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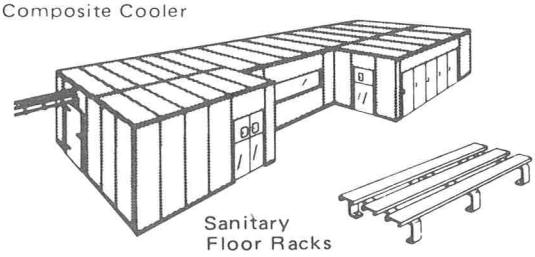

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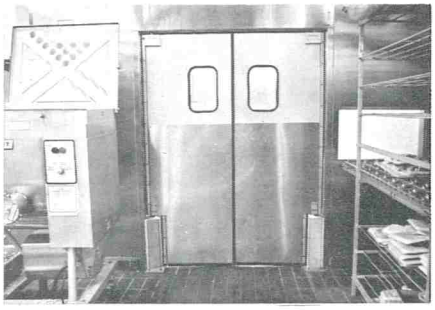

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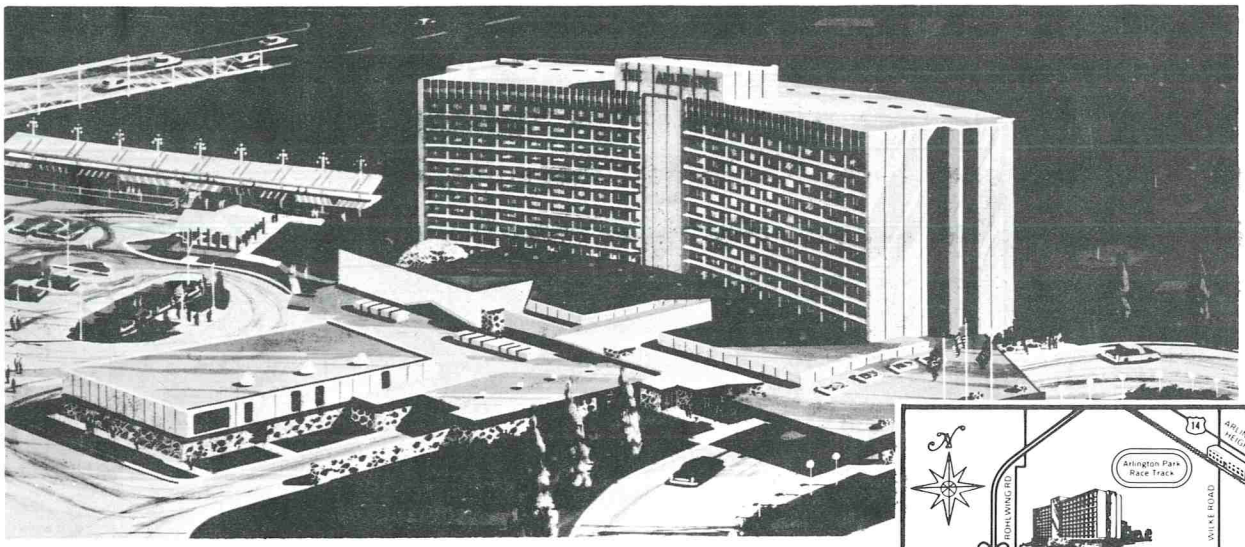

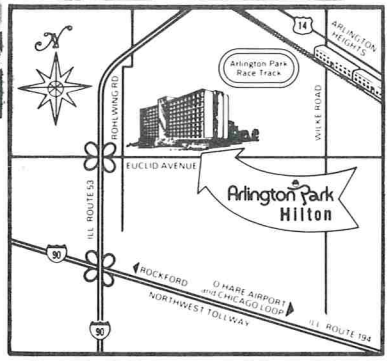

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Tyrosine and Histidine Decarboxylase Activities of *Pediococcus cerevisiae* and *Lactobacillus* Species and the Production of Tyramine in Fermented Sausages

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(Received for publication September 26, 1975)

ABSTRACT

In investigating formation of tyramine and histamine in a model system, it was found that four commercial sausage starter cultures did not exhibit appreciable tyrosine or histidine decarboxylase activity. In addition, other species of *Pediococcus cerevisiae* and *Lactobacillus* did not display appreciable decarboxylase activity. Mixtures of *P. cerevisiae* and *Lactobacillus plantarum* were also unable to produce significant levels of these amines. One species of *Streptococcus* tested was able to produce 34.5 μg of tyramine/hour under the assay conditions. When *P. cerevisiae* and *L. plantarum* were used as starter cultures to prepare sausages, it was found that this treatment resulted in lower tyramine levels (approximately 200 μg/g) than when the *Streptococcus* sp. was used as a starter culture (approximately 300 μg/g). However, the use of *P. cerevisiae* and *L. plantarum* did not result in a significantly lower tyramine level than when no starter culture was used.

Presence of tyramine in fermented sausages has recently been reported (*2, 5, 13*). Tyramine is the major cause of hypertensive crises in patients treated with monoamine oxidase inhibitors (*1*). Symptoms of hypertensive crisis include high blood pressure, headache, fever, and sometimes perspiration and vomiting (*1, 8*). A number of deaths have been reported due to hypertensive attacks (*1*). Presence of tyramine in foods may also give rise to migraine headaches in susceptible individuals (*9*).

Although foods normally contain small amounts of biogenic amines, formation of large amounts has been reported only in aged or fermented products. The factors that govern the formation of amines includes: (*a*) the availability of free amino acids, (*b*) presence of microorganisms that can decarboxylate the amino acids, and (*c*) favorable conditions for the growth of the microorganisms and for the production of decarboxylase enzymes. It is well known that, during the ripening of cheese, liberation of amino acids occur. Dierick et al. (*5*) reported an increase in free amino acids during the ripening of dry sausages, however, little change in the concentration of free tyrosine was observed. Cantoni et al (*2*) showed that free amino acids increased from 0.622% to 1.949% in the aging of a Compagnolo salami and a complete disappearance of free tyrosine was also observed. Both of these workers reported an increase in the presence of tyramine during sausage ripening. These reports indicate that although free tyrosine is produced, it is decarboxylated to form tyramine. Microorganisms commonly found in sausage fermentations include *Pediococcus, Lactobacillus, Streptococcus,* and *Micrococcus* (*3*). Bacteria belonging to the group D streptococci are well known producers of tyrosine decarboxylase. The purpose of this research was to investigate the role of the fermenting bacteria in the production of tyramine, emphasizing bacteria normally used in sausage starter cultures. Histidine decarboxylase was assayed in these bacteria because of reports implicating *Pediococcus cerevisiae* as the source of histamine in sauerkraut (*11*) and reports of high levels of histamine in fermented sausages (*5*).

MATERIALS AND METHODS

Cultures

A list of the microorganisms used in the tyrosine and histidine decarboxylase assays and their sources is shown in Table 1. In addition to testing pure cultures, mixtures of *P. cerevisiae* and *Lactobacillus plantarum* from both Chr. Hansen's Lab., Inc. (Milwaukee, Wisconsin) and Merck & Co., Inc. (Rahway, New Jersey) were assayed, since these starter cultures may be mixed in sausage production. All cultures were carried on Bacto Micro Assay Culture Agar (Difco Laboratories, Inc., Detroit, Michigan), buffered with 1% $CaCO_3$. The *Streptococcus* sp. (probably *Streptococcus faecium*) identified by the authors, was included in this study for comparative purposes. This *Streptococcus* sp. is a good producer of tyrosine decarboxylase and was isolated as a contaminating bacterium in a preliminary decarboxylase study.

TABLE 1. *Cultures examined for decarboxylase activity*

Microorganism	Source
Pediococcus cerevisiae[a]	Merck & Co., Inc.
P. cerevisiae[a]	Chr. Hansen's Lab., Inc.
P. cerevisiae	ATCC 10791
P. cerevisiae	ATCC 8042
Lactobacillus plantarum[a]	Merck & Co., Inc.
L. plantarum[a]	Chr. Hansen's Lab., Inc.
L. plantarum	ATCC 8014
L. acidophilus	Food Science Dept., Univ. of Georgia
L. casei	Food Science Dept., Univ. of Georgia
Streptococcus sp.	Authors' isolate

[a]Used as commercial starter cultures.

Assay of tyrosine and histidine decarboxylase

Bacteria for decarboxylase assay, grown in decarboxylase assay media for 24 h at 30 C, were prepared for inoculation by washing the

centrifuged cells once with saline (0.85%) solution and resuspending in saline solution. Decarboxylase assay media was prepared by adding 10 g tryptone, 10 g yeast extract, 10 g glucose, 0.1 g tyrosine, and 0.1 g histidine to 1 liter of distilled water. One milliliter (0.5 ml for each culture for mixed cultues) of the saline cell suspension was inoculated into 100 ml of the above medium in a 250-ml Erlenmeyer flask. Cultures were incubated at 30 C and assays were done on duplicate flasks. At time intervals of 0 (immediately after inoculation), 12, 24, and 48 h, 10.0 ml of medium was withdrawn with a sterile pipet. In addition, at each time interval, 1.0 ml was withdrawn to determine viable cell counts and 5.0 ml were withdrawn and frozen for a later determination of tyramine or histamine. Plate counts were done by using a medium containing 1% tryptone, 1% yeast extract, 1% glucose, and 2% agar.

The pH of each 10-ml aliquot was measured using a pH meter equipped with a single probe electrode. Each aliquot was centrifuged for 20 min, the cells washed with 5 ml of saline solution, centrifuged again, and resuspended in 1.0 ml of saline solution. An aliquot of 0.5 ml was used for each assay of tyrosine and histidine decarboxylase.

Tyrosine and histidine decarboxylase were assayed by a modified procedure of Levine and Watts (*10*). The enzyme source (0.5 ml of cells in saline solution) was added to the reaction vessel containing 2.9 ml acetate buffer (1.0 *M*, pH 5.5) containing 3.7×10^{-5} *M* pyridoxal phosphate. The reaction was initiated by introduction of 0.1 ml of the substrate, [^{14}C] carboxyl-L-histidine or [^{14}C] carboxyl-L-tyrosine (Calatomic, Inc., Los Angeles, Ca.), 5×10^{-3} *M* and approximately 0.1 μCi. The substrate was kept frozen until ready for use. All reactions were carried out at room temperature in a Dubnoff Metabolic Shaker (Precision Scientific Co., Chicago, Ill.). The reaction vessel was stoppered and incubated for 1 h. At the end of the incubation period, 2.0 ml of 1.2 *N* perchloric acid was injected through the side of the vial and the resulting hole covered with tape. The solution was allowed to incubate for additional 30 min to allow liberation of the CO_2 and absorption onto a rolled filter paper (1 × 3 cm, 3 MM Whatman) containing phenylethylamine (Packard Instrument Co., Downers Grove, Ill). The filter paper rolls were attached to a No. 2 rubber stopper via a wire clip and were dipped into the phenylethylamine just before initiation of the reaction.

After completion of the incubation period, the wire clips containing the filter papers were added to polyethylene scintillation vials containing 10.0 ml of toluene Omnifluor (0.4%, New England Nuclear, Boston, Mass.). The ^{14}C was monitored for 5 min in a Beckman Model LS-100 C liquid scintillation counter (Fullerton, Ca.). Controls consisted of adding 0.5 ml of saline solution to the reaction mixture in place of the cell suspension. The counting efficiency was determined using standard [^{14}C] benzene (Radiation Safety Officer, Safety Services Dept., University of Georgia) with 4,150 disintergrations/min (dpm). The resulting activities were expressed as nmoles of CO_2 evolved/h.

Histamine analysis of decarboxylase assay media

To verify histidine decarboxylase activity by bacterial cultures, the decarboxylase assay media were assayed for histamine by a modification of the method of Shore (*14*). One milliliter of the medium was mixed with 4.0 ml of 0.4 *N* $HClO_4$. A 2.0-ml aliquote of the mixture was transferred to a centrifuge tube containing 5.0 ml of n-butanol:chloroform (3:2, vol/vol), 0.25 ml 5 *N* NaOH, and 0.75 g NaCl. The tube was shaken for 5 min and centrifuged to remove any free histidine. The butanol:chloroform layer was transferred to a second tube containing 2.5 ml of NaCl-saturated 0.1 N NaOH, shaken for 1 min, and centrifuged. A 4-ml aliquot of the washed butanol extract was transferred to a third tube containing 2.5 ml of 0.1 *N* HCl and 7.5 ml of n-heptane. The tube was shaken for 1 min, centrifuged, and the organic phase was removed by aspiration. To 2.0 ml of this acid phase containing histamine was added 0.4 ml of 1 *N* NaOH, followed by 0.1 ml of o-phthalaldehyde (OPT) (10 mg/ml in methanol). After 4 min at room temperature, 0.2 ml of 3 *N* HCl was added. The contents of the reaction tubes were mixed using a vortex mixer following each addition.

The fluorescence at 450 nm, resulting from activation at 360 nm, was measured on a Turner Model 430 Spectrofluormeter (G. K. Turner Associates, Palo Alto, Ca.). Histamine concentration was determined by comparing the fluorescence of the samples to the fluorescence of a standard containing 1 μg/ml histamine.

Tyramine analysis of decarboxylase assay media

Tyramine was quantitated in the decarboxylase assay media in a similar manner to that described for tyramine extraction and quantitation in sausages (*13*). Five milliliters of the media were adjusted to pH 10.0 with solid Na_2CO_3. The liquid was saturated with NaCl (about 1.5 g), 3.75 ml of n-butanol were added, and the mixture was shaken for 10 min using a wrist action shaker. After centrifugation the tyramine in the butanol layer was determined as previously reported (*13*).

Preparation of sausages

To further test the ability of starter cultures to produce tyramine, sausages were made under identical conditions using the following variation with respect to the addition of cultures to sausage mixtures: (*a*) no culture added, (*b*) *L. plantarum* (Merck), (*c*) *P. cerevisiae* (Merck), (*d*) *Streptococcus* sp., and (*e*) *L. plantarum* (Merck plus *Streptococcus* sp. For *L. plantarum* and *P. cerevisiae,* the 6 oz cans of frozen concentrated cultures were thawed by placing in 21-24 C water. Three millimeters of the culture were then added to 6.0 ml of sterile saline. This suspension was added to the sausage preparation at the appropriate time. For studies involving the inoculation of the *Streptococcus* sp., the culture was grown for 24 h at 30 C in Bacto Micro Inoculum Broth (Difco). Cells were harvested by centrifugation, washed with saline solution, and resuspended in approximately 3.5 ml of saline solution. Three milliliters of the saline suspension of *Streptococcus* sp. were used for inoculation into the sausage preparation. For the mixed culture, i.e. *L. plantarum* plus *Streptococcus* sp., only 0.1 ml of the saline-washed *Streptococcus* suspension was used.

Sausages were prepared in a manner similar to that described by DeKetelaere et al. (*4*). Composition of the sausage mixture is given in Table 2. The frozen meats were first cut with a band saw into approximately 2-4 cm cubes. The remaining ingredients were added to the meats and mixed by hand. After the meat had softened, the mixture was passed through a 3/16-inch plate. After remixing by hand, the mixture was divided into five equal lots. At this point the starter cultures, as described above, were added. Sausage mixtures were stuffed into 688-mm diameter smoke permeable fibrous casings (Tee-Pak, Inc., Danville, Ill.) using an E-Z Pak Automatic Sausage Stuffer (E.F. Zuber Engineering & Sales, Co., Minneapolis, Minn.). Sausages were placed in a 22 C incubator (approximately 65% relative humidity) and the temperature was lowered at 18 C over a period of 5 days. On the sixth day sausages were smoked for 4 h with a heavy smoke at 43 C and 80% relative humidity. Sausages were then placed in a drying chamber at 16 C and 70% relative humidity for 30 days.

TABLE 2. *Composition of sausage mixture*

Ingredients	Amount
Beef trimmings	6.8 kg
Pork trimmings	4.1 kg
Pork hearts	2.15 kg
Lean pork	1.25 kg
Salt (NaCl)	425 g
Glucose	105 g
Cervelat seasoning[a]	91 g
$NaNO_2$	1.05 g

[a]A. C. Legg Packing Co., Birmingham, Alabama

Tyramine content of the sausages was determined as described previously (*13*). Duplicate samples were extracted and the butanol extracts were spotted twice and compared to standard amounts of tyramine spotted on the same plate.

RESULTS AND DISCUSSION

No tyrosine decarboxylase activity for the various *P. cerevisiae* test stains was detected throughout the 48-h culturing period. Viable cell counts showed that the

bacteria had achieved the stationary phase within this time. This is important, since Gale (*7*) reported that bacteria primarily produce amino acid decarboxylases towards the end of the growth cycle. All the microorganisms had produced low pH values (4.41-4.95) within the assay period. Again, this is important since the production of amino acid decarboxylases is highest at low pH (*7*). No tyramine was detected in the media at any time. It appears from these data that *P. cerevisiae* strains are not able to decarboxylate tyrosine when grown under these conditions.

TABLE 3. *Activity of histidine decarboxylase in cultures (nmoles CO^2 evolved/h)*

Culture	Age of culture (hours)			
	0	12	24	48
P. cerevisiae (Merck)	0.637	0.181	ND[a]	ND
P. cerevisiae (Hansen)	ND	0.397	ND	ND
P. cerevisiae (ATCC 10791)	ND	ND	ND	ND
P. cerevisiae (ATCC 8042)	ND	0.139	ND	ND
Streptococcus sp.	ND	0.105	ND	ND
P. cerevisiae (Merck) and *L. plantarum* (Merck)	ND	ND	ND	0.175
P. cerevisiae (Hansen) and *L. plantarum* (Hansen)	ND	ND	ND	0.030

[a]ND-Not detected.

Table 3 shows the histidine decarboxylase activities of the *Pediococcus* strains. Low, variable activity was observed. The activities detected under these conditions are too low to give rise to any appreciable quantities of histamine. The highest activities (0.637 nmoles CO_2/evolved/hr) could give rise to only 3.5×10^{-3} μg histamine/10^6 cells/h. Assay of the media for histamine showed that less than 3μg/ml of histamine was formed. It is apparent that under these conditions strains of *P. cerevisiae* are unable to produce substantial amounts of histamine.

As with *P. cerevisiae,* no tyrosine decarboxylase activity was detectable for any of the *Lactobacillus* spp. tested during the 48-h incubation period. It is apparent from the viable cell counts that all the bacteria had achieved stationary phase after 48 h of incubation. The final pH of the *Lactobacillus* media was, in all instances, quite low (pH 4.46-4.58). In addition, negligible levels of tyramine were found in the assay media. It appears that these *Lactobacillus* spp. are unable to produce tyramine under these conditions.

No histidine decarboxylase activities were detectable for any of the lactobacilli during the 48-h culturing period. When the media was assayed for histamine, less than 3 μg/ml of histamine was detected. It is apparent that, under the assay conditions, the *Lactobacillus* spp. cannot produce histidine decarboxylase.

Table 4 shows the results of the tyrosine decarboxylase assays for the *Streptococcus* sp. This microorganism showed high decarboxylase activity at all assay times. From the viable cell counts, it can be seen that the bacteria had reached stationary phase in 12 h. Unlike the other organisms tested, the pH of the media was 4.80 after only 12 h. The ultimate pH obtained (4.36) was also

TABLE 4. *Activity of tyrosine decarboxylase in cultures (nmoles CO_2 evolved/h)*

Culture	Age of culture (hours)			
	0	12	24	48
Streptococcus sp.	2.458	255.1	254.8	177.2
P. cerevisiae (Merck) and *L. plantarum* (Merck)	ND[a]	ND	ND	1.527
P. cerevisiae (Hansen) and *L. plantarum* (Hansen)	ND	ND	ND	0.905

[a]ND = Not detected.

lower than that obtained by the other microorganisms. Tyramine levels of up to 300 μg/ml were detected in the decarboxylase assay media. A decarboxylase activity of 250 nmoles CO_2 evolved/h is equivalent to 34.5 μg of tyramine/h. Given ample substrate and sufficient numbers of these organisms (10^8 cells/g), tyramine levels of 300-900 μg/g could be formed within 9-26 h at the above rate of amine formation. Pederson (*12*) has reported the presence of a *Streptococcus faecalis*-like bacteria that reached a level exceeding 10^8 cells/g after 16 h at 41 C in a Chorizo-type sausage. It is possible that the *Streptococcus* sp. was a strong tyramine producer and that *S. faecalis*-like bacteria are common contaminates in sausages. The tyrosine content of sausage is reported to be 4.54 mg/g (*6*). Assuming a 10% release of tyrosine during proteolysis and 100% conversion to tyramine, it is possible that a tyramine level of 344 μg/g may arise. Tyrosine levels of sausages probably vary widely due to differences in moisture and fat content. Sausages with low fat contents may be able to attain much higher tyramine levels.

Table 3 also shows the histidine decarboxylase activities for the *Streptococcus* sp. A very slight decarboxylase activity was detected at 12 h. The fact that no activities were detected at any other times would tend to decrease the significance of the histidine decarboxylase activity detected at 12 h. It seems that the *Streptococcus* sp. would not be capable of producing appreciable quantities of histamine.

The tyrosine decarboxylase activities for the mixed cultures (*P. cerevisiae* and *L. plantarum*) (Table 4) indicate the absence of this enzyme except at 48 h. Both mixed cultures showed very slight activity at this time. Assay of culture media for tyramine showed no appreciable build-up of this amine. The viable cell counts of the mixed cultures indicated that the microorganisms had attained stationary phase between 12-24 h. The pH of the culture media had dropped to 4.51-4.67 at the end of the 48-h time period. It seems that, although slight tyrosine decarboxylase activity was detected, no appreciable levels of tyramine could accumulate under these conditions.

Table 3 indicates the histidine decarboxylase activities of the mixed cultures. Again, no activity was detected except at 48 h. Both mixed cultures produced slight histidine decarboxylase activity at this time. Analysis of the assay media for histamine showed the presence of less than 3μg/ml of histamine. It seems unlikely that, under these conditions, the mixed culture of *P. cerevisiae* and

L. plantarum could produce appreciable levels of histamine.

TABLE 5. *Tyramine contents of experimental sausages*

Treatment	Tyramine (g/g)
Control	211[a]
Lactobacillus plantarum	212[a]
Pediococcus cerevisiae	160[a]
Streptococcus sp.	298[b]
L. plantarum and *Streptococcus* sp.	285[b]

Values with the same superscript are not significantly different from each other, while values with different superscripts are significantly different ($p < 0.05$).

The tyramine contents of experimental sausages is presented in Table 5. These values were calculated assuming a recovery of 73% (*13*). All of the sausages contained tyramine, while none of the samples contained exceedingly large amounts of the amine (i.e greater than 400 μg/g). Both sausages prepared with the *Streptococcus* sp. were significantly higher in tyramine than the other sausages. It appears from these data, that the *Streptococcus* sp. was able to produce greater amounts of tyramine than in the control and other treatments, regardless of whether it was initially present in high or low populations. It is possible that the *Streptococcus* sp. was more competitive than *L. plantarum* for the available nutrients. Even in the presence of a good tyrosine decarboxylase microorganism (*Streptococcus* sp.), relatively low levels of tyramine were found. It is possible that the production of tyramine was partially inhibited by some environmental factors or that the amount of substrate was limiting. Some ingredients, e.g. spices, may have inhibited the decarboxylase activity. It is also possible that the level of tyramine may have been decreased by catabolic pathways.

The other sausages produced with only *P. cerevisiae* or *L. plantarum* did not show a significantly different amount of tyramine than that found in the control. The source of tyramine in these sausages and in the control is probably the decarboxylase activity of contaminating bacteria. It appears that use of starter cultures did not serve to control their growth and the production of tyramine. It is also possible that tyramine may be produced synergistically with the bacteria present in the natural flora.

The main factors that govern production of tyramine in sausages are the presence of tyrosine decarboxylase-producing microorganisms, such as the Group D *Streptococcus* and the availability of free tyrosine as a substrate. The low acid conditions that occur in sausage fermentations are favorable for the decarboxylation of amino acids. The presence of decarboxylating bacteria may be controlled, at least in part, by proper methods of sanitation and hygiene in production plants. Use of short fermentation times and cooking of the product to kill decarboxylating bacteria may also help to control tyramine levels in sausages.

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Effect of Water Activity and Temperature on Aflatoxin Production by *Aspergillus parasiticus*

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ABSTRACT

The effect of water activity (a_w) on growth and aflatoxin production by *Aspergillus parasiticus* NRRL 2999 was determined using submerged cultures in which the a_w was adjusted by addition of glycerine, glucose, or a mixture of salts. At a sub-optimal a_w aflatoxin production was low in the glycerol and glucose media while no strong inhibition of mycelial growth occurred. A similar effect was obtained in surface cultures on agar media in which the a_w was adjusted by addition of glycerine or sucrose. The effect of a sub-optimal temperature was the reverse; compared to inhibition of mycelial growth in surface cultures, the effect on aflatoxin production was slight. No detectable quantities of aflatoxin B_1 were formed at 0.83 a_w and at 10 C nor at four combinations of higher a_w and temperature. The a_w was measured by a recently developed device using the dewpoint principle.

Fungi of the *Aspergillus flavus* group including *Aspergillus parasiticus* can produce aflatoxins under certain conditions. The most important factors determining growth and aflatoxin production by these molds are moisture and temperature. The effect of these two factors on growth of several *A. flavus* strains was studied by Ayerst (*1*), and the effect of temperature alone on growth and toxin production of two strains was investigated by Schindler et al (*5*). Little research has been done on the combined effects of these two factors on both growth and toxin production. Such information is essential as minimal, optimal, and maximal temperature permitting growth and toxin production are dependent on the moisture condition prevailing in the substrate.

The availability of water to microorganisms can be measured by the water activity (a_w), i.e. the ratio of the water vapor pressure of the substrate to the vapor pressure of pure water at the same temperature and under the same pressure (*6*). At a low a_w water is bound by salts, sugars, proteins, and other solutes. Thus growth of microorganisms cannot take place as water is not present in an available form.

The effect of a_w on growth and toxin production was studied by other workers with substrates that had been equilibrated with the water vapor above saturated salt solutions. In this investigation the a_w was adjusted by adding various amounts of glycerine, glucose, or salts to the growth medium. The a_w of the media was then measured by a dewpoint measuring device.

In the first part of the investigation the influence of a_w was examined in submerged cultures. In the second part the effect of both a_w and temperature was evaluated in surface cultures.

MATERIALS AND METHODS

Organism and spore suspension

A. parasiticus (strain NRRL 2999) was maintained at 4 C on malt extract agar. Subcultures grown for 1-2 weeks on malt extract agar were washed with 0.6% Tergitol-7 and the spore suspension obtained had about 10^6 spores/ml.

Submerged cultures

To study the effect of a_w on mycelial growth and aflatoxin production, peptone (1%)-glucose (2%) medium at different a_w values was inoculated with mycelial pellets. These were obtained by inoculating 100 ml of peptone (1%)-glucose (2%) medium in 300-ml Erlenmeyer flasks with 0.5 ml of spore suspension and then shaking the cultures at 120 rpm for 3 days at 24 C. The mycelial pellets were filtered off on sterile cheese cloth and washed first with 400 ml of distilled water and then with 300 ml of medium with the desired a_w. The pellets were transferred to graduated cylinders and the appropriate medium was added to make a total volume of 100 ml. Inoculated media were then poured into 300-ml Erlenmeyer flasks and shaken at 120 rpm for different periods at 24 C. After incubation, cultures were stored at −18 C until dry weight or aflatoxin content was determined. The dry weight of mycelium was determined to serve as a measure of growth. Mycelial pellets were rinsed with 600 ml of distilled water, dried in a ventilated oven for 24 h at 70 C and then weighed. Different a_w values were obtained by different amounts of glycerine (PGY-series), glucose (PG-series), or a mixture of NaCl, KCl and Na_2SO_4 at a molarity ratio of 5:3:2 (PGS-series). The a_w of the medium was measured after inoculation. After incubation the a_w and pH of the filtrate were determined.

Surface cultures

Various a_w values in malt extract agar (Oxoid) were achieved by adding sucrose (MES-series) or glycerine (MEG-series). Four agar plates were poured for each a_w-temperature combination and then dried for 3 days at 37 C before inoculation and the determination of a_w. Two of the four plates were inoculated with a spore suspension in three areas with an inoculation needle. The two inoculated plates, and one non-inoculated plate which served as a control on the a_w after incubation, were enclosed in a 0.3-liter polyethylene bag (gauge 0.04 mm). The fourth plate was used for measurement of the a_w at the time of inoculation. Incubation temperatures were 10, 13, 16, 24 and 32 ± 0.3 C. The growth rate of the mycelium was determined by daily measurement of two right-angled diameters of the colony which was enlarged seven times on a screen by an overhead projector. Plates were placed at − 18 C on the day that the average diameter of the three

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colonies reached 3 cm or on the 45th day of incubation when they did not reach this size.

Extraction and analysis of aflatoxin

In all submerged cultures 40 ml of the filtrate was extracted with 40 ml of chloroform. In a separate series the mycelium was also extracted with 40 ml of chloroform. Extraction was then repeated twice with 25 ml of chloroform. Surface cultures were extracted by shaking the contents of the two plates in 40 ml of chloroform and by subsequent homogenization of the agar in 40 ml of chloroform with an Ultra Turrax. After centrifugation the extracts were combined. A preliminary visual determination of the aflatoxin B_1 concentration was carried out by thin-layer chromatography (TLC). The sample solution was evaporated or diluted depending on its concentration. The sample extract and 2.5 ng of B_1 standard were spotted on a silicagel G-HR chromatoplate (Machery and Nagel) and developed in chloroform: acetone (9:1 vol/vol). The intensities of fluorescence of the separated B_1 spots from the sample and the standard were measured densitometrically. The recorded intensity values for aflatoxin B_2 and G_1 were divided by 2.2 or 0.55, respectively, since the fluorescence ratio of aflatoxin B_1:B_2:G_1 is 1:2.2:0.55 under the conditions used. Aflatoxin M_1 was measured after evaporation of the extract resulting in a final concentration of about 0.1 μg M_1/ml. The fluorescence of M_1 from a sample was compared with the fluorescence of the M_1-standard after the two dimensional TLC development which employed diethyl ether: Methanol:water (94:4.5:1.5 vol/vol/vol, saturated tank) in the first direction and chloroform:aceton:methanol (90:10:2 vol/vol/vol, unsaturated tank) in the second direction.

a_w-measurement

A scheme for the a_w measurement device is presented in Fig. 1.

