JUNE, 1967
Vol. 30 P. 171-202 No. 6

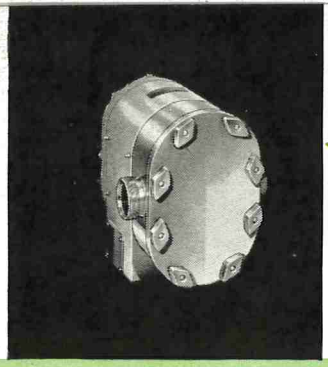
*Journal of*

# MILK and FOOD TECHNOLOGY

**54TH ANNUAL MEETING**
**August 14, 15, 16, 17, 1967**
**Americana Hotel**
**Miami Beach (Bal Harbour) Florida**

*Official Publication*

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



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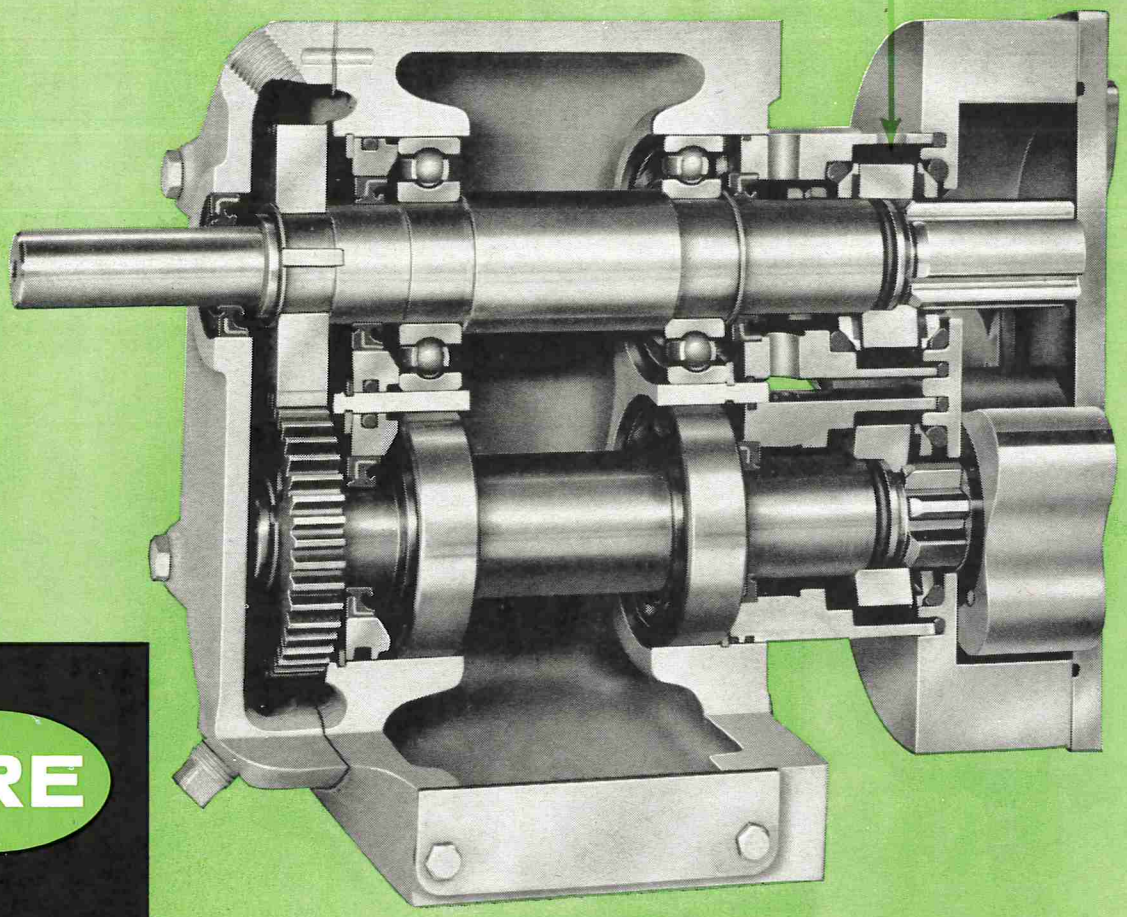

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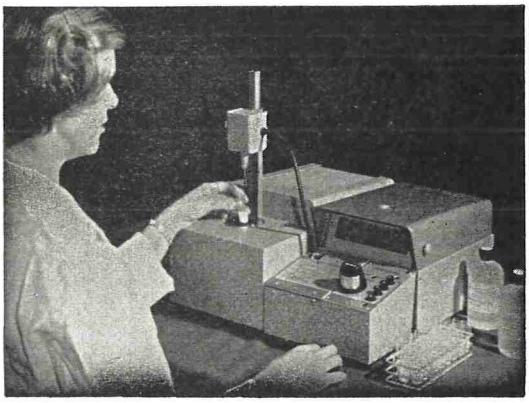

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The Journal of Milk and Food Technology is issued monthly beginning with the January number. Each volume comprises 12 numbers. Published by the International Association of Milk, Food and Environmental Sanitarians, Inc. with executive offices of the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

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

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*Journal of*

# MILK and FOOD TECHNOLOGY

INCLUDING MILK AND FOOD SANITATION

*Official Publication*
International Association of Milk, Food and Environmental Sanitarians, Inc.
Reg. U. S. Pat. Off.

Vol. 30 June, 1967 No. 6



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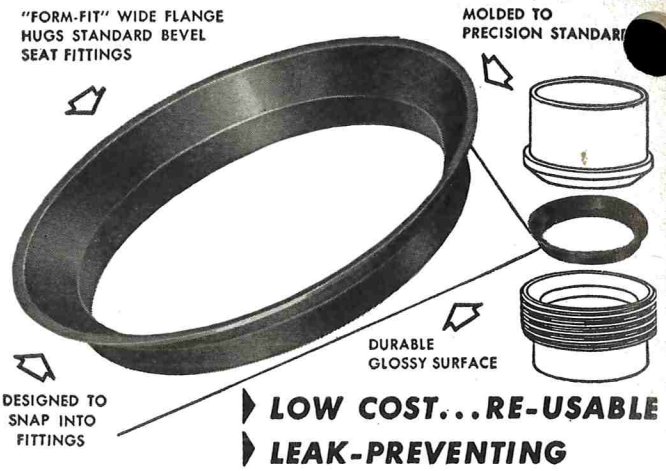


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# QUANTITATIVE MICROBIAL EXAMINATION OF SOME MILKS AND MEAT PRODUCTS DURING STORAGE BY A REPLICA PLATING TECHNIQUE[1]

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*Animal Science Department,*
*Texas A&M University, College Station*

(Received for publication January 23, 1967)

SUMMARY

A replica plating method is described for a quantitative analysis of the microbial flora of certain milk and meat products. Species of *Pseudomonas, Achromobacter, Alcaligenes, Flavobacterium* or *Cytophaga* were present at the beginning of refrigerated storage. After holding for 10-20 days, species of *Pseudomonas* type I and II predominated in the milk samples. The microbial flora of the stored meats included species of greater number of genera.

In many foods, including milk and meat products, mixed microbial populations are the rule rather than the exception. The type(s) of microorganisms that will affect the quality of a food under refrigerated storage depends on *(a)* the initial population level of the various types, *(b)* the ability to grow under the conditions of storage, *(c)* their physical-biochemical activities and *(d)* possible microbial interactions both inhibitory and stimulatory. A quantitative analysis of the microbial flora during the early part of the storage period coupled with some knowledge of the growth characteristics and biochemical activities of the various types present can be useful in predicting shelf life and possible spoilage pattern(s). When carried out at different stages of the storage period such analysis can reveal marked changes that may have taken place in the distribution of the various species. In many instances, levels and type of initial population and ability to grow under the conditions of storage will be the determinative factors with respect to the level and types of microbial population after storage. However, studies of this type may contribute, indirectly, information about microbial interactions in food.

A quantitative microbial analysis of a food is usually accomplished by plating aliquots on or in certain selective or differential media or by analysis of the flora from isolated colonies. This procedure is laborious and time consuming. In recent years, several investigators have used replica plating techniques to examine large numbers of isolates. Corlett et al. *(2, 3)* applied this technique to examine the microbial flora of normal and irradiated Dover sole and ground beef, and Stotzky *(15)* for a study of microbiological interactions in soil. In the present study a replica plating technique was used to determine *(a)* the level and types of the initial microbial population and *(b)* the rate of development of these types during refrigerated storage. Emphasis was placed on gram-negative asporogenous bacteria in milk and meat products.

## EXPERIMENTAL METHODS

*Samples.*

Five raw milks were obtained from dairy farms in Brazos County. Three pasteurized milks were purchased from local grocery stores. The two samples of meat products (ground beef and pork sausage) were from the Meats Laboratory of Texas A&M University.

*Microbiological examination.*

A quantitative examination of the microbial flora of the samples stored for 10 to 20 days at 5 C was made at 5-day intervals. The total viable count was determined by a surface plating technique *(9)* on plate count agar *(1)* with plate incubation for 3 days at 25 C. The colonies from countable plates (30-300) were transferred with sterile glass rods (length 6 cm, diameter 1 mm) to master plates with plate count agar (PCA). The bottom of each plate was marked with a grid of 20 individual positions. Following incubation of the master plates, the colonies were placed on various test media by a replica plating technique *(7)*. For this purpose a piece of velvet was tightly wrapped over the bottom of a replica plate jar (Houston Glass Fabricating Co.) and fastened with waterproof tape. The jars were wrapped in paper bags and autoclaved. Replica plates were prepared on *(a)* Pseudomonas agar F, *(b)* Olson's medium *(8)*, staphylococcus medium 110 *(4)*, SS agar, violet red bile agar, PCA with 10% skimmilk, PCA with 0.4% gelatin and phenol red glucose agar. Size, shape, color, and oxidase reaction *(14)* were recorded from the colonies on the master plate. Plate incubation was at 25 C for 3 days, with examination of the plates after 2 and 3 days. After each sampling period, the predominant types were tested for the Gram reaction *(13)*, presence of flagella by the Baily method *(13)*, motility (hanging drop method), sugar utilization by the Hugh-Leifson procedure *(6)*, nitrate reduction, and production of $NH_3$ from arginine under anaerobic conditions *(16)*. The sensitivity of the cultures to penicillin (2.5 I.U.), streptomycin (80 μg.), chloramphenicol (100 μg.) and oxytetracycline (10 μg.) was tested by the paper disc method.

[1]Journal Paper No. 5728 of the Texas Agricultural Experiment Station, College Station.

## RESULTS AND DISCUSSION

In preliminary studies, samples of raw and pasteurized milks were plated on plate count agar and Olson's medium (plate count agar with 1 ppm crystal violet). Few if any gram-positive organisms appeared on Olson's medium. The number of gram-negative isolates on the two media were very similar. In the present studies, only the isolates appearing on Olson's medium were considered for further grouping, the others were counted and discarded. No one simple scheme exists for the identification of gram-negative asporogenous rods from foods. Basically, the scheme described by Shewan et al. *(10, 11)* was used. It was supplemented with additional features reported by Thornley *(16)*, Thornley et al. *(17)*, Steel *(14)*, and Corlett et al. *(2, 3)*.

The gram-negative isolates were divided into four groups *(a)* a polar-flagellate, oxidase positive group, *(b)* a peritrichous flagellate, oxidase negative, non-pigmented group with a fermentative action upon glucose in Hugh-Leifson medium, *(c)* a non-motile, non-pigmented group and *(d)* a non-motile pigmented group. On the basis of the presence or absence of fluorescence and reaction in Hugh-Leifson medium *(10)* group *a* can be sub-divided into: *Pseudomonas* type I, with fluorescin and oxidative action on glucose; *Pseudomonas* type II, without fluorescin and oxidative action on glucose; *Pseudomonas* type III, without fluorescin and alkaline action on glucose; *Pseudomonas* type IV, without fluorescin and no action on glucose; *Aeromonas*, fermentative action on glucose, without fluorescin, insensitive to pteridine compound 0/129; and *Vibrio*, similar to *Aeromonas* but sensitive to compound 0/129.

Group *b* consisted of species of the family *Enterobacteriaceae*. Group *c* included species of *Achromobacter* and *Alcaligenes*. They were predominantly short, stout rods. Their action on glucose (Hugh-Leifson medium) was variable. Most strains were sensitive to penicillin, whereas the majority of *Pseudomonas* strains were not. Species of *Flavobacterium* and *Cytophaga* were placed in Group *d*.

Steel *(14)* reported that species of *Aeromonas, Alcaligenes, Pseudomonas* and *Vibrio* were predominantly oxidase positive, *Escherichia, Proteus,* and *Serratia* oxidase negative, and species of *Achromobacter* and *Flavobacterium* oxidase variable. Thornley *(16)* showed that $NH_3$ production from arginie under anaerobic conditions was typical for *Pseudomonas* species. This test then is useful to separate *Pseudomonas* strains from the gram-negative bacteria that utilize glucose oxidatively, particularly *Achromobacter* species. Shewan et al. *(12)* also reported on the antibiotic sensitivity pattern of *Pseudomonadaceae* and *Achromobacteraceae*. *Pseudomonadaceae* were resistant to penicillin (2.5 I.U.) but were sensitive to streptomycin (80 $\mu$g.) and chloramphenicol (100 $\mu$g.) *Achromobacteraceae* were sensitive to these three antibiotics. Pigment-producing pseudomonads were resistant to oxytetracycline (10 $\mu$g.); non-pigment producing strains were sensitive.

Corlett et al. *(2)* reported that *Escherichia coli* and *Aerobacter aerogenes* grew as bright-pink colonies on SS agar. *Pseudomonas* type I also grew on this medium and produced yellow colonies. The same was true for about 50 percent of the type II strains tested. *Pseudomonas* type III and IV and many others including *Achromobacter* and *Flavobacterium* did not grow on this medium. Staphylococcus medium 110 supported growth of *Achromobacter* species and was effective in separating these species from other gram-negative asporogenous rods. They also reported that species of *Pseudomonas* were resistant to penicillin (3 I.U). Results with *Achromobacter* were variable.

In the present studies major primary characteristics were reactions on Olson's medium, oxidase reaction, production of fluorescin, growth and color on SS agar, growth on staphylococcus 110 medium, color on plate count agar and production of acid from glucose on phenol red agar medium. Gelatin liquefaction and casein hydrolysis were included to check proteolytic activities of the isolates.

The isolates from a pasteurized milk (Table 1) can be placed in four groups represented by the following characteristics: *(a)* Ox+, F+, SS+, St 110–, *(b)* Ox+, F–, SS+, St 110–, *(c)* Ox–, F–, SS–, St 110+, and *(d)* Ox–, F–, SS–, St 110–. The first two groups are represented by cultures A and B (Table 2). Characteristics such as presence of oxidase, oxidative utilization of glucose, production of $NH_3$ from arginine under anaerobic conditions, polar flagella and resistance to penicillin are typical for species of *Pseudomonas* type I and II. On the basis of presence of a diffusible pigment, those represented by culture A were classified as type I *(10)*. Those similar to culture B were identified as *Pseudomonas* type II. Both groups showed distinct proteolytic activities. Those of the group represented by culture C were tentatively identified as species of *Achromobacter*. They grew on St 110 medium, consisted of non-motile short stout rods and produced some acid from carbohydrates. Species of *Alcaligenes* were excluded because Steel *(14)* reported them to be oxidase positive. The antibiotic sensitivity pattern was similar to that reported by Shewan et al. *(12)*. Except for its reaction to oxytetracycline, the sensitivity pattern was also similar to that reported by Corlett et al. *(2)*. Cultures of the group represented by the characteristics 01+, Ox–, F–, SS–, St 110– had a yellow pigmentation and were assigned to the genus *Flavo-*

TABLE 1. VIABLE COUNT AND CHARACTERISTICS OF MICROBIAL FLORA OF A PASTEURIZED MILK SAMPLE

| Characteristics[e] | | | | | | | | | | Viable count after | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | 0d | 5d | 10d |
| 01 | Ox | F | SS | St | G | Ch | Col | Pr | VRB | x10² | x10⁵ | x10⁷ |
| +[a] | + | + | + | – | + | + | W | – | + | 35 | 98 | 78 |
| +[b] | + | – | + | – | + | + | W | – | + | 70 | | |
| +[c] | – | – | – | + | – | – | W | + | – | 3 | | |
| +[d] | – | – | – | – | – | + | Y | + | – | 5 | | |
| + | – | – | – | – | – | – | Y | + | – | 3 | | |
| | | | | | | | | | Olson's + | 116 | 98 | 78 |
| | | | | | | | | | Olson's – | 2 | | |
| | | | | | | | | | TOTAL | 118 | 98 | 78 |

[a, b, c, d] Represent cultures A, B, C, and D described in Table 2.

[e]Olson's medium (01); oxidase (Ox); fluorescence (F); Salmonella-Shigella medium (SS); Staphylococcus medium 110 (St 110); gelatin liquefaction (G); casein hydrolysis (Ch); color (Col), white (W), yellow (Y); phenol red medium (Pr); violet red bile agar (VRB).

