals appear in foods (71).

*First*, naturally-occurring carcinogens often become incorporated into the food supply. An important example is bracken fern, a plant commonly used as a salad green in various parts of the world and canned and sold in the northeastern United States and Canada as "fiddlesticks." In Turkey, bracken fern is used as the principle forage crop for cattle and 50 to 90% of such cattle have been observed to develop cancer of the urinary bladder. Feeding bracken fern to many different kinds of animals is now known to produce bladder or intestinal cancer in high incidence (46, 52), and the obvious potential hazard to humans using bracken fern in their diets can be easily avoided by discontinuing the use of bracken fern as a foodstuff.

Cycasin, a naturally-occurring glucoside found in the seeds and roots of cycad plants of tropical and

subtropical areas, is a strong carcinogen showing activity in a number of rodent species (37). Hepatotoxic and hepatocarcinogenic pyrrolizidine alkaloids are present in a number of botanical species. The use of such plant materials for medicinal and food purposes may be causally related to the high incidence of liver cancer and chronic liver disease observed in the inhabitants of the tropics (Fig. 5) (57).

Likewise, safrole, a natural component of many essential oils, has been shown to produce cancer of the liver in rats. Safrole has been widely used as a flavoring agent in root beer and in certain pharmaceutical preparations (28). In addition, evidence exists that carcinogenic polycyclic aromatic hydrocarbons may be a naturally-occurring component of certain plant tissues used as foodstuffs (28).

Since the discovery of the carcinogenic effects of the aflatoxins, metabolic products of the ubiquitous *Aspergillus flavus*, as a result of epidemic poisonings of domestic turkey and trout populations (35, 58), fungal contamination of foodstuffs has received attention as a possible factor in the etiology of cancer. A number of fungal products possess carcinogenic activity (4, 35, 72), and further studies in this area will no doubt lead to a more complete understanding of the causes of human cancer.

*Second*, hazardous chemicals can appear in foods as contaminants produced in some phase of food production, processing, or storage. As an example, we can recall the production of carcinogenic polycyclic aromatic hydrocarbons as a result of the incomplete combustion of organic materials in the process of preparing certain smoked foods (40). Similarly, the uncritical utilization of nitrites as preservatives may contribute to the presence of carcinogenic dialkyl-nitrosamines in certain fish and meat foodstuffs (17, 38).

*Third*, a great many chemicals are intentionally added to foods during processing or manufacturing. The potential hazards of such chemicals have been recognized and their uses are, in general, regulated by the Food and Drug Administration in attempts to minimize public health hazards.

#### THE IMPORTANCE AND RELEVANCE OF SAFETY TESTING

In 1958, the Food and Drug Administration, with the help of scientists, compiled a list of substances that were *generally regarded as safe* (GRAS) for human use on the basis of studies existent at that time. However, more thorough and improved tests developed and utilized since that time have often suggested that some of the chemicals on the GRAS list may not be safe. Figure 6 shows the structures of a few chemicals that were used or proposed for

use by humans and that were later shown to possess significant carcinogenic activity.

As an example, we can consider the case of the cyclamates which have been used as artificial non-caloric sweeteners. At first, cyclamates were used primarily by diabetics, but in the last few years, they have been incorporated into diet foods and other pre-sweetened foods used extensively by the general consumer. In October of 1969, the Secretary of Health, Education, and Welfare ordered that cyclamates be removed from the GRAS list on the basis of more recent studies. Utilization of the pellet implantation technique, which involved the surgical placement of a small (25 mg) pellet of cholesterol containing 20% by weight of sodium cyclamate into the lumen of the mouse urinary bladder, with two groups of 58 and 49 mice demonstrated the capability of cyclamate to produce bladder cancer in 78 and 61% of the animals respectively (9). In addition, feeding a diet containing large amounts of a mixture of cyclamates and saccharin to 80 rats produced bladder tumors in 8 of these animals (51). Because of the revised 1962 Delaney clause of the Food Additives Amendment (68), which states very clearly that an additive which produces cancer in man or animal cannot be utilized in the food of man, the use of cyclamates was promptly restricted by law.

As might be expected, the restriction of a potentially hazardous chemical after it has come into common use by the community and industry is attendant with much criticism. It can be, and is, immediately asked what relevance animal studies have for the human situation and we are constantly reminded that "there is no evidence that cyclamates have been harmful to man" (32, 51, 64). We all hope this to prove true, but unfortunately, carcinogens produce tumors only after enough time has passed to encompass the latent period. In humans, carcinogens such as 2-naphthylamine and benzidine (Fig. 3), which are known to produce bladder cancer in man and experimental animals, have an average latent period of over 20 years and in some instances, the latent period can be as long as 40 years (14, 30). Thus, the cyclamates may not have been in general use long enough for possible carcinogenic activity in man to be observed at this time. In addition, because of the extremely widespread use of cyclamates by the general public, any increase in the occurrence of human bladder cancer might be scattered throughout the country and diluted out by the occurrence of bladder cancer of other causes. Consequently, epidemiological studies on the role of cyclamates in the human cancer problem would be very difficult to carry out and, in fact, have not been conducted at this time.

Since studies with hazardous chemicals in humans

are both immoral and impractical, studies utilizing experimental animals are all that are available, and the determination of the safety of a chemical for human use is fraught with uncertainties (11, 26). All organic chemicals known to produce cancer in man also produce cancer in one or more animal species, and if a chemical produces cancer in one or especially in more than one species, there is no reason to believe that man is immune to these effects (7, 41). Unfortunately, because of economic or other reasons, some members of the community and industry, often and understandably, impose pressure on governmental agencies to delay or prolong the attempts to remove potentially hazardous chemicals from the environment until the damaging effects have been proven beyond all shadow of doubt by unfortunate public example. This attitude may not at all be desirable and in many instances is clearly unacceptable. Recalling some of our past mistakes with chemical carcinogens and chemical teratogens, it would appear that the lessons to be learned are done so with considerable difficulty.

Another common criticism of animal tests is that the doses of chemical administered to the animals are often far greater, on a per weight basis, than those to which humans are exposed (32). It is interesting to note, that if this argument were at all valid in itself, there would be no reason to restrict the use of thalidomide (23). Because of high costs, animal studies are usually conducted on only 20 to 100 animals, making it impossible to detect tumor incidences of only a few per cent or less in most instances. There is, consequently, a possibility that these tests will miss the detection of a weak but definitely active carcinogen, an outcome not too unusual in animal studies (58). To avoid this possibility, chemicals are usually tested in high doses to insure an effect will be observed if, indeed, the chemical is carcinogenic. This is most important. It is often pointed out that if humans were exposed to a weak carcinogen in doses capable of producing only a 0.1% tumor incidence in the human population, and if nearly everyone in the United States were so exposed, we might realize nearly 200,000 cases of cancer after a suitable latent period. This result would be most appalling if, unlike cigarette smokers, these victims had been exposed to a carcinogen through no choice of their own. It should be mentioned that the detection of a weak carcinogen producing only a 0.1% incidence of cancer in a mammalian population would be nearly impossible with existing animal safety tests. It is also worthwhile to note that some food additives such as the artificial sweeteners are utilized by an estimated 75% of the population of the United States (45).

The establishment of tolerance levels for carcinogens is quite unrealistic since the determination of the dose of a carcinogen that would be safe for a large population of humans is next to impossible. If one accepts as "safe," a dose level which would affect only one individual in a population of 100 million, then that dose level, as determined by mathematical extrapolations, is often vanishingly small. For example, the "safe" dose of the carcinogen, 3-methylcholanthrene (Fig. 2), in mice is on the order of  $9 \times 10^{-8}$  mg per mouse (41). Studies of dose-tumor multiplicity relationships have been conducted with the interesting result that graphical plots of dose versus the number of tumors produced per individual often extrapolate back through the origin (5, 6), suggesting that in susceptible individuals, any dose gives rise to a finite probability of tumor development.