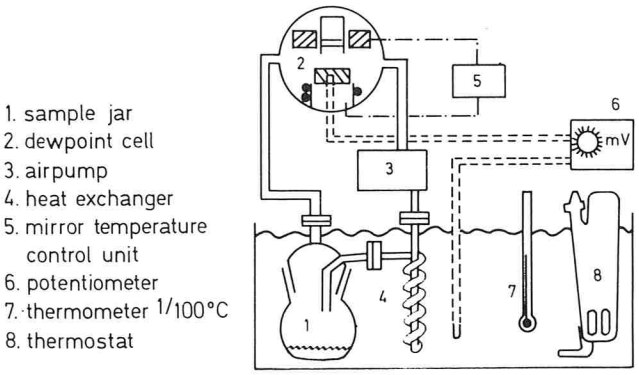

Figure 1. *Scheme of the wateractivity measuring device.*

About 20 ml of medium or culture filtrate is transferred to the 500-ml sample jar. This jar is connected via glass, polyethylene, and stainless steel tubing to a dewpoint cell (Aqmel type HCP 1 s, 50 C), a metal bellows air pump (Metal Bellows Corp.) with a flow rate of 80 liters/h, and to a glass heat exchanger. The jar and the heat exchanger are attached to a movable frame which provides room for six jars which are placed in a waterbath controlled by a thermostat. Each jar can be connected by glass valves to the dewpoint cell. The tubing and the dewpoint cell are heated to a temperature higher than that of the waterbath to prevent condensation. During equilibration of the sample with the circulating air dew droplets are formed on the gold mirror in the dewpoint cell. The mirror is constantly cooled by a Peltier element. Light reflected by the mirror is received by a photoelectric cell that electronically controls the heating of the mirror. In this way the mirror attains a temperature at which an extremely thin layer of dew droplets is maintained. This dewpoint temperature which is dependent on the relative humidity in the sample jar and the temperature of the waterbath is measured by copperconstantan thermocouples in the mirror and in the waterbath. These are connected with a potentiometer. The relative humidity is determined when the dewpoint temperature is constant, as is indicated by a mV recorder. The device is calibrated with distilled water and has a standard deviation of 0.003 a_w. All measurements were done at 25 C.

RESULTS

Submerged cultures

The a_w of the medium during incubation. The a_w of the medium increased in some instances after adding the washed mycelial pellets but it subsequently decreased slightly during incubation. On the fifth day it was at the most 0.005 a_w higher than the a_w of noninoculated medium, on the 24th day it was at the most 0.007 a_w lower. Hence it was considered sufficiently accurate to report the averaged and rounded off a_w-values on the 5th day of incubation in all experiments.

Short term cultures. The mean-value and standard error of triplicate cultures which were incubated for five days are shown in Fig. 2-4. The mycelial growth is the

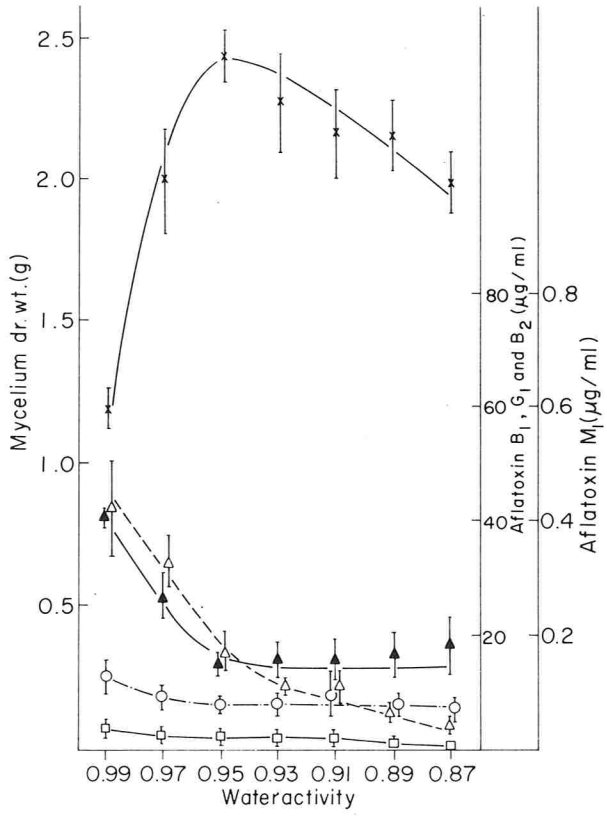

Figure 2. *Growth and aflatoxin production in peptone-glucose-glycerine medium at various water activity values after 5 days of incubation. Average initial weight of dried mycelium was 0.58 g. (Mycelium ×, aflatoxin B_1 △, G_1 ○, B_2 □, M_1 ▲)*

difference between the dry weight at the end and at the start of the experiment, the latter being the average dry weight of five batches of mycelial pellets. Growth was dependent on both the a_w and the medium. In the growth stimulating PGY-medium, the maximal production mycelium occured in the range of 0.96-0.91 a_w. An a_w of 0.87 still permitted a remarkable increase in weight. In the PGS-medium the maximum growth was

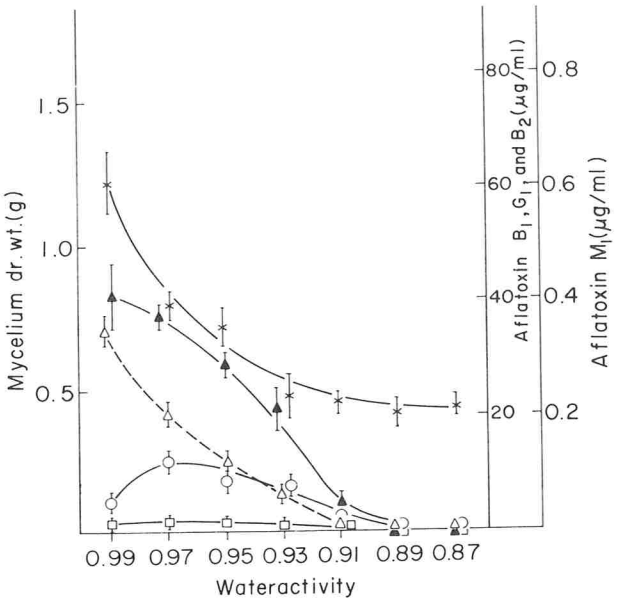

Figure 3. *Growth and aflatoxin production in peptone-glucose-salts medium at various water activity values after 5 days of incubation. Average initial weight of dried mycelium was 0.51 g. (Symbols see Fig. 2.)*

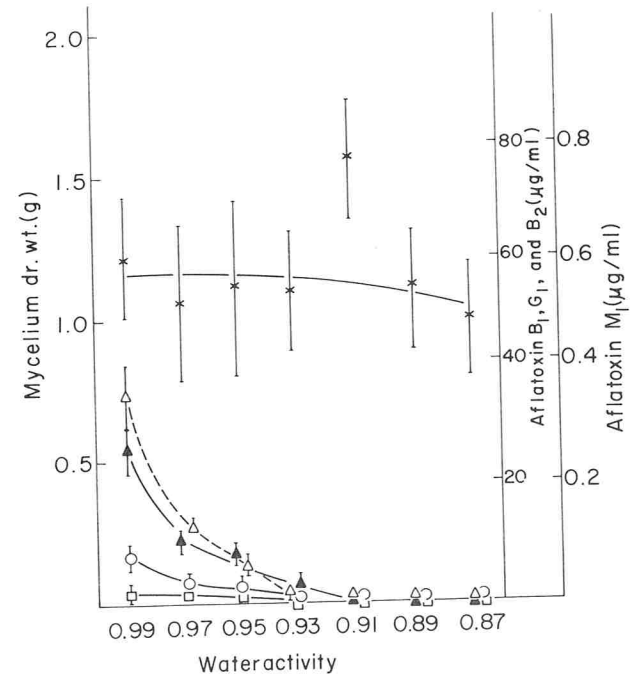

Figure 4. *Growth and aflatoxin production in peptone-glucose-medium at various water activity values after 5 days of incubation. Average initial weight of dried mycelium was 0.38 g. (Symbols see Fig. 2.)*

reached at 0.99 a_w while no growth was observed at 0.91 a_w and below. In the PG-medium growth occurred at all measured a_w-values but it varied widely.

Aflatoxin production was strongly inhibited at low a_w-values in all media. In the PGY-medium the production at 0.87 a_w was 4 µg aflatoxin B_1 per ml (7% of the maximal production). In the PGS-medium only 0.1 µg aflatoxin B_1 per ml was detected at 0.87 a_w. In the PG-medium the production at 0.87 a_w was 0.2 µg of aflatoxin B_1 per ml. The aflatoxin M_1 production followed the same pattern as aflatoxin B_1 at various a_w. However, in PGY the M_1 production was constant at a_w-values below 0.95 a_w. The same was valid for the aflatoxin B_2 and G_1,. Although the production of B_2 and G_1 was low at high a_w, the decrease in production at low a_w was considerably less than for B_1 and M_1. The washed mycelial pellets used for inoculation contained only 5 µg of aflatoxin B_1. If released into the medium this quantity would have resulted in a concentration of only 0.05 µg aflatoxin B_1 per ml of culture filtrate.

Although no growth was measured in the PGS medium at low a_w, the pH declined from 7.4 to 4.0 during incubation. This indicates that the acid producing metabolism was not affected by a low a_w.

The percentages of aflatoxins B_1 and G_1 remaining in the mycelium after separation from the culture filtrate are shown in Table 1. Depending on the medium used,

TABLE 1. *Percentage*[a] *of aflatoxin B_1 and G_1 present in the mycelium after 5 days of incubation in peptone glucose media with glycerine (PGY), salts (PGS), and glucose (PG) at various water activities*

	PGY		PGS		PG	
a_w	B_1	G_1	B_1	G_1	B_1	G_1
0.99	52	27	52	40	35	25
0.97	39	29	26	15	23	13
0.95	42	27	25	11	40	25
0.93	34	19	28	10	30	18
0.91	30	15	44	26	9	6
0.89	26	15	23	10	15	12
0.87	18	11	23	6	8	1

[a]Percentage of the total amount of toxin detected in the culture filtrates and the mycelium.

35-52% aflatoxin B_1 was retained by the mycelium at a high a_w. At a low a_w the percentage was lower. This can be explained by a higher diffusion rate of the toxin into the medium. The same pattern emerged for aflatoxin G_1. This indicates that the greater amount of toxin detected in the culture filtrate at a high a_w does indeed reflect a greater amount of toxin produced, and not merely a greater release of toxin from the mycelium into the medium.

Longterm cultures. In the PGS-medium the mycelial dry weight at low a_w increased slightly over 29 days (Fig. 5). Despite the low growth rate production of relatively large amounts of aflatoxin B_1 occurred at 0.93 a_w. In the PG-medium as like in the 5-day cultures, the mycelial dry weight varied widely. In both media aflatoxin B_1 production started within the first few days. While the aflatoxin B_1 concentration in PG medium decreased after 12 days, that in PGS medium kept increasing.

Surface cultures

The regression lines of colony diameter on days after inoculation were calculated for each a_w-temperature combination. The regression coefficient for all lines was at least 0.98. At low a_w or low temperature the data curves showed two stages of growth (Fig. 6). The line

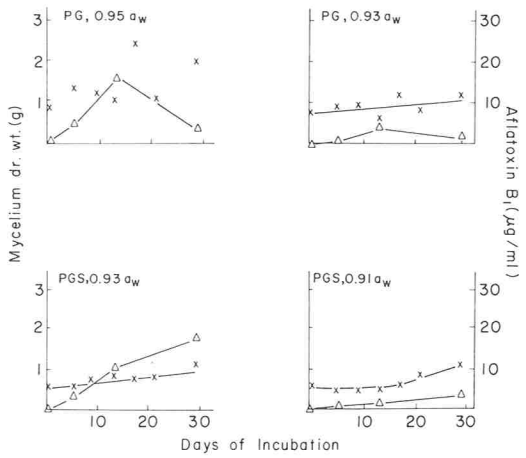

Figure 5. *Growth and aflatoxin B_1 production in peptone-glucose and peptone-glucose-salts media at various water activity values (Mycelium ×, aflatoxin B_1 △).*

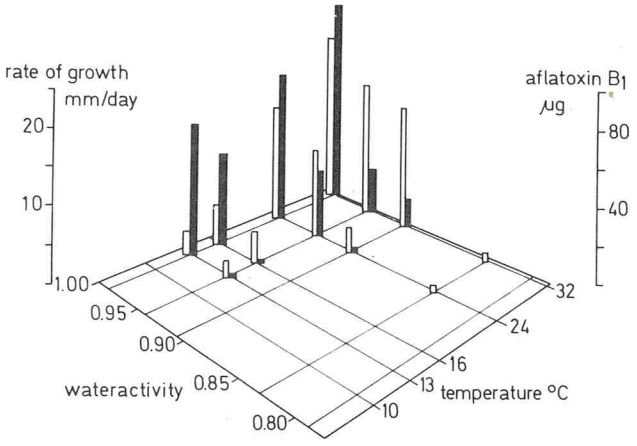

Figure 7. *Growth and aflatoxin B_1 production on malt extract-sucrose agar at various water activity values and temperatures (rate of growth-white column, average aflatoxin B_1 production of 6 colonies-black column).*

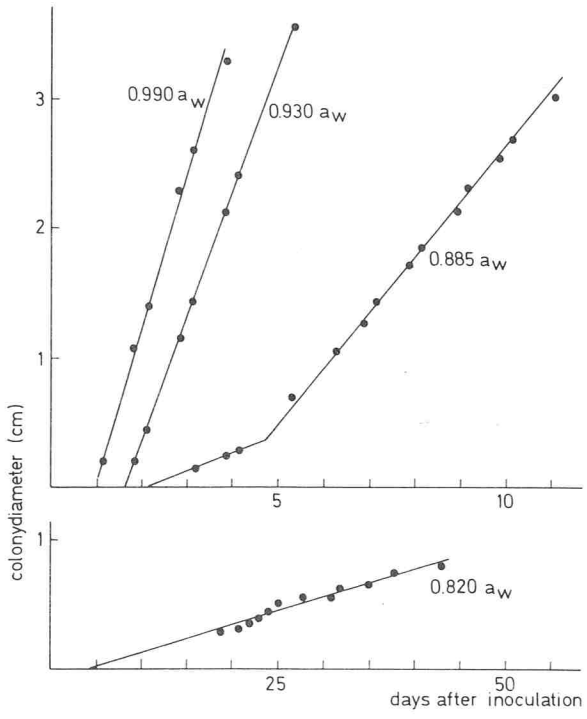

Figure 6. *Increase of colony diameter on malt extract-glycerine agar at various water activity values at 24 C.*

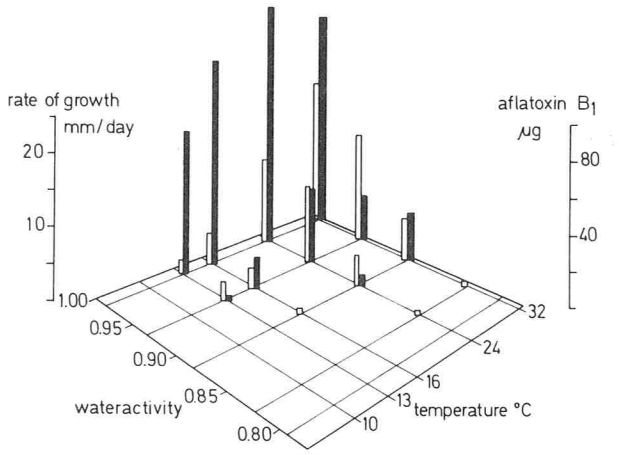

Figure 8. *Growth and aflatoxin B_1 production on malt extract-glycerine agar at various water activity values and temperatures (rate of growth-white column, average aflatoxin B_1 production of 6 colonies-black column).*

through the first three points was extrapolated to the x-axis to determine the germination time and the line through the remaining points was used to determine the growth rate. The average growth rate of colonies at each combination of temperature and initial a_W is shown in Fig. 7 and 8. On agar media the minimal temperature for growth was between 10 and 13 C. At high a_W and suboptimal temperature, toxin production was strikingly less affected than growth. Both figures show that the optimal temperature at 0.94 a_W was 24 C. This optimum was higher at lower a_W. Maximal growth was found at the highest a_W and incubation temperature. Growth appeared to be possible without demonstratable toxin production at 0.83 a_W on MES and 0.82 a_W on MEG. The detection level was 0.02 μg per colony.

The germination time was also affected by suboptimal temperature and a_W. Under favorable conditions the growth rate exceeded 5.5 mm per day and the germination time was less than 2 days. Under unfavorable conditions it was less than 0.2 mm per day and the germination time exceeded 4 days.

The a_W of the agar media decreased only slightly during the incubation period. This was probably a result of diffusion of water vapor through the enclosing plastic bag. The decrease amounted to 0.008 a_W after 8 days and to 0.015 a_W after 40 days.

DISCUSSION

The most striking observation made in the studies with both the submerged and surface cultures was that the a_W did not have the same effect on aflatoxin production as it did on growth. With regard to growth the a_W-optimum

for *A. parasiticus* in submerged cultures was dependent on the medium. The optimum lay at a lower a_w in the media containing glycerine and glucose than in the medium containing salts. This may be related to the fact that growth is influenced not only by a_w but also by composition of the substrate. However, in the surface cultures containing glycerine or sucrose the optimum for growth was close to 0.99 a_w. This was also found for *Rhizopus* and *Aspergillus niger* (*3*), and for *A. flavus* (*1*) in studies where similar methods were used. The different a_w values found for optimal growth rate suggests that a correlation does not always exist between the growth rate measured by the surface culture method and that measured by the submerged culture method. This cannot be explained simply by a depletion of oxygen in the surface culture because of the high oxygen permeability of the polyethylene bags.

In studying aflatoxin B_1 production in peanuts, Diener and Davis (*2*) found an optimum at 0.95 a_w while no significant quantity of aflatoxin was found at 0.85 a_w and lower. Hunter (*4*) found a minimal a_w of 0.84 in corn. In contrast, using the methods described here an optimum value exceeding 0.99 a_w was found in all media. A low a_w suppressed aflatoxin production in all media to about the same degree, yet at 0.87 a_w toxin production still occurred.

Besides influencing toxin production, the a_w also influenced the release of aflatoxin from the mycelium into the medium. As relatively more aflatoxin migrated into the medium at low a_w the inhibition of the aflatoxin production at low a_w is greater than may be concluded from the concentrations measured in the medium. In contrast, Shih and Marth (*7*) found that the percentage of aflatoxin in the medium increased at increasing concentrations of sodium chloride. As they used a stationary culture in yeast extract medium containing 20% sucrose and no mixture of salts other factors may have affected the outcome.

Although there was little or no mycelial growth in the medium with salts at low a_w, a slow increase in aflatoxin B_1 concentration occurred. This indicates that the aflatoxin B_1 producing enzyme system had formed before the mycelium was transferred into the medium.

The most important factor for mycelial growth was observed to be the temperature whereas for aflatoxin production it is the a_w. Slight growth is possible at low a_w without demonstrable formation of aflatoxin. Different optimal temperatures for aflatoxin production and growth was also described by Schindler et al. (*5*). The relatively high aflatoxin production at suboptimal temperature might be explained by the longer incubation period necessary to obtain the desired colony diameter. However, this long incubation period was also used for growth at a suboptimal a_w and since under these conditions relatively small amounts of aflatoxin were produced this explanation cannot be upheld. The explanation should be sought in specific influence of a_w and temperature on the aflatoxin producing mechanism.

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The Aerobic Plate Count, Coliform and *Escherichia coli* Content of Raw Ground Beef at the Retail Level

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ABSTRACT

Nine hundred fifty-five samples of raw ground beef obtained from supermarkets throughout the United States were examined for coliforms, *Escherichia coli,* and Aerobic Plate Count (APC). The results were compared with existent standards for *E. coli* in raw meat in New York and Oregon. Lack of homogenious distribution of *E. coli* in fresh ground beef was demonstrated. Observations were made that indicate that a 2 day-two step procedure will detect the same number of *E. coli* as the more time consuming four step MPN procedure 98% of the time. A comparison of the APC obtained by incubating plates at 20 C and 35 C showed there to be an average 10-fold difference with the 20 C incubation always higher. Questions are raised about the necessity of microbial standards for raw meat and the validity of incorporating *E. coli* in such standards.

The question of microbiological standards for fresh meats is both current and controversial. Several states and counties have already established standards and others are seriously considering them. Oregon has set legal maxima of 5×10^6/g and 50/g for aerobic plate count (APC) and *Escherichia coli,* respectively. Exceeding these limits in three successive samples makes the supermarket manager liable to criminal prosecution. New York State has even more stringent limits of 1×10^6/g (APC) and 10 *E. coli*/g.

Proponents of standards for raw meat cite protection of public health and improvement in product quality as benefits to be derived from their promulgation. Opponents of standards argue that public health will not be altered by the standards and that the number of bacteria in a product that supports microbial growth cannot be used as an indication of sanitation. It hardly needs saying that the ultimate aim of all concerned is to benefit the consumer. What is at issue is how this may best be attained.

A recent publication (*1*) issued by the Center for Disease Control (CDC) has summarized available data on outbreaks of food poisoning attributable to ground beef, cold cuts, and frankfurters. This is particularly timely for it speaks directly to the issue of public health as this is influenced by products for which standards are being considered. Data covering the period 1966-73 showed that in 1827 outbreaks in which a food vehicle could be identified only nine times was an etiologic agent found in ground beef. In eight of these nine instances, mishandling and abuse occurred after purchase of the ground product. The report states that this product cannot be considered a high risk food.

Although some standards are already existent there are relatively few recent published data on the microbial content of retail level ground beef (*5, 7*). This has caused some to wonder what an extensive examination of ground beef obtained from a wide geographic area would show in relation to the standards described above.

To provide these data, a survey was undertaken. Samples were procured from 10 supermarket chains having outlets in the East, Midwest, and West Coast of this country. Samples were obtained from numerous individual outlets of each chain, frozen immediately in dry ice, and airshipped directly to three independent commercial testing laboratories. The results of that survey are presented in this paper.

MATERIALS AND METHODS

Samples

All samples were retail packs usually about 1 lb. in weight which were taken directly from the market display case. Samples were placed unopened on dry ice in styrofoam containers and shipped by air to one of the three testing laboratories. Samples were taken over a period of weeks from multiple outlets of a given chain in that city. Only samples which were received frozen were analyzed by the participating laboratories.

Laboratories

Each of the laboratories was an independent commercial testing laboratory which was respected for its ability in the food testing field. Each laboratory followed exactly the same testing protocol so that the derived data are comparable.

Sample preparation

Upon receipt by the laboratory, frozen samples were removed from the shipping carton and held in a freezer until analyzed. Before analysis, samples were thawed, by holding in refrigerator (5 C) overnight or by placing in watertight plastic bags and immersing in cold water for 1-2 h. When the meat was pliable enough to be worked, the packages were opened and representative portions were taken aseptically.

Test methods

Fifty grams of meat were placed in a Waring blender with 450 ml of sterile 0.1% peptone water and homogenized for 1-2 min. Further decimal dilutions were made in 0.1% peptone water.

The aerobic plate count was done by seeding appropriate dilutions into duplicate petri plates and mixing with plate count agar which had been tempered to 45-50 C before pouring. Plates were incubated at 20-22 C for 72 h. Duplicate plates containing 30-300 colonies were counted; results were averaged and reported as the aerobic plate count. (APC).

Coliforms (presumptive) and *E. coli* were enumerated by the three-tube Most Probable Number (MPN) procedure. Aliquots of the

TABLE 1. *Aerobic Plate Count and coliform levels in 955 samples of raw ground beef*

Aerobic plate count/g	% of samples	Coliforms/g[1]	% of samples	*E. coli*/g	% of samples
< 100,000	13	< 10	8	< 10	38
100,000- 500,000	17	11- 100	41	10- 50	26
500,000-1,000,000	10	101-1,000	30	51-100	10
1,000,000-5,000,000	26	1,001-10,000	17	101-500	16
> 5,000,000	34	> 10,000	4	> 500	10
> 50,000,000	5				

[1]Computed from gas positive LST tubes, but not further confirmed.

homogenate representing 10^{-1} through 10^{-4} dilutions of meat were seeded into triplicate tubes of lauryl tryptose broth (LST). The LST tubes were incubated at 35 C for 48 h. Gas-positive LST tubes were recorded and the pattern of positive and negative tubes yielded the MPN of coliforms in the meat. A loopful of culture from gas-positive tubes was transferred to EC broth and incubated at 45.5 ± 0.05 C for 24-48 h. A loop was used to streak from gas-positive EC broth tubes to EMB agar plates which were incubated at 35 C for 24 h. Colonies typical of or most closely resembling *E. coli* on EMB were picked and checked by the methyl red test. Methyl red-positivity was taken as confirmation of the presence of *E. coli* in the EC broth tubes. The pattern of positive and negative tubes formed the basis for the MPN of *E. coli.*

RESULTS

In total, 955 samples were examined. The APC, coliform, and *E. coli* levels in these samples are shown in Table 1. Of particular interest is the percentage (36) of samples that exceeded the level of 50 *E. coli*/g. These samples would be considered adulterated in Oregon. Even more alarming is that New York State would have considered 62% of the samples adulterated because the *E. coli* content exceeded their arbitrarily chosen limit of 10/g.

As shown in Table 1, the total (APC) counts ranged from less than 100,000/g to greater that 50,000,000/g. Most samples had APC values greater that 1,000,000/g. Despite these counts, none of the laboratories reported finding any samples with noticeable off odors. This was true even for those samples having an APC of greater than 50,000,000/g.

It is not possible to assess how these samples would fare when measured against the APC standards of New York and Oregon since these two states incubate the test plates at 35 C rather than 20-22 C as was true in this study. To get an estimate of the difference in counts obtained when APC plates were incubated at 20 C and 35 C the following experiment was done on nine samples of fresh ground beef. Fifty grams of each sample was homogenized as described in Materials and Methods and the subsequent dilutions were seeded into four plates. After the agar was poured and mixed the plates were divided into two sets. One set of duplicates was incubated at 35 C for 48 h while the other set was incubated at 20 C for 72 h. The results are depicted in Table 2. The counts obtained from plates incubated at 20 C were always higher than those from plates held at 35 C. This is hardly surprising since the predominant flora of raw meat are psychrotrophic organisms incapable of growth of 35 C (*3, 8, 11*). Counts at the two temperatures averaged about 10-fold or one $\log_{10}$ difference but ranged from one-half to one and three-fourths $\log_{10}$ in difference. This wide range would preclude attempting to predict what the APC count at 35 C would be, based on data generated by incubation of plates at 20 C. The count at 20 C more accurately reflects the true microbial content of raw ground beef and provides a better estimate of the keeping quality of the product. These results strongly underscore the importance of incubation temperature on counts obtained and this should weigh prominently in the thinking of those who promulgate microbial limits for raw meats.

TABLE 2. *Influence of plate incubation temperature on the Aerobic Plate Count (APC) of raw ground beef*

Sample No.	APC at 35 C	APC at 20 C
1	730,000	7,900,000
2	1,130,000	10,600,000
3	68,000	2,100,000
4	930,000	14,600,000
5	136,000	430,000
6	119,000	870,000
7	26,000	190,000
8	61,000	3,400,000
9	17,000	290,000

Average $\log_{10}$ difference = 0.98
Range of $\log_{10}$ differences = 0.49 – 1.74

During the course of this study a number of observations were made concerning the distribution and detection of *E. coli* in raw meat. These observations by themselves are significant enough to raise question about the inclusion of *E. coli* among the organisms for which microbiological standards are considered.

Regarding distribution, it is well known that the three-tube MPN is an imprecise assay method having extremely broad 95% confidence limits (*4, 12*). The method was developed for testing water, a menstruum in which bacteria are assumed to be distributed homogeneously. Little or no knowledge is existent regarding the distribution of bacteria in many food products. Contamination by *Salmonella* is often spoken of as spotty or 'heterogeneous' but what about *E. coli* in a comminuted product such as ground beef? To test this, two individual 1-lb. packages of retail ground beef were purchased. Eight 50-g samples were taken from each package and examined for *E. coli.* The results of the 16 analyses are shown in Table 3. In trial 1, a variation of 30-fold existed between samples from the same package. In trial 2 the extent of variation between samples cannot be determined since the sensitivity of the assay was only 3/g and four of eight analyses fell below this level. Larger variations were encountered in analyses of frozen beef patties (unpublished data). The point is made, however,

table 3. E. coli *MPN values on 50 g samples taken from 2 pound retail packages of ground beef*

Sample No.	Trial 1	Trial 2
1	23	< 3
2	23	< 3
3	3.6	3.6
4	3.6	3.6
5	23	< 3
6	43	3
7	93	< 3
8	43	9.1

that *E. coli* are not homogeneously distributed in ground beef and because of this no single sample is representative of any given batch of product. The imprecision of the assay and the lack of homogeneity in bacterial distribution is sufficient reason to reconsider including *E. coli* among those organisms for which there are fixed-number limits.

The procedure followed in enumerating *E. coli* in ground meat samples is a long and cumbersome one. Many tubes and much manipulation is involved. An answer however imprecise is arrived at some 6 days after analyses are begun. During this study, two of the participating laboratories noted a phenomenon that raises a question on the necessity of the extended incubation periods in the first two steps. Laboratory A studied 130 tubes of LST broth that were negative for gas formation after 24 h. Of these 130, 40 became positive at 48 h of incubation. When loopsful of culture were transferred from these positive tubes to EC broth, none of the 40 cultures formed gas at 45.5 C. Similarly, laboratory B found that 0 of 27 LST 24 h-negative, 48 h-positive cultures formed gas in EC broth at 45.5 C. These 67 such cultures were not the only occurrences during this study but were chosen for examination partway through the survey when it became apparent this was occurring with some frequency. This is far from sufficient evidence to openly advocate that the method be changed but it does strongly suggest that this be further investigated with a view toward shortening the procedure as it applies to raw meats. In this same vein, all of the laboratories reported that EC broth positive tubes became positive in the first 24 h and no instances of 24 h-negative, 48 h-positive were encountered. If this step were shortened to 1 day of incubation in conjunction with 1 day in LST (should this prove feasible), an overall saving of 2 days in this time consuming analysis would be effected. Of the 955 analyses, 933 or 98% showed complete agreement between EC positive broth tubes and presence of *E. coli* confirmed by the methyl red test. This would indicate that a two step-2 day procedure could give the same information about 98% of the time. It would seem that 4 additional days for 2% more accuracy is a poor investment.