TABLE 2. CHARACTERISTICS OF CULTURES ISOLATED FROM PASTEURIZED MILK SAMPLE

| Characteristics[a] | Cultures | | | |
|---|---|---|---|---|
| | A | B | C | D |
| Olson's medium | + | + | + | + |
| Oxidase | + | + | – | – |
| Fluorescence | + | – | – | – |
| SS medium | + | + | – | – |
| Staphylococcus medium 110 | – | – | + | – |
| Gelatin liquefaction | + | + | – | – |
| Casein hydrolysis | + | + | – | + |
| Nitrate reduction | – | – | + | – |
| Phenol red medium | – | – | + | + |
| VRB agar | + | + | – | – |
| Leifson-aerobic | + | + | – | – |
| Leifson-anaerobic | – | – | + | – |
| Arginine-Anaerobic | + | + | – | – |
| Motility | + | + | – | – |
| Flagella | Pol | Pol | – | – |
| Gram reaction | –R | –R | –R | –R |
| Penicillin | R | R | S | S |
| Chloramphenicol | S | S | S | S |
| Streptomycin | S | S | S | S |
| Oxytetracycline | S | S | S | S |
| Morphology | CCoE | CCoE | P | P |
| Color | W | W | W | Y |

[a]Flagella polar (Pol), peritrichous (Peri); antibiotic resistant (R), antibiotic sensitive (S) morphology, punctiform (P), circular (C), convex (Co), and entire (E).

*bacterium.* Both oxidase positive and oxidase negative species of *Flavobacterium* are reported in the literature *(14)*. The antibiotic sensitivity pattern, however, differed from that reported by Corlett et al. *(2)*. They reported resistance to penicillin, streptomycin and chloramphenicol, and sensitivity to oxytetracycline. Cultures of group D in this study were sensitive to these four antibiotics. This pattern, however, agreed with that reported for *Cytophaga* species *(2)*. Although Shewan et al. *(10)* reported that species of *Cytophaga* on agar plates have a diffuse margin, spreading outwards and into the agar, Hayes *(5)* has shown that the differentiation of the two genera is not simple. Hence, some may have been classified as *Flavobacterium*. *Pseudomonas* type I was the species that predominated after holding of the milk for 10 days.

The major characteristics of the two groups of bacteria that predominated in a raw milk (Table 3) after 15 days of holding were *(a)* Ox+, F+, SS+, St 110– and *(b)* Ox+, F–, SS+, St 110–. Additional characteristics for two cultures (A, B) representative of these two groups are presented in Table 4. On the basis of these characteristics cultures represented by culture A were assigned to *Pseudomonas* type I. The antibiotic sensitivity pattern, agreed more closely with that of *Pseudomonas* type IV *(2)*. However, type IV does not utilize glucose oxidatively, lacks diffusible pigment, and does not grow on SS agar. The characteristics of cultures represented by culture B were in many aspects similar to those of culture A. Because of the absence of diffusible pigment, they were assigned to *Pseudomonas* type II. Types III and IV could not be considered because the culture utilized glucose oxidatively.

At the beginning of the holding period, there was a rather large group ($17 \times 10^4$) represented by the following characteristics: Ol+, Ox+, F–, SS– and St 110–. Eight of the seventeen colonies were yel-

low. These were tentatively identified as species of *Flavobacterium*. Although the appearance of the colonies on the plates resembled species of *Flavobacterium*, it is possible that some *Cytophaga* species were included in this genus. The other nine of this group represented by cultures C and D (Table 4) were tentatively assigned to the genus *Alcaligenes*. The isolates consisted of short stout rods and were oxidase positive. The following genera were excluded from consideration: *Achromobacter*, because of lack of growth on St 110 medium; *Flavobacterium*, because of lack of color; *Aeromonas*, because glucose was not utilized fermentatively; *Pseudomonas*, because of absence of flagella and motility; *Escherichia*, *Aerobacter* and *Proteus*, on the basis of oxidase reaction, absence of flagella, and lack of growth on SS agar.

The group represented by the major characteristics 01+, Ox–, SS– and St 110+ were placed in the genus *Achromobacter*, primarily because of the oxidase reaction and their ability to grow on St 110 medium. They also produced some acid from glucose. Another group represented by eight colonies were 01+, Ox–, F–, SS– and St 110–. On the basis of these major characteristics, *Pseudomonas*, *Aeromonas*, *Vibrio*, *Achromobacter*, *Escherichia*, *Aerobacter*, *Flavobacterium* and *Alcaligenes* were not considered. The three cultures that produced acid from glucose could be species of *Proteus*.

Table 5 shows the viable count and characteristics of the microbial flora of ground pork before and after holding at 5 C for 20 days. The isolated colonies can be arranged in seven groups. The major characteristics of these groups are *(a)* Ox+, F+, SS+, St 110–, *(b)* Ox+, F–, SS+, St 110–, *(c)* Ox+, F–, SS–, St 110+, *(d)* Ox+, F–, SS–, St 110–, *(e)* Ox–, F–, SS+, St 110–, *(f)* Ox–, F–, SS–, St 110+, and *(g)* Ox–, F–, SS–, St 110–. The first two groups are represented by cultures A, B, C, and D (Table 6). Detailed characteristics of these cultures indicate that they are typical pseudomonads. This is particularly evident from the following characteristics: presence of oxidase, oxidative carbohydrate utilization, production of $NH_3$ from arginine under anaerobic conditions, motility, polar flagella and resistance to penicillin. Culture A was classified as *Pseudomonas* type I, the others as *Pseudomonas* type II. This was based on the presence or absence of diffusible

TABLE 3. VIABLE COUNT AND CHARACTERISTICS OF MICROBIAL FLORA OF RAW MILK SAMPLE

| Characteristics | | | | | | | | | | Viable count after | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | 0d | 5d | 10d | 15d |
| 01 | Ox | F | SS | St | G | Ch | Col | Pr | VRB[c] | x10⁴ | x10⁶ | x10⁸ | x10⁹ |
| +[a] | + | + | + | – | + | + | W | – | + | 59 | 146 | 36 | 20 |
| + | + | – | + | – | + | + | W | – | + | 1 | | | |
| +[b] | + | – | + | – | – | – | W | 1+<br>206 – | + | 10 | 122 | 53 | 22 |
| + | + | – | – | – | + | + | Y | – | – | 2 | | | |
| +[c, d] | + | – | – | – | – | – | W | 1+[f]<br>8 – | 1+(1)<br>8 – | 9 | | | |
| + | + | – | – | – | + | – | Y | – | – | 3 | | | |
| + | + | – | – | – | – | – | Y | – | 2+(2)<br>1 – | 3 | | | |
| + | – | – | – | + | + | + | W | + | – | 2 | | | |
| + | – | – | – | + | – | – | W | + | – | 2 | | | |
| + | – | – | – | – | – | + | W | 2+<br>1 – | – | 3 | | | |
| + | – | – | – | – | + | – | W | – | – | 1 | | | |
| + | – | – | – | – | – | – | W | 1+<br>3 – | 1+<br>3 – | 4 | | | |
| | | | | | | | | | TOTAL | 99 | 268 | 89 | 42 |

[a, b, c, d] Represent cultures A, B, C, and D described in Table 4.

[c] Number of pink-red colonies in parenthesis.

[f] Pink-red colony on VRB medium.

TABLE 4. CHARACTERISTICS OF CULTURES ISOLATED FROM RAW MILK SAMPLE

| Characteristics | Cultures A | B | C | D |
|---|---|---|---|---|
| Olson's medium | + | + | + | + |
| Oxidase | + | + | + | + |
| Fluorescence | + | – | – | – |
| SS medium | + | + | – | – |
| Staphylococcus medium 110 | – | – | – | – |
| Gelatin liquefaction | + | – | – | – |
| Casein hydrolysis | + | – | – | – |
| Nitrate reduction | – | – | + | – |
| Phenol red medium | – | – | – | – |
| VRB agar | + | + | – | – |
| Leifson-aerobic | + | + | + | – |
| Leifson-anaerobic | – | – | – | – |
| Arginine-anaerobic | + | + | – | – |
| Motility | + | + | – | – |
| Flagella | Pol | Pol | – | – |
| Gram reaction | –R | –R | –R | –R |
| Penicillin | R | R | R | S |
| Chloramphenicol | S | S | S | S |
| Streptomycin | S | S | S | S |
| Oxytetracycline | S | S | S | S |
| Morphology | CCoE | CCoE | CCoE | CCoE |
| Color | W | W | W | W |

pigment *(10)*. Differences in the antibiotic sensitivity pattern were observed between cultures A, B, C, and D and those reported by Corlett et al. *(2)*, and Shewan et al. *(12)*. Group c and f, characterized by the reactions Ox±, F–, SS–, St 110+ were assigned to the genus *Achromobacter*, primarily because of their Gram reaction, morphology (short, stout rods) and growth on St 110 medium. Steel *(14)* also reported both oxidase positive and negative species of *Achromobacter*. It is possible that some of the oxidase-positive isolates were species of *Alcaligenes*. Group d, characterized by Ox+, F–, SS–, St 110–, was tentatively identified as species of *Alcaligenes*. The following genera were excluded for one or more reasons: *Achromobacter*, because of lack of growth on St 110 medium; *Pseudomonas* and *Aeromonas* because the cultures were oxidase negative and did not grow on SS medium and *Flavobacterium*, because of lack of pigmentation. Culture E (Table 6) represents the group characterized by Ox–, F–, SS+, and St 110–. The colonies on SS and VRB agar were red pigmented. They were identified as species of *Escherichia* or *Aerobacter*. The fermentative utilization of carbohydrates and the type of flagella supported this decision. The antibiotic sensitivity pat-

TABLE 5. VIABLE COUNT AND CHARACTERISTICS OF MICROBIAL FLORA OF GROUND PORK

| Characteristics | | | | | | | | | | Viable count after | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | 0d | 5d | 10d | 15d | 20d |
| O1 | Ox | F | SS | St | G | Ch | Col | Pr | VRB | x10⁵ | x10⁸ | x10⁸ | x10⁸ | x10⁹ |
| +[a] | + | + | + | – | + | + | W | – | + | | 15 | 4 | 1 | 5 |
| +[b] | + | – | + | – | + | + | W | – | + | 140 | | | | 3 |
| +[c] | + | – | + | – | + | – | W | – | + | | 7 | | | 1 |
| + | + | – | + | – | – | + | W | – | + | | | | 1 | |
| +[d] | + | – | + | – | – | – | W | 1+ 143– | + | 48 | 43 | 30 | 11 | 12 |
| + | + | – | – | + | – | – | W | – | – | | 4 | 2 | | 2 |
| + | + | – | – | – | – | – | W | – | – | | 2 | | | |
| +[e] | – | – | +(16)[i] | – | – | – | W | 15+ 5– | +(16)[i] | | 4 | | 12 | 4 |
| + | – | – | – | + | + | + | W | – | – | 2 | | | | |
| + | – | – | – | + | – | – | W | + | – | | 1 | | | |
| +[f, g] | – | – | – | – | + | + | 22W 8 Y | – | – | 30 | | | | |
| +[h] | – | – | – | – | – | – | W | 26+ 2– | – | 2 | 8 | 8 | 1 | 9 |
| | | | | | | | | | | Olson's + 222 | 84 | 44 | 26 | 36 |
| | | | | | | | | | | Olson's – 10 | | | 3 | |
| | | | | | | | | | | TOTAL 232 | 84 | 44 | 29 | 36 |

[a, b, c, d, e, f, g, h] Represent cultures A, B, C, D, E, F, G, and H described in Table 6.
[i] Pink-red colonies.

TABLE 6. CHARACTERISTICS OF CULTURES ISOLATED FROM GROUND PORK

| Characteristics | Cultures | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | H |
| Olson's medium | + | + | + | + | + | + | + | + |
| Oxidase | + | + | + | + | – | – | – | – |
| Fluorescence | + | – | – | – | – | – | – | – |
| SS medium | + | + | + | + | + | – | – | – |
| Staphylococcus medium 110 | – | – | – | – | – | – | – | – |
| Gelatin liquefaction | + | + | + | – | – | + | + | – |
| Casein hydrolysis | + | + | – | – | – | + | + | – |
| Nitrate reduction | – | – | – | – | – | + | – | – |
| Phenol red medium | – | – | – | – | + | – | – | + |
| VRB agar | + | + | + | + | + | – | – | – |
| Leifson-aerobic | + | + | + | + | + | – | – | + |
| Leifson-anaerobic | – | – | – | – | + | – | – | + |
| Arginine-anaerobic | + | + | + | + | – | – | – | – |
| Motility | + | + | + | + | + | – | – | – |
| Flagella | Pol | Pol | Pol | Pol | Peri | – | – | – |
| Gram reaction | –R | –R | –R | –R | –R | –R | –R | –R |
| Penicillin | R | R | R | R | R | S | S | S |
| Chloramphenicol | S | S | S | S | S | S | S | S |
| Streptomycin | S | S | S | S | S | S | S | S |
| Oxytetracycline | S | S | S | S | S | S | S | S |
| Morphology | CCoE | CCoE | CCoE | CCoE | CCoE | P | P | CCoE |
| Color | W | W | W | W | W | W | Y | W |

tern agreed more closely with that of *Aerobacter* than of *Escherichia* species as reported by Corlett et al. *(2)*. The cultures in the group Ox–, F–, SS–, St 110– are represented by cultures F, G, and H in Table 6. The eight yellow pigmented isolates represented by culture G probably were species of *Flavobacterium* or *Cytophaga*. The other did not fit some of the principal characteristics of the major genera of gram-negative asporogenous bacteria. Although some other genera were represented at the end of the storage period, the majority of the population belonged to the genus *Pseudomonas*.

A summary of the major oxidase-positive and oxidase-negative groups of bacteria of the ten food samples is presented in Table 7 and 8. With respect to the oxidase-positive groups, two groups represented by the characteristics Ox+, F+, SS+, St 110– and Ox+, F–, SS+, St 110– were predominant both before and after holding at 5 C. The first group is typical of *Pseudomonas* type I, the second of *Pseudomonas* type II. With respect to the oxidase-negative groups, few were present after holding at 5 C for the entire length of the storage period. The two groups most frequently present before holding were represented by the characteristics Ox–, F–, SS–, St 110+; and Ox–, F–, SS–, St 110–. The former are most likely species of *Achromobacter* or *Alcaligenes*, those of the latter could belong to various genera such as *Proteus*, *Serratia* or *Flavobacterium*.

## CONCLUSIONS

This study showed that changes in the number and type of gram-negative asporogenous bacteria in foods could be followed with the replica-plating technique. In nearly all samples, considerable increases in total viable population took place during refrigerated storage. Exceptions were one raw milk and the ground beef sample. This milk sample was the only one in which species of *Achromobacter* were predominant both before and after holding. In the sample of ground beef, the total viable count before holding was relatively high ($91 \times 10^8$) and conditions inhibitory to further increases in viable population may have been present. As was expected, the initial level of viable population varied greatly from sample to sample. In seven out of the ten samples both gram-negative (01+) and gram-positive (01–) species were isolated. Gram-positive species made up a considerable portion of the initial population in three raw milk samples and one pasteurized milk sample. In two other milk samples (one raw milk and one pasteurized milk) and in ground pork they were only a minority. In the other three samples,

TABLE 7. DISTRIBUTION OF MAJOR OXIDASE-POSITIVE MICROBIAL GROUPS AMONG SAMPLES OF MILK AND MEAT

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Oxidase | | + | + | + | + | + | + | + | + |
| Fluorescence | | + | + | + | + | – | – | – | – |
| SS medium | | + | + | – | – | + | + | – | – |
| Staph. 110 | | + | – | + | – | + | – | + | – |
| Raw milk | 1 | | ○[a] ●[b] | | | | ○ | | ○ |
| | 2 | | ○ ● | | | | ○ ● | | ○ |
| | 3 | | ○ | | | | | ○ | ○ ● |
| | 4 | | ○ ● | | ○ | ○ | ○ | | ○ |
| | 5 | ○ | ○ ● | ○ | ○ | ○ | ○ ● | ○ | ○ |
| Past. milk | 1 | | ○ | | | | ○ ● | | |
| | 2 | | ○ | | | | ● | ○ | ○ |
| | 3 | | ○ ● | | | | ○ | | |
| Ground pork | | | ● | | | | ○ ● | ● | |
| Ground beef | | | ○ ● | | | | ○ ● | | |

[a]Present at beginning of storage period.
[b]Present at end (10-20 days) of storage period.

TABLE 8. DISTRIBUTION OF MAJOR OXIDASE-NEGATIVE MICROBIAL GROUPS AMONG SAMPLES OF MILK AND MEAT

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Oxidase | | – | – | – | – | – | – | – | – |
| Fluorescence | | + | + | + | + | – | – | – | – |
| SS medium | | + | + | – | – | + | + | – | – |
| Staph. 110 | | + | – | + | – | + | – | + | – |
| Raw milk | 1 | | | | ○ | | | | ○ |
| | 2 | | | | | | | ○ | ○ |
| | 3 | | | | | | | ○[a] ●[b] | ○ ● |
| | 4 | | | | | ○ | | ○ | ○ |
| | 5 | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
| Past. milk | 1 | | | | | | | | ○ |
| | 2 | | | | | | | ○ | ○ |
| | 3 | | | | | | | ○ | ○ |
| Ground pork | | | | | | | ● | ○ | ○ ● |
| Ground beef | | | | | | | ● | ○ ● | ○ ● |

[a]Present at beginning of storage period.
[b]Present at end (10-20 days) of storage period.

no gram-positive species were observed. However, after holding at 5 C for the entire storage period, gram-positive species were observed only in two raw milk samples. In only one of these did they make up a considerable portion of the flora. This was the raw milk sample in which a relatively large number of *Achromobacter* species were present both before and after holding at 5 C. Thus gram-positive isolates were obtained from seven samples before holding and from only two samples after holding at 5 C. Several factors may be responsible for this phenomenon. First, gram-positive species usually do not multiply as readily at 5 C as compared with gram-negative species. Secondly there may have been some inhibitory action exerted by gram-negative species. It also should be pointed out, however, that the total viable population usually increased during storage. Hence, higher dilutions had to be used to obtain countable plates. Even with some increase in the population of gram-positive species, they may have been diluted out in the preparation of dilutions for plating. Before holding of the milk and meat products the microbial flora usually consisted of species of *Pseudomonas* type I and II, *Achromobacter*, *Alcaligenes*, *Flavobacterium* or *Cytophaga*. After holding for the entire storage period only species of *Pseudomonas* type I and II were isolated from 7 out of the 8 milks. These species are known to grow relatively fast at 5 C and therefore can be expected to outnumber many species of other genera. In the other sample, a considerable portion of the flora consisted of *Achromobacter* species. In the two meat samples the flora after holding was more varied. A comparison of colony formation on SS and VRB agar showed that those on SS agar were also present on VRB agar. Pink-red colonies on SS agar showed the same color on VRB agar. The predominant species in four of the stored samples were proteolytic, in five samples both proteolytic and non-proteolytic types were present. An examination of this and additional characteristics of the isolates, for example lipolysis, may be useful in predicting the type of damage (proteolysis, lipolysis) that can be expected in a food during storage.