As is true for other drugs, dose-response relationships can be constructed for carcinogens. However, it has been concluded that while a no-effect level with chemical carcinogens may be determined for a small population of animals, this level is valid only for a specific experimental protocol (68). As the dose of a carcinogen is decreased, the latent period is merely lengthened and the final tumor incidence observed is a function of the total dose, the sum of all individual doses, of compound administered (16, 68). This is no doubt a reflection of the rather unique way in which chemical carcinogens are believed to elicit a carcinogenic response in susceptible tissues.

Most drugs exert their effect in a reversible manner and are metabolized and excreted so that the initial dose need not impinge upon subsequent later doses. However, carcinogenic organic chemicals with few, if any, exceptions, exist as or are metabolically converted to electrophilic reactants capable of attacking, via the formation of firm covalent bonds, low molecular weight tissue nucleophiles and nucleophilic centers in biologically important macromolecules (42). It is generally believed that such irreversible alterations brought about by alkylation or arylation of DNA, RNA, and protein initiate the carcinogenic process by giving rise to a cell or cells which at some later time, and subject to many modifying factors, may give rise to a malignancy (5, 50).

The carcinogenic effect of all individual doses can persist over the life span of the animal, although the agent is no longer present, and eventually sum up to formation of a cancer (16). It has often been suggested, and it seems quite probable, that nearly all humans would develop malignancies if they lived long enough to receive a sufficient exposure to diverse exogenous carcinogenic stimuli and to sufficiently encompass the corresponding latent period. Success in cancer prevention might only require a de-

crease in human exposure to chemical carcinogens to a degree such that the corresponding latent period is significantly greater than human life-expectancy. In this regard, it is vitally important that children who have a long life-expectancy be spared unnecessary exposure to carcinogenic hazards (16). This becomes even more important since many carcinogens can act in an additive or synergistic manner whenever the target tissue or tissues and the mechanism of tumor formation are the same. It is becoming more and more obvious that two or more chemical substances can interact in the living organism to produce totally unexpected types and degrees of response. In addition, many non-carcinogenic agents including such mundane substances as naturally-occurring phenols (34), and vegetable fats and oils (63), as well as components of tobacco smoke (66) can act as cocarcinogens in the process of carcinogenesis by bringing about an increase in tumor yield, a shortening of the latent period, or both. When weakly active carcinogens act in conjunction with non-carcinogenic cocarcinogens, the result is often similar to that observed with strong carcinogens (3). As such, cocarcinogens may be another factor important in the human cancer problem and the implications of cocarcinogenesis may have to be considered in cancer prevention (2).

Because of the vast number of carcinogens present in the environment, because of their apparent irreversible modes of action, and because of possible additive, synergistic, and cocarcinogenic complications of the problem, the background occurrence of carcinogens in the environment must be taken into account when evaluating the safety of a proposed or existing chemical commodity.

#### THE WEIGHING OF RELATIVE BENEFITS AND RISKS

It is obvious that many of the food additives and other chemical commodities presently being used are essential to our way of life in the United States. However, it is said that the last ten years has seen a 50% increase in the number of food additives (36), many of which we know little about. Past results with chronic toxicity tests suggest that as many as one of every six biologically foreign organic chemicals tested will display carcinogenic activity (61), and the need for adequate safety testing is obvious. Although present methods of testing chemicals for carcinogenic activity are far from perfect, test systems have been described which provide us with a reasonably high degree of protection (12, 23, 61, 69, 73). It has recently been urged that many of the newer model systems for studying chemical carcinogenesis should "not be accepted as standard tests until more is known about the possibility of obtaining false positive

results" (26). While false positive results are not desirable, it is perhaps more important to avoid those test systems prone to providing false negative results. Nevertheless, when evaluating the safety of chemical commodities, we must use some common sense in weighing the relative benefits and risks of potentially hazardous substances. The policy suggested by the National Academy of Sciences-National Research Council (12) would seem appropriate for food additives as well as the many other chemicals to which humans are exposed. They suggest that "because of the vagueness of present knowledge concerning quantitative aspects of the carcinogenic process, use of any amount of a carcinogen as a food additive probably is justified only if (a) values to the public are such that banning the use would constitute an important loss or hardship, and (b) there is no reasonably good noncarcinogenic alternative".

With this in mind, when the safety of a proposed or existing food additive or chemical commodity is questioned on the basis of sound data, it would seem only good sense to assume that the chemical is guilty until proven innocent unless the benefits of that chemical far overshadow possible risks. As an example, we can again consider the case of the cyclamates and their sister sweetener, saccharin (Fig. 6).

Recently, utilization of the pellet implantation technique to administer small doses of saccharin to two groups of 66 and 64 mice has been shown to produce bladder cancer in 47 and 52% of the animals, respectively (10). Recalling that rats fed a mixture of saccharin and cyclamate developed bladder cancer (51), the case against these artificial sweeteners seems to be equally stated. Until further tests are conducted, the use of both of these sweeteners should be restricted to those older people whose medical needs definitely require them. The benefits to the general public of an artificial sweetener used to replace sugar in even non-diet foods simply do not overcome the potential hazards in the case of cyclamate and saccharin.

One must keep in mind that the benefits of many food additives, particularly food preservatives, differ from region to region. In highly developed countries, with facilities for refrigerated transportation and storage, the need for chemical preservatives might be greatly reduced. Similarly, the use of chemical agents which only make a product appear of better value have minimal benefits except in an economic sense (15).

As a specific example, nitrites, which have been used in the preservation of fish, are believed to react with trimethylamine which is normally present in fish tissue, to produce the strong carcinogen dimethylnitrosamine. In addition, nitrite is capable of reacting with secondary amines present in the food sup-

ply possibly leading to the production of other carcinogenic nitrosamines. Thus, a good deal of consideration is deserved of the possibility that "Reduction of human exposure to nitrites and certain secondary amines, particularly in foods, may result in a decrease in the incidence of human cancer" (38). Human exposure to nitrites could be easily decreased in view of the fact that the use of nitrites in the preservation of fish, for example, is said to have no functional value except in lieu of good manufacturing and handling practices (15).

It must be pointed out at this time that the intent of this review was not to alarm, chastise, or indict anyone. However, we should all be aware of the existing and the potential problems besetting our way of life and we should remain open-minded and work constructively to maintain a safe and pleasant environment. As mentioned by Shimkin (58), a completely safe environment may not be desirable since "Life is a hazard, and death is inevitable for all biological material in order to continue and to improve the species." Realizing the existence of relative values and competing objectives, this in no way absolves us from the task of improving and maintaining public health at a level that actually does lend to a continuation and improvement of the species. This is proving to be a difficult enough task without our apparent willingness to take risks with our environment.

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## THE FUTURE OF EDIBLE OILS

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lb. of milk daily. It has closed circuit television. Most of the process equipment automatically sets temperatures, flow, pressure, etc. with the aid of a digital computer. The computer checks the position of 278 zephyr valves, 42 flo-splitters, 70 purge valves, 110 pumps, 33 drain valves, 38 contents levels, 54 pressure valves, 14 flow rates, 43 temperatures and densities, and 22 other services.

How the Canadian processor operating under the aegis of the free enterprise system will voluntarily consolidate his facilities still remains somewhat of a mystery. But amalgamate he must or die.

The story of the British creamery can fit every phase of Canadian dairying. Given adequate volumes of throughput, modern technology can reduce unit labor costs to all-time lows—with consistently higher quality levels. Even if this problem of plant rationalization can be resolved and a degree of governmental participation will probably be required to effect it, several other road blocks will still remain.