Each of the laboratories independently observed a trend in which those samples with a high APC had a greater likelihood to have fewer than 50 *E. coli*/g. A typical example of this is shown in Table 4. Samples of meat having an APC of less than 10,000,000/g were almost twice as likely to exceed 50 *E. coli*/g as those with

TABLE 4. *Percentage of samples exceeding 50* E. coli*/g as this relates to APC of ground meat*

APC	% samples > 50 *E. coli*/g[1]
$< 10^6$/g	65
1×10^6-1×10^7	69
$> 10^7$/g	36

[1]Data from 188 samples from a single supermarket chain.

APC values exceeding 10,000,000/g. This finding suggests that the major meat flora is influencing either viability or detectability of *E. coli* in that meat. Whether this influence is exerted in the meat itself or in the initial stages of the test procedure and the identity of the causal agent are currently under investigation.

DISCUSSION

The issue of public health as this may be affected by ground beef has been discussed in a recent publication (*6*). Questions of sanitation and wholesomeness as may be measured by microbial content have been spoken to but remain unresolved. It is often stated that the APC and *E. coli* content of raw meat are a reflection of the sanitation of the premises in which the meat has been prepared. This would seem to be an oversimplification of a situation probably in part due to extrapolation from systems in which this proposition has merit, i.e. water and pasteurized milk. It must be remembered that both water and milk receive treatments of a bactericidal nature that reduce the APC and eliminate coliform bacteria. It is thus a reasonable interpretation that finding coliforms indicates abuse, recontamination or insanitation after treatment. This is not true for raw meat. Raw meat is raw. No treatment has been given to cause elimination of the microbial flora or any component of it. Because of this, the presence of coliforms or large numbers of bacteria in meat has a much different meaning than it would in pasteurized milk or water.

Raw meat is held at refrigeration temperatures. Bacteria can grow at these temperatures. The presence of any level of bacteria in a product which supports bacterial growth cannot be a measure of sanitary quality but rather is a measure of microbial quality (*10*). Microbial quality becomes important if there is a demonstrable cange in the organoleptic properties or wholesomeness of a product. None of the samples examined in this study including those with total plate counts in excess of 50,000,000/g evidenced organoleptic deterioration. That is to say 'spoiled' meat was not encountered. Discussions with officials in states where standards are in existence revealed that consumer complaints about purchasing spoiled meat did not occur even before promulgation of the standards.

Wholesome is defined as promoting health or well-being of mind, spirit, and body (*2*). The presence of bacteria in raw meat does not detract from the product's ability to conform to the definition of wholesome. If large numbers of microorganisms in foods automatically rendered that food unwholesome, we should have to

consider all fermented foods unwholesome. This, of cose, would be foolish.

E. coli is an organism which thrives in the intestinal tract of animals. Often forgotten is that it thrives about as well outside the intestine. The presence of *E. coli* in raw meat does not mean there are feces in the meat. For that matter the presence of *E. coli* in meat does not indicate that there are pathogens in the meat. A search of the literature has failed to reveal any published documentation of a correlation between the presence of *E. coli* and the presence of *Salmonella* in raw beef. The primary souce of *E. coli* (and other bacteria) on beef carcasses is the hide of the animal. (*8*). Efforts to significantly reduce carcass load by washing the animal before slaughter have not been overly effective (*9*). Given this circumstance and the lack of correlation between presence of *E. coli* and *Salmonella* the value of *E. coli* as an indicator of insanitation or much else is questionable.

The data generated in this survey perhaps have raised more questions than they have answered. Finding 36% of the samples exceeding one state's standard and 62% exceeding another state's is alarming. Are these standards too tight for what technology and good manufacturing practice can achieve today? What is to become of these "adulterated" products? Can the purveyor continue to chance selling ground meat when there is this high a risk of fine or incarceration? How can the market manager know the *E. coli* content of his product if the test takes 6 days to complete? Can a test lacking precision be applied to a product in which the bacteria are non-homogeneously distributed and the result compared to a fixed-number limit? Do we really need to test for *E. coli?* If the political answer is yes, must we use a 6-day test? Most certainly the product would have been consumed by the time results were obtained. Could we not better do with a 2 day (i.e. LST-24 h → EC-24 h) test that gives an estimate of a group we just define as "those organisms able to produce gas in EC broth at 45.5 C" without alluding to origin of the organisms? What are we going to do when methods become more sensitive; for example, when we are able to detect injured cells that undoubtedly arise during freezing of the sample? Are we prepared to raise or eliminate the standard in that case?

Lest we lose sight of the ultimate aim of all of us concerned, i.e. benefiting the consumer, will microbial standards on raw meat really protect him/her? From what?

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Microbiological Evaluation of Retail Ground Beef: Centralized and Traditional Preparation[1]

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ABSTRACT

An evaluation of the microbiological quality of retail ground beef prepared in a centralized operation (which has nearly statewide distribution) and in four local stores was undertaken. Forty retail samples were analyzed for total aerobes (22 C and 35 C), yeasts and molds, *Clostridium perfringens, Staphylococcus aureus,* total coliforms, *Escherichia coli,* enterococci, *Salmonella,* and fat content. Products prepared in the centralized operation exhibited trends toward better microbiological quality than that of traditionally prepared products. *Salmonella* screening of the samples resulted in the identification of *S. infantis* in one traditionally prepared sample.

Centralized processing of meats has been shown to be economically feasible (*8*). Movement of the meat industry toward centralization and the need to determine the "state of the art" in centralized operations warrant further investigation.

Geer et al. (*4*) recognized that application of a standard could not be accomplished without considering the processing and handling to which the product is subjected. Rogers and McCleskey (*7*) found that among markets categorized as small, medium, and large there was a trend toward less contamination with increased size. This general trend was attributed to large markets obtaining meat from large volume meat packers and also having a more rapid turnover of meats. Such a trend points to the potential of better microbiological quality of products prepared in a centralized processing operation. In 1973, Duitschaever et al. (*1*) found that the majority of the samples of refrigerated ground beef they studied were unable to meet a standard of 1.0×10^7 bacteria per gram nor were they able to comply with liberal standards for staphylococci and coliform counts.

Arguments for and against microbiological standards for foods have been discussed by numerous authors and researchers. In 1960, Elliot and Michener (*2*) reviewed microbiological standards and handling codes for chilled and frozen foods before that time. The emphasis on total aerobic plate counts was found to be quite prevalent. In addition, the literature appears to be devoid of references to centralized processing operations; apparently no specific data-gathering studies have been conducted to establish the microbiological quality of ground beef prepared in this manner. This study was undertaken to contrast the retail microbiological quality of ground beef prepared in a centralized operation to that prepared in the traditional manner.

[1]*Florida Agricultural Experiment Stations Journal Series No. 6039.*

MATERIALS AND METHODS

Media used for analyses were products of either Difco Laboratories or Baltimore Biological Laboratory. Methods of microbiological analysis used in this study conform to those published in the *Bacteriological Analytical Manual for Foods* (BAM) (*3*) except where modifications were deemed advantageous. Forty retail samples, eight from each of five sources, were analyzed. The sources were: a retail outlet for a centralized processing operation, two large sized supermarkets, a medium sized supermarket, and a small meat market. The samples were collected at random over a 6-month period.

After purchase, samples were transported under wet ice storage and analyzed within 60 min after arrival at the laboratory. Four sub-samples from each ground beef sample were used for analysis. One 50-g sample was homogenized in 450 g of 0.1% peptone sterile dilution water for 2 min at low speed (8000 rpm) in a Waring blendor. Serial dilutions of this homogenate were used for the following microbiological analyses: total aerobic plate counts on Plate Count agar incubated at 35 C for 48 h and at 22 C for 5 days; yeast and mold counts on Plate Count agar with added antibiotics at 22 C for 5 days (*6*); *Clostridium perfringens* counts on Sulfite Polymyxin Sulfadiazine agar (SPS) and Trypticase Sulfite Neomycin agar (TSN) incubated at both 35 C and 45 C, respectively, for 24 h using the Gas Pak® system. Sulfite-reducing colonies were inoculated into tubes of iron milk, fluid thioglycollate broth, and motility nitrate medium and incubated at 35 C for 24 h. Isolates were considered to be *C. perfringens* if they gave a stormy fermentation in iron milk, and were gram positive, nitrate-reducing, non-motile rods. *Staphylococcus aureus* was isolated using a three-tube MPN series of 10% NaCl Trypticase Soy broth at 35 C for 48 h followed by streaking loopfuls of broth from growth-positive tubes onto plates of Baird-Parker. Typical colonies were subjected to a coagulase test (*3*). Total coliforms were determined with the MPN procedure using Lauryl Sulfate Tryptose broth (35 C for 48 h) followed by confirmation (gassing) in Brilliant Green Lactose broth (35 C for 48 h); presence of *Escherichia coli* followed BAM procedures including IMViC analyses. Counts of enterococci were made on KF streptococcus agar incubated at 35 C for 48 h.

Two 25-g subsamples were used for *Salmonella* analyses. The first 25-g subsample was homogenized in 225 g of Gram Negative broth in a Waring blendor for 2 min and transferred to a sterile flask for incubation. The second 25-g subsample was treated identically using Selenite Cystine broth as the selective enrichment broth. Both of these subsamples were incubated at 35 C for 24 h. Selective plating was accomplished using Brilliant Green agar and Xylose Lysine Desoxycholate agar incubated at 35 C for 24 h. Suspect colonies were individually cultured in an "ENTEROTUBE" (Roche Diagnostics, Nutley, N.J.) followed by further biochemical testing and serological identification. A fourth subsample of 57 g was used directly in a Hobart Fat Percentage Indicator to determine percent fat (± 1%).

RESULTS AND DISCUSSION

"Traditional preparation" of ground beef is best

defined as cutting, grinding, packaging, and other preparatory operations done on the premises of a retail store. By contrast, the centralized operation may be defined as an autonomous facility in which all preparatory operations are completed before the packaged product is transported to retail outlets. In this particular instance, the centralized processing operation was located in northeast Florida and the product was delivered fresh throughout the state, with the farthest point requiring approximately 5 h of travel time.

Despite the frequent use of total counts as measures of sanitation and quality, Elliot and Michener (*2*) and Silliker (*9*) have referred to this procedure as ineffective and thus of limited value. That is, high numbers of viable aerobes do not necessarily indicate a potential hazard from pathogenic organisms nor do they reflect poor sanitary conditions. Furthermore, total counts have not always correlated well with spoilage, especially when considering that alteration of a food could come either from multiplication of organisms to large numbers or from the initial presence of large numbers of contaminants. The historical usage of total counts and the prevalence of existing and proposed standards employing total aerobic counts necessitated their inclusion in this study.

Results of analyses for total aerobes at 35 C and 22 C from all 40 samples obtained from 5 sources are presented in Fig. 1 and 2, respectively. In these and subsequent figures, the bars enclose the entire range of counts from each source. Figure 2 depicts total aerobic counts at 22 C and the same general trend that was shown in Fig. 1 is evident.

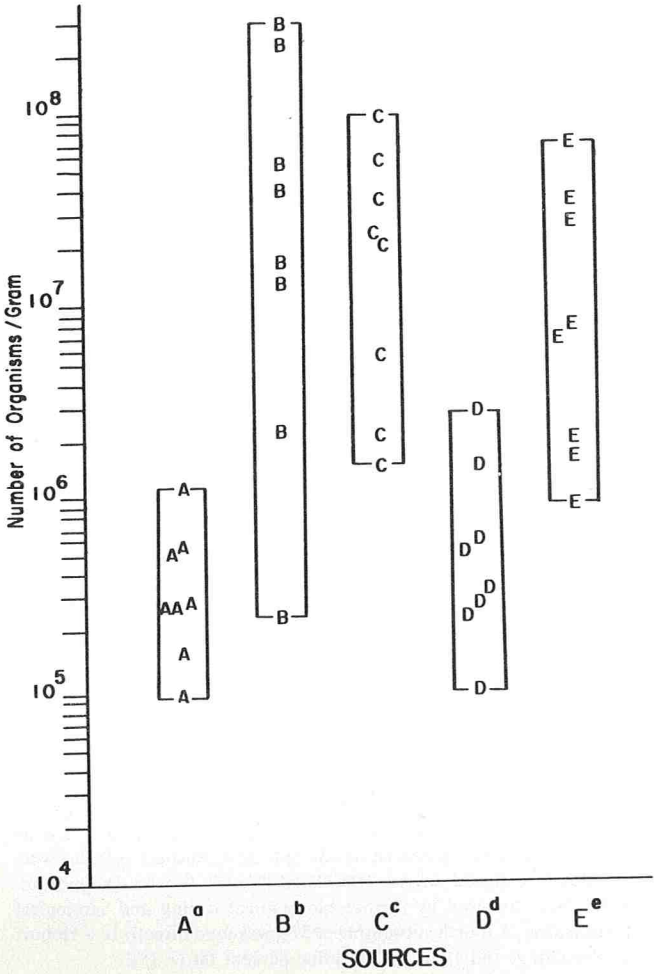

Figure 1. *Total aerobic plate counts at 35 C. A^a, centralized processing operation; B^b, large sized supermarket; C^c, small meat market; D^d, large sized supermarket; E^e, medium sized supermarket.*

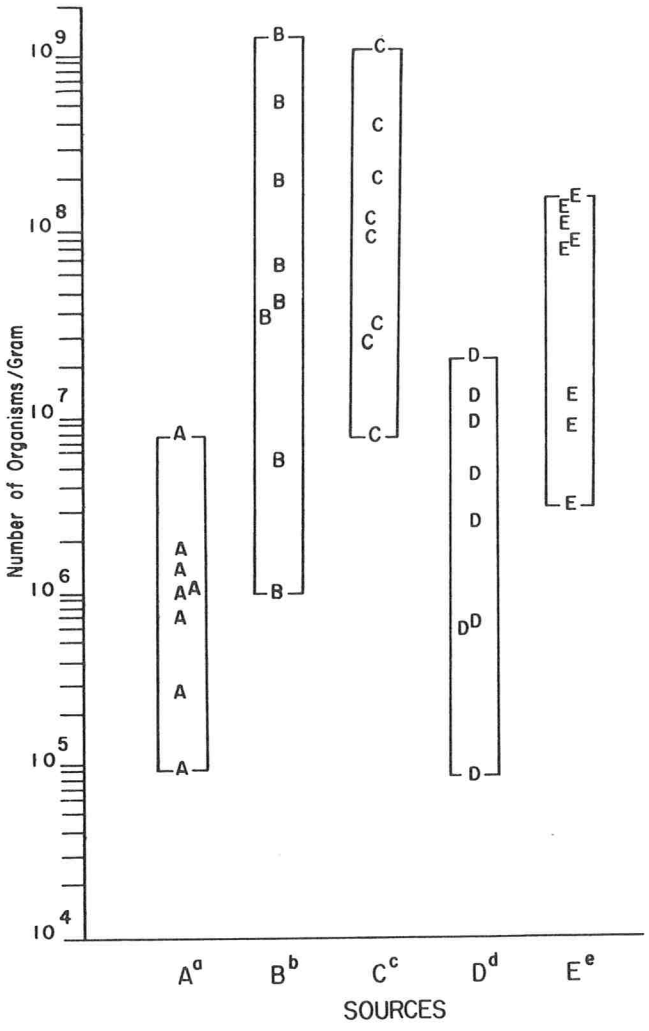

Figure 2. *Total aerobic plate counts at 22 C. See Fig. 1 for identification of code letters for sources.*

Although isolated from meats, yeasts and molds have been considered to be of little significance in spoilage of ground beef. Results of our analyses are depicted in Fig. 3. The minor role these organisms play in the spoilage of ground beef is exemplified when it is noted that 75% of all the samples had CFU/g counts which were less than 0.5% of the total aerobic plate count populations.

The results obtained from analyses for *C. perfringens* were not presented in a figure because of the generally small numbers present. Less that 50 *C. perfringens*/g were found in 30 samples. Of the remaining 10 samples, the highest count was 400/g in one traditionally prepared

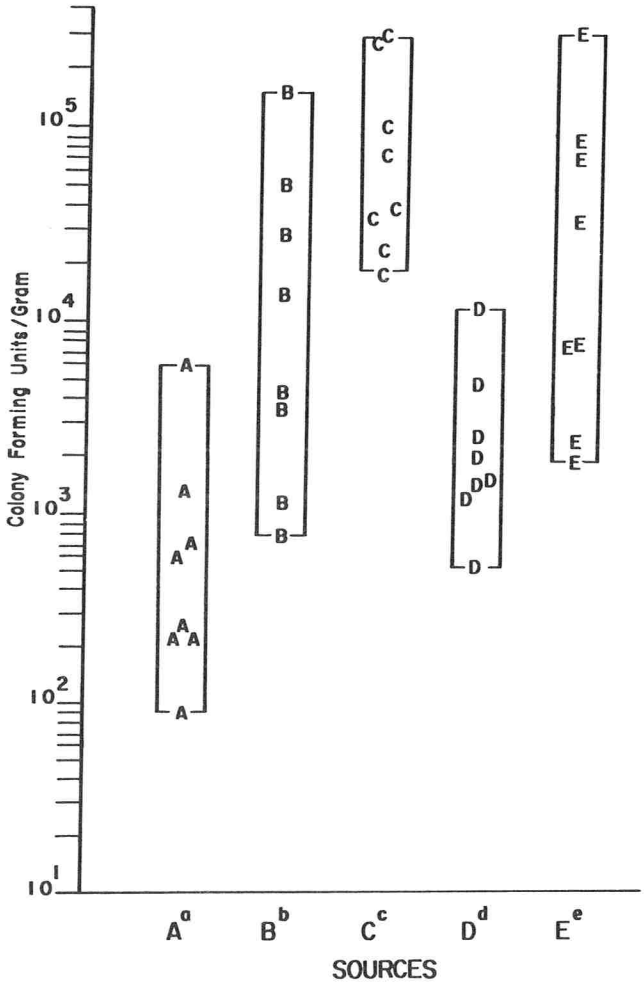

Figure 3. *Yeast and mold counts at 22 C. See Fig. 1 for identification of code letters for sources.*

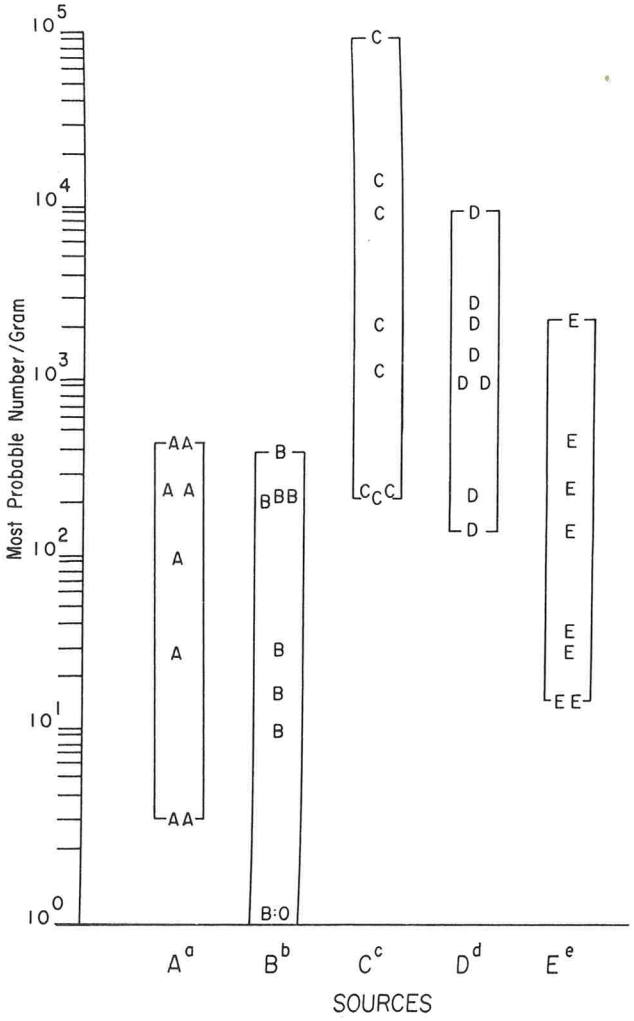

Figure 4. *Coagulase positive* Staphylococcus aureus *MPN/g. See Fig. 1 for identification of code letters for sources.*

sample. In 25 samples, the two media employed (SPS and TSN) showed similar counts of less than 10 *C. perfringens*/g. Nine of the remaining 15 samples showed close correlation between counts on the two media while six samples exhibited divergent counts with TSN being the higher.

Recoveries of coagulase positive *S. aureus* (MPN/g) are shown in Fig. 4. *Staphylococcus aureus* lacks the ability to compete in a highly contaminated product such as ground beef; however, poor handling which would introduce large numbers as secondary contamination presents potential hazards of food poisoning. The hazards rest on the fact that many strains of this organism are capable of producing heat-resistant enterotoxins (5).

Figure 5 depicts the results of coliform analyses. Only three of the 40 samples of ground beef showed levels of less than 100 coliforms/g (MPN/g).

Figure 6 depicts results of analyses for *E. coli* at 45.5 C. As can be seen in the figure, only one sample of centrally prepared ground beef exceeded a level of 50 MPN/g.

Results of analyses for enterococci are depicted in Fig. 7. The centralized processing operation products were substantially less contaminated with enterococci than those prepared in the traditional manner.

Of the 40 samples of ground beef analyzed, only 1 was found to contain *Salmonella.* The organism was identified as *Salmonella infantis.*

Table 1 shows results of fat determinations in the 40

TABLE 1. *Ranges, means*[1] *and standard deviations for percent fat**

Source	Range	Mean	Standard Deviation
A[a]	22.0-29.5	25.1 x	2.4
B[b]	14.4-27.1	18.0 w	4.0
C[c]	27.3-39.4	32.2 z	3.9
D[d]	25.0-37.0	29.4 y	3.8
E[e]	16.0-33.5	25.0 x	5.8

[1]Means with different superscripts are significantly different ($P < 0.01$).
* As determined by the Hobart Fat Percentage Indicator (± 1%).
[a]Centralized processing operation.
[b]Large sized supermarket.
[c]Small meat market.
[d]Large sized supermarket.
[e]Medium sized supermarket.

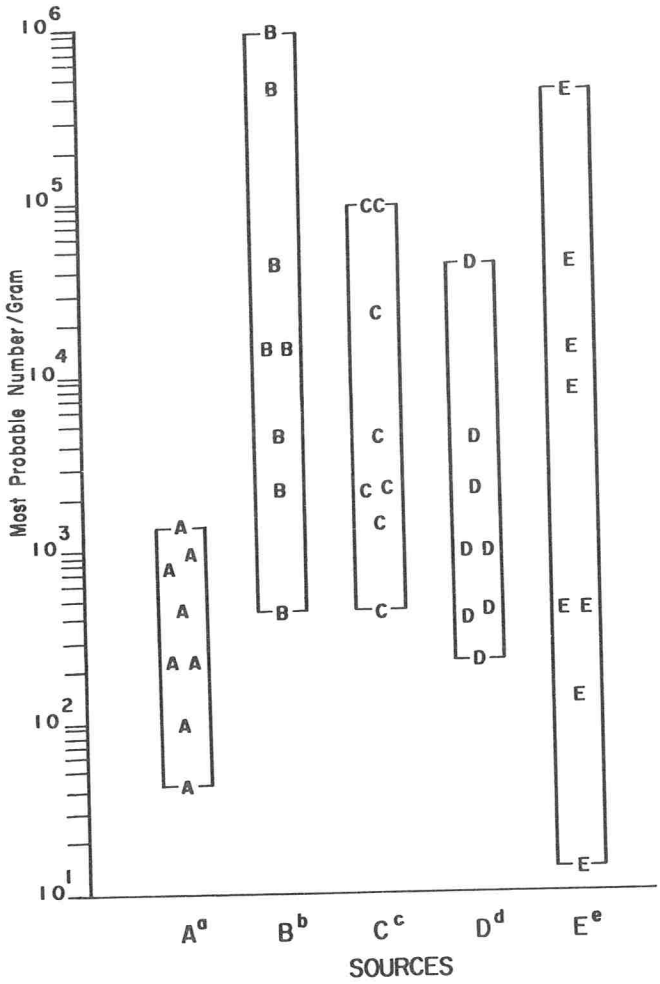

Figure 5. *Total coliforms MPN/g. See Fig. 1 for identification of code letters for sources.*

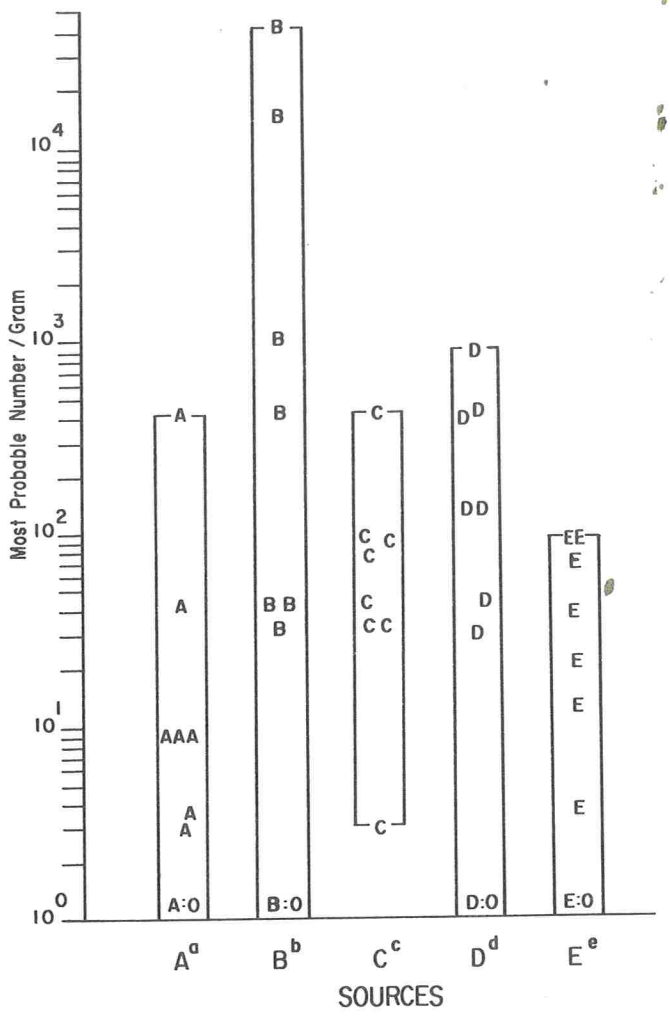

Figure 6. Escherichia coli *MPN/g at 45.5 C. See Fig. 1 for identification of code letters for sources.*

samples of ground beef. Federal law stipulates 30% fat content as the maximum limit in ground beef. The product prepared in the centralized operation was monitored automatically (Anyl-Ray, Anyl-Ray Corp., Waltham, MA) to determine percent fat whereas the final product composition in traditional preparation is usually a personal judgement by the meat cutter based on experience. This accounts for the wider variation of fat content and the numerous samples exceeding the legal limit that were found in 3 sources of traditionally prepared ground beef.

CONCLUSIONS

Based upon data obtained in this study of 40 samples of retail ground beef, products prepared in a centralized processing operation were of better microbiological quality than traditionally prepared ground beef. This is particularly important when one considers that products prepared in the centralized operation were obtained at the retail level after shipment from the production facility.

It is imperative to recognize that such a few samples from a random selection of retail outlets do not lend themselves to extensive statistical interpretation. Quite naturally, consideration of sanitation practices and quality control programs are of major importance in the preparation, distribution and marketing of such a product or, indeed, any food product.

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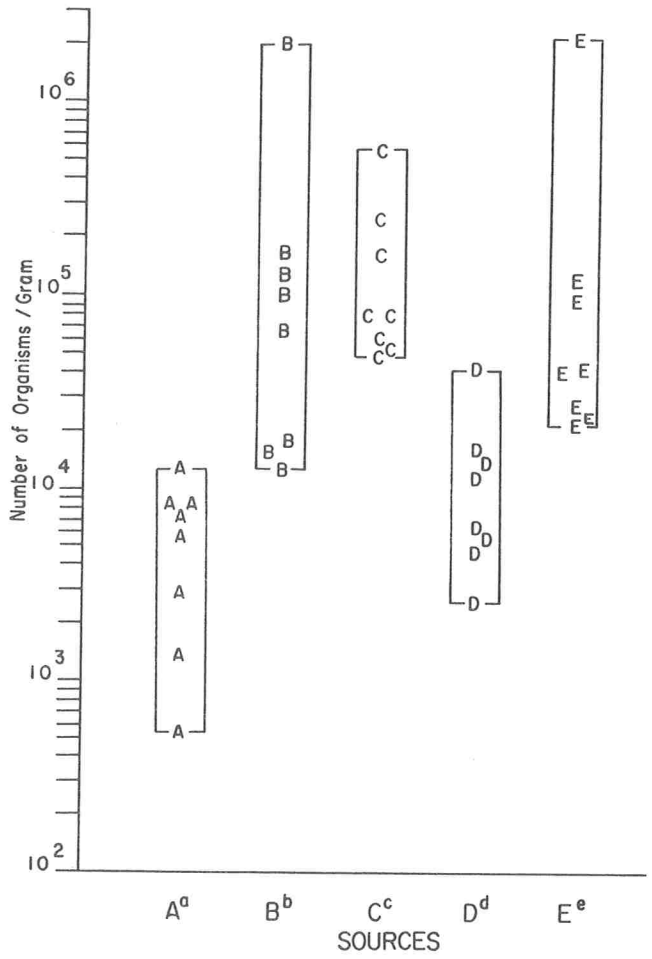

Figure 7. *Plate counts of enterococci. See Fig. 1 for identification of code letters for sources.*

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Cultural and Nutritional Factors that Control Rubratoxin Formation

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ABSTRACT

Penicillium rubrum P3290 was grown in a glucose-salts broth with various supplements and cultures were incubated under different conditions. Maximum mold growth and toxin production was favored by 20% glucose. The highest yield of rubratoxin A appeared in 6 days and of rubratoxin B in 12 days. Rubratoxin A disappeared from cultures after 22 days of incubation; rubratoxin B was gone after 26 days. All amino acids tested supported synthesis of rubratoxin B; highest yields were obtained when the medium contained asparagine or glutamic acid. Glycine, methionine, and serine supported good yields of rubratoxins A and B (3.3 to 6.8 mg of rubratoxin A/100 ml; 36.0 to 46.2 mg of rubratoxin B/100 ml), whereas aspartic acid, lysine, tyrosine, and tryptophan allowed production of only rubratoxin B. Yields of toxin were enhanced by 0.2-0.3% ammonium sulfate and mold growth was maximal when 5% was in the medium. Inorganic phosphate generally enhanced toxin production; it was optimal at 0.6% and inhibitory at 9.6%. Zinc and iron were required for toxin production but manganese and magnesium were not. A temperature of 25-28 C was optimal; reducing it to 18 C caused a 65% decline in toxin production. When the temperature was increased from 28 to 37 or 45 C, a 60-85% decline in, or complete inhibition of, toxin synthesis occurred because the fungus grew poorly at the higher temperatures. An initial pH value of 4-5 enhanced toxin synthesis, although limited toxin production occurred at pH 10 but not at pH 1. A relative humidity of 67% allowed rubratoxin formation in 7-day old cultures, of 77% supported highest yields of toxin, and 85% allowed accumulation of moderate amounts of rubratoxin.