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## NATIONAL INDEX REVEALS LITTERBUGS LOSING STEAM

Litterbugs are slowing down, according to the National Litter Index published annually by Keep America Beautiful, Inc. The national litter-prevention organization said that while the 1966 Index showed a small increase in the amount of littering, the rate of increase was less than half that of the previous year.

The 1966 Litter Index stands at 106.8, a rise of 1.9 per cent over the revised 1965 Index, which stood at 104.8, according to Allen H. Seed, Jr., executive vice president of KAB. The Index was established in November, 1964 as a method of periodically measuring the litter problem. It is determined by KAB from vehicle miles traveled on primary state highways and the annual cost of clearing the litter from these same highways.

"The slowdown in the increase of littering indicated by the 1966 Index is significant in view of the national beautification programs sponsored by the Administration in Washington and the thousands of anti-litter programs conducted by civic-minded groups at the state and local levels across the country," Mr. Seed said. "The new Index shows that these programs, largely based on public education, law enforcement and provision for adequate collection and disposal facilities, are getting results. All litter fighters should take heart."

He pointed out that the current rise in the National Litter Index is partly attributable to the increased cost of labor and the mechanical equipment used to de-litter highways. "Another factor contributing to the rise is the growing awareness of litter," Mr. Seed said. "A greater effort is being made to keep our highways clean, with the result that more money is being spent, sometimes in areas where previously little effort was made."

The fifty states reported that the cost of removing litter from their primary highways in 1965 was $23,187,476. Total vehicle miles traveled on primary highways was 332 billion. The year before, the clean-up figure was $21,706,427, with 316 billion miles traveled.

# STERILIZATION REQUIREMENTS FOR SPACE EXPLORATION[1]

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Summary

Planetary landing space hardware is required to be sterilized. The probability of obtaining sterile space hardware is enhanced considerably when the level of microbial contamination of the hardware is kept very low prior to terminal sterilization. Such control requires constant and efficient monitoring of the hardware and the intramural environments where flight hardware is assembled, tested, and encapsulated before terminal sterilization. This study demonstrates that the levels and types of microbial contaminants recovered from space hardware, or from test surfaces exposed within assembly areas, depend upon the degree of environmental and personnel control. Operating personnel was the chief source of contamination. However, the intramural environment may become a reservoir of contamination, especially when environmental control measures are inadequate. It appears that one of the best means for maintaining microbial contamination at a low level is by use of vertical laminar flow clean rooms.

Guidelines have been developed *(2, 3, 9-12, 15)* and national policy has been established for the Planetary Quarantine Program of the National Aeronautics and Space Administration (NASA) *(8, 23, 25, 28)*. Briefly, the program of the NASA requires that planetary landing space hardware must be sterilized to prevent terrestrial organisms from contaminating Mars, Venus, and other planets of biological interest. The introduction of terrestrial contamination by unsterile space hardware might result in growth of the implanted organisms. This could alter the ecology of the planet and seriously affect future investigations of its biota, and, in turn, preclude the demonstration of extraterrestrial life. By application of well-defined techniques, the practicality of the sterilization requirement can be corroborated and sterility can be demonstrated with predictable reliability.

The basic premise in the development of procedures for implementing the planetary quarantine program is that the probability of achieving sterile space hardware is enhanced markedly when the level of microbial contamination is maintained at an extremely low level prior to terminal sterilization. Consequently studies were initiated to evaluate the influence of clean room operations and conditions on the levels of microbial contamination present within areas employing various degrees of environmental control *(5, 6, 16, 21, 22, 26)*.

In this paper data will be given on the levels of microbial contamination in areas ranging from those having no environmental control, through several types of industrial clean rooms, to a vertical laminar flow clean room. Such data are necessary in order to make proper selections of facility conditions required to produce space hardware containing a level of microbial contamination at or below that specified by the NASA for a particular sterilization cycle.

## Materials and Methods

The sampling procedures used were similar to those recently described in "Standard Methods for the Microbiological Examination of Space Hardware" (NASA, 24). A brief resumé of the sampling methods used is presented below.

*Microbiological Sampling of Air.*

Volumetric air samples were collected with Reyniers slit samplers.[3] Sampler calibration, operation, and incubation of collected samples were performed as previously described *(16)*.

*Microbiological Sampling of Surfaces.*

Selected surfaces were sampled with the Rodac plate.[3]. Details of Rodac plate preparation, sampling, incubation, and counting were described previously *(16)*.

[3]Commercial names are used for identification only and their mention does not constitute endorsement by the Jet Propulsion Laboratory, the Public Health Service, U. S. Department of Health, Education, and Welfare, or the National Aeronautics and Space Administration.

Stainless steel (SS) strips were used to measure the levels of airborne microbial contamination accumulating on surfaces exposed within different environmentally controlled areas. Details of SS strip preparation, sterilization, and use, as well as microbiological assay and incubation after use, have been described previously *(16)*.

*Survival of Microorganisms on Surfaces Exposed to Various Environments.*

Stainless steel was used as a test surface for conducting microbial die-away studies. Details of strip inoculation, ex-

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[1]Presented at the 53rd Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Inc., at Minneapolis, Minnesota, August 15-18, 1966.

[2]Present address: Lovelace Foundation for Medical Education and Research, Department of Microbiology, Albuquerque, New Mexico.

posure and assay after exposure were described previously *(16-20)*.

*Areas Included in the Survey.*

The areas sampled ranged from open factory areas (manufacturing areas A and D) through areas having little environmental control (Mariner and Surveyor spacecraft assembly areas) to industrial clean rooms (Class II, III, or IV) *(1)* and to horizontal and vertical laminar flow clean rooms, including the vertical laminar flow clean room *(7)* of the Experimental Assembly and Sterilization Laboratory (EASL) at the Jet Propulsion Laboratory (JPL).

## RESULTS

*Microbiological Sampling of Air.*

Table 1 contains a general summary of typical results obtained during the air sampling phase of this study. As shown, wide fluctuations in the number of airborne viable particles were detected. From these data, it is apparent that as the degree of environmental control was increased from the open factory areas to the laminar flow clean rooms, there was a corresponding decrease in the number of airborne viable particles.

Typical examples of sequential air sampling data are presented in Figures 1-3. Figure 1 contains the results of air sampling performed in a Class III clean room in the Los Angeles area. One sampling site

TABLE 1. GENERAL COMPARISON OF THE LEVELS OF AIRBORNE VIABLE PARTICLES WITHIN EACH AREA SAMPLED

| Area sampled | Range of viable particles recovered per cubic foot of air |
|---|---|
| Manufacturing areas | |
| "A" (P)[a] | 6.5 to 26.0 |
| "D" (P) | 5.5 to 16.0 |
| Spacecraft facilities (LA)[b] | |
| Outside corridor | 2.0 to 22.0 |
| Mariner high bay | 0.5 to 6.0 |
| Surveyor high bay | 0.5 to 6.5 |
| Industrial clean rooms | |
| Class II (LA) | 0.5 to 10.5 |
| Class II (P) | 0.5 to 5.5 |
| Class III (LA) | 0.0 to 0.5 |
| Class III (P) | 0.0 to 2.0 |
| Class IV (LA) | 0.0 to 2.0 |
| Laminar flow clean rooms | |
| Horizontal filter wall (P) | None detectable when filter intact |
| Horizontal exhaust wall (P) | 0.0 to 3.5 |
| Vertical (LA) | 0.000 to 0.001 |

[a]Facility located in the Phoenix area.
[b]Facility located in the Los Angeles area.

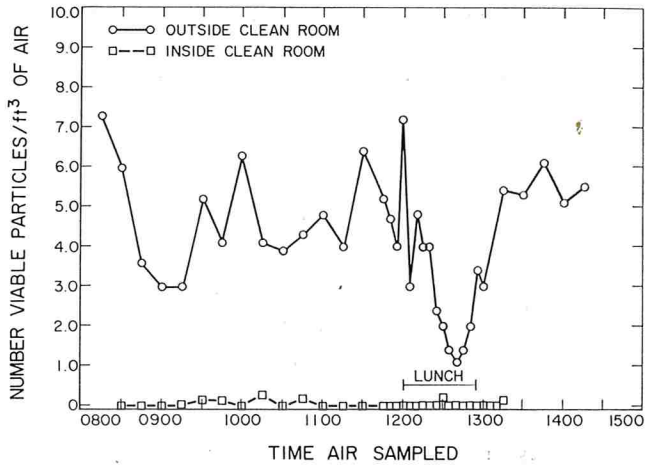


Figure 1. Results of sequential air sampling conducted in a Class III clean room located in the Los Angeles area.

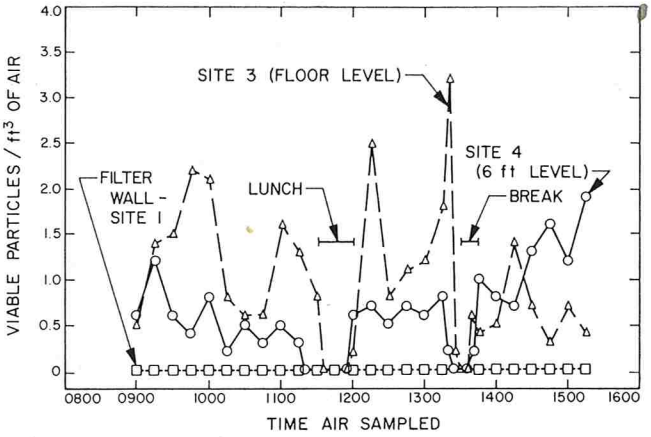


Figure 2. Results of sequential air sampling conducted in a horizontal laminar flow clean room in the Phoenix area.

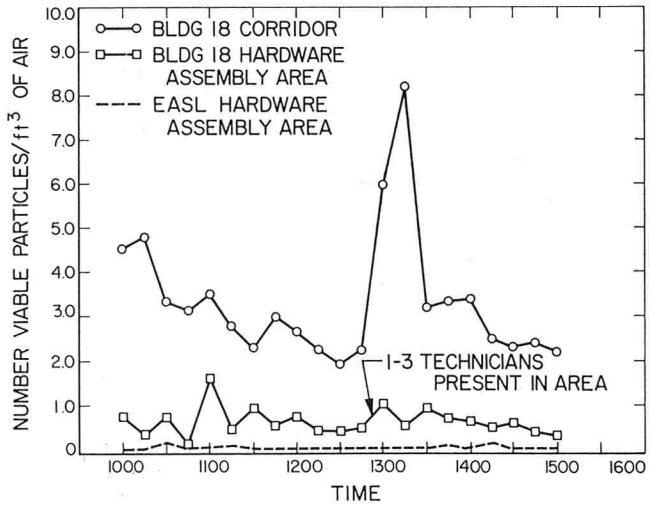


Figure 3. Results of sequential air sampling conducted in three different areas employing various degrees of environmental control (corridor, least; EASL area, greatest).

was located inside the clean room and the other site was located in an uncontrolled area of the building outside of, and adjacent to, the clean room. During the lunch period both areas were vacant and there was a marked drop in the level of airborne viable particles collected at the sampling site outside the clean room. As operating personnel returned from their lunch period, the level of airborne microbial contamination outside the clean room rose again and was roughly proportional to the degree of personnel traffic in the area. In fact, fluctuations in the level of airborne viable contamination were quite discernible at this site throughout the sampling day. However, inside the clean room the level of airborne viable contamination rarely approached an average of 0.5 viable particles per cubic foot of air and sharp changes in the number of microbial particles in suspension inside the room were not detected.

Figure 2 contains typical results obtained during sequential air sampling studies conducted within a horizontal laminar flow clean room located in the Phoenix area. Site 1 was at the filter wall and sites 3 and 4 were located at the exhaust wall. Again fluctuations in the level of airborne viable particles may be correlated with the degree of personnel activity. As operating personnel left the room, the level of airborne microbial contamination fell sharply. When the personnel returned, the level of airborne viable particles rose and roughly paralleled the degree of personnel activity throughout the day.

In Figure 3, the comparative results of sequential air sampling from three different areas at the JPL are shown. The areas sampled employed various degrees of environmental control, the corridor having the least. The area for routine assembly of space hardware had a stricter degree of control than the corridor, and the vertical laminar flow areas of the JPL EASL had the highest degree of environmental control. Differences in the levels of airborne viable particles were noticeable when the three areas were compared (Figure 3) with the Class 100 vertical laminar flow clean room of the EASL which had an extremely low level. Quite frequently viable particles were not recovered from any of four sampling sites within this area during 4 to 5 hours of sequential air sampling, representing volumes of 240 to 300 cubic feet of sampled air.

*Microbiological Sampling of Surfaces.*

Figure 4 contains the results of surface sampling studies conducted inside the environmentally controlled area of the JPL spacecraft assembly facility (SAF). Each plot represents the average value from six SS strips. The level of microbial contamination accumulating on the SS strips stabilized between 1000 to 9999 ($10^3$) aerobic mesophilic microorganisms per square foot of surface during the entire 25-week exposure period. Results similar to those shown in Figure 4 were obtained in the Surveyor SAF. This leveling-off or "plateau" *(6, 16, 27)* in the level of microbial contamination accumulating on surfaces was detected also in the other areas surveyed.

Table 2 contains a general summary of the surface sampling results obtained in the other areas included in the study being reported. These data show that the degree of microbial contamination accumulating on surfaces was proportional to the degree of environmental control enforced in the area. The level

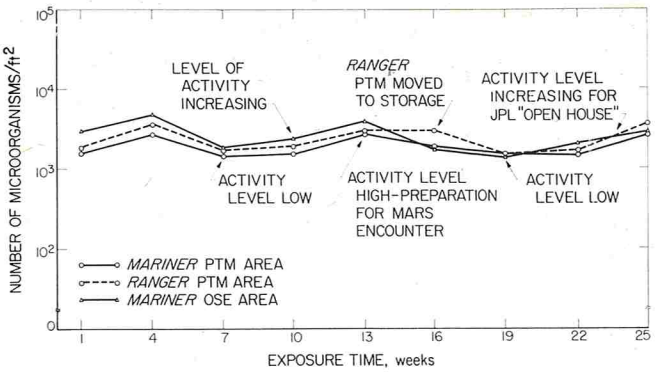


Figure 4. Results of surface sampling studies conducted in the JPL Spacecraft Assembly Facility.

of microbial contamination decreased as the degree of environmental control increased. However, a plateau in microbial contamination on surfaces was observed in all of the areas sampled. The nature and reason for this plateau is the subject of current investigations *(6, 19, 27)*.

Table 3 contains the types of microorganisms found in the intramural air (slit samplers) and upon the bench top surfaces in a Class III clean room located in the Phoenix area. The predominant types of microorganisms detected by both methods were gram-positive cocci (67 to 82%, and diptheroids (10 to 17%). Aerobic sporeforming microorganisms were not detected by air sampling and were present in low numbers (4%) in Rodac plate samples taken from working surfaces within the room.

A comparison of the types of microorganisms isolated from SS strips exposed in three areas that employed different degrees of environmental control is presented in Table 4. In the manufacturing area, the predominant types of microorganisms were fungi and members of the genus *Bacillus*, which are common soil microorganisms. Similar results were obtained from SS strips exposed in manufacturing area A. However, the Class II and Class III clean rooms were more rigidly controlled and the predominant types of microorganisms found were gram-positive cocci and diphtheroids, organisms common to the

TABLE 2. SUMMARY OF SURFACE SAMPLING RESULTS

| Area sampled | Sampling interval | Total exposure of test surfaces | Range of aerobic mesophilic microorganisms per square foot of surface | Plateau level |
|---|---|---|---|---|
| Manufacturing area | | | | |
| "D" (P)[a] | 3 wks | 21 wks | 3000 to 30,000 | $10^4$ |
| Industrial clean rooms | | | | |
| Class II (LA)[b] | 3 wks | 25 wks | 6000 to 25,000 | $10^4$ |
| Class II (P) | 3 wks | 21 wks | 5000 to 26,200 | $10^4$ |
| Class III (LA) | 3 wks | 25 wks | 100 to 400 | $10^2$ |
| Class III (P) | 3 wks | 21 wks | 2800 to 9200 | $10^3$ |
| Class IV (LA) | 3 wks | 25 wks | 100 to 500 | $10^2$ |
| Laminar flow clean rooms | | | | |
| Horizontal filter wall | 1 wk | 7 wks | None detectable | |
| Horizontal exhaust wall | 1 wk | 7 wks | when filter intact | |
| | | | 10,000 to 50,000 | $10^4$ |
| Vertical | Twice weekly | 8 wks | 0 to 40 | $10^1$ |

[a]Phoenix area.
[b]Los Angeles area

TABLE 3. COMPARATIVE TYPES OF MICROORGANISMS RECOVERED FROM THE AIR AND SURFACES WITHIN A CLASS III CLEAN ROOM[a]

| Type of microorganism | Air % | Surfaces-rodac plate % |
|---|---|---|
| Gram positive cocci | 82.1 | 67.4 |
| *Bacillus* spp. (Sporeformers) | 0.0 | 3.6 |
| Diphtheroids | 9.5 | 16.9 |
| Gram negative rods | 3.6 | 1.2 |
| Fungi | 1.2 | 2.4 |

[a]Clean room located in the Phoenix area.

body surfaces, hair, and respiratory tract of humans. Few sporeformers and other microorganisms associated with soil were isolated from these clean rooms.