### EXCISE TAX AND EDIBLE OIL ACT

What about the Federal Excise Tax? At present,

"milk, buttermilk, condensed milk, evaporated milk, powdered milk, cream, prepared whipping cream, butter, cheese, ice cream, and yogurt" are exempt from the tax. The tax, by the way, is approximately 12% of the wholesale selling price. Any dairy food that does not qualify under one of these categories is technically subject to the tax. Under this restriction what incentive is there to develop new dairy foods? Surely it is time for the dairy processor to realize that he is a food manufacturer and actively support efforts to remove this legislative road block.

And I predict that the Edible Oil Act of Ontario that ostensibly was designed to aid the producer and processor will, if not amended, do precisely the opposite. Let's re-examine this Act in the light of the seventies. Then the road block of nutritional quality must be examined.

Our Federal Food and Drug Directorate has consistently demonstrated a sympathetic and understanding policy. One could only surmise that when the technologists can synthesize an equivalent counterpart to the natural elements of milk that this will prove to be a rather minor road block.

Perhaps I have attempted to answer this question of the future of edible oils in the dairy industry in

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# GROWTH OF *STAPHYLOCOCCUS AUREUS* IN ACIDIFIED PASTEURIZED MILK<sup>1</sup>

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## ABSTRACT

The effect of gradually reducing the pH of pasteurized milk with acetic, citric, hydrochloric, lactic, and phosphoric acids over periods of 4, 8, and 12 hr on growth of *Staphylococcus aureus* 100 in this substrate was determined. In addition, 1:1 mixtures of lactic acid and each of the other acids, and of acetic and citric acids were evaluated for their effect on growth of this organism. To achieve a 90% reduction in growth over a 12 hr period, a final pH value of 5.2 was required for acetic, 4.9 for lactic, 4.7 for phosphoric and citric, and 4.6 for hydrochloric acid. A 99% reduction during a 12 hr period was obtained with a final pH value of 5.0 for acetic, 4.6 for lactic, 4.5 for citric, 4.1 for phosphoric, and 4.0 for hydrochloric acid. A pH value of 3.3 was required for a 99.9% reduction with hydrochloric acid, whereas the same effect was produced at a pH value of 4.9 with acetic acid. Correspondingly lower pH values were required to inhibit growth within 8 and 4 hr periods. Mixtures of acids adjusted to pH values at the borderline for growth (12 hr period) exhibited neither synergistic nor antagonistic effects between two acids.

Several reports in recent years have alluded to a direct relationship between pH and degree of growth by or survival of *Staphylococcus aureus* in dairy products. It is widely accepted that starter culture growth and activity in fermented dairy products has an inhibitory effect on *S. aureus* (1, 2, 4) but the contribution by pH has not always been clearly demonstrated. Zehren and Zehren (8) examined manufacturing records for 378 vats of cheese of which 59 were found to contain staphylococcal enterotoxin A and the authors concluded that a relationship existed between subnormal acid development and toxin production. Growth of staphylococci in various kinds of cheese was investigated by Tuckey et al. (5) who suggested that a pH value above rather than below 5.3 was more favorable for proliferation of the bacteria. According to Walker et al. (6), no correlation existed between staphylococcal populations and pH values of 5.1 to 5.75 in Colby cheese during ripening for 120 days.

The present study was conducted to define more clearly the relationship between acidity in pasteurized milk and growth of *S. aureus*.

## MATERIALS AND METHODS

Two series of experiments were performed. The first dealt with the effect on staphylococcal growth of gradually adding to milk a selected quantity of a single acid over a 4, 8, and 12 hr period. The second series involved a comparison of the effect of two acids acting independently with that obtained when the acids were mixed (1:1) and added gradually over a 12 hr period.

### Apparatus

Each experiment was performed using the apparatus illustrated in Fig. 1. The basic unit of the system is a 2-liter, jacketed, three-necked flask (ST 24/40 joints) equipped with a 6 mm bore stopcock for draining (Corning Glass, No. 94002). The center neck is fitted with a mercury-seal stirrer bearing and a glass stirring rod (9 mm in diameter) with an attached Teflon blade (93 mm wide; Kontes, No. K-780530). The stirrer is driven by a 55 watt, 1550 rpm Tal-boy T-line motor (No. 105). The neck on the right of the flask is equipped with a 25 ml burette and the neck on the left is fitted with a thermometer (and also serves as a port through which the milk is added and small samples removed). Four flasks are employed in each experiment, permitting the simultaneous study of three variables while one of the flasks serves as the vessel for a control. The flasks rest on padded rings connected to a 36 by 30 inch frame. Each flask is held firmly in place by two large clamps.

The stirring motors are all connected to a single rheostat. Stirring speeds are regulated by measuring and controlling the total amperage output from the rheostat. The relation between amperage output and rpm of the revolving stirrer shafts has been determined. A stirring rate of 300 rpm (with a variation of  $\pm 10\%$  between flasks) was used throughout this study.

Temperature-controlled water is provided by a Tecam Temp Unit (A.S. LaPine, No. 414-60) appropriately attached to a large stainless steel beaker. The water is circulated to the flasks through 0.5 inch (inside diameter) tubing connected to an Eastern centrifugal pump driven by a 1/15 hp, 5,000 rpm motor operating at nearly full capacity. Rather than connect the flasks in series, we have provided a single tube with branches to simultaneously feed water to the bottom of the jackets of all the flasks and a single branched tube to return water from the tops of the jackets to the steel beaker. Since circulation of water is rapid, we have been unable to detect differences in temperature between flasks.

Between experiments flasks were cleaned-in-place, then sanitized-in-place with a 200 ppm hypochlorite solution, and allowed to drain dry overnight before use.

### Addition of acids

The following acids were used: acetic, glacial, A.C.S. reagent (Allied); citric, granular, analytical reagent (Mallinckrodt); hydrochloric, reagent (DuPont); lactic, USP 85% (Mallinckrodt); and phosphoric, A.C.S. reagent (Allied).

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



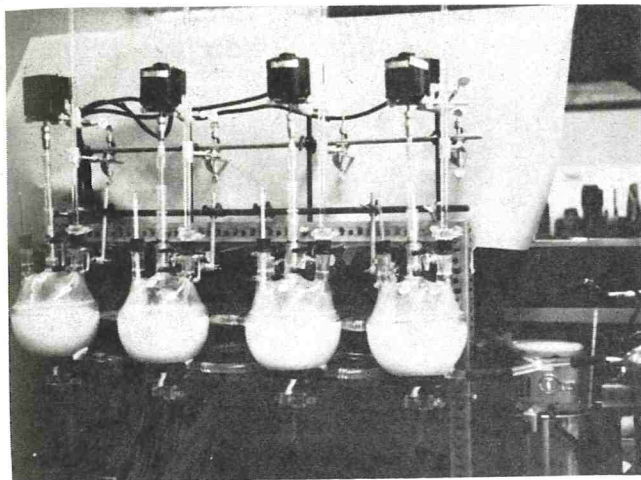


Figure 1. Apparatus used for growth of *Staphylococcus aureus* in pasteurized milk.

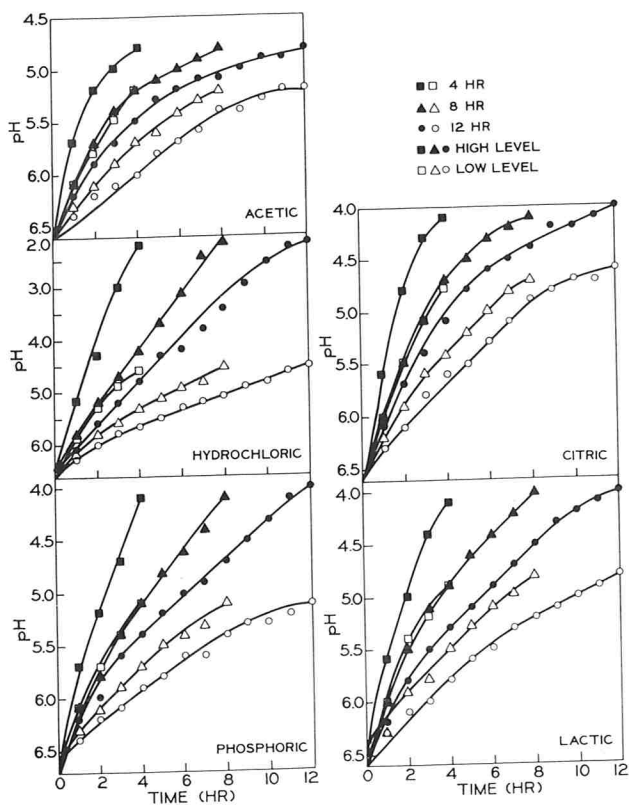


Figure 2. Change in pH of pasteurized milk achieved through the addition of two levels of acetic, citric, hydrochloric, phosphoric, and lactic acids independently over periods of 4, 8, and 12 hr.