Rubratoxin, a hepatotoxic agent, is one of several metabolites produced by *Penicillium rubrum* when it grows on different agricultural commodities, feeds, and laboratory media (*1, 11, 13, 31*). Thus far, two forms of rubratoxin, designated A and B, have been shown to be hepatotoxic to pigs, cattle, dogs, and laboratory animals (*10, 25, 31*). Research reports on the public health significance of the rubratoxins include those of Bamburg et al. (*2*) who described the synergism between rubratoxin and aflatoxin, a potent mutagen and carcinogen (*8, 15, 25*). Hood et al. (*14*) recently described the teratogenicity of rubratoxin B in mice. While rapid progress has been made in some phases of mycotoxin research, such as development of improved strains of molds for increased production of metabolites (*41*), and analytical techniques for rapid isolation and identification of mycotoxins (*27, 32*), the quantitative aspects of environmental influences on mold metabolism (including toxin formation) have not been sufficiently emphasized, especially for toxigenic molds other than the aspergilli.

Since: (*a*) the aerobic fungal biota contains a large component of *Penicillium* spores, (*b*) *P. rubrum* can produce toxic metabolites, (*c*) *P. rubrum* and other penicillia have been implicated as allergenic agents in pulmonary diseases (*16, 30*), and (*d*) penicillia cause widespread spoilage of corn, rice, and soybeans, we initiated experiments to learn how different cultural conditions affect growth of and rubratoxin production by *P. rubrum*. Results are described in this paper.

MATERIALS AND METHODS

Preparation of inoculum

The test organism used in these experiments was *P. rubrum* P3290 (obtained as an agar slant culture from the Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, Illinois). Inocula of the fungus were prepared by using 0.006% of a wetting agent, Leconal (Arthur Thomas, Philadelphia, Pa.), solution to remove spores from *P. rubrum* subcultures grown on corn slants. Appropriate media (50 ml/300-ml Erlenmeyer flask) were inoculated with 1 ml (4×10^5 conidia) of a suspension of conidia of *P. rubrum* P3290. Samples were prepared in duplicate and were incubated quiescently at 38 C for 14 days, unless otherwise indicated.

Medium

The basal medium was a glucose-salts broth containing the following ingredients in grams/liter: glucose, 75; $(NH_4)_2SO_4$, 1.75; KH_2PO_4, 0.75; KCl, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5; $CaCl_2 \cdot 2H_2O$, 0.2; $FeSO_4 \cdot 7H_2O$, 0.05; $ZnSO_4 \cdot 7H_2O$, 0.04; $MnCl_2 \cdot 4H_2O$, 0.02; $Na_2B_4O_7 \cdot 10\ H_2O$, 0.01; $(NH_4)_6\ M_7O_{24} \cdot 4H_2O$, 0.01; asparagine 10; and distilled water. This medium was used in all experiments described in this paper, except where otherwise stated.

Cultural conditions

P. rubrum was grown in the glucose-salts broth containing: 0-20% (wt/vol) glucose, 1% (wt/vol) of different amino acids, 0-10% (wt/vol) ammonium sulfate, 0.9.6% (wt/vol) of monobasic potassium phosphate, vitamins B_1 and C (10^{-5} M/sample), or trace metals (10^{-5} M/sample). In one experiment, the initial pH of the medium was adjusted to 1-10 using NaOH or HCl. Cultures were incubated at 18-45 C for up to 30 days. Some cultures were exposed to different relative humidities (*37*), whereas other cultures were grown in the glucose-salts medium that contained different acounts of "essential" minerals such as iron, zinc, magnesium, and manganese.

Determination of mycelial dry weight

When cultures had grown for designated periods, they were harvested and mycelia were recovered by filtration of growth materials through Whatman No. 1 filter paper at reduced pressure, washed four times with distilled water, dried in an oven at 45 C for 24 h, and weighed.

Rubratoxin analysis

Rubratoxin was recovered from culture filtrates by ether extraction, resolved by thin-layer chromatography, and determined spectrophotometrically according to the method of Alderman et al. (*1*).

RESULTS AND DISCUSSION

Concentration of glucose and rubratoxin formation

Growth of the mold, as determined by dry weight of the mycelium, increased from 0.1 to 2.2 g/100 ml as the glucose concentration increased from 0 to 20%. Production of mycelium was maximal (mg dry weight/g glucose) when the medium contained 0.5% glucose (Table 1).

TABLE 1. *Effect of glucose concentration on rubratoxin formation in a chemically defined medium by* Penicillium rubrum *P3290 grown quiescently at 28 C for 14 days*

Glucose concentration (%)	Dry weight of mycelium (g)	Rubratoxin (mg/100 ml)		Final pH
		A	B	
0	0.1	ND[a]	0.05	6.1
0.5	0.2	ND	4.5	7.2
1.0	0.3	2.16	9.0	6.0
2.5	0.7	2.70	16.5	5.2
5.0	1.3	2.88	21.4	4.2
7.5	1.7	3.75	26.4	3.4
10.0	2.0	10.25	50.6	2.5
20.0	2.2	9.18	60.5	2.0

[a]ND-None detected.

Twenty percent glucose in the medium also favored maximal production of rubratoxin B and total rubratoxins but 10% glucose favored maximal production of rubratoxin A. Changes in the pH of cultures paralleled the amount of glucose present in the medium (*5*). Thus in cultures which contained less than 2.5% glucose, the final pH was 5.0 or greater; cultures containing more than 5% glucose (wt/vol) had final acid pH values (Table 1) probably because of the larger amounts of acid produced from glucose during mold growth (*15*).

TABLE 2. *Bioproduction of rubratoxin in glucose-mineral salts medium by* Penicillium rubrum *P3290 grown quiescently at 28 C from 1 to 30 days*

Incubation (days)	Dry weight of mycelium (g)	Pigments[a]	Slime	Rubratoxin (mg/100 ml)		Final pH
				A	B	
1	NG[b]	None	—	ND[c]	ND	6.1
2	0.3	S,y,sl	—	ND	0.33	3.3
3	0.4	S,y,sl	—	ND	1.35	3.0
4	0.7	S,or	—	2.12	4.50	2.7
5	0.8	S,y	—	4.91	5.86	2.9
6	0.8	D,r,S,y	—	9.21	10.95	2.8
7	1.0	D,r,S,y	sl	8.40	17.88	5.5
8	1.1	D,r	+	6.55	22.50	5.8
9	1.1	D, S, br	++	7.18	35.71	3.8
10	1.0	r,br,S,D	++	5.85	42.10	6.1
11	0.9	r,br,S,D	++	5.30	48.75	6.9
12	1.0	r,br,S,D	+++	5.85	51.73	6.7
13	1.0	r,br,S,D	+++	4.41	38.90	6.8
14	0.9	D,r-b	+++	4.81	45.61	6.8
16	0.8	D,r-b	++	4.13	43.74	7.4
18	0.6	D,r-b	++	3.84	25.00	7.6
20	0.4	D,r-b	++	2.05	18.20	7.2
22	0.4	D,r	+++	1.41	12.35	8.2
24	0.6	D,r	++	ND	8.20	8.3
26	0.5	D,r	++	ND	4.25	8.0
28	0.5	b,D	++	ND	ND	8.0
30	0.5	D,r-b	sl	ND	ND	8.0

[a]Characterization of pigments:
S-surface pigment
D-bottom pigments (released into medium)
a-glucose concentration 5% (w/v)
sl-slight
y-yellow
br-brown
r-b-reddish-brown
or-orange
r-red

[b]NG-Growth not detected.

[c]ND-None detected.

Time of incubation, mold growth, and toxin formation

How length of incubation affected mold development and rubratoxin formation are shown by data in Table 2. Maximum accumulation of rubratoxin A occurred after 6 days, whereas the greatest yield of rubratoxin B was obtained after 12 days of incubation. Rubratoxin A was no longer present after 22 days of incubation; rubratoxin B disappeared from cultures held for more than 26 days. Slime appeared on the second day of growth and persisted through the fifth day of incubation. Surface pigments (in the mycelium) also appeared on the second day of growth and continued through 13 days of incubation. Bottom mycelial pigments were released into the medium on the sixth day and persisted and deepened in color through 30 days of incubation. The amount of toxin recovered varied inversely with the intensity of color of mold pigment.

The relationship between pigment and rubratoxin formation has not been established. Our results suggest that the intensity of medium coloration is inversely proportional to toxin formation. Thus the true secondary metabolites of *P. rubrum* are released into the medium during the stationary phase of vegetative mycelium development, as has been reported for other fungal secondary metabolites (*7, 8, 24, 31*). Cultural pH changes were most rapid through 10 days of incubation, remaining above 7.0 after 16 days of incubation. Glucose consumption (*5, 7*) influences primary and secondary synthesis in fungi (*5, 24, 31*), the rate of glucose metabolism being most rapid in "young" (log phase) cultures, at low glucose concentrations in aerobic cultures. Consumption of glucose usually decreases as the stationary phase of growth approaches when both autolysis of mycelia, production of staling substances, and secondary biosynthesis begin (*5, 15, 31*). Degradation of mycotoxins by the producing organism has been reported; our results suggest that such degradation also may have occurred in this instance, although Ciegler et al. (*6, 22*) have described aflatoxin degradation as nonenzymatic.

Nitrogen source and mold growth and rubratoxin formation

Amino acids. All of the amino acids tested (Table 3) permitted rubratoxin B formation; highest yields were obtained when the medium contained glutamic acid or asparagine. Serine, methionine, and glycine gave good yields of both rubratoxins A and B (3.3-6.8 mg/A and 36.0-46.2 mg B/100 ml) while aspartic acid, lysine, and tryptophan allowed production of only rubratoxin B. The effect of combinations of amino acids on rubratoxin

TABLE 3. *Production of rubratoxin in a glucose[a]-mineral salts medium containing different amino acids by* Penicillium rubrum *P3290 grown statically for 14 days at 28 C*

Amino acid[b] (10 g/l)	Dry weight of mycelium (g)	Rubratoxin (mg/100 ml) A	Rubratoxin (mg/100 ml) B	Final pH
Control[c]	1.3	ND[d]	13.50	1.6
Asparagine	1.9	7.20	54.00	4.5
Aspartic acid	0.7	ND	39.50	7.1
Arginine	2.1	1.26	29.60	2.4
Cystine	1.7	5.40	28.30	2.3
Glycine	1.8	4.50	46.15	5.9
Glutamic acid	1.7	5.04	52.80	5.3
Lysine	1.6	ND	36.60	1.9
Methionine	1.4	3.30	45.00	2.1
Phenylalanine	1.9	3.50	19.20	7.2
Serine	1.5	6.84	36.00	1.5
Tyrosine	1.8	ND	16.20	1.5
Tryptophan	1.6	ND	10.20	1.2

[a]Glucose concentration 7.5% (w/v).
[b]Constituted entire nitrogen source.
[c]No amino acid was added to medium.
[d]ND-None detected.

production is not known. However, it has been reported (*6a*) that some fungi, notably *Venturia inequalis,* grow better in media containing casein hydrolysate rather than in those containing single amino acids.

Heating glucose and amino acids. Autoclaving amino acids and glucose together (in the culture medium) (Table 4) rather than separately caused an average

TABLE 4. *Influence of autoclaving of culture medium on rubratoxin formation by* Penicillium rubrum *P3290 grown quiescently at 28 C for 14 days*

Medium[a]		Dry weight of mycelium (g)	Rubratoxin (mg/100 ml) A	Rubratoxin (mg/100 ml) B	Final pH
Glucose-asparagine	A[b]	3.2	5.1	36.0	3.2
Glucose-asparagine	S[c]	2.2	6.3	50.4	4.2
Glucose-arginine	A	1.6	4.1	19.8	2.8
Glucose-arginine	S	1.4	3.2	29.6	2.8
Glucose-cystine	A	1.4	ND[d]	21.6	2.4
Glucose-cystine	S	1.4	ND	45.6	2.4
Glucose-lysine	A	1.8	1.9	34.2	2.5
Glucose-lysine	S	1.6	ND	43.2	2.6

[a]Glucose 7.5% (w/v); amino acid 1% (w/v).
[b]A-Glucose and amino acid autoclaved together.
[c]S-Glucose and amino acid autoclaved separately.
[d]ND-not detected.

reduction of 25% in rubratoxin A formation; formation of rubratoxin B was reduced by 45%. Growth of the mold was always enhanced by autoclaving glucose and amino acids together.

The effects of nitrogen sources on toxin formation should be interpreted in relation to pH changes in the course of fungal development. Cultures grown in the medium that contained either glutamic acid or asparagine had final pH values reported as being favorable for toxin formation (*13, 15, 23*). The relatively poor mold growth observed with cultures which received aspartic acid may have resulted from the low solubility of this amino acid. Low yields of toxin (0-5.4 mg of rubratoxin A/100 ml, 10.2-28.3 mg of rubratoxin B/100 ml) were recovered from mold cultures exposed to cystine, tyrosine, tryptophan, and phenylalanine even though these amino acids supported good mold growth. The reason for this occurrence is now known; however, it is known that at a pH between 4 and 9 cystine and tyrosine are only sparingly soluble because of the preponderance of their zwitterions. This may decrease the amount of nitrogen from these two amino acids that was absorbed by the fungus.

The direct role of amino acids in regulation of rubratoxin biosynthesis has not been established. Detroy and Freer (*8*) have reported that aflatoxin formation is controlled by methionine because of a requirement for C1 methylation. Whether the same is true for rubratoxin is unclear since rubratoxin is nonaromatic but it is worth investigating. In this regard both serine and glycine should support formation of large quantities of toxin; that this did not happen might have been caused by an insufficiency in carbon fragments supplied by these compounds (*5, 19*). Many nitrogenous materials, especially amino acids, are precursors of mycotoxins (*31*). The large quantities of rubratoxin recovered from cultures which received asparagine may have resulted from the extra amide nitrogen supplied by this amino acid. Furthermore, according to the pathway for rubratoxin formation proposed by Moss et al. (*31*), both asparagine and aspartic acid should support rubratoxin formation since they are metabolites derived from oxaloacetic acid, a proposed precursor of rubratoxin.

Ammonium sulfate. Highest yields of toxin resulted when the medium contained 0.1 to 0.3% ammonium sulfate (Table 5). Maximal mold growth occurred when the medium contained 5% ammonium sulfate.

TABLE 5. *Effect of ammonium sulfate on growth and rubratoxin synthesis by* Penicillium rubrum *P3290 in a chemically defined medium at 28 C for 14 days*

Ammonium sulfate[a] concentration (%)	Dry weight of mycelium (g)	Rubratoxin (mg/100 ml) A	Rubratoxin (mg/100 ml) B	Final pH
0	0.2	ND[b]	ND	2.1
0.1	0.8	9.3	42.6	4.9
0.2	1.0	7.2	57.1	3.1
0.3	1.1	6.9	48.6	3.7
0.5	1.1	6.8	30.5	1.5
1.0	1.3	6.3	19.8	1.5
2.0	1.4	6.0	18.0	1.6
3.0	1.5	4.5	17.5	1.4
5.0	1.6	7.0	17.5	1.2
7.5	1.5	ND	20.5	1.2
10.0	1.1	ND	20.5	1.2

[a]Ammonium sulfate was the only nitrogen source in culture medium.
[b]ND-Not detected.

Generally inorganic nitrogen, either as NH_4-N or NO_3-N, is assimilated rapidly with a resulting acid pH of the medium (*19, 23*). A high C:N ratio (in the culture medium) enhances initial vegetative morphogenesis, a phase of maximum toxin accumulation (*3, 5, 19, 23*). A low C:N ratio is associated with secondary biosynthesis which results in production of phoenicin, among other pigments (*31*).

Effect of zinc, iron, magnesium and manganese on mold growth and rubratoxin formation

Data in Tables 6, 7, 8, and 9 illustrate the effect of

TABLE 6. *Effect of zinc on growth and production of rubratoxin by* Penicillium rubrum *P3290 in a glucose-mineral salts medium at 28 C for 14 days*

Concentration of zinc (M/1)	Dry weight of mycelium (g)	Rubratoxin (mg/100 ml)		Final pH
		A	B	
0	0.6	ND[b]	ND	3.3
6×10^{-5}	2.0	ND	10.2	4.4
1.2×10^{-4}[a]	2.3	6.8	51.3	2.1
2.4×10^{-4}	2.5	4.7	43.2	4.0
4.8×10^{-4}	2.3	4.0	31.8	4.9

[a]Normal concentration in medium.
[b]ND-None detected.

TABLE 7. *Effect of iron (Fe^{2+}) on growth and production of rubratoxin by* Penicillium rubrum *P3290 in a glucose mineral salts medium at 28 C for 14 days*

Iron ($FeSO_4$) concentration (M/1)	Dry weight of mycelium (g)	Rubratoxin (mg/100 ml)		Final pH
		A	B	
0	2.1	ND[b]	ND	5.7
9×10^{-5}	1.8	ND	12.3	7.0
1.8×10^{-4}[a]	2.1	11.3	27.6	2.4
3.6×10^{-4}	3.0	7.2	31.2	7.1
7.2×10^{-4}	2.5	4.8	23.8	7.0

[a]Normal concentration in medium.
[b]ND-None detected.

zinc, iron, magnesium, and manganese on growth and rubratoxin formation by *P. rubrum*. Zinc (Table 6) and iron (Table 7) are required for rubratoxin formation since no toxin was formed in the absence of either metal. A concentration of 1.2×10^{-4} M of zinc supported maximum accumulation of rubratoxins A and B. Growth of the mold was greatest when the concentration of zinc was 2.4×10^{-4} M. Doubling the normal concentration of iron in the medium (Table 7) also caused maximum mold growth. Neither magnesium (Table 8) nor manganese (Table 9) appreciably affected toxin formation, since substantial quantities of rubratoxin were recovered at all concentrations of either metal. Growth of the mold was approximately the same when the medium contained 1×10^{-3} M magnesium or greater. Similar results were obtained when the medium contained manganese (5×10^{-5} M or greater).

TABLE 8. *Effect of magnesium (Mg^{2+}) on growth and production of rubratoxin by* Penicillium rubrum *P3290 in a glucose mineral salts medium at 28 C for 14 days*

Conctration of magnesium ($MgSO_4$) (M/1)	Dry weight of mycelium (g)	Rubratoxin (mg/100 ml)		Final pH
		A	B	
0	0.8	8.1	54.3	4.1
1×10^{-3}	2.6	8.4	55.2	5.9
2×10^{-3}[a]	2.3	7.2	47.7	2.3
4×10^{-3}	2.5	8.7	46.2	5.1
8×10^{-3}	2.6	4.8	27.3	3.5

[a]Normal concentration in culture medium.

Mineral elements are important to microbial development (*19, 33, 34, 35*) especially since many of them are constituents of biosynthetic enzyme systems (*5, 17, 19*). Thus a high concentration of either iron or zinc will inhibit an NAD-lined dehydrogenase or enolase, required for production of metabolic intermediates

TABLE 9. *Effect of manganese (Mn^{2+}) on growth and production of rubratoxin by* Penicillium rubrum *P3290 in a glucose-mineral salts medium at 28 C for 14 days*

Concentration of manganese ($MnSO_4$) (M/1)	Dry weight of mycelium (g)	Rubratoxin (mg/100 ml)		Final pH
		A	B	
0	1.7	12.1	42.3	4.9
5×10^{-5}	2.5	5.9	41.9	4.6
1×10^{-4}[a]	2.5	4.5	42.6	3.3
2×10^{-4}	2.7	ND[b]	38.6	5.2
4×10^{-4}	2.6	ND	36.5	5.1

[a]Normal concentration in culture medium.
[b]ND-None detected.

and/or precursors (*17*). Magnesium and manganese at all concentrations tested resulted in good yields of toxin, suggesting that they are interchangeable, at least in this instance, as has been reported (*24*).

Effect of vitamins and trace metals

Copper, ascorbic acid, cobalt, barium, and thiamine did not markedly affect rubratoxin formation (Table 10). Growth of the mold was reduced by all the additives and

TABLE 10. *Production of rubratoxin by* Penicillium rubrum *P-3290 grown at 28 C for 14 days in a glucose-mineral salts medium fortified with vitamins and trace metals*

Concentration of supplement (M x 10^{-5})	Dry weight of mycelium (g)	Rubratoxin (mg/100 ml)		Final pH
		A	B	
Control[a]	1.5	6.0	54.6	3.7
Thiamine hydrochloride	0.7	4.5	26.4	3.3
Ascorbic acid	0.7	ND[b]	13.5	5.7
Cobalt chloride	0.8	3.6	27.6	4.8
Copper sulfate	0.8	1.5	16.2	5.0
Barium chloride	0.9	ND	12.6	4.9

[a]Did not receive any supplement.
[b]ND-Not detected.

the reduction ranged from 43% when the medium contained barium to 65% when thiamine was added to the basal medium.

Although many fungi grow in the absence of vitamins, it is not clear why both vitamins B_1 (a cocarboxylase cofactor) and C caused a reduction in mold growth and toxin production. Inhibition of rubratoxin formation by ascorbic acid may be the result of glucose antagonism while inhibition by vitamin B_1 may be the result of supersaturation of enzyme sites, thus reducing enzyme-substrate complex formation or accelerating its decomposition (*20*). Cocarboxylase (thiamine pyrophosphate, TPP) is the biologically active form of vitamin B_1. Thus inhibition of toxin formation by vitamin B_1 may be the results of the inability of *P. rubrum* to esterify the vitamin. Copper, cobalt, and barium may be toxic to the fungus at the concentrations tried; it may also be that at such high concentrations (10^{-5} M vs. 10^{-7} M) they limited the availability of other nutrients (*5, 17, 24*).

Temperature effects on mold growth and toxin formation

A temperature of 25-28 C (Table 11) was optimal for rubratoxin formation as well as mold growth. Rubratoxin A increased from 0 to 18 C to 4.8 and 5.4 mg/100 ml at 25 and 28 C, respectively; this toxin was

TABLE 11. *Effect of temperature on rubratoxin formation by* Penicillium rubrum *P3290 grown in a glucose mineral salts medium at 28 C for 14 days*

Temperature (C)	Dry weight of mycelium (g)	Rubratoxin (mg/100 ml)		Final pH
		A	B	
18	0.7	ND[b]	4.5	4.6
25	2.0	4.8	52.8	3.3
28[a]	2.2	5.4	54.9	2.9
37	1.2	ND	ND	5.3
45	1.2	ND	ND	2.8
28-18[c]	1.3	3.2	18.2	4.8
28-25[c]	1.6	4.2	51.0	5.4
28-28[a,c]	1.9	5.1	53.1	2.7
28-37[c]	1.2	0.9	22.8	4.1
28-45[c]	1.1	ND	10.2	4.4

[a]Control.
[b]ND-None detected.
[c]All cultures were grown quiescently at 28 C for 3 days after which they were incubated at the higher or lower temperature indicated for 11 days.

not detected in cultures held at 37 or 45 C. Rubratoxin B increased from 4.5 mg/100 ml in cultures held at 18 C to 52.8 and 54.9 mg/100 ml in cultures held at 25 or 28 C, respectively; rubratoxin B was not detected in cultures held at 37 or 45 C. When the incubation temperature was shifted down from 28 to 18 C, toxin production decreased by 65%; increasing the incubation temperature to 37 or 45 C resulted in a 60 to 85% decline in, or complete inhibition of, toxin formation.

The influence of temperature on fungal activity has been widely reported (*5, 7, 9, 13, 35*). It has been postulated (*7, 35*) that narrow temperature limits (usually suboptimal for growth) are more favorable to secondary biosynthesis than to mold growth. However, rubratoxin is not a true secondary metabolite and in our experiments temperatures that permitted maximum fungal growth also permitted maximum toxin formation. Temperatures higher than the optimal growth temperature (25-28 C) (*5, 7*) enhanced fungal growth probably because of increased sugar consumption. Holding cultures at 37 or 45 C for 14 days probably accelerated rate of growth (as a result of increased glucose consumption) but also increased production of staling (waste) substances whose toxicity probably caused a reduction of growth and blocked toxin formation (*5*).

Effect of inorganic phosphate on mold growth and toxin formation

Inorganic (monobasic potassium) phosphate (Table 12) at low concentrations enhanced toxin formation; it was optimal at 0.6%, but inhibitory at 9.6%. Similar results were evident for mold growth. Phosphate controls sugar utilization; similarly, temperature controls phosphate metabolism. High phosphate concentrations are inhibitory to mold development because they depress phosphotransferase and phosphatase enzyme activities (*7, 29, 35*). Our results are in agreement with this hypothesis. The low yields of rubratoxin and mycelial dry weight recovered in cultures incubated in the presence of high phosphate concentrations may also have resulted from the low solubility of phosphate at the incubation temperature tried.

TABLE 12. *Effect of inorganic phosphate concentration on rubratoxin formation by* Penicillium rubrum *P3290 grown in a glucose-mineral salts broth at 28 C for 14 days*

Potassium phosphate (KH_2PO_4) (g/1)	Dry weight of mycelium (g)	Rubratoxin (mg/100 ml)		Final pH
		A	B	
0	0.1	ND[a]	4.0	3.0
0.75	2.2	8.1	51.0	4.5
1.5	2.5	15.3	57.6	3.3
3.0	2.8	14.9	62.5	4.2
6.0	3.4	18.2	84.5	3.2
12.0	0.9	12.6	0.4	5.8
24.0	0.7	0.90	22.5	6.1
48.0	0.5	ND	12.1	5.5
96.0	0.5	ND	8.4	4.1

[a]ND-None detected.

Effects of pH on mold growth and toxin formation

An initial pH of 4 to 5 enhanced rubratoxin production although some toxin was recovered from cultures whose initial pH was as high as 10 (Table 13). Even after incubation for 28 days, fungal growth was severely limited and toxin formation completely blocked when *P. rubrum* was placed in a medium whose initial pH was 1.0.

TABLE 13. *Growth, culture pH change, and rubratoxin formation in a glucose*[a] *mineral salts medium by* Penicillium rubrum *P3290 grown at 28 C for 14 days*

Initial pH[b]	Dry weight of mycelium (g)	Rubratoxin (mg/100 ml)		Final pH
		A	B	
1[c]	NG[d]	ND[e]	ND	1.9
2	0.5	ND	0.15	1.7
3	1.3	1.8	9.3	3.4
4	1.3	4.5	42.6	3.9
4.7[f]	1.5	5.4	51.0	3.9
5	1.6	5.4	52.8	4.2
6	1.6	5.8	40.8	4.5
7	1.2	7.8	38.4	5.4
8	1.0	2.4	24.6	5.8
9	0.9	0.7	16.8	6.5
10	0.9	ND	7.2	7.3

[a]Concentration 5% (w/v).
[b]Initial pH achieved by adjusting medium pH with either 2 N HCL or 2 N NaOH.
[c]No rubratoxin was recovered from cultures whose initial pH was 1.0 even after incubation for 28 days.
[d]NG-No growth.
[e]ND-None detected.
[f]Control; pH not adjusted.

The effect of pH on rubratoxin production has been the subject of a few reports (*13, 15*) but has been studied more extensively with respect to aflatoxin formation (*6, 15, 31*). In general, a pH near neutrality is optimal for mold growth, a pH of 7.0 is optimal for production of rubratoxin A, and a pH of 5.0 is optimum for production of rubratoxin B. Ideally, buffered media are used in some antibiotic fermentations and production of mycotoxins for animal studies (*13, 40*). Although cultural pH changes affect enzyme stability and denaturation, conformational changes, enzyme-substrate decomposition kinetics, and degrees of ionization of substrate components, such antibiotic or mycotoxin fermentations

do not consider the substantial pH drift normally associated with mold growth (*4, 20*). We feel that the influence of pH on rubratoxin formation should be studied further.

Effect of relative humidity on toxin production

Relative humidity was optimal at 77% (Table 14) for toxin formation in cultures grown at 28 C for 7 and 14 days. Mold growth also was substantial under these conditions. *P. rubrum* cultures grown in a humidity chamber whose relative humidity (RH) was 67 or 85% produced only moderate amounts of rubratoxin. In all instances mold growth was affected less than toxin formation.

TABLE 14. *Effect of moisture*[a] *(equilibrium relative humidity) on rubratoxin formation by* Penicillium rubrum *P3290 grown in a glucose-mineral salts broth at 28 C*

Relative humidity (%)	Incubation (days)	Dry weight of mycelium (g)	Rubratoxin (mg/100 ml) A	Rubratoxin (mg/100 ml) B	Final pH
Control[b]	7	1.9	5.1	41.7	3.5
	14	2.4	7.6	57.2	4.1
67	7	1.3	4.2	26.4	2.5
	14	2.5	4.2	21.6	2.7
77	7	1.2	4.9	31.5	3.0
	14	1.4	5.4	49.8	3.1
85	7	1.7	2.5	28.8	4.5
	14	1.5	4.9	31.2	5.5

[a]Culture medium-glucose mineral salts broth.
[b]Culture not incubated in humidity chamber.