*Microbial Survival on Environmentally-Exposed Surfaces.*

Figures 5-8 contain the results of studies on the effects of environmental conditions on surface-exposed vegetative cells. Three different areas of environmental control were included in the tests: the Class 100 clean room (vertical laminar flow) in the JPL EASL; the operational support (OSE) area of the EASL, and the JPL SAF. Both the OSE and SAF areas have conventional air control systems, whereas the Class 100 clean room in the EASL is controlled to 20 to 25 C and 40 to 45% relative humidity (RH). From the data in Figures 5-8, it appears that survival of all four species of vegetative cells was poorest on surfaces exposed to the vertical laminar flow Class 100 clean room of the EASL. This area has a RH near 50% and earlier studies have shown that humidities at and above 50% are rapidly lethal to surface-exposed microorganisms *(5, 17-20, 29)*. Three of the four microorganisms tested (*Pseudomonas aeruginosa* was not included in the SAF study) survived longest on surfaces exposed in the JPL SAF. Environmental

TABLE 4. A GENERAL COMPARISON OF THE TYPES OF MICROORGANISMS ACCUMULATING ON STAINLESS STEEL SURFACES

| Type of microorganism | Area sampled[a] | | |
|---|---|---|---|
| | Class II clean room | Class III clean room | Manufacturing area D |
| | (%) | (%) | (%) |
| Gram positive cocci | 34 | 55 | 14 |
| *Bacillus* spp. | 18 | 10 | 31 |
| Diphtheroids | 36 | 28 | 10 |
| Gram negative rods | 0 | 0 | 2 |
| Fungi | 7 | 4 | 27 |

[a]All areas located in Phoenix.

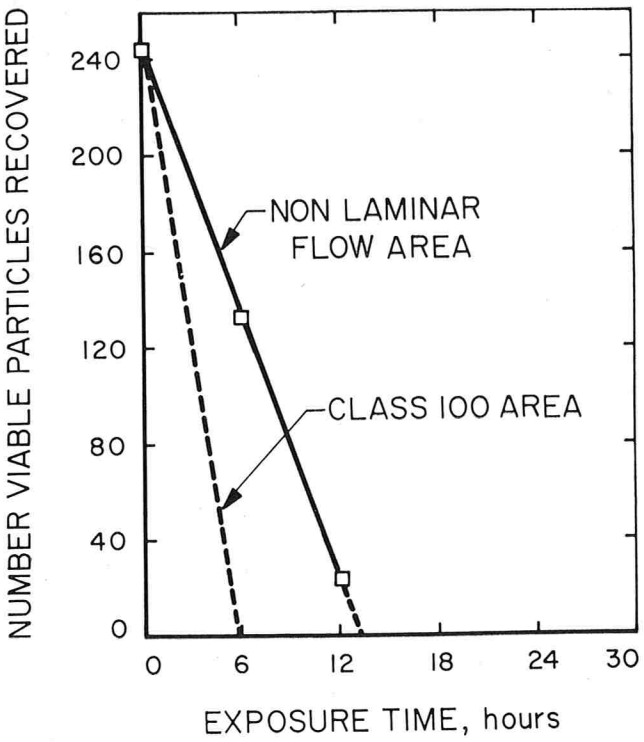


Figure 5. Survival of *Pseudomonas aeruginosa* on stainless steel surfaces exposed to different environmental conditions.

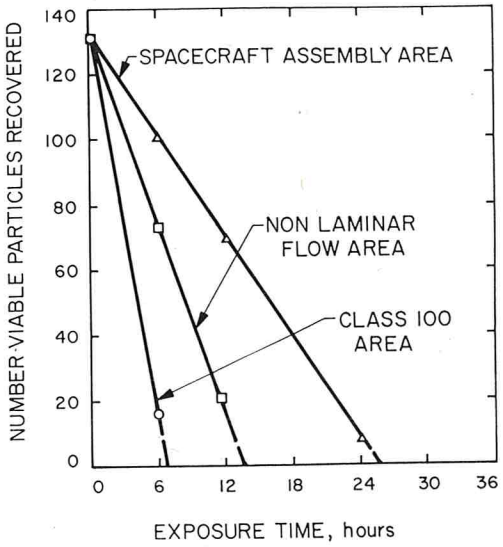


Figure 6. Survival of *Escherichia coli* on stainless steel surfaces exposed to different environmental conditions.

conditions in this facility were poorly controlled and the RH was frequently below 50% during the study. Surface-exposed microorganisms have been shown to survive for relatively long periods of time at low (10 to 15%) relative humidities *(17-20, 29)*. However, in all cases the gram-positive microorganisms survived exposure under all conditions for longer periods than did the gram-negative test organisms. These preliminary results *(26)* have prompted further investigation concerning the effect of moving air at different relative humidities on surface-exposed microorganisms.

## DISCUSSION

In general, the air sampling results indicate that volumetric sampling of air provided a good indication of the levels of microbial contamination when the number of airborne viable particles was high. Differences in the levels of airborne microbial contamination were sharp and correlated with concurrent activities. But when the degree of airborne microbial contamination was low (EASL Class 100 clean room), the sensitivity of the volumetric air-sampling method was quickly lost. In fact, the available sampling devices did not appear to be adequate to insure a representative sample when these instruments were used to sample vertical laminar flow clean rooms.

The level of environmental contamination recovered was dependent also on the microbiological sampling method used. As personnel density increased in the vicinity of a volumetric air sampler, "clouds" of microbial aerosols could have been generated. Collection of such aerosols would result in proportionately high levels of airborne viable particles. However, the deposition of such airborne viable particles onto SS strips might yield an entirely different set of results. Environmentally exposed microorganisms are subjected to a number of conditions that may have an effect on the survival of a given species. A number of studies *(17-20)* have shown that variables such

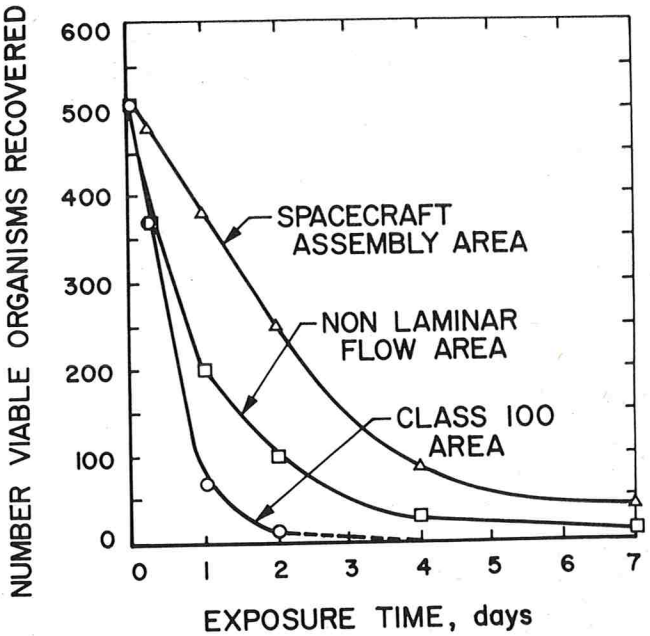


Figure 7. Survival of *Staphylococcus aureus* on stainless steel surfaces exposed to different environmental conditions.

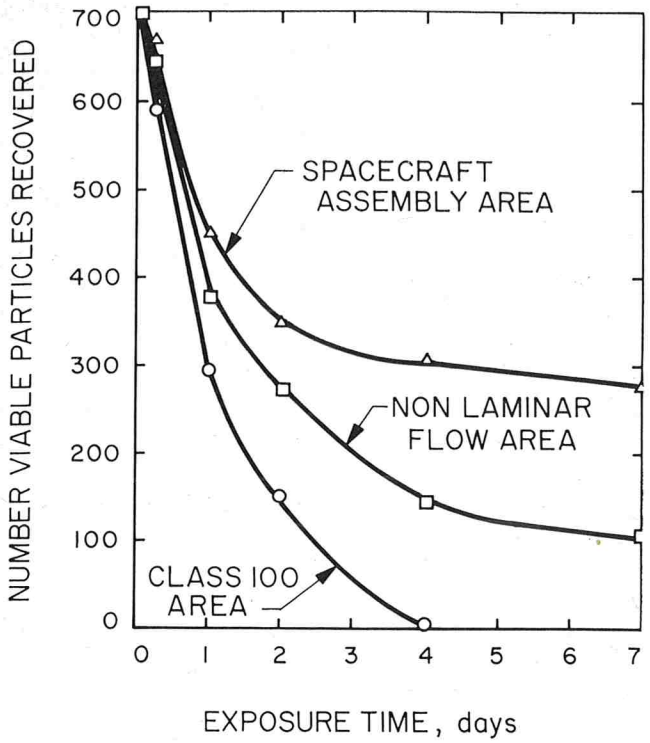


Figure 8. Survival of *Streptococcus faecalis* on stainless steel surfaces exposed to different environmental conditions.

as temperature and relative humidity, desiccation, exposure time, species, age, and number of exposed microorganisms, as well as the availability of nutrient materials, all play a role in the survival or die-away of microorganisms exposed in the environment.

Comparison of the results obtained from air-sampling studies with the data obtained in the surface-sampling studies is not possible. It must be realized that the two methods of sampling microbial contamination are different. The volumetric air sampler measures the number of viable particles that are suspended in air during a specific sampling interval. The SS strips measure the number of viable particles that sediment from airborne suspensions, as well as the subsequent survival and accumulation of such sedimented particles. Furthermore, the methods of microbiological assay do not produce the same type of results. If, for example, large particles containing many viable microorganisms were shed into the environment by clean room personnel, such particles would, if large enough, rapidly settle out of airborne suspension. If such a large particle was collected with a volumetric air sampler, only one colony would result, even though the particle contained many viable microorganisms. However, if the same particle landed on a SS strip, subsequent analysis by shaking the SS strip in a liquid medium might break the large particle into clumps of smaller particles, each containing viable microorganisms. After plating and incubation, an increased number of colonies would be detected from the SS-strip sample. Therefore, the air-sampling data are expressed as the number of *viable particles* per cubic foot of air and the SS-strip data are reported as the number of *viable microorganisms* per square foot of surface.

Use of the SS-strip method was quite reliable. In fact, SS strips have proven to be fairly sensitive in detecting changes in low levels of microbial contamination in the laminar flow areas of the JPL EASL facility as well as in some of the clean rooms included in the Phoenix studies. At present, use of a surface sampling procedure such as the SS-strip method seems to be the only reliable tool to measure the level of microbial contamination within vertical laminar airflow clean rooms. Furthermore, use of a surface sampling technique such as the SS strips is quite logical, for the primary interest in monitoring the level of intramural microbial contamination is to determine what level and species of microorganisms fall onto and accumulate on space hardware. Thus, it would seem that the SS-strip method is a valuable tool for use in monitoring the level of microbial contamination that accumulates on surfaces within facilities involved in the assembly or test of space hardware.

It is obvious (Table 1, Figure 3) that as the degree of environmental and personnel control was increased, the levels of airborne microbial contaminants decreased. Whenever personnel activity was high, there was a corresponding rise in the number of viable particles. Consequently, one can assume that the main source of microbial contamination was from clean room personnel. This concept was strengthened by identification studies (Table 3) which showed that most of the microorganisms collected by air sampling were indigenous to humans. Very few microorganisms associated with soil were found.

Studies on the accumulation of airborne microorganisms on SS strips exposed to the intramural environment of several types of clean rooms and manufacturing areas showed that the lowest levels of contamination occurred in those areas employing the laminar flow system. This was especially evident in the case of the JPL EASL. The types of microorganisms found to accumulate depended, to a certain extent, on the degree of personnel and environmental control. Wherever the controls were not stringent, high numbers of microorganisms associated with dust and soil were detected. On the other hand, where the environment was controlled adequately and where personnel wore full protective clothing, most of the contaminants were those indigenous to human skin, hair, and respiratory tract.

The results obtained in studies concerned with the survival of microorganisms on sufaces were most

interesting. It appears from the results obtained to date that the death rates for microorganisms on stainless steel strips depend, to a great extent, on the species involved. Non-sporeforming species exhibited a definite die-away during exposure to environmental conditions. In all cases, die-away of surface-exposed vegetative cells was accelerated in the Class 100 clean room (vertical laminar flow) and less pronounced in the non-laminar flow area and the SAF (Figures 5-8). Aerobic bacterial spores did not appear to be significantly affected during exposure to any of the three environmentally controlled areas. The effect of moving air on the survival of surface-exposed microorganisms may influence the die-away rates. Studies to evaluate this possibility are in progress.

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# A SURVEY OF TEMPERATURES INVOLVED IN BOTTLING MILK IN PAPER CONTAINERS

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(Received for publication February 18, 1967)

## Summary

The temperature of milk during bottling in half-pint knock-down and preformed paper containers at ten dairy plants ranged from 43 to 56 F with an average of 47 F. The effects of these temperatures on the shelf life of the product are discussed.

The rise in the temperature of milk during bottling always has been the concern of industry personnel and regulatory authorities. The advent of knock-down paper containers increased this concern because of the high temperatures involved during their formation in the milk plant and the possibility that they might retain heat at time of filling. The transfer of this heat to the milk could result in a sufficient rise in temperature of the milk to affect its keeping quality.

The National Conference of Interstate Milk Shippers has a committee studying the practicability of a temperature requirement of not to exceed 45 F for bottled pasteurized milk. Some processors feel that they could not comply with this requirement using the present methods of bottling. A survey within the industry of the temperatures involved during bottling of milk and their effects on the keeping quality of the product would be of value to the industry.

The objective of this survey was to obtain information on the temperature rises involved during processing and bottling of milk in knock-down and pre-formed paper containers and the effects of these rises on the keeping quality of the milk during storage.

## Experimental Procedure

The survey included duplicate sampling on each of five dairy plants using knock-down plastic laminated and five using pre-formed waxed paper containers. State inspectors obtained the temperature data and collected the samples from the various milk plants. Temperatures recorded were *(a)* raw milk storage, *(b)* high temperature–short time pasteurization temperature, *(c)* product leaving the plate cooler of the pasteurizer, *(d)* milk in the pasteurized surge tank, *(e)* the product in the container within one min after filling, and *(f)* the milk in the container after holding at room temperature for 15 min. The half-pint milk samples collected at the time of filling were packed in ice in Styrofoam coolers and transported to the laboratory. Sufficient samples were collected from each dairy plant for storage at 35, 40, 45, 50, and 55 F for 0, 3, 6, 9, 12, and 15 days. At the conclusion of each storage period, the milk samples were analyzed for standard plate counts (SPC) using 32 C for incubation *(1)* and checked for flavor by two experienced judges.

Table 1. Temperatures Involved During Processing and Bottling of Milk in Paper Containers

| Source of temperature | Pre-formed carton | | Knock-down carton | |
|---|---|---|---|---|
| | Range | Mean[a] | Range | Mean[a] |
| | (°F) | (°F) | (°F) | (°F) |
| Raw milk storage | 37-41 | 39.6 | 40-43 | 41.0 |
| Product during pasteurization | 167-172 | 169.6 | 165-174 | 169.0 |
| Product leaving press | 37-41 | 38.6 | 36-43 | 38.8 |
| Pasteurized surge tank | 38-43 | 40.9 | 39-42 | 40.8 |
| Container after filling | 43-47.5 | 45.4 | 45-56 | 48.8 |
| Container after 15 min | 49-52 | 50.7 | 50-64 | 53.2 |

[a]Mean of two replicates on each of five milk plants.

## Results and Discussion

The temperatures involved during processing and bottling of milk in preformed and knock-down paper containers are presented in Table 1. The mean temperature of the milk in the pasteurized surge tank was practically the same for the two types of container using plants, but in the container it was 3.4 F

Scientific contribution No. 264, Agricultural Experiment Station, University of Connecticut, Storrs.

higher in the knock-down than in the pre-formed containers. Although not a direct comparison of the same milks placed in each type of container under identical conditions, this observed mean difference suggested that, on the average, milk in knock-down containers retained more heat at the time of filling.