Pasteurized homogenized whole milk of excellent bacteriological quality (Standard Plate Count of approximately 300 per milliliter) was obtained from the University of Wisconsin Dairy plant on the day preceding each experiment. One quart was used per flask.

Acids were prepared as solutions in distilled water at concentrations so that the desired quantity could be distributed over the course of the experiment in 0.5 ml increments added to milk every 15 min. The acidic solutions were steamed 15 min to reduce microbial contamination and dispensed from

autoclaved 25 ml burettes. Steamed distilled water was added to one of the flasks in each experiment to serve as a control.

Titrateable acidity and pH were monitored hourly in the first series of experiments and every 2 hr in the second series.

**Culture**

A culture of *S. aureus* 100 (a good enterotoxin A producer) was obtained from Dr. K. F. Weiss (Food Research Institute, University of Wisconsin). Cultures were carried on Brain Heart Infusion agar (Difco) slants for up to three months and were transferred twice in Brain Heart Infusion broth before use. All four flasks in each experiment were inoculated with 5 ml of a 1:100 dilution of an  $18 \pm 1$  hr broth culture in phosphate buffer. This resulted in an initial plate count of approximately  $2 \times 10^4$  cells per milliliter in each flask. An incubation temperature of 37 C was provided for optimum growth of *S. aureus*.

**Determination of growth**

Plate counts were performed according to recommendations of *Standard Methods for the Examination of Dairy Products* (7) every 4 hr and in the first series of experiments a count was determined after 2 hr. Plates were poured with Mannitol Salt Agar (Difco) and incubated at 37 C for 2 days. Mannitol-fermenting colonies of proper size and appearance were reported as *S. aureus* and occasionally were confirmed by microscopic examination.

The natural flora of the milk interfered with enumeration of staphylococci when Staphylococcus 110 Medium (Difco)

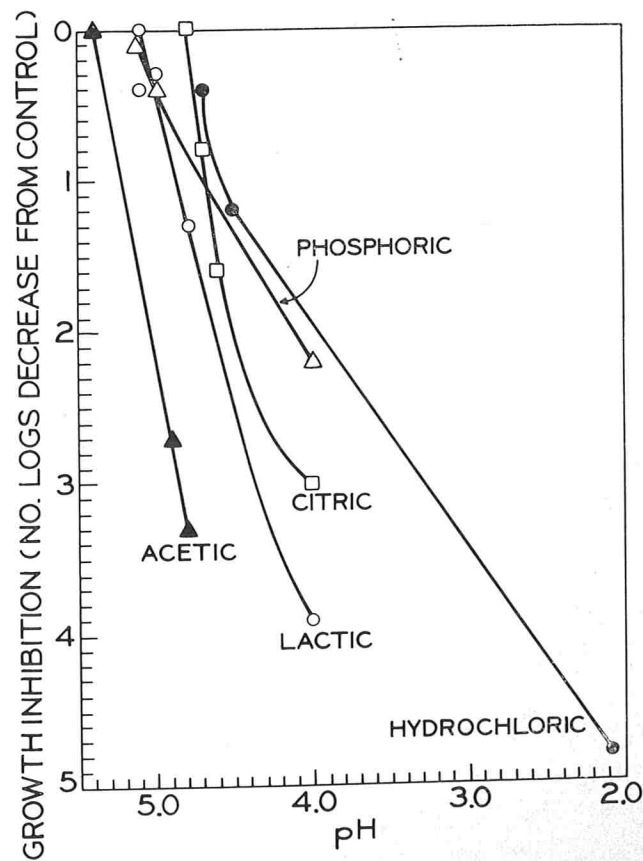


Figure 3. Relationship between terminal pH value of pasteurized milk fortified with different acids and inhibition of *Staphylococcus aureus*.

TABLE 1. GROWTH OF *Staphylococcus aureus* 100 IN PASTEURIZED MILK ACIDIFIED WITH DIFFERENT ACIDS DURING 4 HOURS OF INCUBATION AT 37 C<sup>1</sup>

| Acid         | Final |                     | Decrease in growth from control—No. logs <sup>2</sup> |      | Difference from initial number—No. logs <sup>3</sup> |      |
|--------------|-------|---------------------|---|------|--|------|
|              | pH    | Per cent added acid | 2 hr  | 4 hr | 2 hr   | 4 hr |
| Acetic       | 5.2   | 0.22                | 0.2   | 0.9  | 0.1  | 0.1  |
|              | 4.8   | 0.43                | 0.2   | 0.8  | 0.1  | 0.2  |
| Citric       | 4.8   | 1.03                | 0.3   | 0.7  | 0.2  | 0.0  |
|              | 4.1   | 1.97                | 0.6   | 1.0  | 0.0  | 0.1  |
| Hydrochloric | 4.6   | 0.17                | 0.3   | 0.7  | 0.1  | 0.4  |
|              | 2.2   | 0.42                | 0.4   | 1.5  | 0.1  | -0.4 |
| Lactic       | 4.9   | 0.41                | 0.1   | 0.3  | 0.3  | 0.8  |
|              | 4.1   | 0.70                | 0.3   | 0.9  | 0.1  | 0.2  |
| Phosphoric   | 5.1   | 0.60                | 0.1   | 0.6  | 0.1  | 0.3  |
|              | 4.1   | 1.20                | 0.4   | 1.0  | 0.4  | 0.2  |

<sup>1</sup>Inoculated to contain  $2.1 (\pm 0.7) \times 10^4$  staphylococci per milliliter.

<sup>2</sup>Number per milliliter in control: 2 hr:  $5.2 (\pm 2.1) \times 10^4$ ; 4 hr:  $6.6 (\pm 1.5) \times 10^5$ .

<sup>3</sup>Positive values indicate an increase in numbers and negative values a decrease.

TABLE 2. GROWTH OF *Staphylococcus aureus* 100 IN PASTEURIZED MILK ACIDIFIED WITH DIFFERENT ACIDS DURING 8 HOURS OF INCUBATION AT 37 C<sup>1</sup>

| Acid         | Final |                     | Decrease in growth from control—No. logs <sup>2</sup> |      |      | Difference from initial number—No. logs <sup>3</sup> |      |      |
|--------------|-------|---------------------|---|------|------|--|------|------|
|              | pH    | Per cent added acid | 2 hr  | 4 hr | 8 hr | 2 hr   | 4 hr | 8 hr |
| Acetic       | 5.2   | 0.23                | 0.2   | 0.7  | 1.9  | 0.0  | 0.2  | 0.8  |
|              | 4.8   | 0.43                | 0.2   | 0.8  | 2.9  | 0.1  | 0.2  | 0.3  |
| Citric       | 4.7   | 1.11                | 0.3   | 0.0  | 1.8  | 0.2  | 0.4  | 0.7  |
|              | 4.1   | 2.29                | 0.3   | 0.8  | 2.0  | 0.1  | 0.2  | 0.7  |
| Hydrochloric | 4.5   | 0.16                | 0.0   | 0.1  | 0.9  | 0.4  | 1.0  | 2.1  |
|              | 2.1   | 0.43                | 0.3   | 0.8  | 3.9  | 0.2  | 0.2  | -0.6 |
| Lactic       | 4.8   | 0.41                | 0.1   | 0.1  | 0.2  | 0.2  | 0.9  | 2.1  |
|              | 4.0   | 0.75                | 0.2   | 0.8  | 3.0  | 0.1  | 0.2  | 0.3  |
| Phosphoric   | 5.1   | 0.60                | 0.1   | 0.3  | 0.7  | 0.1  | 0.6  | 1.8  |
|              | 4.1   | 1.20                | 0.4   | 0.2  | 3.0  | 0.4  | 1.0  | 1.0  |

<sup>1</sup>Inoculated to contain  $2.1 (\pm 0.7) \times 10^4$  staphylococci per milliliter.