The influence of moisture on toxin production is very important from the standpoint of food storage and/or preservation. Etchells et al. (*12*) studied the effects of temperature and humidity on microbial, enzymatic, and physical changes in pickling cucumbers and found that a high temperature (27 C) favored mold growth and softening-enzyme production. They also found that a low temperature and high humidity (>95%) were optimal for storage of cucumbers. In our experiments, a controlled environment (> 90% RH) was optimal for toxin formation at either 7 or 14 days of incubation. All other humidities that were tried permitted more toxin elaboration in cultures grown for 14 days, except at 67% RH when more rubratoxin was recovered in 7-day old cultures than in those grown for 14 days. This is understandable since cultures exposed to 67% RH for 14 days would lose more water to the atmosphere in the chamber and would equilibrate less effectively with the resulting high concentrations of minerals in the medium, a situation inhibitory to toxin formation (*20*).

Cultures exposed to 77 or 85% RH would lose less water and would equilibrate with the exogenous environment (*37*). Minor fluctuations in relative humidities within the growth chamber might serve to explain the seemingly contradictory results obtained in relation to rubratoxin formation in cultures exposed to 85% RH (*39*).

We have discussed the relative importance of applying environmental control methods to the management of mycotoxin production, a situation of economic, ecological, and public health significance. The recent increased awareness of mycotoxigenic fungi and their role in food spoilage (*18, 38*), and the isolation of mycotoxins from cultures of some molds used in cheese ripening (*32*) confirm the need for more intensive and exhaustive studies of environmental influences on mycotoxin formation.

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Studies of Scharer's Original Method for Alkaline Phosphatase in Milk with a Modification Utilizing an Organic Buffer

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ABSTRACT

Based on studies of Scharer's original (1938) method for alkaline phosphatase in milk, a modified procedure was developed utilizing two strengths of an organic buffer, 2-amino-2-methyl propanol. This modification, designated the AMP method, has the following advantages: (*a*) elimination of non-enzymatic substrate hydrolysis resulting from immersion in boiling water; (*b*) complete reaction of liberated phenol to form indophenol by control of temperature, pH, and volume of 2,6-dibromoquinonechloroimide; and (*c*) provision of a clear solution for spectrophotometric measurement following protein precipitation and filtration. The method is able to detect less than 0.1% of raw milk in pasteurized milk, and has a precision with a 1-h incubation period equivalent to the Kay and Graham method requiring 24 h incubation. A standard of 1.6 phosphatase activity units is proposed for pasteurized milk on the basis of equivalence to 2.3 Lovibond color units by the Kay and Graham method.

One of the early methods described for determination of alkaline phosphatase in milk as a test for pasteurization was that of Scharer in 1938 (*6*). The test as originally described is no longer used although it provided the basis of modifications which have official acceptance today (*2*). When our own experiences found Scharer's original method to have very poor reproducibility, we undertook some studies of the procedure which led to a modification utilizing an organic buffer. A standard for this modification, referred to as the AMP method, was established by comparison with the Kay and Graham procedure.

MATERIALS AND METHODS

Scharer's original method

Scharer's original method (*6*) for alkaline phosphatase in milk utilized disodium phenyl phosphate as the enzyme substrate, a borate buffer, lead acetate for protein precipitation, and 2,6-dibromoquinone-chloroimide (BQC) for color development following filtration. Certain parameters of this original method were examined in this study, which subsequently led to a modification.

Kay and Graham method

The Kay and Graham method for alkaline phosphatase in milk was employed as originally described for the 24-h incubation test (*5*). Test results were measured spectrophotometrically, representing a departure from the original procedure, in a 10-mm cuvette against a negative control blank prepared with milk heated to 90 C for 1 min. Activities were expressed in Lovibond color units by calculation from the linear regression equation ($Y = 0.08634X - 0.00202$) for a standard curve relating absorbance at 650 nm to Lovibond units of color for phenol standards (Fig. 1). That liquid standard prepared with

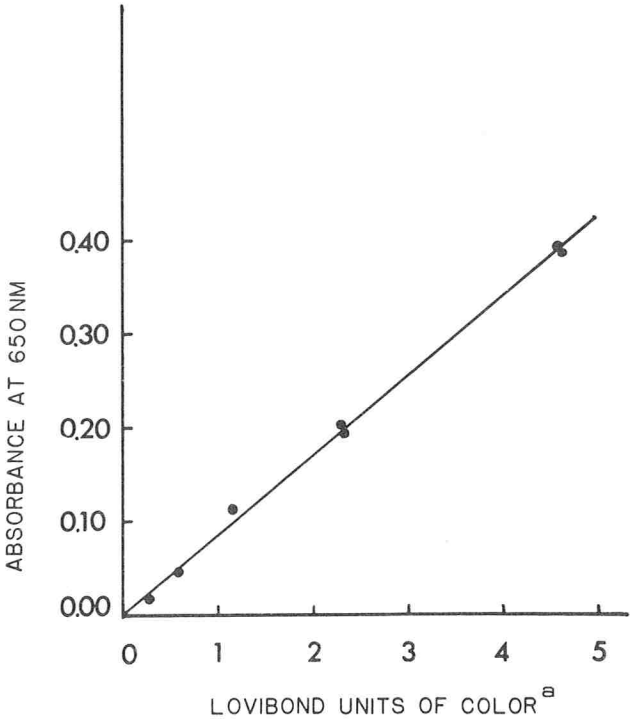

Figure 1. *Standard curve for alkaline phosphatase in milk by Kay and Graham method.* [a]*Based on a "standard color" prepared with 100 ug/ml phenol and accepted as equivalent to 2.3 Lovibond units (Kay and Graham, J. Dairy Res. 5:191, 1935).*

100μg phenol/ml according to the procedure described by Kay and Graham (*5*) was accepted as the equivalent of 2.3 Lovibond color units. This relationship between liquid standards and Lovibond color units was confirmed by comparison against permanent Lovibond color standards in a Tintometer comparator.

AMP method

Reagents. Buffered substrate was prepared by dissolving 1.09 g of disodium phenyl phosphate in 900 ml of distilled water saturated with ether. AMP buffer pH 9.75 (50 ml) was added and the solution diluted to 1 liter. Buffered substrate was prepared fresh each day.

AMP buffer was prepared by diluting 95 ml of 50% (wt/vol) 2-amino-2-methyl propanol (AMP) with about 800 ml of distilled water and adjusting to pH 9.75 with 5 N HCl. The solution was diluted to 1 liter with distilled water. AMP buffer 2× at pH 10.25 was prepared by mixing 100 ml of 50% AMP with about 350 ml of distilled water,

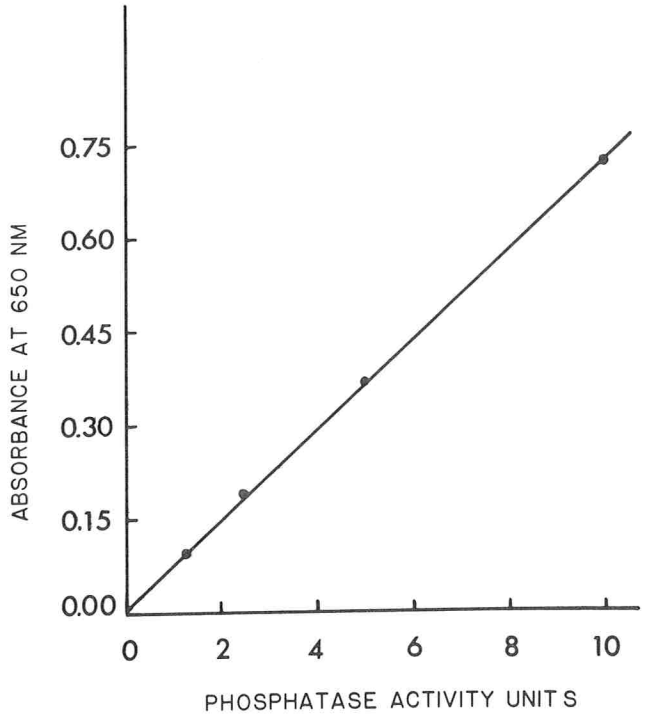

Figure 2. *Standard curve for alkaline phosphatase in milk by AMP method. Means of duplicate determinations.*

adjusting the pH with 5 N HCl, and then diluting to 500 ml with distilled water. The pH of both buffers was checked before each use and readjusted if necessary.

BQC solution was prepared fresh daily by dissolving 40 mg of 2,6-dibromoquinonechloroimide in 10 ml of 95% ethanol.

Basic lead acetate was prepared by dissolving 280 g of the salt in 500 ml of distilled water, then boiling for 5-10 min, followed by cooling and settling. The solution was then filtered and diluted to 500 ml.

Standard curve. A solution of phenol was standardized by iodometric titration to 1 mg/ml. Dilutions were prepared at 0.25, 0.5, 1.0 and 2.0 μg/ml, representing 1.25, 2.5, 5.0 and 10.0 phosphatase activity units, approximately equal to Scharer units, i.e. μg phenol per 5 ml of solution (*1*). AMP buffer 2× pH 10.25 (0.5 ml) and 12 drops of BQC solution were added to tubes containing 10 ml of the standard. Tubes were held at 37.5 C for 5 min then 25 min at 21-23 C before reading absorbances in a 19-mm cuvette at 650 nm against a reagent blank prepared with distilled water. Means of duplicate determinations were used to prepare a standard curve (Fig. 2). All activities by this method were calculated from the linear regression equation for this standard curve ($Y = 0.07172X + 0.00730$).

Procedure. Twenty milliliters of buffered substrate were added per 19 × 150 mm tube. Two milliliters of sample were added, the tubes were stoppered with boiled rubber stoppers, and the contents were mixed by inversion. A negative control consisting of milk heated to 90 C for 1 min was included with each test series and used as the reagent blank for spectrophotometric measurements.

The tubes were incubated in a 37.5 C water bath for 1 h, allowing 3 min for temperature equilibration, then immediately cooled in an ice bath. Basic lead acetate (0.3 ml) was promptly added, and the tubes stoppered and mixed well by inversion. Tubes were then held in the ice bath for at least 5 min before adding 1.1 ml of AMP buffer 2×. The contents of stoppered tubes were mixed by inversion and then replaced in the ice bath for 3 min, followed by filtration (Whatman 2V). Twelve drops of fresh BQC solution were added to 10 ml of filtrate followed by gentle shaking. The tubes were placed in a 37.5 C water bath for 5 min and then held for an additional 25 min at 21-23 C to allow complete color development. Absorbances were measured in a 19-mm cuvette against a negative control blank, and converted to phosphatase activity units by calculation with the linear regression equation for the standard curve.

RESULTS

Substrate stability

The poor reproducibility of Scharer's original method, plus the absence of a colorless negative control in any trial, suggested the possibility of non-enzymatic hydrolysis of the substrate disodium phenyl phosphate. Buffered substrate was prepared as specified for the method and 10 ml placed in each of nine tubes. Three tubes were held at room temperature for 1 h, three were held at 37.5 C for 1 h, and three at 37.5 C for 1 h followed by exactly 5 min in a boiling water bath. Borate buffer and BQC were added to each tube as specified for Scharer's original method. Percent transmittance at 650 nm was measured after color development at room temperature against a distilled water blank. The results presented in Table 1 show that no phenol, indicative of substrate hydrolysis, was liberated at room temperature or at 37.5 C, but a significant amount was released by immersion in boiling water. The modified procedure eliminated this step, relying on immediate cooling in ice water with prompt addition of lead acetate to stop the enzyme reaction.

TABLE 1. *Non-enzymatic hydrolysis of disodium phenyl phosphate in Scharer method for milk phosphatase*

Treatment of buffered substrate	% T at 650 nm[a]		
	Tube 1	Tube 2	Tube 3
Room temperature	99.2	99.0	98.8
37.5 C for 1 h	99.0	99.2	99.0
37.5 C for 1 h plus boiling water for 5 min	92.1	98.0	94.5

[a]Following addition of 0.5 ml borate buffer and 4 drops BQC to 10 ml buffered substrate with color development at room temperature. Read against a reagent blank prepared with distilled water.

A similar experiment was conducted to determine whether any phenol was lost during the required heat treatments. The results demonstrated that phenol was stable under test conditions.

Temperature and indophenol formation

After observing increases in color intensity following the 15 min period at room temperature after adding BQC specified in Scharer's original method (*6*), some alternative conditions were examined. The recommendation of *Standard Methods* (*1*) for 5 min at 40 C after addition of CQC served as a guide. Tubes containing 10 ml of a phenol solution (0.50 μg/ml) at pH 9.75 and 4 drops of BQC were placed at 37.5 C for 5 min and then held at 21-23 C. This technique not only increased the rate of indophenol formation but the final color intensity was greater than for identical solutions held an equal period at 21-23 C only. Twenty-five minutes at 21-23 C following the 5 min at 37.5 C was adequate for complete reaction, producing a stable solution which showed no further changes in color intensity on standing.

Volume of BQC

To observe the influence of BQC volume on indophenol formation, various volumes (drops) from a Pasteur pipet were added to 10 ml of a phenol solution (2 μg/ml) containing 0.5 ml of AMP buffer 2× (pH 10.25). Absorbances were measured after color development against a distilled water blank of equal volume and 16 drops of BQC. The results shown in Fig. 3 indicate a

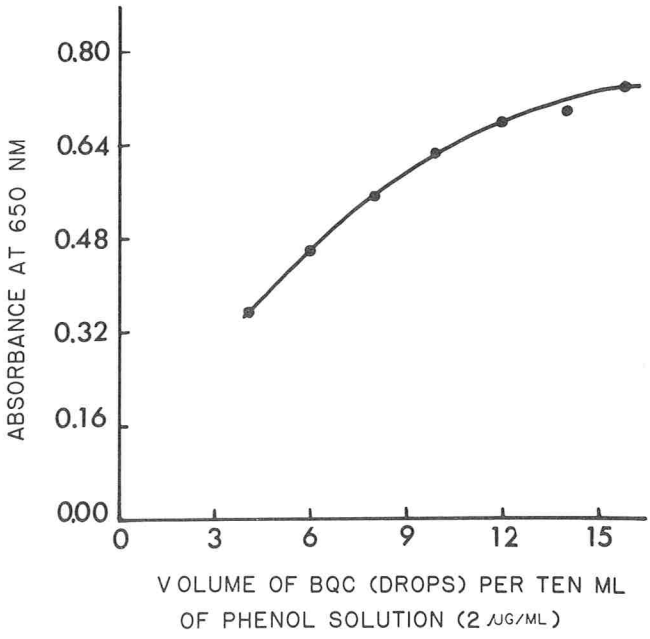

Figure 3. *Effect of BQC volume on development of indophenol.*

rapid change in color intensity with volumes of BQC less than 10 or 12 drops. Twelve drops was selected for the modified method, which produced a slight distortion of the blue color at low phosphatase activities making visual comparison difficult but not interfering with spectrophotometric measurement.

Control of pH

The influence of pH on formation of indophenol was studied by adding 4 drops of BQC solution to a borate-buffered solution of phenol (0.5 μg/ml) and adjusting to various pH values. The means of three determinations of absorbance at 650 nm for each pH are presented in Fig. 4, demonstrating that small differences in pH below 9.6 produce large differences in color intensity.

Addition of borate buffer (pH 9.75) to the filtrate as specified in Scharer's original method gave a solution with a pH in the range of 8.6. The final pH was even lower when the borate buffer was added before filtration in an attempt to eliminate precipitation which frequently occurred with adjustment after filtration. The pH of the filtrate was obviously too low for complete formation of indophenol, and in a range where slight variations would produce large differences in test results (Fig. 4).

Improvement of buffering capacity by increasing the

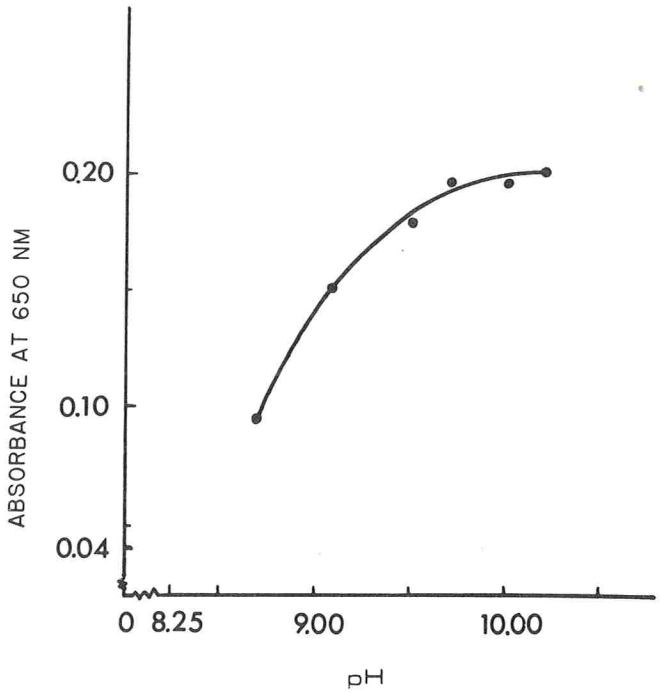

Figure 4. *Effect of pH on development of indophenol. Mean of three determinations at each pH.*

strength of the borate buffer (28.427 g/l) was impossible because of solubility limits. An alternative buffer, 2-amino-2-methyl propanol (AMP), was tried with successful results. Two buffers were required, one adjusted to pH 9.75 for preparation of buffered substrate, and the other a double-strength buffer at pH 10.25 for adjustment before filtration. As shown in Table 2, the first buffer was adequate for holding the pH of the

TABLE 2. *pH adjustment with AMP buffer in Scharer method for milk phosphatase*

Test stage	pH		
	Homogenized milk	2% Milk	10% Cream
Buffered substrate plus sample before incubation[a]	9.60	9.60	9.60
Following precipitation and filtration[b]	9.86	9.80	9.84

[a]Using AMP buffer, pH 9.75.
[b]1.1 ml AMP buffer 2 ×, pH 10.25, added per 20 ml buffered substrate before filtration.

milk sample at 9.60, the optimum for alkaline phosphatase activity (*7*). The second buffer provided a clear filtrate with a pH high enough for complete reaction of BQC with liberated phenol to form indophenol.

Application of AMP method

A pooled raw milk sample was prepared with equal volumes from 100 herd samples, and diluted with pasteurized-homogenized milk heated to 90 C for 1 min to 0.1, 0.25, 0.5, 0.75, and 1.0%. Each sample was analyzed five times by the AMP and Kay and Graham methods. The results are presented in Fig. 5 and 6,

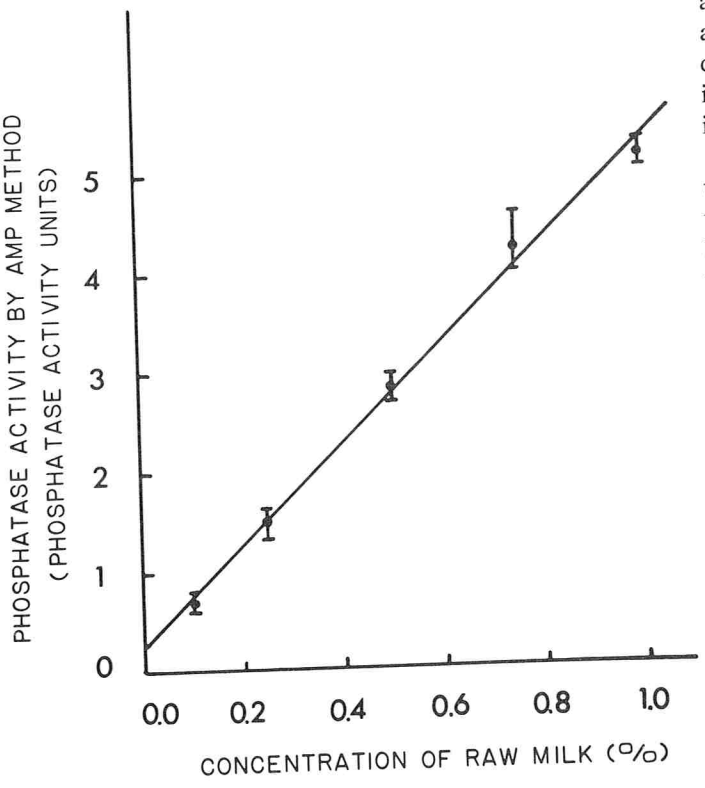

Figure 5. *Phosphatase activity by AMP method at five concentrations of a pooled raw milk sample. Mean and range for five determinations.*

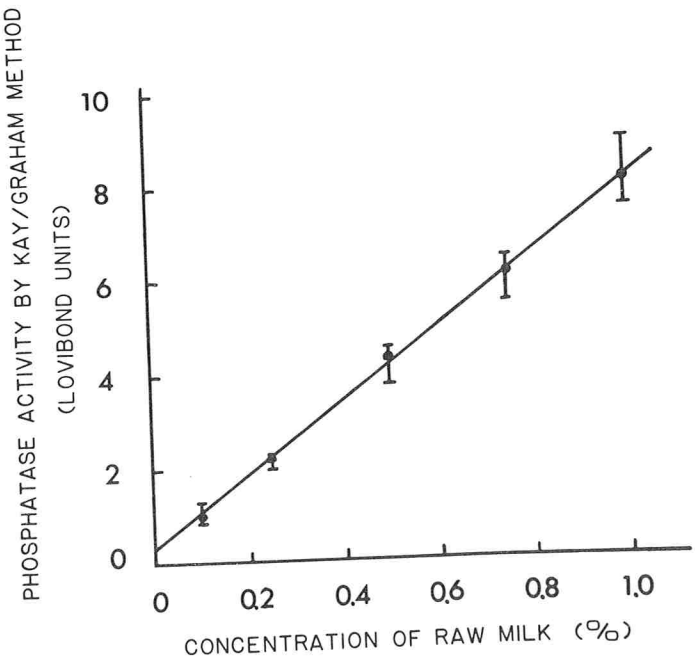

Figure 6. *Phosphatase activity by Kay and Graham method at five concentrations of a pooled raw milk sample. Mean and range for five determinations.*

demonstrating the linearity of the AMP method for increasing enzyme concentration, as well as a precision, indicated by the range for five replicate determinations at each concentration of raw milk, equivalent to the Kay and Graham method. The results shown in Fig. 5 also demonstrate that the AMP method, using a 1-h incubation period, can detect less than 0.1% of raw milk in pasteurized milk.

A standard for judging acceptable pasteurization by the AMP method was established by comparison against the Kay and Graham method, which has a recommended limit of 2.3 Lovibond color units (*5*). The equivalency of the two methods is shown in Fig. 7, indicating that 2.3

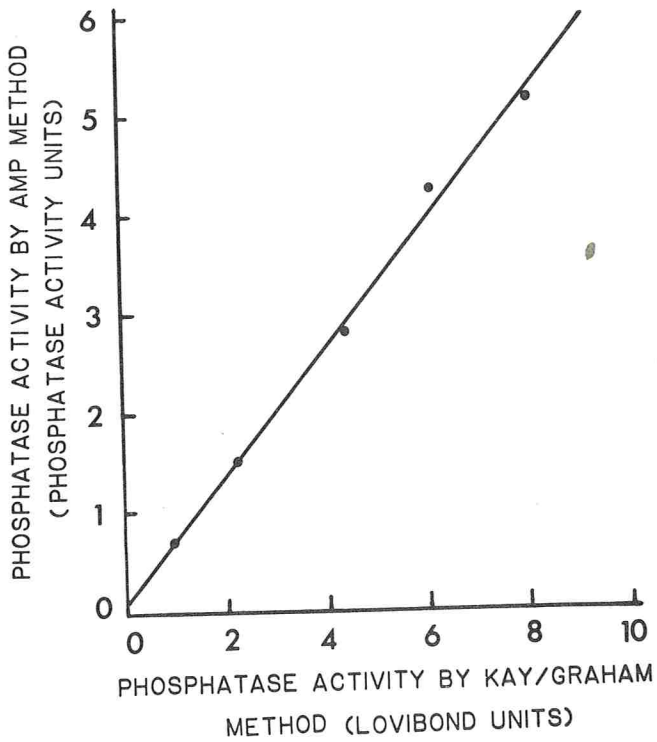

Figure 7. *Relationship of AMP and Kay and Graham methods for alkaline phosphatase in milk. Means of five replicates by each method at five concentrations of raw milk.*

Lovibond units by the Kay and Graham method is equivalent to 1.6 phosphatase activity units by the AMP method.

DISCUSSION

Horwitz (*4*) previously reported studies of some of the same parameters in Scharer's original method described here, and his work led to the modification now identified as Method II in *Official Methods of Analysis* (*2*). Horwitz had also observed that the borate buffer originally used by Scharer could not hold the pH high enough for complete reaction of liberated phenol to form indophenol, which he determined was pH 9.4 or above with 30 min at room temperature. We found that a pH above 9.6 was desirable, and could be obtained without subsequent precipitation problems by addition of AMP 2× buffer at pH 10.25 before filtration. We also observed that color intensity due to indophenol formation was

increased by use of 37.5 C for 5 min with no further changes after an additional 25 min at 21-23 C.

Our study showed that the volume of BQC used by Scharer and retained by Horwitz, i.e. 2 drops per 5 ml of filtrate, is inadequate for complete reaction of liberated phenol at concentrations that can be expected with under-pasteurized milk samples. This ratio was increased to 6 drops per 5 ml, which produced some visual distortion of the blue color at low enzyme activities but did not affect spectrophotometric measurement. BQC was retained in the AMP method, as it has been by *Official Methods of Analysis* (*2*), although the superiority of CQC as an indicator has been described (*3*).

The use of boiling water immersion for 5 min by Scharer, which we found resulted in substrate hydrolysis, was retained by Horwitz as "3 to 5 minutes" but modified in *Official Methods of Analysis* to "nearly 1 min." Our modification relies on immediate immersion in an ice bath and prompt addition of lead acetate to stop the enzyme reaction.

The modification which resulted from studies of Scharer's original method, designated the AMP method, showed good linearity with increasing enzyme concentration and a precision with a 1-h incubation period equivalent to the Kay and Graham method requiring 24-h incubation. The method is able to detect less than 0.1% of raw milk added to pasteurized milk. A standard of 1.6 phosphatase activity units (approximately equivalent to Scharer units, i.e. μg phenol per 5 ml of solution) is proposed for pasteurized milk on the basis of equivalence to 2.3 Lovibond color units by the Kay and Graham method.

ACKNOWLEDGMENTS

We wish to thank R. G. Reynolds for assistance in preparation of certain standard solutions and reagents.

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J. Milk Food Technol. Vol. 39, No. 3, Page 196 (March, 1976)

Microbiology of Mass Feeding Systems: An Introduction[1]

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The feeding systems selected for review in this seminar,—hospitals, military systems, universities, and airlines and cruise ships share many attributes with each other and with other similar systems.

These systems which are designated in-house feeding systems are complete in that the food, while it may be purchased preportioned, or even partially processed is prepared and served in-house and constitutes a main source of food supply for a large number of customers. The populations serviced are quite distinct and the systems are often of high complexity as reflected by the large assortment of menu items prepared. They are responsible for serving the three main meals and, for certain systems, numerous between meal servings are also involved. The physical plant for food processing and distribution is usually an integral part of the facility. It is obvious that additional in-house feeding systems could have been included in this seminar but as seen in the presentations those selected represent the major problems encountered in in-house feeding systems while at the same time certain problems will characterize a particular system.

In hospitals, food is prepared for a larger number of customers having a high susceptibility to infection. In contrast the military serves a generally resistant population but these meals often have to be prepared in submarines, in airplanes, or under field conditions. Airlines depend almost completely on centrally processed prepackaged meals but cruise ships are frequently obligated to serve very extensive menus with limited food preparation facilities.

These systems, while regulated by governmental agencies, in practice are monitored by techniques varying from the sophisticated to the most rudimentary, often but not always reflecting the risk involved to the consumer.

Modern food preparation techniques, to decrease labor costs have utilized more sophisticated labor saving production and distribution technology. These modifications incorporate a greater use of precooked, chilled, frozen items or items transported at elevated temperatures. In addition packaging, transportation, and reconstitution techniques are also undergoing changes in response to economic requirements. Whether or not these changes alter safety remains to be ascertained.

None of the papers at this seminar refer to such terms such as "Good Manufacturing Practices" (GMP) or to "Hazard Analysis—Critical Control Points" (HACCP). These terms are commonly employed in quality assurance programs by the FDA and by industry for more effective monitoring of industrial production facilities. While qualified personnel monitoring in-house feeding systems do in effect attempt to perform a hazard analysis, effectiveness is often negated by a failure to formalize the approach by not issuing written procedures supported by effective training and supervision. One difficulty of applying the HACCP—which is really a preventative approach for industry, to these feeding systems is that the facilities involved were not designed for industrial processing. In industrial facilities each product processing line is a distinct entity producing a single item. In the in-house feeding facilities many items are being produced simultaneously using common equipment. For example, the effective segregation of raw foods from contact with processed foods is a frequent problem in the limited cooking areas of the in-house feeding system.

The purpose of this seminar is therefore to find out to what extent these representative feeding systems are: (*a*) affected by microbiological problems, (*b*) developing procedures to monitor feeding systems, (*c*) deriving benefits from GMP and to what extent a breakdown in GMP must occur in order to result in outbreaks of food poisoning or infections, (*d*) conducting end product analysis and employing microbiological standards, (*e*) maximizing safety in relation to cost, and (*f*) determining the relative contribution of food hygiene and environmental sanitation to producing a safe product.

[1]*A Seminar on Microbiology of Mass Feeding Systems was convened by Gerald Silverman at the the 75th Annual Meeting of the American Society for Microbiology, New York, N.Y., April 27-May 2, 1975. Papers presented at the Seminar include: "Microbiology and Hospital Feeding Systems" by Ruth B. Kundsin and Hollis A. Bodman, "Assurance of Microbiological Safety in a University Feeding Safety" by M. Solberg et al.: "Microbiological Aspects of Certain Military Feeding Systems" by Durwood B. Rowley; and "Food-and Waterborne Disease Outbreaks on Passenger Cruise Vessels and Aircraft" by Michael H. Merson et al. These papers will appear in the* Journal of Milk and Food Technology.