Nearly all of the containers contained milk that exceeded 45 F immediately after filling (Table 1). Only milk in two of the twenty containers represent-

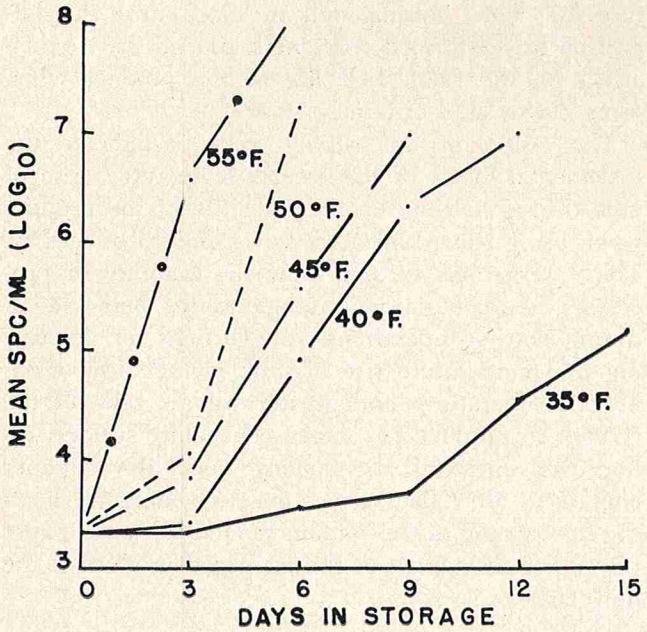


Figure 1. Effect of temperature of storage on bacterial counts of pasteurized milk in pre-formed paper containers.

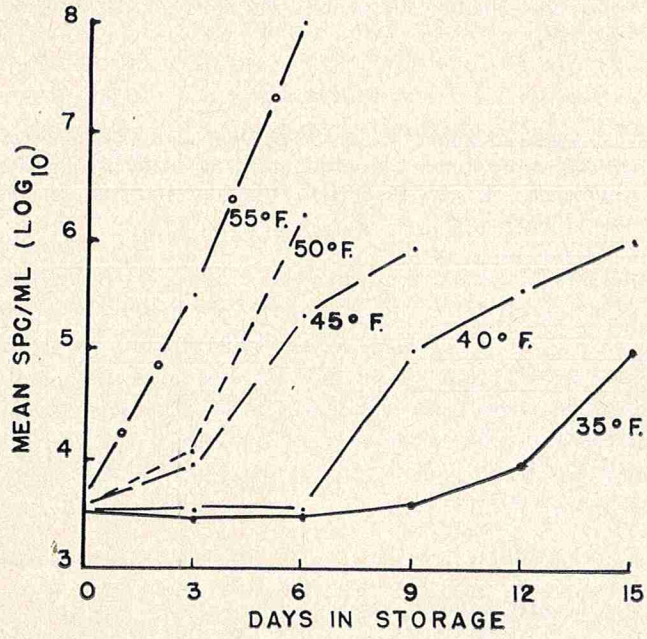


Figure 2. Effect of temperature of storage on bacterial counts of pasteurized milk in knock-down paper containers.

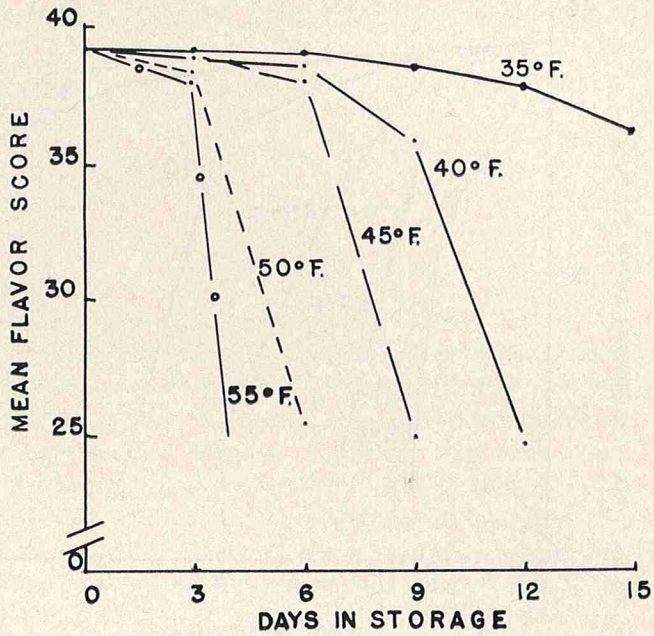


Figure 3. Effect of temperature of storage on flavor scores of pasteurized milk in pre-formed paper containers.

ing ten dairy plants was below 45 F at the time of filling and the containers in these cases were pre-formed. The additional warm-up of the milk upon standing at room temperature (Table 1) indicated the importance of moving the filled containers into the cold room as quickly as possible. All of the plants but one moved the filled containers into the cold room by track within 2 min. In one plant filled cases were placed on dollies and 10 min elapsed before they reached the cold room.

The time required for milk which has warmed-up during filling to cool down in the cold room is also of prime importance. Milk in half-pint waxed pre-formed containers at temperatures of 45, 50 and 55 F required 75, 105 and 135 min, respectively, to reach 40 F when placed in a refrigerator maintained at 35 F. These times indicated that further study is warranted to determine the effect of slow cooling of milk in the container on keeping quality.

The effect of temperature of storage on the SPC of pasteurized milk stored in pre-formed and knock-down paper containers is shown in Figures 1 and 2, respectively. Each figure is an average of the data representing ten samples. A comparison of the two figures indicated that milk with the lower average temperature warm-up (pre-formed containers) had lower bacterial counts at nearly all temperatures during storage than milk with the higher average temperature warm-up (knock-down containers). Furthermore, the milk with the lower temperature in the containers met the State standard of not to exceed 25,000 per ml (SPC) ($\log_{10} = 4.4$) for pas-

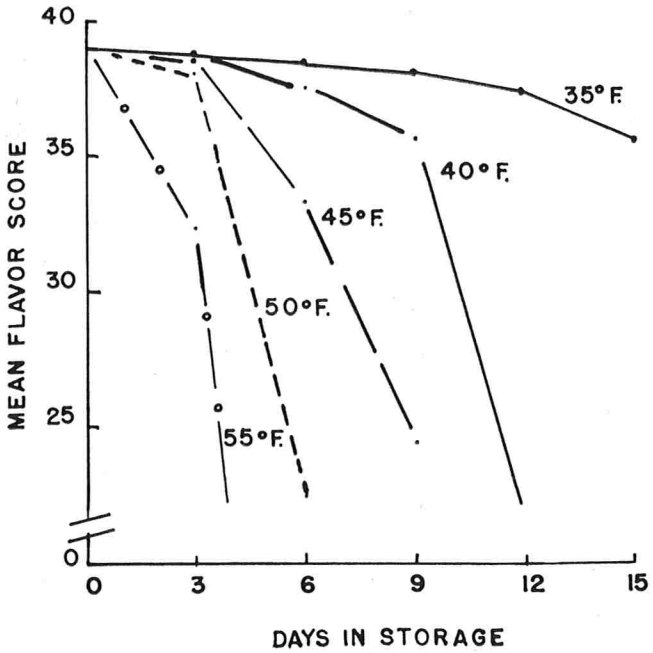


Figure 4. Effect of temperature of storage on flavor scores of pasteurized milk in knock-down paper containers.

teurized approved milk for longer periods of storage. This is exemplified by the milk in preformed containers meeting bacterial requirements for 6 and 12 days of storage at 40 and 35 F, respectively, whereas, milk in knock-down containers met this requirement for only 3 and 9 days of storage at 40 and 35 F, respectively. The Figures 1 and 2 also demonstrated the well recognized fact that the lower the storage temperature, the longer the shelf life.

The flavor scores of the milk stored in pre-formed and knock-down paper containers at various temperatures of storage are presented in Figures 3 and 4, Each figure is an average of the data representing 10 samples. A comparison of the two Figures indicated that the advantage of the pre-formed container with its lower milk temperature over the knock-down container with its higher milk temperature at the time of filling is not as apparent when based on flavor scores of milk as it was for bacterial contents, particularly at the lower storage temperatures. If a score for milk of 36 and above is assumed to indicate a good product, then there does not appear to be a difference in the shelf-life of milk in the two containers. However, there is some evidence at temperatures of 45 and 55 F that milk with the greater warm-up (knock-down containers) deteriorated quicker than that with the lesser warm-up (pre-formed containers). It appeared that the higher temperature rise, when bottling milk in knock-down in comparison to pre-formed containers, did not have much affect on the final shelf life of the product when using flavor as a criteria.

The results of this survey seem to indicate the detrimental effect of the greater temperature rise of milk during bottling on the shelf life of the product when using bacterial counts as a standard of quality. The observations further show the inability of processors to maintain milk temperatures below 45 F during bottling operations. Suggestions for decreasing the temperature rise of milk include use of *(a)* lower temperatures and greater volume ratio of the coolant to product, *(b)* increased cooling surface, *(c)* improved methods for cooling knock-down paper containers after their formation, *(d)* minimum heating for forming of the containers, and *(e)* faster paper fillers and movement of the packaged product to the milk cooler.

### Acknowledgment

The authors are indebted to R. F. Anderson, P. E. Gotthelf, C. W. Jekanowski, W. R. Peckham, W. F. Russell and H. M. Wilson for collecting the samples and obtaining the temperature data.

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# SOLID WASTES: A WORSENING URBAN PROBLEM[1]

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Concern for the urban environment and its effects on human health and welfare is as old as the city itself. From the earliest history of urbanization, man has recognized that urban life, with all its advantages, is not an unmixed blessing. The discoveries of modern medical science and recognition of the importance of sanitation in the prevention and control of disease merely explained what the ancients long ago observed about the problems of the urban environment.

If the contemporary problems of the urban environment are rooted in the dust and rubble of ancient cities, they are, nonetheless, of a complexity which our ancestors never faced. The growth of technology and the explosion of urban population, the end of which is not even in sight, have so magnified the problems of urban environments that our ability to measure and analyze them is constantly shown to be inadequate. And our capacity to meet and resolve these problems is being taxed literally to the breaking point.

We Americans like catch phrases, and we have adopted one for the urban problem. We call it "the crisis in our cities." Now crisis is a strong word. It makes us think of clear and present danger, of perils that threaten our very existence. We think of a serious illness as having a time of crisis when the outcome, whether the patient will live or die, is in the balance. The word crisis seems too awesome to apply to the problems of the urban environment. But is it? I think not.

## NEED FOR WASTE MANAGEMENT

We have reached a stage in the evolution of urbanization at which the sickness of our cities threatens to bring an end to urban life as we know it. I want to devote these remarks to what is beyond question one of the most critical of urban ills; one which, though it can be clearly identified, is so closely tied to a great many urban problems as to be of tremendous importance to all of them. I am thinking of the management of urban solid wastes.

People in cities have always had to contend with the problem of solid waste disposal. The problem must have been born at the same time that human beings banded together to create the first town. Whether this first town was a cave in the side of a mountain or a more or less permanent campfire site, it was a place where wastes were produced and had to be disposed of. Furthermore, disposal had to be accomplished in a way that did not make the cave or the campsite unlivable. If this problem sounds familiar, it ought to. It is exactly the same one cities face today. The only difference is in degree.

But the degree of difference is staggering. What prehistoric peoples must have regarded as a bothersome chore, we must now accept as one of the greatest threats to human health and welfare. The cave dweller could always find someplace else to throw his trash, but we are finding that "someplace else" is rapidly becoming urbanized and that one man's trash heap often is another man's front door.

Yet the difference is far more than a matter of space. The quantity and variety of solid wastes generated in the contemporary urban environment are an equally great cause of deep and growing concern. Today the typical city dweller produces four and one-half pounds of solid wastes every day, more than twice as much as a generation ago. And the nature of these waste materials is constantly shifting. The direction of the change seems to be inexorably toward materials whose disposal is more difficult, more costly, and more hazardous to health and welfare. Our waste disposal facilities and programs are tragically geared to the needs of the last generation, even of the last century. They are simply not equipped to cope with the modern solid waste problem.

Burn or bury. This is what the caveman did, and this is what we are still trying to do. But how do you burn an aluminum can? How do you bury forever a polyethylene bottle? Both of these waste discards, so long as we rely on conventional, outmoded disposal techniques, will be with us for centuries. Man, with his infinite technological ability, has succeeded in burdening himself with a solid waste problem as remote from the past as the supersonic jet is from the oxcart. And despite his technological pro-

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[1]From a paper presented before the Public Works Congress and Equipment Show sponsored by the American Public Works Association, Chicago, Ill., September 10-15, 1966.

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wess, man has not yet adequately turned his talents toward solving the problems of waste management. We are, as someone had said, a generation standing knee-deep in its own refuse, hurling spaceships to the moon.

## THE HIGH COSTS OF WASTES

The handling and disposal of solid wastes cause hazards to health and welfare. The rat and fly infested dump heap in the back alley or at edge of town, the incinerator with its noxious plume of smoke, the stream or lake literally chocked with rotting waste materials—these are health hazards by any standard of common sense as well as by the scientific evidence of their role in causing and spreading disease. By the same token, who can fail to recognize that a mountain of municipal refuse, an auto graveyard, or an offensive waste disposal facility depresses the value of the surrounding area as well as the lives of the people who must live nearby? No one today knows what the economic cost of inadequate handling of municipal solid wastes is. But we do know, thanks largely to studies conducted by the American Public Works Association, that Americans living in cities pay each year roughly $3,000,000,000 for the collection, transportation, processing, and disposal of solid wastes; and for this huge sum they are getting no bargain.

What they are getting is a steadily worsening solid waste disposal problem with increasing pollution of air, water, and land, a rising economic burden, and continuing erosion of the natural beauty of cities and of the open countryside. This is a poor return on a $3,000,000,000 annual investment. And just for the record, we should not overlook the fact that this money is spent for the handling of urban wastes and does not include the cost of disposing of farm wastes or of those industrial solid wastes that do not ordinarily become a part of the urban waste stream, but are definitely a part of the solid waste problem. Agricultural and industrial solid waste production probably equals twice the amount that is collected in cities. With the accelerating trend toward urban sprawl out to and around once predominantly farm and industrial areas, the cities will have to come to grips with an increasing share of the national solid waste management problem for which they now are almost totally unprepared.

Agencies of government at the municipal, county, State, and Federal level must recognize that protecting the environment from the relentless accumulation of unmanaged solid wastes will require a commitment of public resources, the equal of which has never before been brought to bear on this problem. Let me not mince words. By resources I mean public funds and governmental action.

The American people spend $3,000,000,000 a year to dispose of municipal solid wastes. Yet half of the cities and towns in the country are not today carrying on even marginally acceptable waste disposal programs. Who but the public can correct that fault?

## THE NATIONAL SOLID WASTE PROGRAM

We are relying on waste collection and disposal methods that were primitive at the turn of the century. Yet until the Federal Government launched the National Solid Waste Program less than $200,000 a year was being invested in research and development to find and apply better methods. Who but the public can be expected to bear the brunt of the burden of improving the management of solid wastes?

With the passage last October of the Solid Waste Disposal Act, the Federal Government signaled that it was ready to accept its share of the national solid waste management challenge. In less than a year we have gotten under way a broad program of research and training, technical aid to State and local governments and industry, assistance to States in the development of comprehensive solid waste management plans, projects to demonstrate the effectiveness of new and improved solid waste management techniques, and programs to increase the public understanding of a problem which it has unwittingly created and most knowingly undertaken to solve.

The outlines of the Federal Solid Waste Program are known to many and they will be better and more widely known as the program grows and as its accomplishments increasingly are felt on the national effort to deal with the solid waste problem. But I want to emphasize that the Federal Government has not taken on the entire task of bringing an end to the solid waste problem of our cities, or of the nation. This is a job which must be done not by a single level or agency of government. The enormity and complexity of the solid waste management challenge are too great to be left to the unaided hands of the cities, or the States, or the Federal Government. Each has its share of the total job.

Problems of the environment — air pollution, water pollution, pollution of the land itself — these problems and their effects are no respecters of the arbitrary boundaries by which we divide our cities, counties, and States. No lasting solution can be found for a regional solid waste problem unless it is based on a regional approach. It is for this reason that we need greater investments of public funds *and* a greater commitment of governmental action. For the kind of needed regional approach to the solid waste problems of urban areas will require an unprecedented degree of cooperation among cities,

among counties, and among States. Otherwise we will go on trying vainly to cope with the problem by dumping our wastes in our neighbor's backyard or his river. We will go on polluting his air with the smoke from our burning dumps and overburdened incinerators. And he will do the same things to us because, like us, he will have no alternative.

## OUTLOOK FOR THE FUTURE

Now let me say something about the prospects.

Frankly I am optimistic. I think a change is occurring with respect to the solid waste problem which at least contains the seeds of success. For one thing the problem is now recognized as a national issue. Where once apathy prevailed, growing concern now exists. Where once the norm was a desire on the part of the public and of public agencies to sweep this problem under the rug, now we find open discussion, even controversy. Although the process will surely not be a quick one, out of this discussion, out of this controversy will come progress. For the time being the most significant tangible result of this change in attitude is the adoption of new Federal legislation relating specifically and exclusively to the solid waste problem. But this is just the first result. I am confident we will see many others.

I believe we will see the cities and States, with Federal help where it is needed, turning increasing attention to the management of their solid waste problems, trying new approaches, creating new regional programs. I believe we will see the enactment of local and State laws that will encourage and compel the establishment of new programs or the strengthening of existing ones so that solid waste disposal will not continue to be a major source of environmental decay. I believe we will see "new town" projects which will incorporate wholly new and vastly better systems for collecting, processing, and disposing of solid wastes, systems that will make use of advanced technology for the recovery and reuse of valuable materials and resources we are now foolishly and dangerously throwing away. And I believe we will see a much greater awareness by industry of its role in reducing wastes at the source and in designing and producing products that do not needlessly add to the national solid waste burden.