<sup>2</sup>Number per milliliter in control: 2 hr:  $5.2 (\pm 2.1) \times 10^4$ ; 4 hr:  $6.6 (\pm 1.5) \times 10^5$ ; 8 hr:  $9.3 (\pm 12) \times 10^7$ .

<sup>3</sup>Positive values indicate an increase in numbers and negative values a decrease.

was used but created no problem with Mannitol Salt Agar. It also has been reported that both media recover staphylococci from cheese with equal efficiency (5). Consequently Mannitol Salt Agar was adopted for use throughout this study.

## RESULTS AND DISCUSSIONS

### Addition of a single acid

Figure 2 presents data on the change in pH achieved in milk through the addition of two levels of different acids over a 4, 8, or 12 hr period. The effect of these additions on the growth of staphylococci is detailed by data in Tables 1, 2, and 3.

Since the staphylococci increased by little more than one log in the control after 4 hr of incubation, it was difficult to demonstrate a marked difference in growth during a 4 hr period when acids were added (Table 1). If one considers as significant only

those differences of one log or greater, then only the high levels of citric (1.97%), hydrochloric (0.42%), and phosphoric (1.20%) acids were effective [but both levels of acetic and the high level of lactic acid (0.7%) were on the borderline of significance] in retarding growth of *S. aureus*.

Substantial differences were more easily observed when acids were added over an 8 hr period (Table 2). Both levels of acetic and citric and the high levels of hydrochloric (0.43%), lactic (0.75%), and phosphoric (1.2%) acids were highly effective in inhibiting *S. aureus*. None of the levels produced greater than a one log difference after 2 or 4 hr of incubation.

Both levels of all acids, except a low concentration of phosphoric (0.62%), produced substantial differences in growth after 12 hr of incubation (Table

TABLE 3. GROWTH OF *Staphylococcus aureus* 100 IN PASTEURIZED MILK ACIDIFIED WITH DIFFERENT ACIDS DURING 12 HOURS OF INCUBATION AT 37 C<sup>1</sup>

| Acid         | Final |                        | Decrease in growth<br>from control—No. logs <sup>2</sup> |      |      |       | Difference from<br>initial numbers—No. logs <sup>3</sup> |      |      |       |
|--------------|-------|------------------------|--|------|------|-------|--|------|------|-------|
|              | pH    | Per cent<br>added acid | 2 hr   | 4 hr | 8 hr | 12 hr | 2 hr   | 4 hr | 8 hr | 12 hr |
| Acetic       | 5.2   | 0.23                   | 0.0  | 0.2  | 0.6  | 1.9   | 0.2  | 0.7  | 0.8  | 2.1   |
|              | 4.8   | 0.45                   | 0.1  | 0.7  | 2.3  | 3.3   | 0.2  | 0.3  | 0.9  | 0.9   |
| Citric       | 4.6   | 1.26                   | 0.3  | 0.0  | 0.2  | 1.6   | 0.2  | 0.7  | 2.2  | 2.4   |
|              | 4.0   | 2.58                   | 0.2  | 0.4  | 1.8  | 3.0   | 0.2  | 0.6  | 0.8  | 1.2   |
| Hydrochloric | 4.5   | 0.18                   | 0.0  | 0.1  | 0.0  | 1.2   | 0.6  | 1.1  | 3.1  | 3.2   |
|              | 2.1   | 0.43                   | 0.2  | 0.2  | 2.4  | 4.8   | 0.3  | 0.8  | 0.9  | -0.7  |
| Lactic       | 4.8   | 0.42                   | (0.1) <sup>4</sup>                                       | 0.4  | 0.6  | 1.3   | 0.4  | 0.6  | 1.8  | 2.7   |
|              | 4.0   | 0.76                   | 0.2  | 0.7  | 2.3  | 3.9   | 0.3  | 0.3  | 1.0  | 0.4   |
| Phosphoric   | 5.1   | 0.62                   | 0.0  | 0.2  | 0.0  | 0.1   | 0.2  | 0.7  | 2.6  | 3.6   |
|              | 4.0   | 1.26                   | 0.3  | 0.1  | 1.8  | 2.2   | 0.4  | 1.0  | 2.2  | 2.4   |

<sup>1</sup>Inoculated to contain  $2.1 (\pm 0.7) \times 10^4$  staphylococci per milliliter.

<sup>2</sup>Number per milliliter in control: 2 hr:  $5.2 (\pm 2.1 \times 10^4)$ ; 4 hr:  $6.6 (\pm 1.5) \times 10^5$ ; 8 hr:  $9.3 (\pm 12) \times 10^7$ ; 12 hr:  $3.7 (\pm 1.5) \times 10^8$ .

<sup>3</sup>Positive values indicate an increase in numbers and negative values a decrease.

<sup>4</sup>An increase from the control.

3). Only the high levels of all the acids showed a marked reduction in growth at the 8 hr point. There were no differences among the acids at 2 or 4 hr.

#### Addition of mixtures of acids

Mixtures of acids, for the most part, were evaluated at pH values on the borderline of affecting staphylococcal growth (Table 4). This series of experiments also was used to determine the reproducibility of data obtained in the initial trials. Neither synergistic nor antagonistic effects were demonstrated with any of the mixtures which were evaluated.

#### Relationships between pH and growth

The relationship between pH and growth inhibition is summarized in Fig. 3 from the data presented in Tables 3 and 4 (12 hr periods). Acetic acid had the greatest effect on growth in that the threshold for minimum inhibition was pH 5.3-5.4 and a pH value of 4.8 produced more than 99.9% reduction in growth. The threshold for growth-inhibition by lactic and phosphoric acid was pH 5.1-5.2 followed by citric acid at pH 4.8 and hydrochloric slightly below citric. Whereas these thresholds were closely grouped, differences were greater at lower pH values. To achieve a 90% reduction in growth, a final pH value of 5.2 was required for acetic, 4.9 for lactic, 4.7 for phosphoric and citric, and 4.6 for hydrochloric acid. To achieve a 99% reduction in growth, a final pH of 5.0 was required for acetic, 4.6 for lactic, 4.5 for citric, 4.1 for phosphoric, and 4.0 for hydrochloric acid. A pH value of 3.3 was required for a 99.9% reduction with hydrochloric while the same effect was produced at pH 4.9 with acetic acid. The organic acids were thus more effective than the mineral acids.

Results of this study demonstrated that the pH of cultured dairy products may have to approach a value of 4 by the end of the fermentation in order to achieve control of staphylococcal growth during 4 hr fermentations; but, then, growth of the organism is slow during this period of incubation. A final pH value of 4.5 may be effective for control during 8 hr fermentations and a final pH value slightly below 5 may be sufficient for control during 12 hr fermentations. When acids are used for acidification in lieu of starter cultures, acetic acid appears as the best choice for control of staphylococci and hydrochloric the least satisfactory of those acids tested using the conditions established in our experiments. Direct acidification as currently practiced, however, is completed in less than 15-30 min and the pH is rapidly reduced to 4.6 to 5.6. These two conditions minimize the opportunity for staphylococcal growth far more than when starter cultures are used. The effectiveness of lactic acid at higher pH values can be greatly extended by adding acetic acid, but mixtures of other acids would be of little additional benefit.