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Microbiology and Hospital Feeding Systems[1]

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ABSTRACT

Food service in a hospital involves the close collaboration of physicians, dietitians, and microbiologists. The hygienic environment recognized as essential for patient care areas is equally essential for food preparation areas. The food preparation for critically ill patients with individualized nutritional requirements involves not only house diets and their modifications, but food fed in highly specialized ways such as tube feeding via nasogastric tube and hyperalimentation parenterally infused. Opportunities exist for microbial contamination in these unique situations which require alert microbiologic intervention for patient protection.

Hospital patient food service differs from all other types of mass food preparation and service because the individuals fed are the most sick and the most incapacitated members of our society. Staphylococcal enterotoxin, hepatitis virus, and salmonellae will incapacitate a healthy person but can be the final death-dealing infection in already critically ill patients. Contamination of food with potential pathogens is considered to be critical in the outcome of a burned patient, an immunosuppressed patient, or a patient in renal failure.

The Peter Bent Brigham Hospital is a 325-bed hospital providing medical care for acutely ill adults. Because it is a teaching hospital for Harvard Medical School, patients with a wide range of problems are admitted. Specialties, such as cardiovascular and renal surgery, treatment of burn patients, and patients with blood dyscrazias and metabolic disorders, concentrate individuals with arcane and exotic diagnoses in the hospital. This necessitates food systems for highly individualized food preparation. Sixty-three percent to 68% of patients are on modified diets. The menus for the house diet for the remaining 32-37% are recycled every three weeks. As the average duration of patient stay is 11 days, most patients are not exposed to the same menu twice.

Since 1963 the system has been centralized. Dishwashing machines and facilities for food preparation exist in one central area except for three units where patients with special problems are located. These are the Renal Unit, the Critical Care Unit, and The Clinical Center.

Four basic menus are prepared in the main kitchen. On the trayline sample portion sizes are weighed and placed in front of dispensing personnel. Trays with pre-portioned servings are placed in food trucks with hot and cold compartments where hot food is kept between 185-200 F (85-93 C), and cold food is kept between 45-55 F (7-13 C). The truck, with its 20 trays, is delivered by dietary personnel to the patients within one half hour. These trucks are cleaned three times a day with hot water, soap, and ethyl alcohol, and thoroughly scrubbed down weekly with stronger detergent. The four basic diets prepared in the main kitchen are: (*a*) regular or house diet, (*b*) modified diet (calorie or nutrient manipulated), (*c*) sodium restricted diet (frequently adjusted to accomodate Nos. *a* and *b*), and (*d*) modified consistency diet.

The total team of dietitians for in-patient services is 14 full time registered dietitians comprised of the following: five in general patient areas, four in specialty patient areas, three in production, and two administrators, plus 16 dietetic interns. Three full time and four part time additional dietitians are involved with ambulatory or outpatient care. Although this may appear to be an inordinately high number of dieteric personnel, teaching hospitals by their very nature employ more personnel than non-university associated hospitals. Specialized research dietitians are located in the three areas of intensive dietary supervision previously mentioned. These three areas have their own kitchens and receive food in bulk for distribution to special patients.

Certain basic rules are followed throughout the hospital. No raw eggs in any form are served to any patient unless a doctor makes a written request. Raw fruits and vegetables are not served to patients on a minimal bacteria diet such as burned patients or patients awaiting a bone marrow transplant. All unused packaged food items, such as cold cereal, milk, sugar, and jelly, are discarded because of concern for contamination once the items have been distributed to patients. On patient floors, provisions for juice and snacks on each ward are in refrigerators which are cleaned by the housekeeping department on a routine basis. In the main kitchen, dishwashing temperature is 150-160 F (66-71 C), rinse temperature is 180 F (82 C). Washable canvas gloves are used to take dishes from the machine. Plastic disposable gloves are used in the preparation of cold food.

CRITICAL CARE CENTER

In this intensive care center most patients are critically ill and few are consuming a house diet. Patients with a normal gastrointestinal tract, but who are unable or

[1]*Presented in a Seminar on Microbiology of Mass Feeding Systems held at the 75th Annual Meeting of the American Society for Microbiology, New York, N.Y., April 27-May 2, 1975.*

unwilling to take adequate nutrition orally, are fed via nasogastric, gastrostomy, or jejunostomy tubes. The feedings are generally a canned commercially prepared product which must be refrigerated after opening.

Patients who have problems of malabsorption are fed by mouth or via tube defined formula diets consisting of amino acids, glucose, fats, vitamins, and minerals. These are supplied in powder form in cans or sealed envelopes and must be reconstituted with water, blended, refrigerated, and used within 24 to 48 h.

Patients who cannot be orally or tube fed due to malfunction or extensive surgery to the gastrointestinal tract receive total parenteral nutrition. Products used in these intravenous infusions are sterile and are prepared under aseptic conditions in the pharmacy. The nitrogen sources can be natural protein hydrolysates or crystalline amino acids and are combined with concentrated glucose, vitamins, and minerals. Good calorie/nitrogen ratios are desired for optimal nitrogen utilization. Because these solutions are hypertonic, central venous catheters are used for their infusion.

Recently solutions of isotonic amino acids without glucose have been used via peripheral infusions but this technique is not truely "hyperalimentation" or "total parenteral nutrition." Intravenous lipid preparations are not yet available for general clinical use in this country, but they are considered non-toxic to blood vessels and can be administered via peripheral veins.

Recently the discovery of microorganisms in these liquids for infusion and septicemia associated with their administration have caused concern (*2, 3*). These solutions are prescribed by physicians, prepared in the pharmacy, and infused by nurses under physicians' supervision. The research dietitian must, however, be involved as she evaluates patients for parenteral nutrition and monitors their nutrient intake. The microbiologist should also be consulted in the preparation and delivery of these products.

Burned patients are located in this critical care center. They can be fed by mouth, nasogastric tube, or parenteral hyperalimentation. They are followed intensively as to sequential surface bacteriology. In our experience the original surface colonization with *Bacillus* species is followed by gram-positive cocci, and then fungi and gram-negative organisms. *Pseudomonas aeruginosa* is the most dreaded organism on the surfaces of burned patients. Because uncooked fruits and vegetables are known to harbor these organisms as pointed out by Kominos et al. (*6*), they have been eliminated from the diet of burned patients.

THE CLINICAL CENTER

This patient care center has research patients as its predominant population. Some are medically ill, others are normal controls undergoing metabolic studies. Two dietitians are required for the 21 patients.

Metabolic studies entail use of constant diets necessitating that all food items consumed by the patient be weighed on gram scales. To maintain consistency in nutrients, canned fruits and vegetables are used rather than fresh produce. Aliquots of the complete diet are periodically blended and analyzed to determine exact nutrient composition.

Patients undergoing bone marrow transplantation are also maintained within this unit in a special room adapted to provide a benign aseptic environment. Bacterial monitoring of both the patient and his environment is done throughout the period of patient confinement, beginning 11 days before actual transplantation.

The patient's room is thoroughly disinfected immediately before entry. The patient is then admitted to this clean room following bowel and vaginal antibiotic preparation to minimize her (his) own bacterial flora.

Pretransplant the patient receives potentially lethal doses of chemotherapeutic agents or total body irradiation to suppress his existing abnormal bone marrow and promote engraftment. This treatment causes the patient to become extremely susceptible to fungal, viral, bacterial, and parasitic infections.

Personnel involved in patient care and food preparation are cultured for nasopharyngeal and skin carriage of microorganisms. Gram-negative carriers have been excluded from the patient's room, but not gram-positive carriers. All people entering the patient's room wear masks, caps, surgical scrub suits, gowns, and booties. Following bone marrow transplantation, the patient is maintained in the described type of isolation until he begins forming his own leukocytes from the transplanted marrow.

Throughout this critical period the patient receives a minimal bacteria diet prepared in the metabolic kitchen by specially trained dietary personnel using aseptic techniques. Foods are prepared by steam autoclaving, dry heat sterilization, and pressure cooking. A variety of canned fruits, vegetables, entrees, and beverages are frequently utilized to provide interesting and nutritionally adequate meals. No fresh fruits or vegetables are served. Dishes and utensils used for food preparation and service are steam autoclaved or dry heat sterilized. Bacterial contamination is monitored by periodic sampling of all food items in test tubes containing trypticase soy broth. These specimens are later processed in the bacteriology laboratory.

Our results have indicated that two-thirds of the hundreds of specimens sampled are sterile for aerobes and fungi. One-third have predominantly *Bacillus* species, *Staphylococcus epidermidis,* and diphtheroids.

THE RENAL UNIT

Patients in renal failure and on hemodialysis do eat but have a very restricted diet. Portions are monitored for caloric as well as nutritional content. Fluid restrictions are also observed for patients who have a diminished urine output. Once a patient has received a kidney transplant he is immunosuppressed and susceptible to

fungal, viral, bacterial, and parasitic infections just as are the bone marrow transplant patients (*1*). The patient has to be closely watched for oral, oesophageal, or anal lesions. All of these necessitate an alteration in the texture of foods. He also has to be observed for steroid-induced diabetes. Following confirmation of diabetes by serum blood level determinations, the carbohydrate content of the food is altered.

When infection in a patient is noted by the clinical dietitian in the unit, disposable trays, dishes, and utensils are given to the patient so that handling of these contaminated items in the kitchen is eliminated. (This procedure for infected patients is followed throughout the hospital).

Because the risk of acquiring hepatitis is a concern in dialysis units, no eating or smoking is permitted in these units by patients and personnel. This rule is strictly enforced. The recent reports of finding hepatitis antigen in the saliva of patients with antigen in the blood has revised our isolation techniques for these patients from simple glove and needle precautions to maximum precaution techniques. These increased precautions result from a concern for all possible modes of transmission that are associated with mouth secretions and air-borne aerosol formation by sneezing, coughing, and talking (*5*). Hepatitis antigen is also relatively stable on surfaces even after prolonged exposure to ambient environmental conditions (*4*). The hospital Infection Control Committee is currently considering recommendations that hepatitis antibody-positive personnel be assigned to patient care in the dialysis and transplant units.

CONCLUSIONS

The main objective of this discussion is to stress a philosophy of cooperation and collaboration between microbiologists and dietitians rather than to elaborate on the minutiae of monitoring food and dishes. The role of the microbiologist in Hospital Food Service should include the following.

(*a*) Promote ongoing education of all personnel who handle or prepare food with training programs for food service employees by showing finger cultures, stressing good personal hygiene and handwashing.

(*b*) Collaborate with dietitians in programs of random sampling of foods and equipment, such as cultures of food for bone marrow transplant recipients. Dietitians should be alert to foods with a questionable appearance. (A bubbling portion of Chicken a la King was found to be loaded with *Aeromonas hydrophilia*).

(*c*) Communicate with dietitians when concepts of microbial transmission change. The current reports that hepatitis is transmitted by saliva and may be airborne necessitate scrupulous concern for utensils involving oral contact.

(*d*) Be available for questions concerning day to day microbiological problems associated with food service. For example, what to do with trays returned to the kitchen with vomitus on them when no facilities for autoclaving exist on all wards; what kind of germicides to use on equipment when potential contact with food exists.

ACKNOWLEDGMENTS

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Assurance of Microbiological Safety in a University Feeding System

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ABSTRACT

The sanitation surveillance system was developed with standards and follow-up actions as a part of an assurance program for microbiological safety in a university feeding system. The surveillance is based upon an adhesive contact and transfer tape system for surface monitoring and a micropore filter system for monitoring efficiency of the associated laundry operation. Results compiled from 3 years of operation generally show continued reductions in numbers of items testing below fully satisfactory. Specific systems of microbiological data analysis for detection of some mechanical or procedural breakdowns were developed. The overall cost for the entire laboratory part of the food safety assurance program was less than 0.5 cent per meal served.

INTRODUCTION

The major objective of a food safety and protection program is to insure preventive practices such that a food safety breakdown does not occur. To achieve such a state requires a level of effort greater than is required to correct a deficiency which has been exposed through an outbreak of food borne disease. The reward of prevention is much greater since the reputation of the establishment is maintained and the potential law suits are avoided. The complete confidence of the customers should be a major objective of an establishment. A single breakdown of the system is long remembered while the usual day to day successes are expected and go seemingly unnoticed.

The university feeding system is no different from any other in its safety needs but is often considerably different in its clientele. The customer is usually in a high state of physical well-being and at an age of high resistance to disease. However, the customer is often a regular 2 or 3 meal per day, 5 to 7 days per week consumer, thereby creating a great demand for a very large variety of items on the menu, and a relatively long menu cycle. The customer is generally free to leave the system. Many university feeding systems continue to have subscription eaters but almost all have an active cash activity to provide the students with greater freedom of choice. The university student can be vociferous and often has a ready and willing press waiting in the form of numerous student publications, always eager to criticize a university operated or controlled activity.

[1]*Presented in a Seminar on Microbiology of Mass Feeding Systems held at the 75th Annual Meeting of the American Society for Microbiology, New York, N.Y., April 27-May 2, 1975.*

This paper will describe a microbiological safety assurance program which was developed, activated, and is now completing its third year of operation. The program was designed to provide regular surveillance, evaluation, and presentation of facts to persons at all operational and managerial levels to insure that each and every person knows exactly what is expected of them.

The system is based upon strong laboratory support in terms of analyses, interpretation, and communication; and strong support from a convinced management through a trained sanitarian with organizational power. The organizational structure may be seen in Fig. 1. The

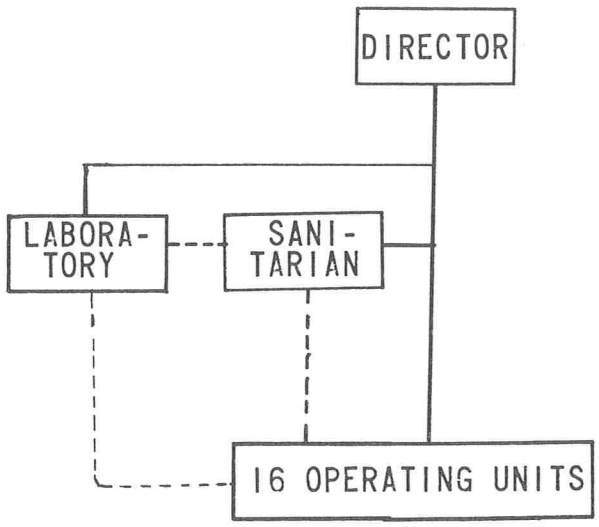

Figure 1. *Organizational chart showing the position of the laboratory and the sanitarian with respect to the operating units and the director of all feeding operations.*

sanitarian is in direct communication with the supervisors of the 16 operating units and with the director whose support is available in every move made with respect to food safety. The success of the system is a function of the combined activities of the laboratory's communications effectiveness which presents interpretations of results with recommended actions to the sanitarian and the sanitarian who then communicates effectively with the operations managers and the operations personnel. The sanitarian also ensures that corrective action is taken as required.

The mass feeding system is composed of 16 units; 15 are feeding operations and the 16th is a laundry. The feeding units vary in size from 1,000 meals per week to approximately 40,000 meals per week. Six of the units are snack bar operations with limited menus. The others are capable of full-scale feeding. During an average week, approximately 140,000 meals are served.

Each unit is visited on a regular basis for microbiological evaluation. The frequency of visitation is regulated by the number of meals being served. Thus, the units which have the greatest number of contacts with customers get the most attention and usually need the most coverage due to the pressures of the larger operations.

TABLE 1. *A breakdown of feeding units by size, frequency of microbiological evaluation and level of samplings based upon meals served*

Evaluation frequency	Activity level (meals/week)	Number of feeding units	Samplings per 1,000 meals
Weekly[a]	> 40,000	1	0.75
Every 2 weeks	12,000-20,000	3	1.00
Every 4 weeks	8,000-12,000	2	1.00
Every 6 weeks	< 8,000	9	0.67-4.25

[a]Double sampling on a biweekly basis due to logistical considerations.

Table 1 relates the size of the unit to the frequency of visitation and classifies each of the currently active, 15 feeding units into a category. There are no units which serve between 20,000 and 40,000 meals weekly. Within the units serving less than 8,000 meals weekly, there are some as low as 1,200 meals weekly and others as high as 7,600 meals weekly. In this category, five of the nine units are small enough to be placed in a less than every 6 weeks evaluation category but it was arbitrarily decided that a lesser frequency would sacrifice too much in effectiveness.

The surveillance targets included in the program are the physical facilities, operational practices, raw materials, and end products. These objectives are accomplished through inspection, training, and testing. Periodic inspections based upon the Retail Food Establishment Code, which is part of the state statutes, are conducted. The sanitarian is trained to do this phase of the program. Equivalent inspection is also accomplished by the state or local health officials on an annual or more frequent basis. Testing procedures include microbiological, instrumental, and sensory evaluations. The instrumental tests are primarily concerned with temperatures of foods and storage facilities. The sensory tests involve observations of the general status of equipment, supplies, and facilities. The personnel training is carried out by formal classroom presentations, of the 1 day or 2 day program type, several times during the year. This, too, is conducted and organized by the sanitarian and takes advantage of the slow periods which occur during student vacations and breaks between regular teaching periods. Lectures, slides, film strips, films, and teaching aides of many types are used. A test has been developed to evaluate the degree of learning which has taken place. This test is composed of a general section and a specialized section. The general section covers aspects of such significance that workers at all levels and in all jobs should know these facts. There are specialized sections for food handlers, including dish and pot washers; and for non-food handlers, such as porters, truck drivers, and maintenance persons. All managerial personnel are examined in all sections of the test.

The remaining functions are those which intimately involve the laboratory and are microbially oriented. Included are testing and the development of microbiological specifications. The laboratory is organized in accordance with Fig. 2. The program may

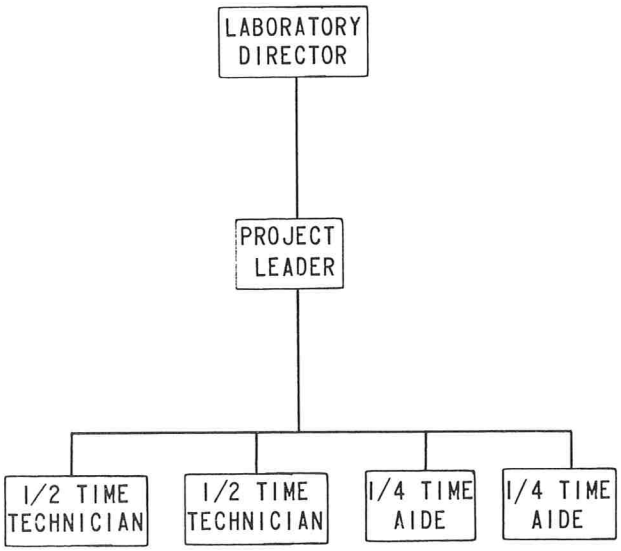

Figure 2. *The organizational chart for the laboratory operations of the food safety assurance program.*

be divided into two segments, each requiring approximately 50% of the work hours available. The first part includes the testing of raw materials, finished product, and development of microbial specifications. This finished product part of the program serves as an audit of the overall effectiveness of the entire microbiological food safety assurance program. Most of the raw material testing is evaluation of new suppliers or new products being proposed for addition to the menu. All of the food evaluation testing has examined products for the common indicators, including total aerobic plate count, coliforms, and *Escherichia coli,* as well as the common food pathogens; *Staphylococcus aureus, Clostridium perfringens,* and *Salmonella.*

The second sgement of the laboratory operation is called the "surveillance program." The surveillance program involves the microbial evaluation of sanitary operation through testing food contact surfaces, ancillary surfaces, and laundry or linen.

PROCEDURES

Surface testing is accomplished with a transparent,

sticky tape (Birko Chemical Co., Denver, Colorado) contact technique utilizing Plate Count Agar (PCA) as the culture medium. Incubation is carried out at 37 C for 20-24 h. Plastic petri dishes are stamp-printed on the bottoms with fast drying ink to divide the dishes into five squares each measuring 1 in^2 and each labeled with a letter from A through E, as may be seen in Fig. 3. The

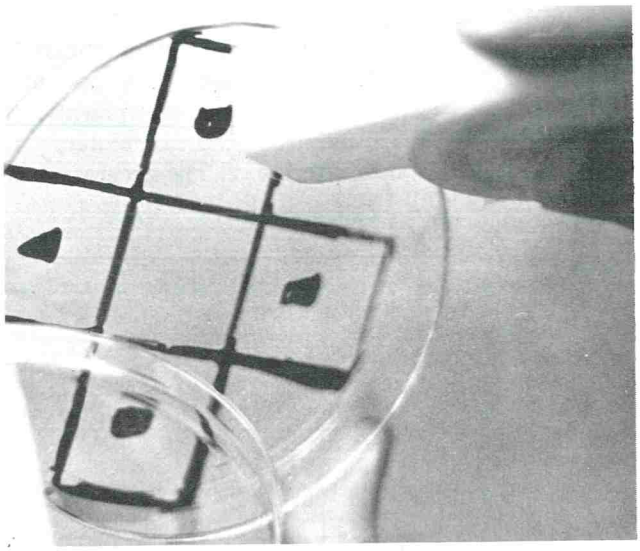

Figure 3. *The adhesive contact tape dispenser and the subdivided Petri dish containing Plate Count Agar.*

petri dishes are prepoured, 18-24 h before use and left inverted at room temperature until they are taken from the laboratory to the test site. The gas sterilized contact tape is ¾inch wide and is in a dispenser designed to permit the tape to be drawn out of the dispenser, looped over a ¾in^2 finger-like protrusion on the dispenser, and then placed on the surface to be tested. If the surface being evaluated is not flat, it is easy to carefully place a finger on the back of the tape and press it onto the irregular surface. This procedure can even be accomplished upon the tines of a fork or on the surface of a beverage spout, a strainer or any other device. The used surface of the tape is then replaced on the dispenser finger and placed in contact with the PCA surface within one of the lettered squares. Each petri dish is numbered on its cover, and a record is kept of each sampling on the "Sanitation Evaluation Survey Report" sheet (see Fig. 4). The petri dish number is placed in column 1. Column 4 is filled in with the actual physical location of the equipment or the implement when it was tested. The part or section of the item being tested is entered in column 5, and a check (√) is placed in column 6 if the item was in use as opposed to clean and ready for use. The remainder of the form is self-explanatory. This system is used for evaluation of both food contact surfaces and ancillary surfaces, such as trays, tables, carts, cabinets, walls, ceilings, and others.

Contamination levels are determined by counting the colonies formed using a Quebec Colony Counter. Routine sampling of a feeding unit involves 30 surface evaluations. There are approximately 50 different items which appear in the test reports. The number could be much larger but is kept small by grouping items which are used and cleaned similarly and which are made of similar materials. Evaluation of the contamination significance is based upon arbitrary standards which are presented in Table 2. For purpose of comparison, 10

TABLE 2. *Surveillance standards for surface contamination as applied for clean and in use items being evaluated by the adhesive contact tape method*

Condition	CFU[a]/3/4 $inch^2$	
	Clean	In use
Acceptable	< 5	< 20
Level of Concern	5-10	20-40
Potential Hazard	> 10	> 40

[a]Colony forming units

colonies from the adhesive contact transfer would be equivalent to 70-80 colonies on an agar contact plate (RODAC) which has a 4 in^2 surface. This relationship is based upon unpublished recovery data obtained using *Bacillus subtilis* spores, artificially inoculated on stainless steel surfaces which indicated approximately 42% recovery using contact tape as opposed to approximately 59% recovery using surface contact agar plates (personal communication with J. S. Rose, Birko Chemical Co., Denver, Colorado).

Table 2 also shows that there are different standards for clean surfaces as opposed to surfaces which are in use. The rationale employed in establishing these standards is that even "in use" equipment must be cleaned if the microbial build-up exceeds some reasonable limit. The actions which result from the evaluations are a routine report for all acceptable items, a special letter report for "level of concern" items with a routine follow-up which involves resampling of the item during the next regular surveillance, and a special report for "potential hazard" items with a special follow-up within 1 week. The special follow-up involves an extensive survey of the problem item or items. If this follow-up indicates a continuing problem, a systematic study will be initiated to determine the cause of the problem, and corrective action will be suggested. All special letter reports are given personal attention by the sanitarian, and the results are communicated to the operations personnel.

A centralized laundry handles all uniforms, aprons, towels, linen, etc. for 13 of the 15 feeding units. The two remaining units use an outside laundry service. The microbiological effectiveness of the laundering operation is monitored every two weeks. Sample items are selected from completed items ready for delivery to users. The samples are asceptically handled after collection. An area of significance, such as the front of an apron or coat, is selected and placed on top of a micropore filter which is in place on top of a fritted glass funnel base inserted into a 500-ml filter flask. A funnel top is placed on top of the garment and a clamp is fitted into place with care

Date of
test completion: _____

Food Science Department
Rutgers The State University
New Brunswick, New Jersey 08903

Initials:
Tester: _____
Facility
representative: _____

SANITATION EVALUATION SURVEY REPORT

Facility _____ Date: _____ Time: _____ Tester(s): _____

(1)	Sample code (2)	Equipment nomenclature (3)	Location (4)	Area sampled (5)	In use (6)	Physical appearance and comments (7)	Contamination level organisms/3/4in^2 (8)	Remarks (9)
	A							
	B							
	C							
	D							
	E							
	A							
	B							
	C							
	D							
	E							
	A							
	B							
	C							
	D							
	E							

Figure 4. *The sanitation evaluation survey report form which is prepared by the laboratory and submitted to the sanitarian for subsequent dissemination to the operational units.*

taken to minimize the amount of fabric within the clamp. The micropore filter is a 47-mm diameter disc with a 0.45-μ pore size (Millipore Corp., Bedford, Mass.), and the filter assembly is the appropriate one from the same source. One hundred milliliters of 0.1% sterile peptone water is poured through the fabric and the filter with suction supplied by a water aspirator. The filter is then removed and placed upon the surface of a prepoured Petri dish containing Tryptone Glucose Extract agar, which is then incubated at 37 C for 20-24 h before counting the colonies formed. Arbitrary standards were set up at ≤ 6 colonies being acceptable. Results are reported to the sanitarian who communicates them to the operations personnel.

RESULTS AND DISCUSSION

A summary of results, broken down by year of operation, appears in Table 3. The first year (1972/1973) was one of gradual buildup with units being added to the program as the procedures and personnel became ready for them. The follow-up totals expressed in percent of total samples are indicative of the effectiveness of the overall program in establishing an awareness of problem areas and the need for continual vigilance on the part of operations level personnel. The steady drop in follow-up total percent may be seen in Fig. 5 with a projected point for the 1975/1976 year. The shift during 1974/1975 of items out of the "hazard" level and into the "concern" level and into the "acceptable" level is evident from Table 3. There is some bias towards higher numbers in these analyses since many of the results are problem area follow-ups which would be more likely to repeat as problems, than would other routine samplings.

TABLE 3. *A three year summary of sanitation surveillance results for approximately 50 categories of surfaces tested expressed in terms of the established standards and the total of items requiring follow-up testing*

Tests and results	72/73		73/74		74/75	
	Number	%	Number	%	Number	%
Tests	3,138	—	6,048	—	5,285	—
Concern	236	7	267	4	234	4
Hazard	466	15	612	10	344	7
Follow-up, total	702	22	879	15	578	11

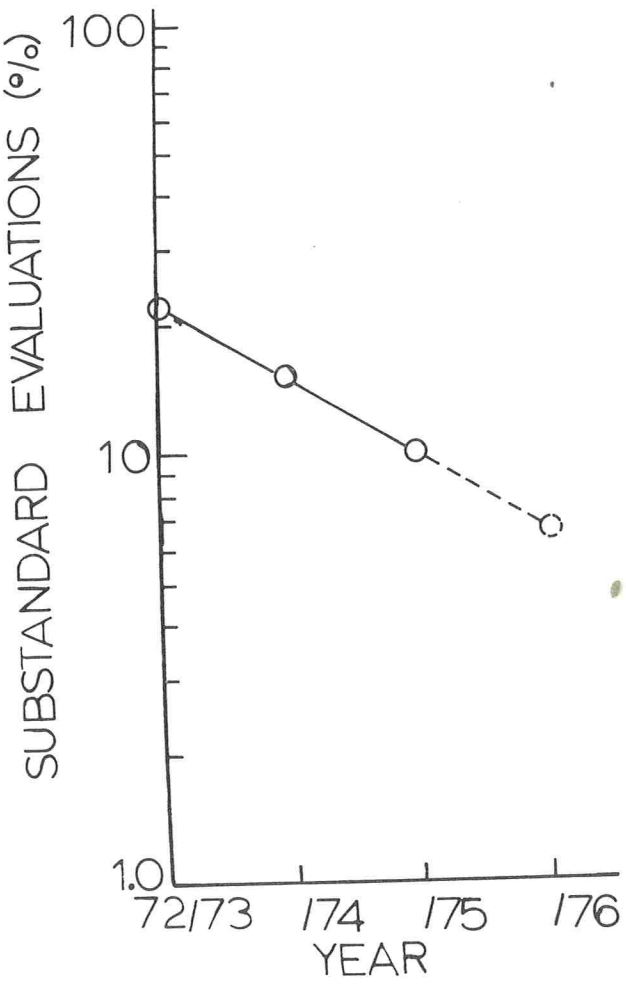

Figure 5. *The percentage of substandard surface surveillance samples encountered on an annual basis with a projection for one year in the future.*

TABLE 4. *The number of substandard samples encountered during testing of various specific items expressed on an annual basis*

Equipment	% Requiring follow-up		
	72/73	73/74	74/75
Vegetable cutter	88	12	11
Slicers	75	17	8
Scales	67	27	13
Cutting boards	66	23	25
Rubber	—	24	26
Wooden	—	10	22
Plastic	—	33	20
Trays-fiberglass	41	21	10
Student	—	9	11
Kitchen	—	36	9
Mixing bowls-st. st.	26	17	11

Table 4 considers some specific items which are among the most troublesome ones encountered. Early in the program, it was observed that fiberglass trays, which are used by students to carry their food from the serving counters to the tables, were showing high contamination levels at a higher than expected frequency. Study of the problem showed that worn trays were the principal offenders. Once these were replaced, the problem diminished considerably. The fiberglass trays provided an additional quantity of unexpected information. The trays are washed in the dish-washing machines in each unit. The fiberglass tray is probably among the least conductive materials washed in the machines. When the trays from one unit yielded a spreading type colony which was a gram positive sporeformer, an operations study was conducted. The study led to the observation that the dish-machine had several clogged spray heads. On another occasion, the isolation of similar organisms led to an improperly functioning mixing valve in a dish-machine. The observance of spreading colonies from tray sampling has become an indicator of dish-machine maintenance problems. Since the trays

probably get the least heat treatment in the washer due to their low thermal conductivity, they provide a margin of safety to other materials going through the same machine. This has been evident since isolations of these typical spreading organisms has not been observed on dishes or silverware passing through the same machine.