If cities are not to continue to be drained of economic and cultural vitality, if they are not to become increasingly rundown, if slum areas are not to grow and become more typical of conditions in the city — if these trends which we see and deplore are not to continue, then one of the problems we will have to learn to deal with is the problem created by the mismanagement of urban solid wastes. We have started this part of our practical education perilously late in the day, but not too late. I think we as a nation have begun to appreciate the full and growing significance of the solid waste problem at a time when our resources and energies are equal to the challenge. But time and the stupendous capacity we have to produce waste are working against us.

There is only one best time to solve the solid waste problem, and it is now.

# AFLATOXINS AND OTHER MYCOTOXINS IN AGRICULTURAL PRODUCTS[1]

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(Received for publication January 12, 1967)

Molds of many types have long been recognized as spoilage agents of many different foods. Growth of molds invariably has been associated with the formation of surface colonies and consequent discoloration of the food. Flavor defects and changes in the physical nature of the product often accompany development of the mold. When molds appear on certain foods such as cheese, the obviously molded area is often removed, and the remainder, unless it is deteriorated, is considered satisfactory for use as a food. Although this practice has been common for years, its safety must be re-evaluated in the light of what has recently been learned about *mycotoxins*. Mycotoxins are toxic metabolites produced by certain molds during growth on a suitable substrate.

There are undoubtedly many unreported instances in which moldy feed or food caused illness in animals or humans. Two of the most dramatic to be reported occurred in Russia and England.

Wartime conditions during the Falls of 1942, 1943, and 1944 resulted in incomplete harvesting of cereal grains grown in the Orenburg district and in some other areas of Russia. After overwintering in the field, grains were harvested the following spring and were used to prepare foodstuffs. Consumption of these food products resulted in frequent and fatal outbreaks of a condition designated as septic angina or toxic alimentary aleukia *(15)*. Production of the toxic substance(s) was associated with the development of certain fungi on the grain while it was covered with snow. Organisms principally responsible for this mycotoxicosis were found to be in the genera *Fusarium* and *Cladosporium (14)*.

Eighteen years later, early in 1960, outbreaks of what seemed to be a new disease caused heavy losses among young turkey poults on a number of farms in southern England. It has been estimated that at least 100,000 poults died during this outbreak. The disease was characterized by depression, a staggering gait, and sudden death. The turkey carcass was usually congested and edematous and the liver was enlarged, pale, and firm. Later outbreaks of a similar nature were reported in ducklings and young pheasants. One farmer alone is believed to have lost about 10,000 ducklings. Ducklings seemed to be very susceptible to the toxic substance, and in addition to liver lesions, many had extensive subcutaneous hemorrhages of the legs, feet, and back. The source of toxic material was found to be peanut meal used in the diet and imported from Brazil. Cultural examination of the peanut meal resulted in the isolation of a strain of *Aspergillus flavus* possessing the ability to produce the toxin present in the peanut meal *(10)*.

These two examples are sufficient to demonstrate that certain molds, under proper conditions, can do much more than merely spoil a product by rendering it moldy. They can, in fact, develop substances with a high degree of toxicity. The present paper will, first, summarize information on a number of mycotoxins which have been isolated, and then will explore in some detail the toxic substances produced by *A. flavus* and designated as aflatoxins.

## Many Mycotoxins Reported

Development of toxins is not limited to one or several species of molds. A partial list of the molds, infected material, toxic substances, susceptible animals and symptoms has been compiled by Friedman *(12)*, and is given in Table 1.

An examination of the data in this table leads to a number of conclusions. First, toxin was produced on a variety of substrates. This might be expected since molds can grow on most feeds and foods provided sufficient moisture is present. Second, the toxic material varied in its nature although some of the toxins have not been characterized. Third, many animals and man were susceptible to some if not all of the toxins. Finally, the toxins generally seemed to be rather potent in that they often caused death after ingestion. Many of them also appeared to affect the liver, an organ which is incapable of regen-

[1]Presented as part of a Symposium on Food-Borne Diseases at the Tenth Anniversary meeting of the Wisconsin Section of the Institute of Food Technologists, December 2, 1966, at Madison, Wisconsin.

TABLE 1. A SUMMARY OF SOME DATA ON MYCOTOXINS[a]

| Mold | Infected Product | Toxin | Reported Susceptibility | Symptoms |
|---|---|---|---|---|
| *Sclerotinia sclerotiorum* | Celery | 8-Methoxy psoralen | Man<br>Rabbits<br>Mice | Blistering lesions on skin exposed to sunlight |
| *Fusarium sporotrichioides* | Cereal grains | Unknown | Man<br>Cat<br>Guinea pig<br>Dog<br>Monkey | "Alimentary toxic aleukia"—Hemorrhages of skin and mucous membranes, necrotic ulcers in oral and pharyngeal tissues, leukopenia, anemia, fever, bone marrow exhaustion |
| *Sporodesmium bakeri* | Rye grass<br>Bermuda grass | "Sporodesium" ($C_{19}H_{22}O_6N_3S_2CR$) | Sheep<br>Cattle<br>Guinea pig<br>Rabbit<br>Mouse | "Facial eczema" in ruminants, hyper-irritability, lacrimation, nasal discharge, photosensitivity, icterus, stenosis, obliteration of bile ducts, cirrhosis |
| *Stachybotrys atra* | Hay<br>Straw<br>Grain | Stable to heat and radiation; destroyed by alkali | Horses<br>Cattle<br>Mice<br>Guinea pig<br>Dogs<br>Man | Stomatitis, inflammation of buccal tissues, thrombocytopenia, prolonged clotting time, fever, leucocytopenia, massive hemorrhages, fatal in 3 to 4 weeks, dermal inflammation in man. |
| *Aspergillus chevaleri* | Hay<br>Grain | Unknown | Cattle<br>Mice<br>Rabbits | Acute—fatal in 4 to 5 days, chronic hyperkeratosis |
| *Aspergillus clavatus* | Pelleted feed | Unknown | Rabbits | Dermal toxicity |
| *Aspergillus fumigatus* | Fodder | Unknown | Cows | Hyperkeratosis |
| *Aspergillus flavus* | Peanuts<br>Grains | Aflatoxins | Turkeys<br>Ducks<br>Swine<br>Calves<br>Rats | Liver parenchymal cell damage, bile damage, bile duct proliferation, hepatoma |
| *Aspergillus flavus* plus *Penicillium rubrum* | Corn | Unknown | Swine<br>Mice | Anorexia, cachexia, icterus, fatal in 1 to 5 days, profuse hemorrhages in all tissues, mortality—25 to 50% |
| *Penicillium toxicarium* | Cereal Grains | Unknown | Higher Vertebrates | Ascending paralysis of CNS |
| *Penicillium citrinum* | Rice | Citrinin | Mice | Acute glomerulonephrosis, liver damage |
| *Penicillium rugulosum* | Rice | Rugulosin | Mice<br>Rats | Fatty degeneration of liver, Kidney damage |
| *Penicillium islandicum* | Rice | Chloride-containing peptide | Rats | Fatty degeneration of liver, bile duct hyperplasia, focal necrosis and hemorrhages of the liver, primary malignant hepatomas |

[a]As reported by Friedman *(12)*.

eration. Consequently, changes in this organ are quite permanent in nature and tend to be rather deleterious to the welfare of the animal or human being.

One of the toxins listed in Table 1 is aflatoxin which is the product of *A. flavus* and *Aspergillus parasiticus*, although the latter mold was not mentioned in Table 1. This toxin recently has received more attention than the others since it has been associated with a food crop—peanuts. This emphasis has resulted in the accumulation of a substantial amount of information on aflatoxin. The remainder

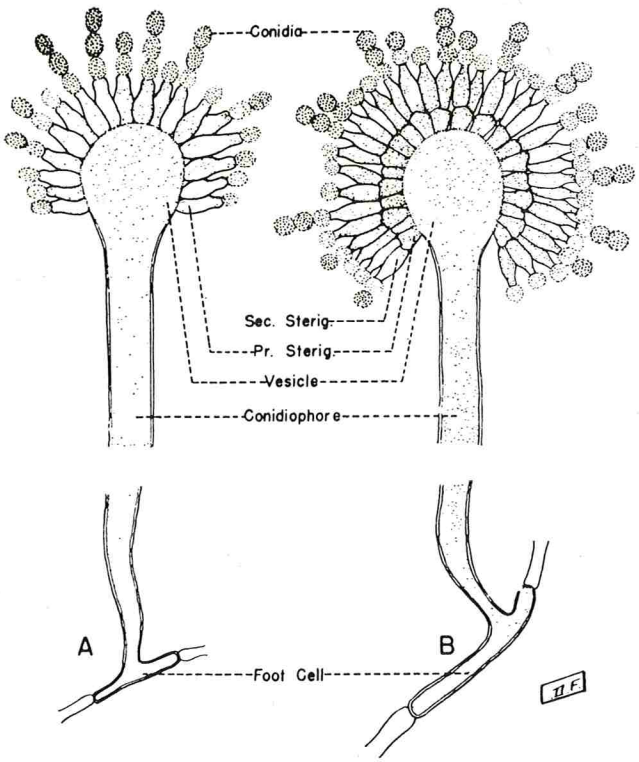


Figure 1. Diagramatic sketch showing principal characteristics of molds in the genus *Aspergillus (20).*

of this paper will be devoted to a consideration of some of this information.

*Aflatoxins.*

It was mentioned earlier that a toxic substance now designated as aflatoxin was first associated with moldy peanut meal which caused the death of large numbers of turkey poults and ducklings after they ingested the feedstuff. The mold recovered from the peanut meal and found able to produce the toxin was *Aspergillus flavus.* Before considering the nature of the toxin and ways it affects animals, some space will be devoted to a description of the mold responsible for the problem.

*Description of Aspergillus flavus.* The genus *Aspergillus* has certain peculiar characteristics which serve to distinguish it from other genera of molds. The vegetative mycelium consists of septate branching hyphae which range from colorless to brightly colored and, in a few instances, are colored in localized areas *(20).* The reproductive or conidial apparatus develops in the form of conidiophores and heads from specialized, enlarged, thick-walled hyphal cells designated as foot cells. Conidiophores, either septate or nonseptate, usually enlarge at the top to form fertile vesicles that in turn bear fertile cells or sterigmata. Conidia (or spores) which may vary in color, size, shape, and markings are produced from the tips of either the primary or secondary sterigmata. Figure 1 *(20)* illustrates the major characteristics of molds in the genus *Aspergillus* as they have just been described.

*Aspergillus flavus* has all of the characteristics just discussed but is designated as a species because it differs from other molds in the same genus with regard to some of its distinctive features. The principal characteristics of *Aspergillus flavus* include: *(a)* the conidia are round or virtually round when mature and may have a rough surface, *(b)* conidial heads are round to radiate or columnar in shape and are very light yellow-green to jade green or cress green in color, *(c)* conidiophores are colorless and usually have a rough surface, and *(d)* vesicles tend to be round and are fertile over most of their surface. These characteristics of *Aspergillus flavus* are illustrated in Figure 2 *(20).*

This brief description of the genus *Aspergillus* and, more specifically, of *Aspergillus flavus,* is sufficient to provide some background on the type of organism responsible for the formation of aflatoxin. The toxic substance itself will now be considered.

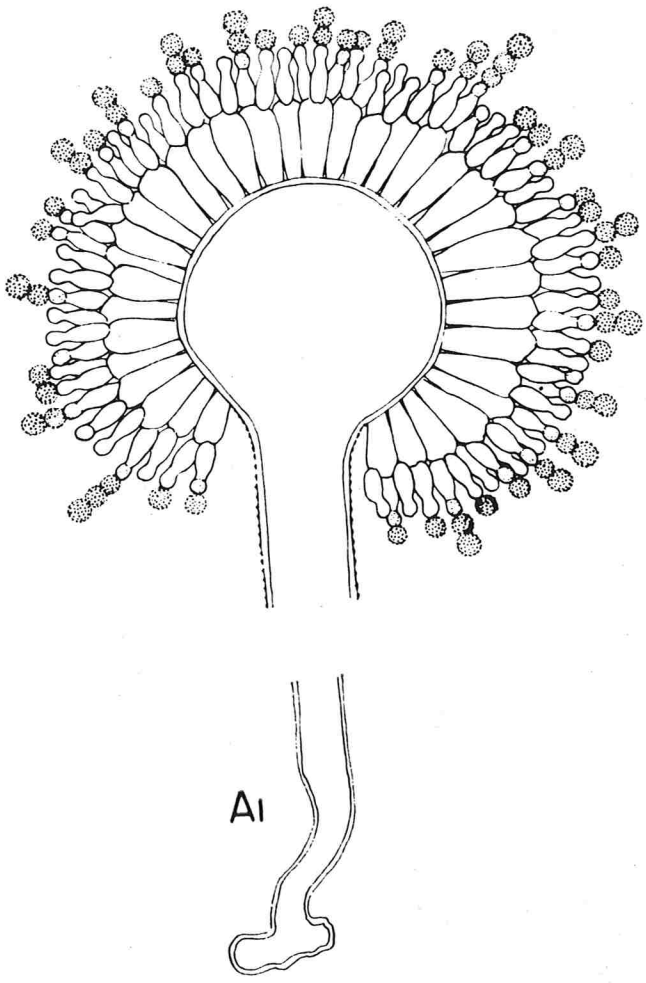


Figure 2. Diagramatic sketch showing principal characteristics of *Aspergillus flavus (20).*

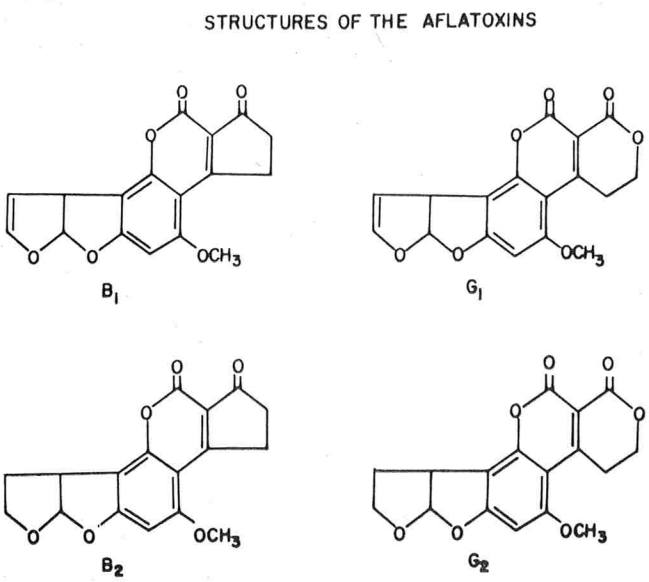


Figure 3. Structures of aflatoxins $B_1$, $B_2$, $G_1$ and $G_2$ *(25)*.

*Chemical and physical nature of aflatoxin.* Aflatoxin consists of four components when viewed under ultraviolet light. Two of these components emit blue visible light and are designated as $B_1$ and $B_2$. The other two fluoresce with a yellow-green color and are called $G_1$ and $G_2$ *(25)*. The amounts and relative proportions of these four compounds present in culture extracts are variable, depending on such factors as mold strain, medium composition, and cultural conditions. Typically, aflatoxins $B_2$ and $G_2$ are present in smallest quantities, whereas the concentration of $B_1$ is usually greatest. These four compounds were originally isolated by investigators in England *(18, 21)* and the Netherlands *(24)*.

The molecular formula of aflatoxin $B_1$ was established as $C_{17}H_{12}O_6$ and of $G_1$ as $C_{17}H_{12}O_7$, whereas aflatoxins $B_2$ and $G_2$ were found to be the dihydroderivatives of the parent compounds and have the formulae $C_{17}H_{14}O_6$ and $C_{17}H_{14}O_7$, respectively *(13)*.

Structures based largely on interpretation of spectral data were proposed in 1963 for aflatoxins $B_1$, $G_1$, and $B_2$ *(4, 5, 9)*. These and the proposed structure for $G_2$ are shown in Figure 3.

These closely related compounds are highly substituted coumarins, and thus are among a large group of naturally occurring compounds with many pharmacological activities. It should be pointed out before concluding the discussion on the nature of aflatoxins that all four are very heat stable. The reported melting points for $B_1$, $B_2$, $G_1$, and $G_2$ are 269, 288, 245, and 239 C, respectively *(25)*.

The discussion just completed has served in part, to describe the toxic metabolites of *Aspergillus flavus* from physical and chemical viewpoints. Attention will now be directed to the effect of these toxins on various animals.

*Effects of aflatoxins on animals.*

The effect of aflatoxins on animals is governed by: *(a)* the dosage administered in the form of moldy feed or in another fashion, *(b)* the kind of animal, *(c)* the length of time that the animal is exposed to the toxin, and *(d)* the age of the animal.

Wogan *(25)*, in a recent review of this subject, approached the problem of the effect of aflatoxin on animals from three points of view: *(a)* acute toxicity associated with ingestion of a lethal dose, *(b)* subacute toxicity associated with consumption of small amounts of toxin, and *(c)* carcinogenic properties of the toxin. The same pattern will be followed in the present discussion.

Table 2. Pathological Changes in Animals That Received Aflatoxin-Contaminated Feed[a]

| Liver Lesions | Calves | Cattle | Swine | Sheep | Duckling | Adult Duck | Poult | Chick |
|---|---|---|---|---|---|---|---|---|
| Acute necrosis and hemorrhage | – | – | + | – | + | – | + | – |
| Chronic fibrosis | + | + | + | 0 | – | + | – | – |
| Regeneration nodules | – | + | + | 0 | ± | + | + | – |
| Bile duct hyperplasia | + | + | + | 0 | + | + | + | ± |
| Veno-occlusive disease | + | + | – | 0 | – | – | – | - |
| Enlarged hepatic cells | + | + | + | 0 | + | + | + | – |
| Liver tumors | 0 | 0 | 0 | 0 | – | + | 0 | 0 |

[a]As reported by Wogan *(25)*.