Subramanian and Marth (3) have conducted studies similar to those described in this paper using *Salmonella typhimurium*. They found citric acid to be most inhibitory to the organism followed in order by lactic and hydrochloric acids when added gradually over a 16 hr period to final pH values of 4.5, 4.25, and 4.0, respectively, in skimmilk held at 37 C.

Results of this work justify further investigations on how other factors together with pH serve to inhibit growth of and enterotoxin production by *S. aureus*.

TABLE 4. GROWTH OF *Staphylococcus aureus* 100 IN PASTEURIZED MILK ACIDIFIED WITH DIFFERENT ACIDS AND MIXTURES OF ACIDS DURING 12 HOURS OF INCUBATION AT 37 C<sup>1</sup>

| Acid         | Final |                     | Decrease in growth from control—No. logs <sup>2</sup> |      |       | Difference from initial numbers—No. logs <sup>3</sup> |      |       |
|--------------|-------|---------------------|---|------|-------|---|------|-------|
|              | pH    | Per cent added acid | 4 hr  | 8 hr | 12 hr | 4 hr  | 8 hr | 12 hr |
|              |       |                     |   |      |       |   |      |       |
| Acetic       | 5.4   | 0.22                | 0.1   | 0.1  | 0.0   | 1.0   | 4.0  | 4.1   |
| Citric       | 4.8   | 0.99                | 0.1   | 0.1  | 0.0   | 1.0   | 4.0  | 4.1   |
| 1:1 Mixture  | 5.1   | —                   | 0.2   | 0.1  | 0.1   | 0.9   | 4.0  | 4.0   |
| Lactic       | 5.1   | 0.35                | 0.4   | 0.7  | 0.0   | 0.9   | 2.1  | 4.0   |
| Acetic       | 4.9   | 0.31                | 0.6   | 1.7  | 2.7   | 0.7   | 1.0  | 1.2   |
| 1:1 Mixture  | 5.0   | —                   | 0.4   | 0.8  | 1.6   | 0.9   | 1.9  | 2.3   |
| Lactic       | 5.0   | 0.39                | 0.1   | 0.7  | 0.3   | 1.1   | 3.3  | 4.0   |
| Citric       | 4.7   | 1.10                | 0.1   | 0.9  | 0.8   | 1.1   | 3.1  | 3.6   |
| 1:1 Mixture  | 4.8   | —                   | 0.1   | 0.9  | 0.3   | 1.1   | 3.1  | 4.0   |
| Lactic       | 5.0   | 0.39                | 0.4   | 0.7  | 0.4   | 1.2   | 3.4  | 3.9   |
| Hydrochloric | 4.7   | 0.14                | 0.4   | 1.0  | 0.4   | 1.2   | 3.1  | 3.9   |
| 1:1 Mixture  | 4.8   | —                   | 0.4   | 0.9  | 0.3   | 1.2   | 3.2  | 4.0   |
| Lactic       | 5.1   | 0.37                | 0.2   | 0.4  | 0.4   | 2.2   | 3.7  | 3.9   |
| Phosphoric   | 5.0   | 0.83                | 0.3   | 0.7  | 0.4   | 2.1   | 3.5  | 3.9   |
| 1:1 Mixture  | 5.0   | —                   | 0.2   | 0.7  | 0.4   | 2.2   | 3.5  | 3.9   |

<sup>1</sup>Inoculated to contain  $2.1 (\pm 0.7) \times 10^4$  staphylococci per milliliter.

<sup>2</sup>Number per milliliter in control: 4 hr:  $6.6 (\pm 1.5) \times 10^8$ ; 8 hr:  $9.3 (\pm 12) \times 10^7$ ; 12 hr:  $3.7 (\pm 1.5) \times 10^8$ .

<sup>3</sup>Positive values indicate an increase in numbers and negative values a decrease.

## ACKNOWLEDGMENT

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## THE FUTURE OF EDIBLE OILS

(Continued from Page 515)

rather an oblique manner. The future deployment of edible oils as an ingredient in food stuffs manufactured by dairy processors is not up to the gods—but only to the politicians. A statesman-like, business-oriented position would be to rid the industry of these artificial restrictions that have not and will not operate to the best long-term interests of either the processor or the producer.

There are a host of reasons why but let me illustrate only two. A few years ago I was talking to an American, who pioneered the vegetable oil coffee whitener business. Originally his formula called for a vegetable oil and milk solids combination. When he discovered that this end-product contravened cer-

tain interstate commerce regulations, he went back to the drawing board and developed a formula totally void of any dairy product. This he could legally move interstate. He advised me that even if it became legal to revert to his original formula he would not do so. His estimate of the potential lost market to the producer of milk solids was astronomical and he only hoped that Canada would benefit by the short-sighted U.S. policy that resulted in the production of the totally non-dairy whitener—and lost a substantial market forever.

Or what about the impact of the elasticity of demand? This phase of market research is receiving a considerable amount of study at present—particularly in the U.S. The University of California has com-

(Continued on Page 523)

# PLANNING, INSTALLATION, AND INSPECTION CONSIDERATIONS OF AUTOMATIC CIP SYSTEMS<sup>1</sup>

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If we are to automate the cleaning of an existing hand washed plant or even a completely new plant, we must first consider the process flow and storage. This is not to say that cleanup is of secondary importance, but something must be number one. After all, we say we have a milk plant or are building a milk plant, not that we have or are building a CIP plant. We build or remodel a milk plant to process milk in the most economical and efficient way possible. An economical and efficient CIP system is a part, a large part, of this overall picture. But the fact remains, you must make the CIP fit the process.

It is assumed that we all agree that a properly planned and installed CIP system is much more trustworthy and efficient than the most dedicated employee doing the work by hand. Therefore we will not spend time here selling automated cleaning to you on the basis of sanitation, or to tell you that the question of hand cleaning versus CIP enters into modern day milk plant planning. As you know, much of the equipment we use today cannot be cleaned by hand. For instance, a 50,000 gallon silo tank that is 60 ft tall or even a 6,000 gallon milk tank truck, are impossible to hand-clean on a daily basis. Our men are no longer geared, mentally, for this type of work. I'm not sure they ever were.

This brings up another major advantage of automated cleaning. Labor savings. I would venture that if you had a plant with equipment 10 to 15 years old, small enough for a man to wash by hand, 5,000 gallon tanks, and joints in the milk lines every 6 to 8 ft, you would need 5 to 7 cleanup men for every one we have today. I'm sure there are many small plants doing a fine job of hand cleaning but this discussion will primarily describe conditions in plants processing 15,000 to 20,000 gallons and up per day.

Also, with your permission, I will primarily consider the installation of a new plant; mainly because if you have a say in how the processing equipment is to be made, you can have a more efficient system. Much of what is said will be applicable to automating existing plants, however. Also it will be phrased as if you are building the plant, not inspecting it.

## JOB TO BE DONE

We must determine the various jobs the system will be called upon to perform. Will we have tanks with burn-on, as is true of vat pasteurizing? Where are our hot milk lines? Where are the cold? What about viscous products such as sour cream? How about the cheese room; can we CIP the cheese vats and the mixer? How many separate systems do we need to handle the peak demands?

We must consider the functional safety of the CIP equipment and also the safety to the equipment to be cleaned. We must protect it from collapse or other damage.

Can the men operate it without danger of "detergent burn" and are they reasonably safe from hot solutions and pipes? Can we automate without endangering our products?

Cost of operation other than labor must be considered. How much soap will it take to do the job automatically as compared to a hand wash? What about water costs? Presently many municipalities charge for the water when it is used and again when this used water goes into the sewer. So water costs are becoming more and more real.