All of the most troublesome items from the first year of the surveillance program were substantially reduced in frequency of required follow-up activity during the second operational year. In the 1972/1973 year, there were few fiberglass trays in use within kitchen areas. As the reports showed the continued high level of cleanliness of the trays, the operations personnel began using more and more of the trays for storage of foods, resulting in much higher soil levels and many more unacceptable microbial evaluations. Control was achieved over this problem by discouraging use and requiring that all fiberglass trays used within the kitchen area had to be wrapped in clean saran.

The dramatic change in follow-up frequency for vegetable cutters resulted from the replacement of machines containing pitted aluminum cutting bowls with new machines manufactured with stainless steel cutting bowls. The marked improvements in sanitary quality of slicers and scales is attributed to the surveillance program and the resulting increased awareness for proper cleaning and maintenance of such equipment since no new equipment purchases were involved.

Cutting boards showed dramatic reductions in the frequency of unacceptable samplings between 1972/1973 and 1973/1974 and a slight increase between 1973/1974 and 1974/1975. The overall improved conditions are the result of constant testing and development of improved cleaning procedures. Cutting boards are now scrubbed by brush with detergent, washed in the dish-machines, sprayed or soaked in sanitizing solution and rinsed clean before storage for subsequent use. Most of the wooden cutting boards were replaced during 1972/1973. It is evident that more of these became worn and uncleanable with additional use. In 1973/1974, a new plastic cutting board was introduced. It was touted as being a super board, and the result was that operating personnel did not give it the attention needed to insure microbial quality maintenance. The problem was brought under control by demonstrating that cleaning processes for this item were as critical as for any other cutting board. During the 1974/1975 year, the plastic cutting boards were in line with the rubber cutting boards.

The data given were selected from the significant problem areas. The lesser problem areas demonstrate similar overall trends. This is evident from Table 3 when it is recognized that approximately 50% of the follow-up total is in items other than those listed in Table 4. Results of surveillance in the laundry operation demonstrate that approximately 80% of all samples are within the acceptable region of ≥ 6 colonies per micropore filter disc. This result was not surprising since the facility was new and all of the equipment was new. The results in the 1974/1975 year showed a sudden increase in unacceptable samples during one month. This was traced to a change in detergent made by the supplier due to a shortage at one time during the year. The entire problem disappeared when the regular detergent was put back into use. This occurance has established a certain level of respect for the surveillance program from the laundry operations personnel who now want to know how good a job they are doing on a more regular basis.

The real effectiveness of the program can be measured only in terms of the safety of the food being served to the customers. Three years of total freedom from illness attributable to food borne pathogens has been the result. The microbiological surveillance program contributed to the food safety through the establishment and continual reinforcement of operations personnel awareness and through the detection of procedural breakdowns so that corrective action can be taken before a serious problem can occur.

The entire cost of the laboratory program including food sampling and sanitation surveillance is less than 0.5 cent per meal served.

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Various Environmental Health Structures in California

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ABSTRACT

Through an administrative approach eight models of environmental health structures are examined with special reference to the Los Angeles County Environmental Management organization. The first six models represent existing California Environmental Health structures. Models I, II, and III are examples of those existing within the health departments or agencies. Models IV, V, and VI represent environmental health structures in comprehensive environmental agencies. Three major developments affected present structural organizations and programatic content of environmental health the most. These are: the California Environmental Quality Act, California Assembly Bill 3197, and Title 17 of the California Administrative Code. Looking ahead, two alternatives for future changes in California environmental health structures are outlined in Models VII and VIII.

Environmental health organizational structure is influenced by its legal basis, tradition, practices, prevailing reorganization efforts, current social trends, and contemporary environmental needs. Traditionally, the authority and responsibility for administering environmental health programs have been associated with health departments and the Health Officer. This is reflected in the fact that most California environmental health structures still exist within health departments or the recently organized health agencies. However, few California environmental health programs are being admistered within the framework of Comprehensive Environmental Agencies. The first study of various environmental health structures in the seventies was conducted by Lynch (*5*) on behalf of the California Conference of Directors of Environmental Health in 1972. Lynch's study was updated by Stone (*10*). And, both studies have been updated by the authors of this paper.

This article does not only investigate the various existing structures but probes further into the major developments influencing them and provides two administrative alternatives.

It is importnat to recognize that there is a reciprocal relationship between what happens in California and what takes place in other states and at the Federal level. Several articles have provided an insight into such relationships. Other publications by El-Ahraf (*3*) and El-Ahraf and Hanson (*4*) examined, *Alternatives in Environmental Health Administration* on national, state, and local levels and defined further steps to formulate viable environmental structures. These steps include: (*a*) establishment of a philosophical foundation for environmental health programs that defines health as "complete state of physical, mental, social, and environmental well-being and not merely the absence of disease, infirmity or eco-pathological conditions", and (*b*) development of sound principles and guidelines for environmental health structures within different organization settings, i.e., The health agency, environmental agency, or an independent agency.

MAJOR CATEGORIES AND ADMINISTRATIVE MODELS OF CURRENT ENVIRONMENTAL HEALTH STRUCTURES

The current structures of the California Environmental Health Programs fall within two categories according to the parent organization. (*a*) *Those within the health department or agency* (Three representative examples are shown in Fig. 1). (*b*) *Those within the Comprehensive Environmental Agency* (The three available are shown in Fig. 2). Following is an analysis of the six examples of existing environmental health structures in California.

Environmental health structure in the health department (or agency)

Because most local Environmental Health Programs are within health departments or agencies, only the three major representative models (I, II, and III) are shown in Fig. 1. This category covers 43 health local environmental health programs.

Both Models I and II represent, basically, the traditional approach to environmental health administration. In structural terms, these models reveal the administrative leadership is provided by a medical health officer or a medically oriented Health Services Director.

The Director of Environmental Health is placed in a

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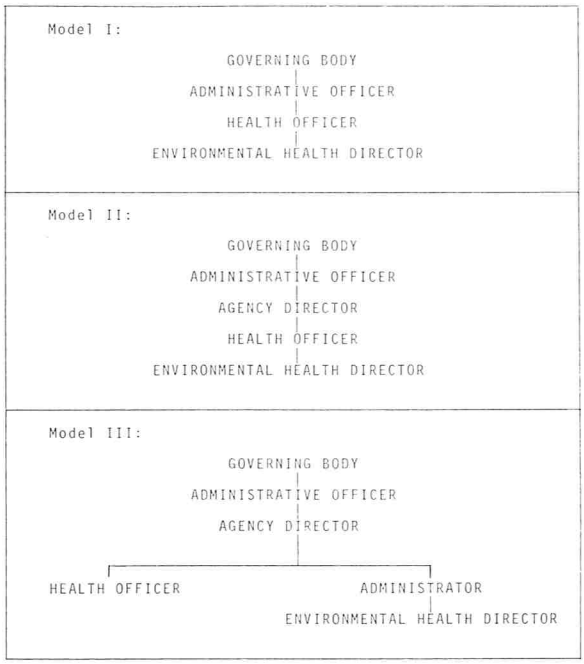


Figure 1. *California environmental health structures within health department or agency.*

position to deny him direct access to the decision makers in the political and public arenas. This fact is further aggravated in Model II where Environmental Health is moved another step in the downward direction.

Also, Models I and II indicate a less than co-equal arrangment between the two wings of public health: environmental health and personal health services. Organization priorities and allocation of resources are often influenced by such administrative arrangements. Furthermore, environmental health programs tend to be narrowed to reflect the major philosophy of the Department of the Agency. Such philosophy is usually based on a narrow definition of health that values curative measures as a primary function of health service. Also, such philosophy is largely influenced by a microbiological and epidemiological reason for preventive measures.

Some local environmental health programs have been able to expand their own philosophical basis of action and subsequently their programatic activities, under the above mentioned arrangements. But these are the exceptions and not the rule.

Model III exists only in one California county and reflects the most undesirable administrative structure of environmental health. In this Model environmental health program activities are constrained by the traditional approach of the health agency. In addition, the immediate direction of the program is in the hands of a non-environmental health administrator.

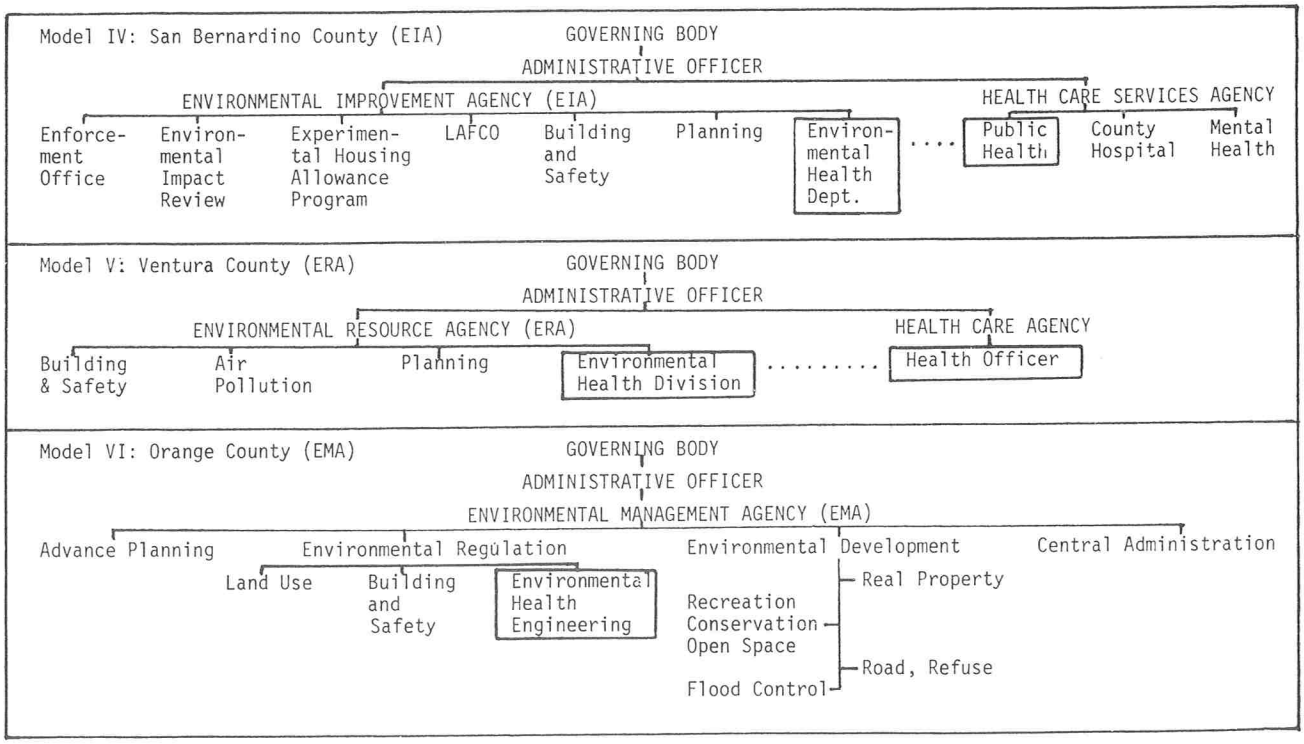


Figure 2. *California environmental health structures within the comprehensive environmental agency.*

Environmental health structure in a comprehensive environmental agency

The term Comprehensive Environmental Agency (CEA) is used here in reference to a local environmental protection agency that includes environmental health and other environmental programs. The actual nonmenclature of the CEA varies from one County to another. The current three types of the CEA in California are: (*a*) the Environmental Improvement Agency (EIA) in San Bernardino County, (*b*) the Environmental Resource Agency (ERA) in Ventura County (*1*), (*c*) the Envrionmental Management Agency (EMA) in Orange County.

Models IV and V provide for the following major administrative and programatic advantages: (*a*) Policy making decisions affecting environmental health are made at their highest departmental level by the Sanitarian, rather than by a medical health officer. The Sanitarian's expertise in both public health and environmental management come into full administrative play. (*b*) Easier access of the Environmental Health Director to the decision-makers and the public. (*c*) Better communication and beneficial, reciprocal, relationship with environmentally related organizations such as Planning. On one hand the Environmental Health input (and consequently public health input) in pure environmental programs has increased. On the other, the philosophical and operational base of environmental health are being expanded in the direction of environmental quality. Sanitarians influence planning and air pollution control decisions while operating the highest developed land use sections in California environmental health programs. (*d*) Medical input does not cease to flow in the environmental health program, but the health input, broadly defined, has a better chance to be intensified.

There are no known major disadvantages of Models IV and V at this time. However, administrators of programs represented by these Models face a major challenge, i.e. making full use of the advantages provided by the new organizational arrangements.

Model VI is advantageous only from the point of view of closer relation to other environmentally related activities. However, there are some serious disadvantages from the administrative and programatic point of view. These are: (*a*) Environmental Health is further removed from the decision making process (to the fourth level of the organization). This can endanger environmental health as a field and its health aspects as a distinctive feature. It also can result in further fragmentation of environmental health activities. One of the objectives behind transferring environmental health to CEA is achieving program visibility, and administrative ability to have direct access to the decision-makers in the public and political arenas. This objective could not be satisfactorily achieved through Model VI. (*b*) Placing Environmental Health under "Environmental regulations" may confine its activities to two major aspects of its functions, i.e. inspection and enforcement at the expense of the third major function of Environmental Health education and community involvement. (*c*) Being a part of an environmental health and engineering

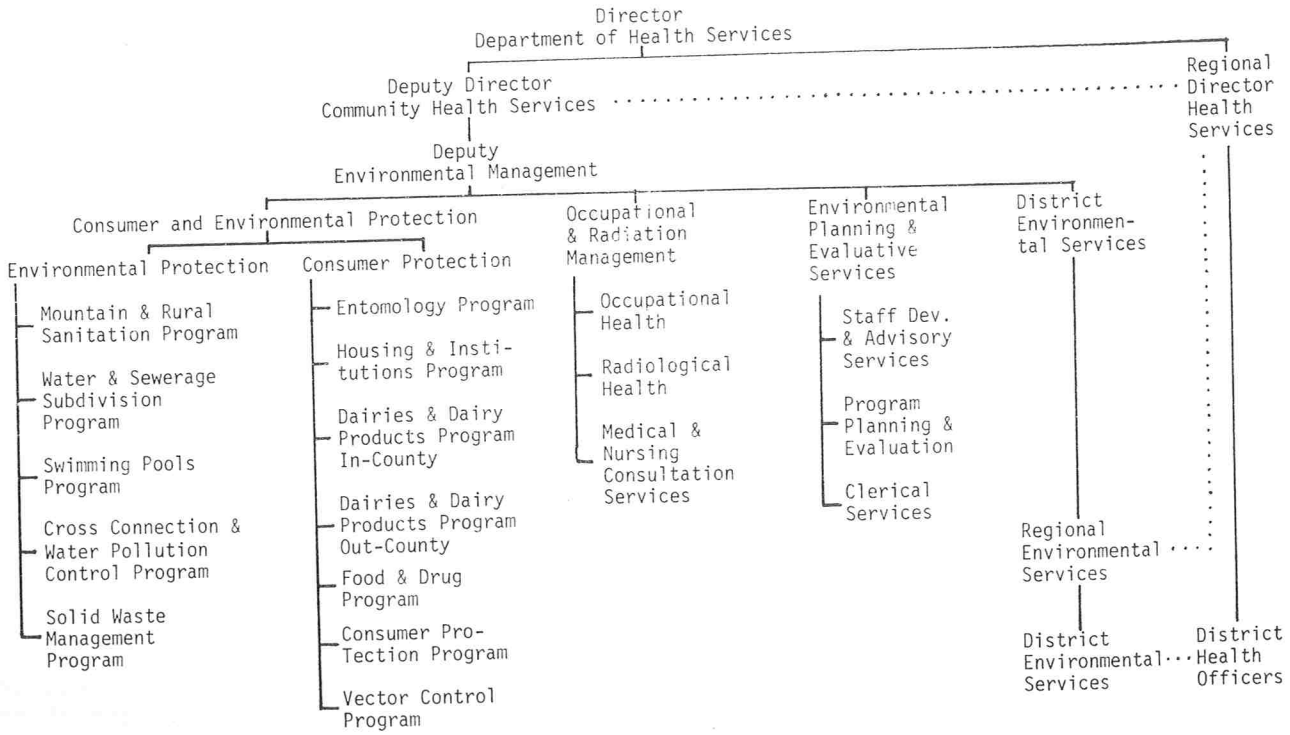


Figure 3. *Environmental management organization chart, County of Los Angeles Department of Health Services.*

component may further de-emphasize another important feature of environmental health, i.e., revolving around daily human health and environmental needs. In addition, it may impose a traditional engineering approach on environmental health. While an engineering approach is effective in solving several environmental problems, it does not provide an acceptable means of solving environmental health issues. (*d*) Generally, Model VI does not appear to give priority to environmental health, nor does it recognize its full potential in public service.

Because of these disadvantages, Model VI is the least desirable structure for environmental health programs transferred to CEA.

Environmental management in Los Angeles County

The current Environmental Management Programatic content and administrative structure have been influenced by the consolidation of the Los Angeles City and the Los Angeles County Public Health Departments on July 1, 1964, and the follow-up formation of the Los Angeles County Department of Health Services in September, 1972. The new Health services Department is composed of the former departments of Public Health, Mental Health, Hospitals, and the County Veterinarian.

Before and following the first consolidation of the City and County Health activities in 1964, several studies were conducted with the purpose of improving services provided by the new department. These included studies related to organization and management—Program Review and Evaluation. One of these studies, the Program Review, had the most impact on environmental health. The Program Review was conducted by the Community Health Action Planning Service of the American Public Health Association (APHA). The final report came to be known as the *Merrill Report* (*6*) in reference to the director of the study, Malcolm H. Merrill. As a result of the Merrill Report, recommendations and other efforts, the Environmental Health Director was designated as an Environmental Management Deputy and the previously fragmented environmental health services were consolidated into one organization.

The 1972 consolidation did not result in any additional significant changes in the internal organization of Environmental Management, but it affected its relation to the entire new organization. Environmental Management became a part of Community Health Services (the equivalent of the old Public Health Department). In the new organizational structure, Environmental Management moved one step further from the upper decision-making levels of local

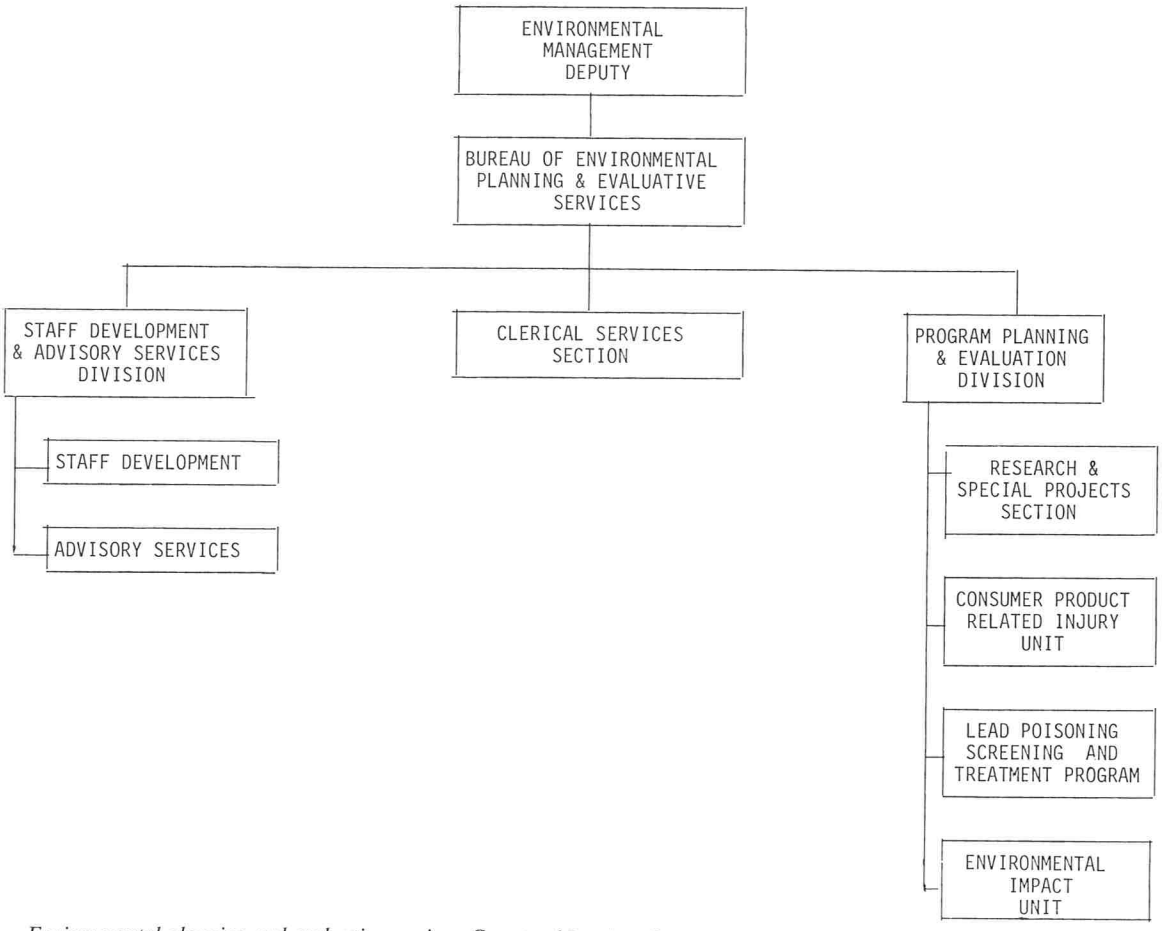


Figure 4. *Environmental planning and evaluative services, County of Los Angeles Department of Health Services.*

government.

In relation to the three prevailing models of environmental health programs inside the health structure, it can be said that Los Angeles County Environmental Management was represented by Model I after the 1964 consolidation and Model II after 1972 consolidation. Measnwhile, the internal structure of Environmental Management continued to improve and services were expanded.

Some distinctive features of the Los Angeles County Environmental Management include: (*a*) It assumes an "environmental management" approach to environmental health activities. (*b*) New programs emphasizing an expanded role of environmental health have been established. Examples are: Environmental Impact Review Unit, an aggressive Consumer Protection Program, a limited but active, Consumer Product Safety Program (Under a Federal Grant) and efforts to secure

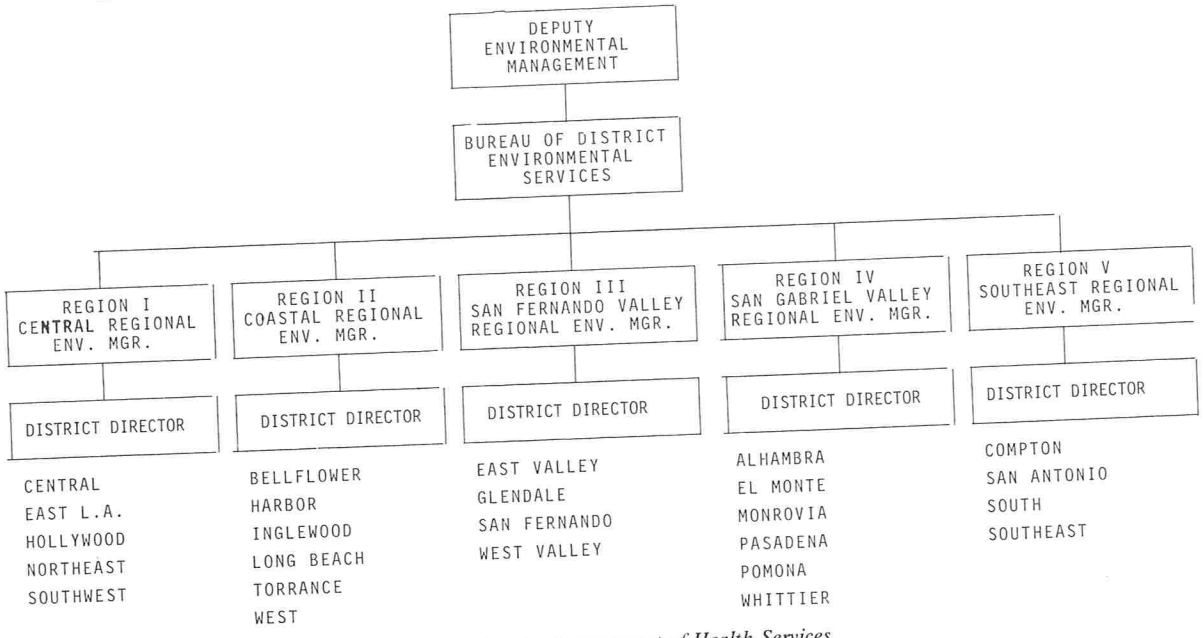


Figure 5. *District environmental services, County of Los Angeles Department of Health Services.*

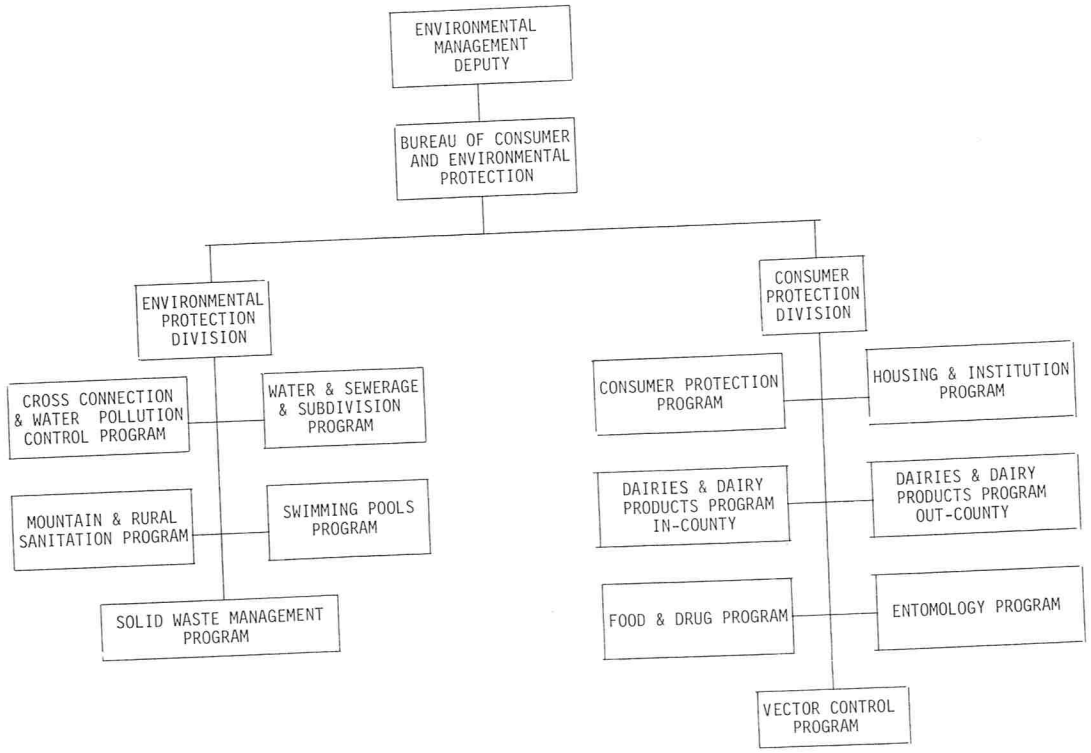


Figure 6. *Consumer and environmental protection, County of Los Angeles Department of Health Services.*

citizen participation. (*c*) Environmental Management is the only Unit in the Department of Health Services that has its own Program Planning, Evaluative and Staff Development Programs. The current administrative structure of the Los Angeles County Environmental Management is illustrated in Fig. 3-7.

MAJOR LEGISLATION AFFECTING ENVIRONMENTAL HEALTH STRUCTURE AND PROGRAMATIC CONTENT IN CALIFORNIA

There is a multiplicity of legal authority for the Environmental Health programs in California, including certain Federal laws, the State Health and Safety Code, other applicable State Codes, County and City Ordinances, and several Bills passed by the State Legislation over the years. However, there are three major legal authorities that affected California Environmental Health in the most significant manner. These are: (*a*) The California Environmental Quality Act of 1970 (*b*) California Assembly Bill 3197—Local Health Department Subsidies, 1974, and (*c*) Title 17 of the California Administrative Code (*7, 9*).

The California Environmental Quality Act (CEQA)

CEQA is patterned after the National Environmental Policy Act (NEPA) of 1969, requiring environmental impact statements on projects significantly affecting the environment. CEQA had its impact on California Environmental Health in two important directions: (*a*) *Internal organization.* The Act gave environmental health administrators the legal basis for establishing Environmental Impact Units in their organizations. These Units have been expanded into Land Use Section in some programs. For a long time, sanitarians felt that they should be involved directly in land use planning. This feeling stems from two reasons—one, they can contribute their environmental health knowledge to sound planning; second, often they are involved in correcting negative environmental influences on human health and comfort resulting from past unsound land use planning. (*b*) *Recognition by local government of the environmental quality role of sanitarians.* This contributed to his acceptance by other environmentalists (Planners, etc.) as part of the environmental team in the newly formed CEA.

The California Assembly Bill 3197

A.B. 3197 was introduced by Assemblyman McDonald of Ventura County primarily to resolve the conflict between San Bernardino County and Ventura County on one had and the State of California on the other. Both Counties transferred their envrionmental health activities to Comprehensive Environmental Health Agencies. This raised questions as to the authority of the local health officers and the eligibility of these environmental health programs for the State Subvention Funds distributed by the State to local health departments. For example, San Bernardino County receives about $160,000 per year in State Health Department Subvention Funds.