*Acute toxicity.* The aflatoxins are acutely toxic to most animal species. Early experimental studies as well as observations in the field suggested that the duckling was the species most susceptible to acute poisoning. The $LD_{50}$ of one-day-old ducklings is approximately 0.5 mg per kg. This value is considerably smaller than those for the rat and hamster. Some tests indicate that the dog, rabbit, guinea pig, and rainbow trout all have $LD_{50}$ values similar to that of ducklings *(6, 25)*.

In most species, death usually occurs within 72 hours after the toxin is administered. Examination of animals after death consistently reveals gross liver damage and occasional hemorrhaging in the intestinal tract and peritoneal cavity.

Animals appear to become less sensitive to the toxin as they grow older. For example, a one-day-old rat has an $LD_{50}$ of 1.0 mg toxin per kg of body weight, whereas after it is 21 days old, the $LD_{50}$ value has increased to 7.0. As a basis for comparison, the $LD_{50}$ of lead arsenate is approximately 500.

The structure of the aflatoxin molecule also affects its toxicity. Aflatoxin $B_1$ is most potent, followed in order by $G_1$, $B_2$, and $G_2$. The presence of the additional oxygen in the G compounds results in a reduction of activity by a factor of two, whereas the unsaturated compounds are approximately 4.5 times as potent as the dihydro-derivatives.

The information just presented becomes a bit more meaningful when it is realized that one of the most toxic peanut meals ever encountered contained approximately 10 ppm. of aflatoxin $B_1$. As little as 1.0 gram of this meal proved lethal to day-old ducks *(21)*.

In another instance calves died when they were fed some of the original Brazilian peanut meal *(17)*. Later experiments were conducted in which other calves received diets containing 18% of a highly toxic peanut meal *(3)*. The calves became unthrifty and died within 16 to 25 weeks. The same toxic meal, when fed to three-to four-year old dairy cattle in a diet containing 20% of the meal, caused a loss of condition. Cows aged eight to ten years showed no clinical symptoms when they received the toxic meal at the same level.

*Subacute toxicity.* Animals which consume sublethal quantities of aflatoxin for several days or weeks develop a subacute toxicity syndrome which commonly includes moderate to severe liver damage. Several types of liver lesions have been observed in different species, and this information is summarized in Table 2 *(25)*. Consideration of the data leads one to conclude that sheep are rather resistant to effects of the toxin, and that biliary hyperplasia (a condition in which there is excessive growth of liver tissue) is the lesion most consistently observed in all species except sheep *(25)*.

Subacute toxic effects of aflatoxins in monkeys have been reported *(23)*. In the experiments young Rhesus monkeys (1.5 to 2.0 kg) were fed either 1.0 mg of aflatoxin per day or 0.5 mg per day for the first 18 days followed by 1.0 mg per day. All animals lost their appetite and died in 14 to 28 days. The principal findings on autopsy included liver lesions similar to those seen in ducklings and which were suggestive of liver cirrhosis.

*Carcinogenic properties of aflatoxin.* Prolonged administration of the toxin at subacute levels leads to formation of liver tumors which are cancerous in nature. This was observed in early investigations on the feeding of toxic peanut meal to rats *(16)*. After feeding a purified diet containing 20% of toxic peanut meal for six months, nine of eleven rats developed multiple liver tumors, and two of these displayed lung metastases. The carcinogenicity of toxic peanut meal has been demonstrated repeatedly since then, and aflatoxin has clearly been shown to be the responsible agent.

Precise dose-response conditions have not yet been established, but some information is available regarding relationships between tumor incidence in rats and aflatoxin content of contaminated peanut meals. Results of several studies have demonstrated a good correlation between liver tumor incidence and dietary aflatoxin in the range of 0.06 to 1.8 ppm *(19)*. Administration of the highest level (e.g., 1.8 ppm.) for 370 days was accompanied by a tumor incidence in excess of 90%. The lowest level of toxin studied (0.005 ppm.) failed to induce liver tumors within a similar time period.

Data accumulated from feeding tests employing the pure toxin have permitted the estimation of the effective dose of aflatoxin $B_1$ for the induction of liver tumors in rats. It has been estimated that this dose is approximately 10 $\mu$g per day *(8)*. When this value is compared with similar estimates for other hepatocarcinogens such as dimethylnitrosamine (750 $\mu$g/day) and butter yellow (9,000 $\mu$g/day), the relative potency of aflatoxin is readily apparent.

The rainbow trout was found to be considerably more sensitive than the rat to the carcinogenic effects of aflatoxin. It has been shown that this fish develops liver tumors at significant rates when fed purified diets containing only 0.5 to 2.0 $\mu$g aflatoxin $B_1$ per kg (i.e., 0.5 to 2.0 ppb) *(6, 7, 22)*. The apparent sensitivity of this fish has suggested that aflatoxin may be an etiological agent of the so-called "trout hepatoma syndrome."

*Metabolic alterations of aflatoxin.*

The discussion on aflatoxins, up to this point, has been concerned largely with the effect of the toxin on a number of animals. There is another side to

the story—the changes that may result in the toxin as a result of its metabolism by animals. Before concluding the discussion, this aspect of the problem will be briefly considered.

Studies with rats and radioactive labeled aflatoxin indicated that 25 to 30% of the toxin was metabolized to $CO_2$, 25% was excreted in the urine, 25% was contained in feces, and six to nine per cent appeared in the liver. The nature of compounds present in urine and feces has not been determined, and the metabolic pathways are not fully understood *(25)*.

In another series of tests, rats were fed a dried, heat-treated culture of *A. flavus* grown on peanuts and also some pure aflatoxin $B_1$ *(11)*. Chromatographic analysis of an extract of milk produced by the rats revealed the presence of a component different from aflatoxin, but one which retained the toxic properties of the mycotoxin. It was concluded that the lactating rat can convert aflatoxin $B_1$ to another still toxic form and secrete it in the milk. Similar observations have also been made on dairy cattle *(1, 2, 11)*. In fact, it has been demonstrated that the toxic component in cow's milk is associated with casein and remains with the milk protein when it is precipitated with rennin and removed from the remaining milk constituents.

Tests on another product of animal origin, namely eggs, revealed the absence of a toxic substance even when the hens that produced the eggs received a diet containing 15 per cent toxic peanut meal *(2)*.

## SUMMARY

Production of toxic metabolites has been associated with the growth of different molds on a variety of substrates including cereal grains, celery, peanuts, hay, and straw. Most of the toxins affect more than one species of animal, and many of them induce pathological changes in the liver.

Most research attention has been devoted to the heat stable aflatoxin produced by *Aspergillus flavus*. Actually, four different aflatoxins are produced. All are highly substituted coumarins and two of them ($B_1$ and $B_2$) fluoresce with a blue color under ultraviolet light, whereas the others ($G_1$ and $G_2$) fluoresce with a yellow-green color.

The aflatoxins are acutely toxic to most animal species and death will result if enough is ingested, especially when the animal is young. When low levels of the toxin are consumed for several days or weeks, symptoms of subacute toxicity develop. These include biliary hyperplasia and hepatomas. A daily intake of 10 $\mu$g of toxin appears adequate for the induction of hepatomas.

Aflatoxin ingested by animals undergoes certain metabolic changes within the animal body. Rats excrete some of the toxin as $CO_2$, some in the urine, and some in feces. Approximately 6-9% of the ingested toxin is retained in the liver. Rats and daily cattle have also been found able to modify and excrete some of the toxin in milk. The milk toxin is associated with the casein fraction and on precipitation by rennin remains with this milk component.

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# ASSOCIATION AFFAIRS

## MISSOURI AWARDS TO BAIRD AND NICKEL

The Missouri Association of Milk and Food Sanitarians at its awards dinner in connection with the 35th Annual Milk and Food Sanitation Conference at Columbia, April 10-12, 1967, selected I. H. Baird for the 1967 Sanitarians Award and presented to Vernon Nickel a 25 year certificate and pin for continuous service to the Association.

I. H. Baird, D.V.M., Director of Laboratory and Milk Control for the St. Joseph Department of Health, has given 45 years service to the health and welfare of his community. First employed as City Bacteriologist he was instrumental in the adoption of the first milk ordinance in St. Joseph in 1929 and by continued revisions and modifications has kept the city's milk program at the highest level.

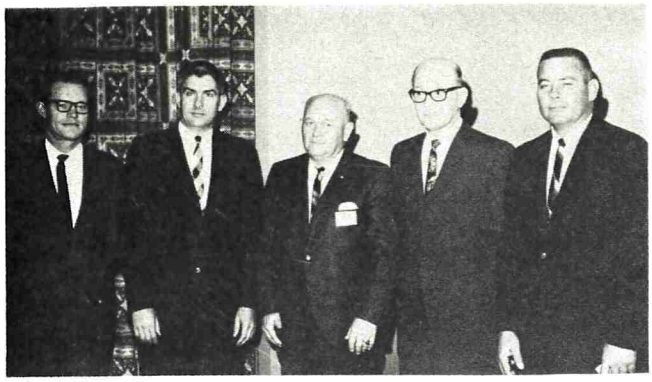
An outstanding achievement was the initiation of the first mastitis control program in the state. Starting without guidelines and no standardized tests, "Doc" devised a program to suit his needs. He introduced test methods, particularly for subclinical mastitis, sometimes over the objections of local veterinarians but his program is now fully supported in the St. Joseph area. He has been an active member of state public health and veterinary medical associations and his work in St. Joseph has contributed substantially to the formation of the Missouri Mastitis Council.

Dr. I. H. Baird (left) receives 1967 Sanitarians Award certificate from Earl White of the Awards Committee.


Vernon Nickel holds 25 Year Sanitation Award certificate and pin.

Vernon D. Nickel, with the St. Louis City Health Division, has long been active in the affairs of the Missouri Association and is presently chairman of its Dairy Farm Methods Committee. "Nick" is also active nationally, serving as the chairman of the Subcommittee on Education of the IAMFES Dairy Farm Methods Committee. His group is responsible for the selection of outstanding farm publications currently being abstracted in the Journal.

The 35th Conference was well attended and an interesting and informative program was presented,



Missouri Association officers for 1967-68 (left to right). E. P. Gadd, Howard Hutchings, C. W. Dromgold, Charles Neighbors and J. I. Kennedy.

covering milk control problems, restaurant and food processing sanitation and general and environmental sanitation topics. Formal presentations on selected subjects were supplemented by panels and discussion periods dealing with specific problem areas.

At the annual business meeting new officers for the 1967-68 period were selected as follows: Charles Neighbors, Kansas City, President; C. W. Dromgold, St. Louis, 1st Vice-president; Howard Hutchings, Columbia, 2nd Vice-president; Erwin P. Gadd, Jefferson City, Secretary-Treasurer. James I. Kennedy, Jefferson City, is Past-President.

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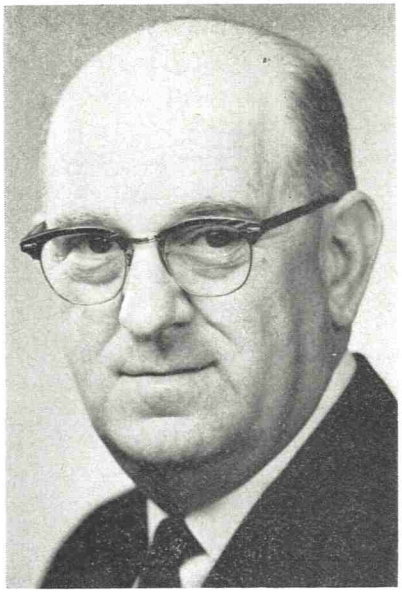
## INTERESTING HIGHLIGHTS OF THE NORTHEAST FOOD INDUSTRY CONFERENCE

One hundred industry and government representatives from the Northeast took a look at developments of the future and some of the problems of the present during the annual Food Industry Conference sponsored by Rhode Island University's College of Agriculture at Kingston on April 26, 1967. Sidney Shepard, executive director of the Rhode Island Quality Milk Association, was honored during the day with a certificate of achievement for his services to the dairy industry. The association is a milk testing organization.

The consumer pendulum is swinging away from supermarkets and back toward the old-style "Ma" and "Pa" neighborhood store, a retailing executive told the annual Conference. Clifford Merrill, president of National Convenience Stores Inc. of New York, described how thousands of dairy convenience stores selling a variety of products are opening around the country. These stores range in size from only 1,000 to 3,000 square feet and they serve the public 12 to 16 hours a day, seven days a week.

Among the problems discussed by various speakers was *salmonellae* in food and what could be done about it. Nevis E. Cook, director of the Boston district of the U. S. Food and Drug Administration, said housewives cannot solve the problem but he asked them to take care in the preparation of food. In preparing meats, particularly poultry, the food should not be taken from the oven and set back on the cutting board that was used in preparing the raw meat, Mr. Cook said, so that the prepared food will not become re-contaminated with any salmonella bacteria that might be present.



Sidney Shepard, Rhode Island Quality Milk Association.

A Harvard nutritionist, Dr. Robert B. McGandy, said there is an increasing pressure for an alteration in the nature of many dairy products and the development of new ones. Dr. McGandy conceded there is plenty of room for controversy about diet and heart disease and said milk and milk products are among the easiest foods to change when it comes to changing diets. It is regrettable that there are not more dairy products that could serve as substitutes.

Dr. McGandy asked why the dairy industry is not at the forefront of the development and marketing of dairy products lower in fat and with a favorable balance of saturated to unsaturated fats. He suggested the possible development of milk, cream and ice cream with vegetable oil substituted for part of the fat.

Allen R. Buller, general manager of Worthington Foods Inc., which specializes in producing meatless meats, described how edible protein products are produced by a spinning process similar to that used

in spinning textiles. He said a slice of simulated meat can be fabricated from plant fibers as readily as a synthetic fabric.

Soybean protein, he said, is spun into 15,000 fibers to form a strip of material to which flavors, binders and colors are added to produce a product that resembles beef, pork, chicken or turkey in looks, flavor and texture. He said these products meet the needs of vegetarians as well as those whose religious beliefs restricts or prohibits the use of meat. They also aid in controlling the amount and type of fat in a diet, he added.

Mr. Buller said that more pounds of simulated meat can be produced on an acre of ground than on the hoof. At present eight ounces of simulated ham slices cost about 69 cents, but the price will eventually be lower as production costs are lowered. It will be possible in the future, Mr. Buller added to produce simulated berries, fruits, nuts, cocoanuts and spaghetti.

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## ROBERTS EVERETT RECEIVES 3-A TOP AWARD

A former executive vice-president of the Dairy and Food Industries Supply Association has received the top honor of the 3-A Sanitary Standards Committees. Roberts Everett was presented with a 3-A bronze plaque April 6 during the spring meeting of the Committees in Miami Beach, Florida.

DFISA Technical Committee Chairman, Gordon A. Houran of The DeLaval Separator Company, made the presentation following introductory remarks by DFISA's present Executive Vice-President Joseph S. Cunningham. The plaque's inscription reads: "Presented to Roberts Everett in appreciation of his services to the 3-A Sanitary Standards Program."

Everett, who now serves as supervisory consultant and secretary for DFISA, is credited with major support of the 3-A activity in its formative period. During that time, he made available to the infant organization many DFISA (then DISA) facilities. His strength and leadership in the movement are widely recognized," states Mr. Cunningham.

Awarded to participants who have made outstanding contributions to the 3-A activity, this plaque is the fourth to be presented since the honor was initiated in 1964. Earlier honorees include the following persons: Dr. Elliott H. Parfitt, formerly with Evaporated Milk Association, who served as Chairman of the Sanitary Standards Subcommittee of the Dairy Industry Committee; T. A. Burress, formerly with The Heil Co., and long-time co-chairman of the DFISA Technical Committee; and C. A. Abele, for 20 years chairman of the Committee on Sanitary Procedures-International Association of Milk, Food and Environmental Sanitarians.

Roberts Everett, former executive vice president of the Dairy and Food Industries Supply Association, as he receives the top award of the 3-A Sanitary Standards Committees. Making the presentation is Gordon A. Houran (l.), Chairman of DFISA's Technical Committee. Joseph S. Cunningham, present Executive Vice President of DFISA and Dean R. Stambaugh (r.), 3-A Sanitary Standards Committees Chairman, look on.

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## SIX NEW AMENDMENTS ADOPTED AT 3-A COMMITTEES MEETING

Six new amendments have been adopted as final by the 3-A Sanitary Standards Committees. This action which reflects the organization's philosophy of technological up-dating of existing standards and practices, involves four Sanitary Standards and two Accepted Practices. The body of user, fabricator, and regulatory segments of the dairy processing industry took final action during its April 6-8 meeting in Miami Beach, Fla.

Amendments to the following 3-A Sanitary Standards were accepted as final:

| STANDARD | SERIAL NO. | SUBJECT OF AMENDMENT |
|---|---|---|
| Silo Tanks | 2200 | Clarification of venting provisions |
| Evaporators and Vacuum Pans | 1600 | Authorization for rolled tubes |
| Multiple-Use Plastics | 2000 | Addition of new plastics |
| Sanitary Fittings | 0800 | Supplement for diaphragm volves |

Also adopted as final were amendments to the following 3-A Accepted Practices:

| PRACTICE | DATE | SUBJECT OF AMENDMENT |
|---|---|---|
| Permanently Installed Pipelines | March, 1966 | New cast surface standard |
| Air Under Pressure | April, 1964 | Inclusion of high pressure air |

This series of amendments will be prepared for signature and publication later this year in the Journal of Milk and Food Technology. Although effective dates for the several amendments may vary, none will exceed one year.