Hence, these are some of the prime considerations in the initial planning of an automated system: (a) process flow; (b) quality of the cleanup; (c) savings—labor and other costs; (d) safety of the equipment, both to the men and product; and (e) the type of cleaning to be done.

We know our raw milk is to be stored over here and our pasteurized over there. In between is a short time unit and out on one end are milk tank trucks bringing in the milk and on the other end are the packaging machines to complete the job. And tying it all together are several hundreds of feet of stainless steel tubing. And we want it all clean, every day, before we go home.

If the following installation features are considered, this daily cleanup can be made in a manner acceptable to all, the plant man and the sanitarian.

## INSTALLATION OF LINES

Of utmost importance is the welding ability of the man installing the valves, lines, etc. Unless you know the workman, you have a need to inspect some

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

of his work; either some special welds he may make for you in your presence, or request a weld or two be cut out for your inspection. Better yet, learn to discover questionable welds by their outward appearance. The best welds are the least noticeable. And poor welds cannot be cleaned.

Check the slope of lines. Don't install lines exactly horizontally because they look better that way. They must drain and an easy way to drain them is by removing the face plate clamp from a pump; but the lines must slope to the pump. Also, return pumps and milk product pumps are far more efficient when lines have more slope. It should be mentioned that air blows of a proper volume, pressure and duration do a faster, more complete and sanitary job of draining lines than the method just mentioned. Why clean a line system properly and then have a man handle a pump face plate to drain clean water from the system?

Speaking of pumps, it is very important that pumps are of adequate size. Many factors must be considered but the main ones are size of vessel to be cleaned, distance the solution must be supplied and returned, and the size of the CIP lines. Today's average size pumps are 5 to 10 HP for supply pumps, and 5 to 7.5 HP for return pumps. No supply line should be less than 2 inches in diameter or return lines less than 2.5 inches to the return pump.

If new equipment is to be ordered, operation will probably be at higher speeds than before and thus there should be considerable slope in the bottoms of the tanks. The 3A standard of 0.75 inch of slope per foot is very good. Also, regarding slope, the receiving area floor should have at least this much.

In every plant, no matter how completely "welded in," there must be some manual connections at storage vessels, filter bowls, etc. These joints must fit easily and completely square or the employee will not do a proper job. The return pumps will cavitate and milk will leak into the joints forming pockets that cannot adequately be cleaned by CIP procedures. Don't hang pipes too rigidly near outlets of tanks and allow the employee some slack when making up manual connections.

Avoid special CIP jumpers. Wherever possible, make the process lines do the job for CIP.

It is amazing how long a dead end may be and still clean properly; especially if the cap is cracked slightly as it should be. However, for safety it should not be over 10 inches.

Some of the best planned plants have automatic valves installed that won't drain completely. They are hard to find, but they are there. They are of least importance as they can be disassembled and hand drained if their installation cannot be avoided.

Automatic air valves are the most troublesome components of an automated system. There are none available that are completely cleanable by CIP methods to the degree required for "sterile" products, or even "extended shelf life" products.

Many, many more things are necessary if we are to have a foolproof system: automatic pH sensing and control; automatic temperature sensing and control (both on the solution return, of course, as close to the solution tank as possible); proper gears for timing programs which are versatile enough to pulse valves, to start and stop pumps and to prevent hydraulic shock; safety devices that preclude the CIP of a storage silo tank with the manhole door closed which could cause severe damage to the vessel; and safety devices that make it impossible to wash a storage vessel that contains a milk product.

In summary, regarding the actual installation and valve arrangements, the items of importance are: (a) welding skill of the worker installing the system; (b) proper slope of lines; (c) equipment designed to facilitate CIP cleaning, proper slopes, design of agitators, etc; (d) proper size of CIP lines and pumps; (e) proper fit of necessary manual connections; (f) valves arranged for positive drainage; (g) adequate safety controls; and (h) pH and temperature controls.

#### PREPARATION OF PLANT

We might benefit from a discussion of what should be done to prepare a new plant for milk processing.

Start with a conservatively timed program for each type of cleaning problem to be encountered. A cold milk vessel should receive 2 or 3 10 to 20 sec burst rinses. This should be followed by enough time to drain this pre-rinse from the vessel; then a 5 to 10 min wash or whatever time it takes should be used to bring the return temperature up to 145 F. All of the solution should be returned and this should be followed by the final rinse. Be sure that the program will properly wash the vessel if all mechanical items are normal. This should be done for line systems, HTST, starter vats, and road tankers.

During washing make sure you actually get a pre-rinse, then soap solution, and that the vessel is properly post-rinsed. Visually inspect and swab surfaces. Disassemble all valves, pull off "O" rings, and check for presence of shipping oil, or welding or polishing debris. Wash all valves at least once by hand or until all traces of foreign material are gone.

Check out safety devices as you go. Dilute the solution to check the pH control and lower the temperature. Improper pH or temperature should sound an alarm and put the system "in neutral" until satisfactory levels are reached. Put some water in the

vessels, all the vessels, to see if the system is rendered inoperative by the presence of liquid in the tank. Can you wash a silo with the manhole closed? Discrepancies must be corrected at once.

Next, get a milk hauler to cooperate. Fill his tanker with water. Receive it, pass it through the short time, the separator, and whatever else you have and put part of it in each downstream vessel. Make sure you can get there from here. If you have fillers installed, package some of it. Take samples and plate them as if they were milk. Wash the plant all over again. By the time all the problems are out, all storage vessels and lines should have been washed 3 or 4 times. Efficiency of the CIP system can be checked from time to time by visual inspection and swabs.

#### MAINTENANCE

Regarding maintenance, there should be little required after the first 3 to 6 months: all pumps that are going to burn out will have done so; all program timing should be reduced to a minimum; electrical components such as relays, switches, etc. that have survived this long will not give you trouble for years; the men have made all possible mistakes in hook ups. Really, the troubles are over.

Valve "O" rings must be replaced from time to time, pumps must be greased, seals changed, but these are very normal. Keep track of the soap inventory so you know how the automatic pH controls are functioning. Spray devices should be inspected routinely.

Everything to this point has been discussed with the hope that it will make your job as sanitarian easier as it pertains to milk plant inspection.

#### OTHER THOUGHTS

The following remarks, while not as factual or objective, are based upon the same experience as the previous discussion. If you can give credence to what has been said up to now, please give some consideration to the following.

(a) Consider discontinuing the mandatory dating of milk products. Milk, when properly processed can be easily held at 40 F for 2 weeks. We have held it for up to 3 weeks at 45 F.

(b) Don't insist on inspection ports in welded lines. They completely nullify much of the value gained by eliminating manual joints. As mentioned before, satisfy yourself that the welding is satisfactory during the installation, then let the various quality checks you have tell the story.

(c) Discontinue the demand for mirror finishes on tubing and equipment. It only raises the cost of doing business and it may very well be that slightly less polished surfaces are more readily cleaned.

(d) Please decide among yourselves who has jurisdiction in any given plant. We, on the other side of the fence, feel that one inspector per plant, quite possibly, is enough.

(e) Don't insist on a 3A stamp. Many small stainless steel fabricators around the country who can't qualify for this emblem have some real innovations and sometimes real cost savings.

(f) Last but not least, assume that we are all interested in producing the best bottle of milk possible and if we try something new in either method or equipment, rest assured that we realize that everyone's reputation is on the line.

#### THE FUTURE OF EDIBLE OILS

(Continued from Page 520)

puted the elasticity of demand on many dairy foods. For example, the demand figure for ice cream is 0.95. This means that for each 1% shift in the selling price there is approximately a 1% (0.95 to be exact) shift in volume off the shelf.