In addition to finalizing the six amendments, the 3-A group reviewed tentative standards for dry milk fillers, and proposed revisions or amendments to the 3-A HTST Practices, Storage Tanks Standards, and Sanitary Fittings. These were referred back to the respective task committees for further changes.

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## ADDITIONAL LIST OF COMMITTEES 1966-1967

### COMMITTEE ON FOOD PROTECTION

*Objectives*

To provide international leadership in the prevention and control of foodborne diseases through:

1. Identification and evaluation of microbial, chemical, radiological and physical hazards associated with the processing, transportation, storage, handling and service of foods and animals feeds;

2. Encourage the conduct of research to provide data needed to develop effective, practical control measures;

3. Promote improved reporting of foodborne disease outbreaks;

4. Encourage development of improved methodology for detection of foodborne pathogens and hazardous chemicals in market foods;

5. Encourage the development of model laws and regulations for the control of food hazards, and promote their uniform adoption and application by State and local regulatory agencies.

6. Promote the development of regional and/or national certification programs designed to assure the safety of foods moving in interjurisdictional shipments;

7. Study existing and new processing and serving practices and techniques to assure the incorporation of new and improved food protection measures;

8. Lend support to agencies and groups concerned with the training of industry and regulatory agency personnel;

9. Assist any agency or group engaged in the eradication of foodborne hazards from market foods; i.e. Salmonellae in eggs, dry milk, cake mixes, etc.;

10. Provide technical and consultative assistance to any segment of the food industry and to regulatory agencies in matters of food protection.

David Kronick, Chairman, Chief, Milk and Food Section, Division of Environmental Health, Philadelphia Department of Public Health, Philadelphia, Pennsylvania 19146.

William V. Hickey, Vice Chairman, Public Health Committee, Paper Cup and Container Institute, New York, New York 10017.

W. A. Fountain, Chief Food Technologist, General Engineering-Sanitation Service, Georgia Department of Public Health, Atlanta, Georgia 30334.

A. E. Abrahamson, Deputy Assistant Commissioner, Environmental Health Services, New York City Department of Health, New York, New York 10013.

Dr. James C. White, Department of Food Science, Cornell University, Ithaca, New York 14850.

Dr. K. G. Weckel, Department of Food Science and Industries, University of Wisconsin, Madison, Wisconsin 53706.

Elmer D. McGlasson, Milk and Food Branch EEFP, Public Health Service, Washington, D. C. 20201.

Robert Back, Chief Food Technology Division, District of Columbia Department of Health, Washington, D. C.

### COMMITTEE ON SANITARY PROCEDURE

Dick B. Whitehead, *Chairman*, 210 Casa Linda Plaza, Dallas, Texas 75218.

C. A. Abele, 2617 Hartzell Street, Evanston, Illinois.

D. C. Cleveland, Dairy and Food Division, Room 505, Municipal Building, Oklahoma City, Oklahoma.

Kenneth Carl, Chief, Dairy Consumer Service Division, Oregon Department of Agriculture, Salem, Oregon.

Dudly J. Conner, State Milk Inspector, Division of Environmental Health, 275 E. Main Street, Frankfort, Kentucky.

P. J. Dolan, Bureau of Dairy Service, State Building, Room 3051, 2550 Mariposa Street, Fresno, California 93712.

Harold Irvin, Omaha-Douglas Health Department, 1202 S. 42nd Street, Omaha, Nebraska.

W. K. Jordan, Associate Professor, Department Dairy and Food Service, Stocking Hall, Cornell University, Ithaca, N. Y.

Joseph J. Karsh, Alleghaney City Health Department, Pittsburg, Pennsylvania.

C. K. Luchterhand, 240 City-County Building, Madison, Wisconsin.

James A. Meany, 8949 S. Laflin Street, Chicago, Illinois.

Sam O. Noles, State Board of Health, P. O. Box 210, Jacksonville, Florida.

O. M. Osten, Assistant Director, Food Inspection Division, Minnesota Department of Agriculture, State Office Building, St. Paul, Minnesota 55101.

Richard M. Parry, Chief, Dairy Division, State Department of Agriculture, State Office Building, Hartford, Connecticut.

H. L. Thomasson, P. O. Box 437, Shelbyville, Indiana.

F. E. Fenton, Chief, Standarization Branch, Dairy Division, Agricultural Marketing Service, U. S. Dept. of Agriculture, Federal Center Building, Hyattsville, Maryland 20781.

# NEWS AND EVENTS

## OHIO HOLDS ANNUAL MID-WEST WORKSHOP IN SANITARY SCIENCE

Approximately 150 individuals representing five states attended the Annual Mid-west Workshop in Sanitary Science at The Ohio State University, March 20-24, 1967. The Workshop program offered in service training for persons having principal responsibilities in milk procurement, handling, processing, or quality control; regulatory work dealing with food service operations and establishments; or regulatory work pertaining to the control and the labeling of hazardous substances.

Many educators, industrialists and public health officials participated in the five-day event. Dr. W. J. Bashe, Univ. of Cincinnati, opened the Workshop with a discussion of the "Epidemiological Aspects of Milk and Food Sanitation" and emphasized the public health reasons for milk and food sanitation programs, the epidemiology and the etiology of some food borne diseases. Other participants included Dr. H. E. Randolph, Univ. of Kentucky; Dr. A. R. Brazis, USPHS; D. A. Seiberling, Klenzade Products; C. Lay, Ohio Dept. of Health; D. J. Hartley, National Automatic Merchandising Assn.; J. W. Steckel, Torco Test Control Co.; Dr. E. H. Marth, Univ. of Wisconsin; S. M. Hart, FDA; Dr. J. H. Russell, Ohio Dept. of Health; W. R. McLean and R. L. Sanders, USPHS; C. M. Crosby, The De Laval Separator Co.; H. Wainess, Consultant in Public Health; T. C. Klapperich, USPHS; and P. A. Freebairn, Pennsalt Corp.

Milk and food personnel participated in concurrent sessions. For milk, there were discussions on "Dairy Farm Practices" dealing with herd health, milking and milk handling equipment, farm inspection and abnormal milk; "Milk Pasteurization—Equipment and Methods" featured pasteurization in review, instrumentation, pumps and equipment and post-pasteurization contamination; and "Automated Cleaning Systems" relating to unit components and the automated scheme. Concerning food, there were actual workshop demonstrations in the areas of "Food Service Operations and Vending Machines" dealing with food operation plan review, food vending sanitation, food equipment evaluation and approval, layout design for food handling areas, environmental conditions for food operations and establishments and water heating for commercial kitchens. "Special Phases of Food Sanitation" were concerned with accident prevention, bakery and poultry products and plant sanitation. The "Hazardous Substances Section" dealt with the labeling act, regulations, inspection and analysis of hazardous substances.


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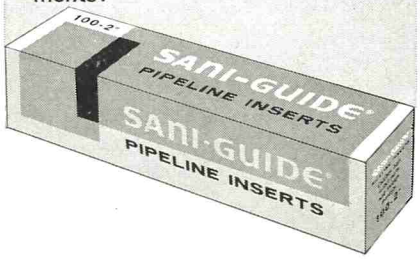


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## FDA POLICY ON SALMONELLA CONTAMINATED FEED INGREDIENTS

In the Federal Register in March, 1967, the FDA published a statement asserting that it will consider to be adulterated and therefore subject to seizure any animal feed ingredients contaminated with *salmonellae.* Listed were such items as bone meal, blood meal, feather meal, crab and fish meal, fish solubles, meat scraps, tankage or similar by-products.

FDA said that investigations had revealed that animal by-products used for animal feed may be contaminated with *salmonellae.* Contamination occurs through inadequate heat treatments of products or improper storage and handling subsequent to processing.

The announcement is issued because of the public health significance of such microorganisms in meat, milk, eggs and other edible products from food-producing animals. FDA states that the announcement creates no new authority with respect to application of the Food, Drug and Cosmetic Act to salmonella contamination in animal feeds.

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## NEW USPHS BOOKLET ON MILK

"What You Should Know About Grade A Milk" is the title of a new booklet issued by the Public Health Service. The booklet is Public Health Service Publication No. 1472 and is different from Public Health Service's "Grade A Pasteurized Milk and Milk Products, Your Best Buy" which was released about a year ago.

The booklet is designed to answer three important questions about milk which should be of concern to all persons. They are:

1. What is the nutrient value of milk and how much should we include in the dairy diet for maximum health?
2. What is being done to safeguard milk to prevent the dissemination of disease through milk and milk products?
3. What is being done about cholesterol, chemical residues and radiological fallout?

Separate sections are devoted to a discussion of each of the questions. With respect to its nutritional value and its importance in the daily diet, the pamphlet emphasizes the excellent quality of the protein in milk, the completeness of its assortment of mineral elements and the proportions in which they occur, the high content of calcium necessary for growth, the presence of indispensable vitamins and the calcium phosphorous ratio important in the building of strong bones and teeth.

The booklet points out that Grade A pasteurized milk must come from healthy animals and be produced, pasteurized, and handled under conditions of strict sanitary control by State and local milk sanitation officials. Effective milk sanitation controls in every State today protect consumers against such diseases. Continued vigilance through adherence to these controls is, however, essential.

As to the third question concerning cholesterol, chemical residues and radioactive fall-out the booklet reviews what is presently known about cholesterol and the position of the Public Health Service on various recommended dietary changes. The booklet discusses the increased uses of pesticides and antibiotics and possible effects and the public concern over radioactive materials in foods. It points out that cooperative plans by industry, and local, State, and Federal authorities have been worked out so as to safeguard and protect the milk consuming public from these harmful substances.

Today, consumers may be assured that pasteurized grade A milk and milk products that they purchase have been properly safeguarded to prevent illness when produced, processed and packaged in accordance with the Grade "A" Pasteurized Milk Ordinance —1965 Recommendations of the United States Public Health Service.

Copies of the booklet are available at 15 cents each from the Superintendant of Documents, U. S. Government Printing Office, Washington, D. C. 20402.

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## AGRICULTURAL ECONOMIST PREDICTS 200,000 DAIRY HERDS IN LATE 1970s

University of Wisconsin Agricultural Economist Truman Graf told the American Dairy Association 1967 annual meeting: "There were slightly more than 500,000 dairy herds in the United States in 1966. This total will be cut by more than one-helf in the next decade. There will be only about 200,000 U. S. dairy herds in the late seventies. Most of these will be in the 60-100 cow category. However, because of greater production efficiencies, these herds will be producing substantially more milk than is being produced now."

In his discussion of dairying trends in the next decade, Dr. Graf made several points. It is true that the number of farms selling milk and cream dropped 56% in the 1954-64 decade. It is true too that the number of milk cows on farms dropped 19% since 1960. It is also true that the shift from farm separated cream to whole milk sales, and the shift from farm use of milk, has been largely completed. The reduction in milk used on the farm resulted in 13 billion pounds of additional milk marketings since 1950.

Nevertheless, the above factors will be more than offset by others which will encourage substantial future increases in milk production. Production per

cow has increased 21% since 1960, and promises to continue to increase considerably above the 1966 level of 8,513 pounds. Also the rapid exodus from dairying in recent years has resulted in fewer, but larger, dairy herds. For example the number of cows per farm increased 40% in the 1959-64 period. Increased herd size results in increased production per cow because of higher grain and concentrate feeding rates, and improved breeding and feeding practices.

Furthermore, the number of commercial dairy farms with gross sales of over $10,000 annually, more than doubled in the 1949-59 decade and has continued upward in the 1960's. It is these commercial dairy farms who will be the big producers of milk in the future. As far back as 1959 these commercial dairy farms accounted for over one-half the total marketings of milk but represented only about 15% of the farms selling milk and cream. What happens on these larger commercial dairy farms, will to a large extent determine what happens to future milk production in the U. S. These commercial dairy farms will be controlling more and more of the total dairy production in coming years, and upward pressure on milk production will increase substantially as control shifts to the larger commercial sized dairy operations.


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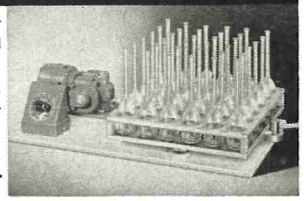

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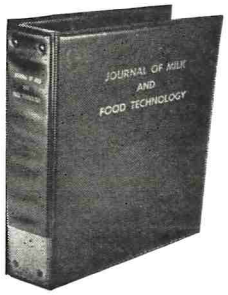


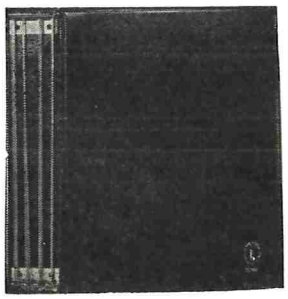

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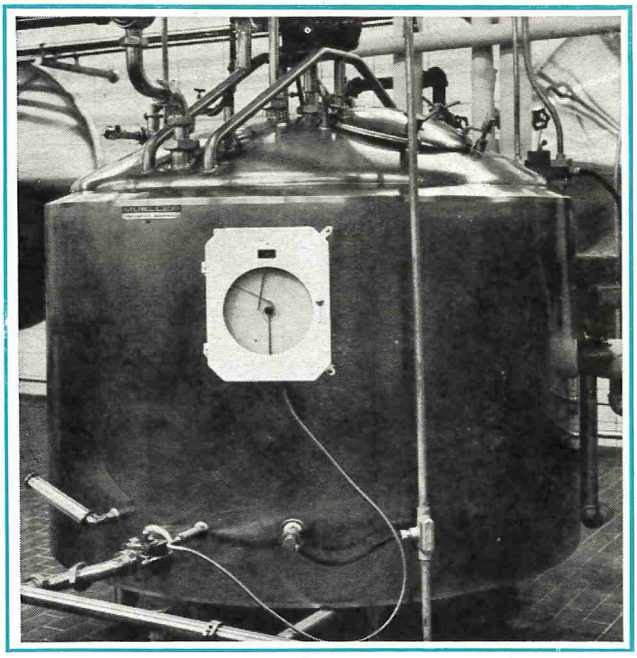

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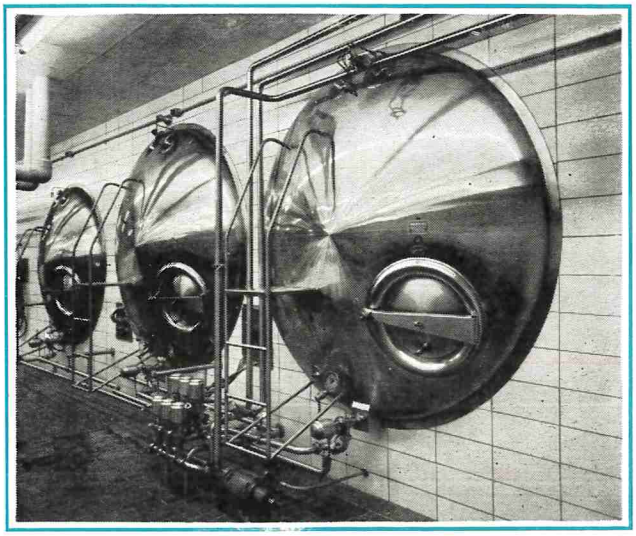
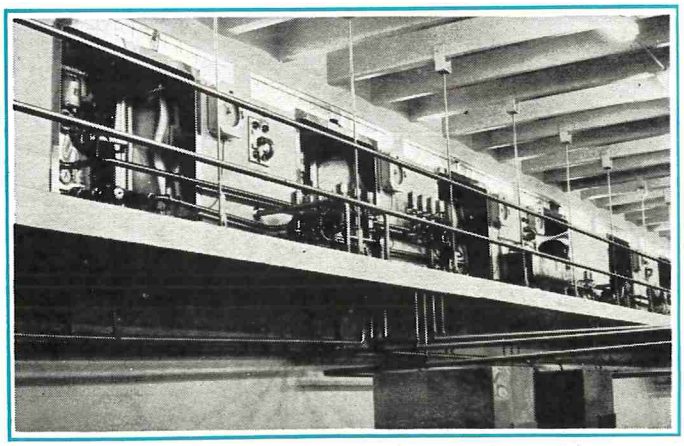


Five Walker Stainless Storage Tanks equipped with Klenz-Sprays are installed at Economy Stores.



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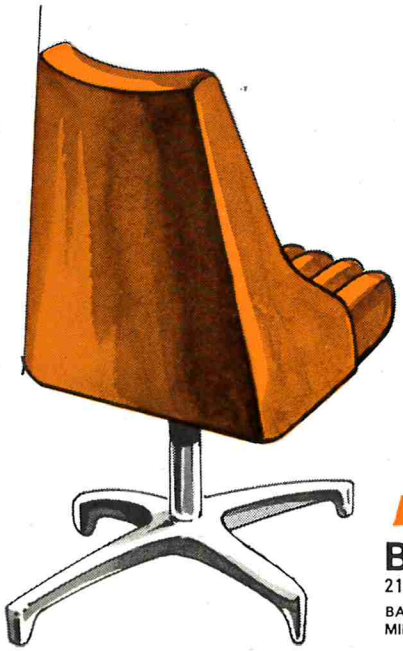

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