The incorporation of lower priced vegetable oils could result in lower selling prices (we are assuming that the 12% sales tax would be eliminated) to the consumer. The lower selling price would increase the sales proportionately. This increase in sales for

many categories could well result in a larger utilization of the producer's milk supply. It is my personal opinion that the attitude of some producers and processors can best be compared to the late President Roosevelt's comment about the depression in the mid-thirties, "All we have to fear is fear itself."

Well, let me conclude by quoting from the position paper that we delivered for the National Dairy Council at the Dairy Policy Conference two years ago. "Unshackle the dairy processing industry—give the processor the right to develop new and in-

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# NEWS & EVENTS

## BOOK REVIEW

BEURRIERIE INDUSTRIELLE: SCIENCE ET TECHNIQUE DE LA FABRICATION DU BEURRE, (Industrial butter making), by H. Poinurier and J. Adda, published by La Maison Rustique, 26 Rue Jacob, Paris.

When the per capita consumption of butter in many countries is decreasing for various reasons, a new book on buttermaking is received with mixed feelings. However, no new authoritative text has been published in this field for many years, despite the appearance of new knowledge and new manufacturing techniques within the butter industry.

The copy of the book received for review had two annoying features. First, the poor quality binding did not suit the authors' suggestion that the book could be used as a student text and reference book for industry and professional people. Second, the table of contents was placed at the end of the text after the index. Hopefully the publisher could easily correct both of these defects.

Both the authors and the publisher should be congratulated for the format of the text. Judicious use of heavy type, italics, and other devices to delineate portions of the text, make the contents very easy to read. Furthermore, the authors have divided the text into three distinct parts: basic biochemistry, surface chemistry, and bacteriology relevant to buttermaking; the manufacturing techniques for butter and other high milkfat products; and finally sanitation, economics, and regulations.

In the first chapter a lucid presentation has been prepared on the types of lipid present in milk, organization of fat globules, and influence of the cow's feed intake on the fatty acid composition of milkfat. Nevertheless, there were two places where the text was deficient. In the section on fatty acids that introduced generic naming, positional and geometric isomers of unsaturated fatty acids, there was no mention of the counting system to assign the position of the double bond(s). Any discussion of fatty acid nomenclature must include this numbering system.

Later in the first chapter, the concept of the milkfat globule membrane and structure was presented. Only King's model (1955) was presented. Surely a book published in 1969 could have offered at least a summary of more recent hypotheses proposed for the membrane's structure.

The second part of the book, beginning at chapter four, gives detailed descriptions of butter manufacturing techniques from maturation of cream to the storage of butter. There is useful information on batch and continuous churning of cream, along with chapters on techniques to control the physical properties of the finished product. A strong emphasis on the production of "acid flavored" butter is maintained.

One aspect of good quality butter production that received very little attention was the deodorization of cream. Problems with "feed flavors" have frequently occurred in the Southern Hemisphere and in U.S.A., whether the cows were fed on pasture, with supplementary feed, or completely fed indoors on harvested crops. Is "feed flavor" removal from cream such a minor problem in Europe?

The quantities of "acid flavored" and "sweet cream" butter produced in the world are similar. Hence it would be desirable for the authors to extend their discussion on "sweet cream" butter to give readers a more balanced view of the types of butter production.

One portion of the book was disappointing—the chapter on other types of high milkfat products. For example, the

section on butter powder consisted mainly of a list of manufacturing techniques, without mentioning the physico-chemical problems inherent in the manufacture of this product.

In general, the book presents most of the information available on "acid flavored" butter very well and uses a good selection of references to promote an understanding of the operations used in the manufacture of butter. Despite a French language text, some people may wish to purchase this book for its coverage of "acid flavored" butter manufacture.

If the book is revised or an English language version printed, then I would recommend major revisions to improve the discussion on deodorization of cream, manufacture of "sweet cream" butter, compositional control of continuously churned butter, and high fat products other than butter.

A. T. GRIFFIN

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Victoria, Australia.

## BOOK REVIEW

BOTTLE WASHING, by H. Burton (ed.), Society of Dairy Technology 17 Devonshire Street, London, W 1. England, 1968, Price 4/-.

This pamphlet forms part of a continuing effort by the Society of Dairy Technology of Great Britain to provide information on subjects of interest to its members.

Although there is very little milk sold in the U.S.A. in glass containers under 1 gal size, there is a movement to coerce food processors to use returnable containers, rather than one-way containers. Hence this pamphlet may interest professionals in the food beverage industry, although the pamphlet was designed for the dairy industry.

Bottle washers have been designed to handle milk bottles ranging from 1/3rd pint to 1 quart sizes. Essentially, the washing process provides a system where the dirty bottles are rinsed-soaked and/or soaked in hot detergent solutions and finally rinsed with high quality water. The pamphlet gives a clear description of the types of bottle washers and recommended techniques for successful operation. One of the very desirable features of the text is the information on control of detergents and the selection of suitable water supplies for the operation of the washers. Diagrams of washer types are very useful.

The standard of editing could have been improved. As an example, the reader is referred to page 15 on page 1 to ascertain the acceptable levels for bacteria after the bottles have been washed; yet after a clear warning regarding the temperature differential between washing treatments to prevent bottle breakage, the reader was given neither the figure for the maximum temperature difference nor a reference to find this information in the pamphlet. (It is found in the section on Detergent Solutions on page 6).

One small point that readers may ponder. While the processor is provided with procedures for testing the strength of his detergents, and the bacteriological quality of the washed bottles, no procedure is recommended to demonstrate that the bottles have been adequately rinsed of detergent solution.

As a basic source of information on bottle washers, their operation and control techniques for the efficient washing of



bottles, this pamphlet can be highly recommended. Industry personnel and educators conducting training courses should find this pamphlet very useful.

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### MICROBIOLOGICAL STANDARDS FOR POULTRY PRODUCTS

The Poultry Science Association, at its 1969 annual meeting, held a symposium on microbiological standards for poultry products. Copies of the proceedings of the symposium are available from the Institute of American Poultry Industries, 67 E. Madison St., Chicago, Ill. 60603. The publication is a 50-page (8.5 x 11 inch size) spiral-bound book which can be purchased for \$7.50.

The proceedings include: "Microbiological Standards for Poultry Products" by Margaret Huston, a list of references on microbiological standards for foods, "Microbiological Criteria in USDA Regulatory Programs for Meat and Poultry" by R. Paul Elliott, "Significance of Various Bacteria Considered for Microbiological Standards for Poultry and Poultry Products" by A. A. Kraft, "The Pros and Cons of Microbiological Standards: A Poultry Processor's View" by R. B. Thompkin, and questions and answers at the discussion which followed presentation of the papers.

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### THE FUTURE OF EDIBLE OILS

(Continued from Page 523)

teresting products that have the profit margin adequate to promote them properly and the total market will be expanded. Frankly, the dairy processor is rapidly becoming a food processor per se. He has the tools to convert these new synthetics now being introduced into new marketing opportunities. The battle against margarine was lost because it was totally negative—100% defensive. The best defense is a strong offense. Let the research and product development capabilities within the dairy processing segment be the bench mark, our guide line, and our road to progress."

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H. L. Thomasson, Managing Editor

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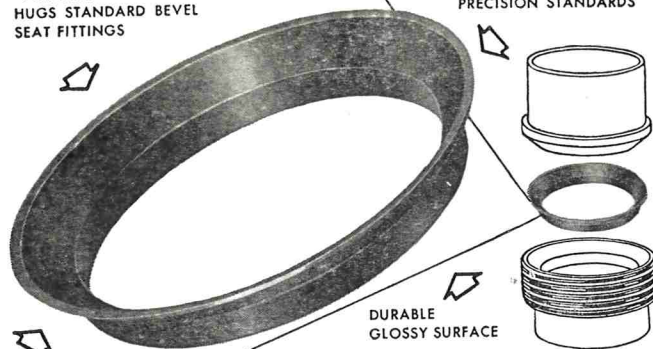
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