After several discussions among the author of the Bill and interested parties, an amended Bill received the support of the California Conference of Directors of Environmental Health, California Conference of Local Health Officers, the California Environmental Health Association, and others. The amended Bill was passed

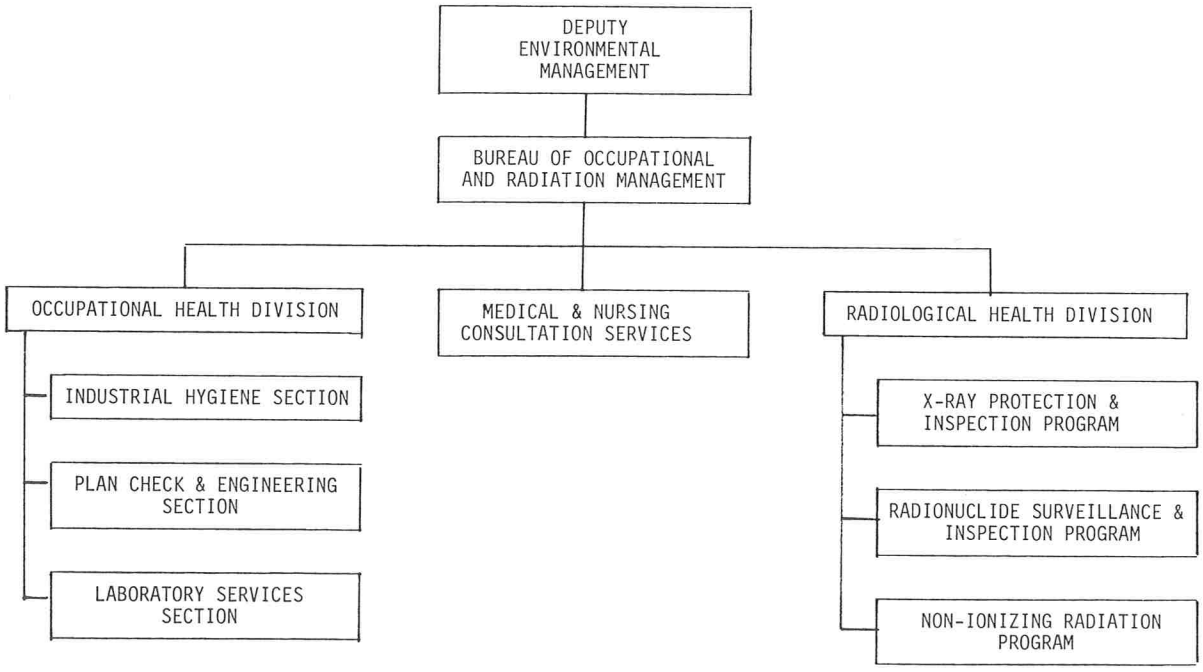


Figure 7. *Occupational and radiation management, County of Los Angeles Department of Health Services.*

and signed by the Governor on September 29, 1974. Besides resolving the financial issue, the Bill provided for the following: (*a*) An opportunity for local government to explore other models of environmental health administration in particular, and environmental quality in general. (*b*) When desired, the local government can move the environmental health program to a Comprehensive Environmental Agency, provided that (i) the entire environmental health program is transferred—thus preventing fragmentation. (ii) the program is under the direction of a Registered Sanitarian—thus recognizing his/her administrative role, and (iii) the program is supported by sufficient staff—thus preventing any reduction or elimination of services. (*c*) Communication with the health department is maintained through formal and informal means. The State Health Department has the authority to review and approve the annual plans of the transferred Environmental Health Programs.

In case of emergencies, the Health Officer has authority to assume direction and authority over the environmental health program. A detailed description of A.B. 3197 and related developments has been reported by Eich (*2*).

Title 17 of the California Administrative Code

Title 17 of the California Administrative Code sets the standards for Environmental Health and Sanitation Programs in California. Currently, Article 2, Section 1276 (e) of Title 17 states: "Services in Environmental Sanitation, to include appropriate activities relating to water, food, air, wastes, vectors, housing, bathing places, and safety."

However, this section is being revised to reflect contemporary changes in the field. A document prepared by the State Department of Health, dated July 9, 1975, is currently being processed for adoption in the California Administrative Code, Title 17. This document is expected to be adopted substantially as finalized in this July 9 draft for public hearings. The document details mandated and non-mandated programs. Also, it is expected to result in uniformity of program standards throughout the State.

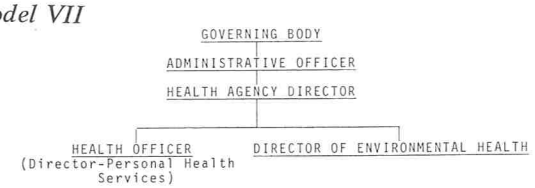
In addition to Title 17, the State Health Department document, *Elements in Environmental Health and Sanitation Programs* contains recommended program guidelines and a description of the elements of environmental health by goal, activities, and statutory authority and responsibility. This document is being retitled *Services in a Local Environmental Health Program.* The "Services" document was developed by the Administrative Practices Committee of the California Conference of Directors of Environmental Health and the Sanitarian Services Section of the State Health Department. Currently, the 1975 edition is being reviewed for final adoption.

ALTERNATIVES

The previous discussions indicate that environmental health programs in California are well developed. To be sure there are several programs that need to be included in environmental health services to achieve the desired degree of comprehensiveness. However, the major point of discussion, now, is one of administrative structure within the framework of increasing program effectiveness.

Generally, there is an agreement among environmental health professionals that environmental health structure within traditional health departments should change. The authors of this paper wish to offer the following two Models (VII and VIII) as alternatives to traditional or conventional models of environmental health administration in California.

Model VII



Alternative Model number VII is the most desirable structure for environmental health within the health agency. Model VII has distinctive and intrinsic administrative values.

(*a*) It gives credibility to the "health team" concept through elevating environmental health to its appropriate level in the health agency as a co-equal of personal health services.

(*b*) The Health Officer and the Director of Environmental Health have equal access to the Agency Director, if not a direct access to the decision-makers. In the frame work of such relationship, "Comprehensive Health Services" can be realistically planned and implemented. The Environmental Health Director has a better chance of forwarding the new concepts of environmental health organization and its practice to decision makers, without being screened first by the medical officer. Thus environmental health could be broadened to express new trends in environmental quality and contemporary definitions of health.

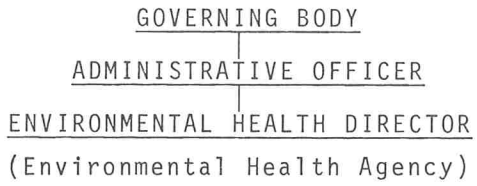
(*c*) Such co-equal relationship may strengthen environmental health to the degree of expanding its activities into more meaningful area of preventive medicine, beyound the microbiological theory. This reduces the economic and human impact of curative services needed to cure otherwise environmentally preventable illnesses.

(*d*) These benefits are achieved at a minimum of administrative dislocation in the health agency and other county organizations.

The benefit of Model VII would be fully realized only within a health agency that subscribes to a non-traditional philosophy of health services. This will require recognition of the role of environmental management as a true partner in the health team, and its potential in promoting health and well-being as well as disease prevention.

However, in pragmatic terms, such a model is beneficial to Environmental Health even within health agencies with traditional philosophies. This is because of two reasons: (*a*) it is definitely better than Model I or II, particularly as far as accessibility to decision-makers is concerned; and (*b*) it prepares Environmental Health for better administrative position if transferred to a comprehensive environmental health agency. This has been true of Ventura County, which had such an arrangement in 1973. As it is clear from Model V, Ventrua County Environmental Health Program gained equal status with the other Divisions after its transfer to the Environmental Resource Agency in 1974.

Model VIII



This 'Model represents another possible alternative for large environmental health programs in major counties. Model VIII has the basic advantages associated with Model VII, and in addition it has the following advantages.

(*a*) It provides the environmental health director with an opportunity for maximum contacts with the highest decision-makers in the governmental and private sector.

(*b*) It recognizes the identity of field of environmental health as the field that deals equally and simultaneously with problems of human health and human ecology.

(*c*) It avoids possible domination of Environmental Health Services with a medical or an engineering approach to problem solving.

(*d*) It provides for open communications with medical health services and environmental engineering services where an effective coordination of activities can be established.

CONCLUSION

An administrative structure for environmental health should reflect a combination of several factors. These include providing for the best possible public service, the full utilization of the potential of the sanitarian/environmentalist at the highest executive level of the organization, program flexibility, program viability, and broadening the philosophical and operational base for environmental health activities. If these objectives are to be achieved, then sanitarians must take an active and knowledgeable leadership role in present and future organization efforts.

ACKNOWLEDGMENTS

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Fate of Animal Viruses in Effluent from Liquid Farm Wastes

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ABSTRACT

Various aspects of viral pollution of the environment associated with disposal of liquid farm manure on agricultural land are being investigated. Techniques have been developed for concentration and recovery of animal viruses from various field samples. Seventeen of 22 samples of liquid manure from a swine fattening house yielded enteroviruses, adenoviruses, and a coronavirus. One enterovirus was isolated from six samples of waste from a swine farrowing house, but no virus was isolated from 18 samples of liquid cattle manure obtained from a dairy farm. A swine enterovirus was isolated from surface soil samples collected up to 8 days after liquid manure was spread on agricultural land. A swine enterovirus was also isolated from 2 of 26 samples of surface run-off collected from sites at which liquid pig manure was routinely spread on agricultural land. Thirty three samples of surface water and 36 samples of ground water were collected in areas in which liquid pig manure was routinely spread on farm land, and a swine enterovirus was isolated from one surface water sample. Field and laboratory experiments indicated that enteroviruses are more rapidly inactivated in aerated liquid manure than in untreated manure.

During the past two years I have been investigating some aspects of environmental viral pollution which may be associated with the disposal of liquid farm manure on agricultural land. Collection and disposal of manure from farm livestock in liquid form is, for economic reasons, an increasingly widespread practice in the livestock industy, and may represent a viral pollution hazard since the opportunities for thermal inactivation of viruses are less for liquid manure which is stored for a relatively short period than for composted solid manure which is more readily stored for longer periods before disposal on the land. Farm animals are known to excrete a variety of viruses in their feces (*3*). Many of these agents are pathogenic in farm livestock, and some may be transmissible to man. The extent to which infectious viruses may spread in the environment in association with the disposal of liquid manure depends mainly upon their stability, firstly in the liquid manure itself, and secondly in water to which they may gain access from surface run-off from farmland. Preliminary studies which we have completed, and other work which is currently in progress, suggest that certain enteric viruses, such as swine enteroviruses and bovine adenoviruses, are sufficiently stable in the liquid manure or water environment to represent a pollution hazard. While techniques are available for the recovery of certain human viruses from domestic sewage and from water, techniques had not been described for concentration and recovery of animal viruses from liquid manure and from water. The initial phase of our investigation related to selection of suitable techniques for this purpose. We then examined a small number of samples of swine and cattle liquid manure for the presence of viruses, and followed this by a search for viruses in surface soil samples from land on which liquid manure has been spread, in surface run-off samples, and in surface and ground water collected in areas in which liquid manure was routinely spread on farm land. Finally, I wish to discuss the effect of aeration of liquid manure on the infectivity of viruses which it may contain. Certain aspects of these studies will be published in detail elsewhere; the present paper is an attempt to bring together in abbreviated form some of the findings which are of more general interest.

CONCENTRATION TECHNIQUES

Samples of distilled water, river water, or liquid pig manure were seeded with a swine enterovirus, and various concentration methods were compared for recovery of the virus (Table 1). Pervaporation was not pursued as a concentration technique because of the long

TABLE 1. *Concentration of a porcine enterovirus from water and liquid manure*

Concentration method	Mean % recovery of virus from 6 samples		
	Distilled water	River water	Liquid Manure
$Al(OH)_3$ adsorption	93	83	0
PE-60 adsorption	95	100	74
Hydroextraction	73	45	0
Pervaporation	52	not done	not done
Centrifugation	19	not done	not done

period required to achieve sufficient concentration from large volumes of water, and ultracentrifugation was discarded because of the practical difficulties of dealing with large volumes in the ultracentrifuge. Aluminium hydroxide adsorption (*7*) and hydroextraction (*2*) satisfactorily concentrated the virus from water, but were unsuccessful on liquid manure because of the extreme cytotoxicity of the concentrated samples to cell cultures. Adsorption with the polyelectrolyte PE-60 (*6*) gave satisfactory recoveries of the swine enterovirus from both water and liquid manure. In subsequent tests, PE-60 adsorption was found not to be satisfactory for

concentration and recovery of bovine or porcine adenoviruses from liquid cattle or pig manure, or from water. Another method which we tried for concentration of viruses from water was by adsorption to layers of a mixture of talc and celite, based on the technique developed by Westwood and Sattar (*8*), and this worked well for enteroviruses in water, but not for adenoviruses, and it was unsuitable for use with liquid manure. For the recovery of adenoviruses from water we had more success with membrane filter adsorption (*1*), and this technique also gave satisfactory recoveries of a porcine enterovirus from seeded water samples. In our subsequent investigations we used PE-60 adsorption for liquid manure and soil samples, membrane filter adsorption for suface run-off samples, and talc-celite adsorption for surface water and ground water. Talc-celite adsorption enabled relatively large volume samples to be tested, and it worked well if the water was reasonably clean.

VIRUSES IN LIQUID MANURE

Liquid manure was collected from four sites. One site was a liquid manure tank which received effluent from a swine fattening house, the second from a swine farrowing house, and the remainder were liquid manure tanks which received waste from adult dairy cattle and calves, respectively. The numbers of samples collected at each site are shown in Table 2, together with the virus isolation results obtained by direct inoculation of the samples onto suitable cell cultures, and those after concentration of each sample by PE-60 adsorption. It will be seen that the major source of positive samples was the liquid manure from the swine fattening house, which yielded 17 isolations from 22 samples. Only a single isolation, of a swine enterovirus, was made from the sow waste samples, and this lower prevalence corresponds with the lower excretion rate of enteric viruses from adults compared with recently weaned piglets (*4*). Viruses were not isolated from the cattle waste samples. Two factors may be relevant to the lower prevalence of viruses in cattle waste compared with pig waste. Firstly, adult cattle and older calves excrete viruses less regularly than recently weaned piglets. Secondly the cattle waste tanks were emptied at longer intervals (2-3 months) than the pig manure tank (1-2 weeks), so that while the pig waste was always relatively fresh, the cattle manure had been stored for a significant period, during which thermal inactivation of any viruses present might reasonably be expected to occur.

TABLE 2. *Isolation of viruses from liquid manure*

Source of manure	No. of samples	No. of positive samples	
		Direct isolation	PE-60 concentration
Swine fattening house	22	9	17
Sow farrowing house	6	0	1
Dairy cattle barn	12	0	0
Calf rearing unit	6	0	0

The identity of the viruses isolated from the manure from the swine fattening house is given in Table 3. The large number of enteroviruses isolated is probably related to the high excretion rate of these viruses by piglets (*4*), to their rapid multiplication in cell cultures, and to the efficiency of the PE-60 adsorption procedure in concentrating enteroviruses from pig waste. Other studies which we have in progress have demonstrated also that enteroviruses are relatively stable in liquid pig manure. Quantitation of some of the positive samples in pig kidney cell cultures revealed that the titre of enterovirus present in the manure ranged from about one million median tissue culture infectious doses ($TCID_{50}$) per liter in two samples from which the enterovirus was isolated directly, to an estimated 50 to 100 $TCID_{50}$ per liter in samples which yielded enterovirus only after PE-60 concentration. These results clearly indicated that, on occasion, liquid manure may contain relatively large amounts of virus which could be discharged in infectious form on agricultural land.

TABLE 3. *Identification of viruses isolated from swine liquid manure*

Virus group	No. of isolations	
	Direct	PE-60 concentration
Enterovirus	6	14
Adenovirus	2	3
Coronavirus	1	0

FATE OF VIRUSES AFTER MANURE SPREADING

The next phase of the investigation was to attempt to determine the fate of viruses contained in liquid manure which was spread on farm land. Since we had isolated viruses fairly regularly from pig manure, but not from cattle manure, we concentrated our efforts on disposal sites for liquid pig manure, and because we found that enteroviruses were most likely to be present in pig manure, we used techniques suitable for recovery of enteroviruses from soil and from water. The results obtained are summarized in Table 4. Sites for the collection of surface run-off were established in 13 locations on farms in Southern Ontario on which liquid pig manure was routinely spread on sloping ground. At the foot of the slope in each location, a collection device, consisting of plastic sheeting providing drainage into a 4-liter capacity plastic container buried beneath the soil surface, was installed. Since the collection of a suitable sample was dependent upon the occurrence of local rainfall, sampling was intermittent, but in the period between July and November, 1974, a total of 26 run-off samples of 2 to 4 liters was obtained. Each sample was concentrated in the laboratory by membrane filter adsorption, and the concentrates were tested for viruses on pig kidney monolayer cell cultures. Two confirmed isolations of swine enterovirus were obtained from these

TABLE 4. *Isolation of swine enteroviruses from soil, surface run-off, surface water and ground water*

Sample	No. of samples tested	No. of samples positive
Soil	6	3
Surface run-off	26	2
Surface water	33	1
Ground water	36	0

samples (Table 4), each from a different location, and both collected at the end of July.

Additional observations, involving the collection of surface soil samples were made at one of the above sites following the spreading of liquid pig manure which had been shown to contain a porcine enterovirus. The manure was spread on hay stubble at the rate of 15 tons per acre and at intervals after spreading, samples of surface vegetation and soil to a depth of 1 inch were collected from the site. The samples were suspended as a slurry in distilled water, and after centrifugation of the slurry the supernatants were collected and concentrated by PE-60 adsorption. The concentrates were then assayed for virus in pig kidney cell cultures. The soil samples collected 1, 2, and 8 days after spreading yielded a swine enterovirus, while the samples obtained 3, 15, and 22 days after spreading were negative. Surface run-off was collected at this site 8, 17, 23, 35, and 55 days after the manure was spread, each sampling after the occurrence of heavy rain in the area, but no virus was isolated from these run-off samples.

The findings just described indicate that a porcine enterovirus can remain viable in surface soil for at least 8 days after deposition in contaminated liquid manure. The virus can also, on occasion, be eluted from the surface by rain water, and may be present in a viable form in concentrations of at least 1 $TCID_{50}$ per 20 ml of run-off. This concentration of infectious virus in the run-off was calculated from the observations made on the two positive samples recorded just previously, and assumes 100% recovery of the virus by the membrane filter adsorption technique and no inactivation of the virus in the collection vessel before concentration. Since some viral infectivity was probably lost at both of these stages, the actual concentration of virus in fresh run-off may well have been at least 10 times greater than this.

Twelve of the farms which were used for the collection of surface run-off were utilized for collection of samples of surface water and ground water. On 11 of these farms, surface water was available from ponds, streams, or rivers which received run-off from the sites used for the spreading of liquid pig manure, and on each of the 12 farms, ground water was available from a well which provided the supply of farm water. Three samples of surface water and ground water were collected from each farm. Each sample consisted of a volume of 20 liters, which was concentrated by talc-celite adsorption and hydroextraction, and then tested for virus by inoculation on pig kidney cell cultures. The only virus isolated from this material was a swine enterovirus from a surface water sample (Table 4). The titre of virus in this sample was calculated to be about 1 $TCID_{50}$ per 40 ml, assuming 10% recovery by the concentration procedure which was used. This finding suggests that, on occasion, surface run-off from agricultural land on which liquid pig manure is spread may result in the presence of detectable amounts of infectious animal viruses in surface water.

AERATION OF LIQUID MANURE

The findings just outlined indicate that the disposal of raw liquid manure on agricultural land can represent a significant viral pollution hazard, and we next turned our consideration to possible ways in which this hazard might be reduced. Aeration of liquid manure, with the main objective of odor control, may become a widespread practice in the future. Pilot aeration studies, at the University of Guelph (5), and elsewhere, have given encouraging results, and it seemed worthwhile to investigate whether aeration of liquid manure had any effect on the infectivity of viruses which it might contain. This was studied under both field and laboratory conditions.

The field studies utilized an experimental liquid manure aeration tank at the University of Guelph. At weekly intervals, samples of raw pig waste were collected from a liquid manure tank, immediately before the contents were transferred to the aeration tank. At the same time intervals, composite samples of aerated waste were obtained from the aeration tank. This sampling procedure was continued for 10 weeks, at which time the aeration tank was emptied. Each sample of raw or aerated waste was concentrated by PE-60 adsorption, and viruses were isolated from the concentrates on pig kidney cell cultures. All the samples collected, of both raw and aerated waste, yielded viruses, and each was subsequently identified as a swine enterovirus. Cytopathic effects usually appeared more rapidly in the cell cultures inoculated with raw waste concentrates, suggesting a higher titre of virus in these samples. When the concentrates prepared from the raw and aerated waste collected in the first, fifth, and ninth weeks of the experiment were titrated for infectivity, the results given in Table 5 were obtained. It will be seen that the titre of

TABLE 5. *Titration of concentrates of raw and aerated liquid pig manure*

Sample week no.	Infectivity titres (Log_{10} $TCID_{50}$ per ml)	
	Raw manure concentrate	Aerated manure concentrate
1	4.25	1.0
5	4.0	1.0
9	4.75	1.0

enterovirus in the raw manure was always at least one thousandfold greater than in the aerated material. This suggested a significant degree of viral inactivation in the aeration tank, in spite of the addition of fresh, raw, liquid manure at weekly intervals. Whether the inactivation which occurred was specifically associated with the aeration procedure, or whether it represented thermal inactivation on storage could not be readily determined under the available field conditions, and this problem was investigated in the laboratory studies to be described.

In our laboratory studies, raw liquid pig manure was seeded with a swine enterovirus, and continuously aerated and magnetically stirred for 71 days at room temperature (22C). A similar volume of seeded,

untreated manure was included in the experiment for control purposes. Samples of the raw and aerated manure were collected twice weekly for up to 10 weeks for attempted viral isolation in pig kidney cell cultures. A rather high seeding rate was chosen to obviate the need for concentration of the samples for recovery of the virus. The seeded enterovirus was isolated from the aerated manure at each sampling interval up to 14 days after seeding, but the same virus was isolated from the control waste up to 71 days after seeding. It appeared from these findings that the swine enterovirus was inactivated more rapidly in aerated manure than in untreated manure held at the same temperature. The mechanism of inactivation is not known, and further studies with additional manure samples and other viruses are required, but on the basis of our field and laboratory results, aeration would appear to hold some promise for reducing the viral pollution hazards associated with liquid manure.

CONCLUSIONS

(*a*) Techniques have been established for concentration and recovery of enteric viruses from liquid manure and from water.

(*b*) Liquid farm manure may contain infectious animal viruses when it is discharged on agricultural land.

(*c*) Animal viruses have been detected in soil samples, surface run-off, and surface water in areas in which liquid pig manure is sprayed on to agricultural land.

(*d*) Aeration of liquid manure affords some hope of reducing the viral pollution hazards associated with the disposal of this material.

ACKNOWLEDGMENTS

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Food Safety in the Seventies

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ABSTRACT

There is presently more concern about food safety than ever more, even though our foods are now much safer than they have ever been. As a regulatory agency, the Health Protection Branch wishes to ensure that all foods are safe for all Canadians all the time. The presence in foods of potentially hazardous chemicals, microorganisms, or products of microbial metabolism constitute some of the major hazards associated with foods. The acceptability to society of a given hazard (risk) and the cost to eliminate it must be considered when regulatory programs are developed.

I wish to discuss—in broad terms—some aspects of the safety of the nation's food supply. It is particularly appropriate to do so at this time, in light of continued public concern, bordering almost on anxiety, about the safety of the foods we eat each day.

At first glance, it may seem paradoxical that there is presently more concern about food safety than ever before, even though objective evidence indicates our foods are now much safer than they have ever been. A constellation of competing forces is at work: (*a*) an increasingly well-informed public is concerned about the adequacy of safety measures; (*b*) a vigorous industry is concerned about economic costs of food safety procedures; and (*c*) a watchful regulatory agency is concerned about a rational balance between risk and benefit as these apply to food safety.

As a regulatory agency we wish to ensure that all foods are safe for all Canadians all the time. This is our ultimate goal and we believe we are coming close to that mark; but food safety will probably never be absolute. There are simply too many complicating factors. I would like to review some which make it extremely difficult to ensure absolute food safety, and consider those forces that are brought to bear by us in attempts to assure that Canadians will enjoy the highest possible standard of food safety.

Public interest in food safety has, as you all know, occupied public attention for many years. Look at these questions from a popular magazine article published in the *Ladies Home Journal* in December, 1916: (*a*) What is the effect upon the stomach of saccharin used in place of sugar in tea or coffee? (*b*) What is the effect of coffee on the nervous system? (*c*) What is the effect of drinking coffee three times a day? Do these sound familiar? Many readers will recognize the author of the article, Dr. Philip B. Hawk who also co-authored *Practical Physiological Chemistry,* a textbook that is still with us over 70 years after its first publication.

The picture of the happy baby on the front of the magazine in which Dr. Hawk's article appears suggests that food wasn't all that bad in the good old days. At least it looks as if there was enough of it. The smiling cherub does not, of course, tell us all we need to know about the safety of the nation's food supply in time of yore. The truth is, and we can prove it for our records, that the nation's food supply suffered from many safety-related deficits a century or even a few decades ago.

The vast majority of the citizens of Canada today, as did their ancestors 100 years ago, consider it a proper function of government to take whatever steps are necessary to assure the public that their food is wholesome, safe, and effective when properly used and that foods are not fraudulently misrepresented. Having accepted this function, the government must provide a service to carry it out and, further, one that keeps pace with the vast and rapid changes in the food and related industries. Let us, for a few moments consider some of the hazards known to be associated with foods.

CHEMICALS IN FOODS

What are the sources of potentially hazardous *chemicals* in food? The world's chemical industry has a great capability to synthesize new compounds, and to produce not only these but also the enormous number of established chemicals in staggering quantity. Fortunately, only a tiny fraction of these chemicals is actually intended for direct addition to food. A somewhat larger proportion is intended for legitimate uses related to food, for example, fertilizers, veterinary drugs, pesticides, packaging materials, boiler water additives, or for use on food machinery and utensils. A vast array of other chemicals are produced by man for still different uses—fuel for heating and transport, pharmaceuticals, plasticisers, organic monomers for polymerisation to fibers, pigments for paint, and so on.

Whatever the intention, all these chemicals and their breakdown products have the potential for passing from one compartment to another of the system which has been called "Spaceship Earth." Contamination of the

food compartment of the system is always a potential threat.

We should not overlook those chemicals which were present in the "spaceship" long before man came aboard. There is more mercury occurring naturally in the ocean than has ever been refined from ores by man. The annual loss of mercury from rocks into water is of the same order as the current industrial production of mercury.

Nor should we overlook the great diversity of chemicals which are inescapably part of food, however "naturally" it is grown. Many of these can have adverse effects on man. Numerous episodes of poisoning from roots, leaves, and berries throughout history have shown us that some natural foods are "unfit for man." There are many others which should be eaten only in limited quantity or after special preparation.

Finally, we must remember that man requires the proper balance of a variety of foods. Although there may be "soul food" that satisfies, man has not yet devised the perfect, safe, sole food. Indeed, it is hard to say what food is safe in unlimited quantity—for adults, probably not animal fat; for young boys, certainly not green apples.

It is against this complex background that we must attempt to achieve safe foods for the people of Canada.

We are not the first to appreciate that such a worthy goal cannot be reached in isolation. The information in Fig. 1 is from a book published in 1820.

TREATISE

ON

ADULTERATIONS OF FOOD,

AND CULINARY POISONS.

Philadelphia:

PRINTED AND PUBLISHED BY AB'M SMALL

1820.

vi Preface.

for which it is published; or if it should tend to impress on the mind of the Public the magnitude of an evil, which, in many cases, prevails to an extent so alarming, that we may exclaim with the sons of the Prophet,

"THERE IS DEATH IN THE POT."

For the abolition of such nefarious practices, it is the interest of all classes of the community to co-operate.

FREDRICK ACCUM.

LONDON.

1820

Figure 1. *An excerpt from an early book on adulteration of food.*

It is noteworthy that the author recognized—"There is death in the Pot" and that "for the abolition of such nefarious practices it is in the interest of all classes of the community to co-operate".

Regulatory approach

To ensure safe foods for Canadians there must be a coordinated effort, with a true spirit of cooperation between consumer, industry, and government. Although their motives inevitably are somewhat different, those involved in the food safety enterprise are bound together by a common goal—different production of enough safe food. In no industry is there more need for high standards than in the food industry. Consumer and government hold it accountable for safe useful products, honestly promoted. The vast majority of food processors accept this as their duty: so do most organizations and companies dealing with chemicals whose uses are relevant to food. A strong and effective regulatory agency is in the best interest of responsible industrialists; it is their guarantee that their *good* products will not be undercut by *bad* products produced by a careless, ignorant, and venal competitor. Adulteration of foods will not be tolerated by the Branch. Although George Bernard Shaw opined that silence is the most eloquent expression of scorn, we usually do more than that. If necessary, we are quite willing to go to the mat, the legal mat if need be, to contest manufacturers who persist in violating the law. We have less patience with adulterers than Moses had.

However, punishment is not the major objective of an effective regulatory agency. We realize that the Food and Drugs Act and Regulations is a complex document to understand, especially for the small producer and manufacturer. We attempt to deal informally, quickly, and sympathetically with the problems of enquirers. More than that, we attempt to assist them to comply wherever we can. In particular, we are concerned with the problems raised by the importation of food from less-developed countries where the will to meet Canadian standards is present, but the necessary expertise may not be. Our food scientists have been able to help here, both in bilateral exchanges and through international organizations.

The Branch also cooperates wholeheartedly with other Departments of the Canadian government—particularly the Department of Agriculture—in examining requests for uses of chemicals that might lead to residues in food so that in suitable cases "tolerance" of certain chemicals (such as insecticides) can be established. However, the presence of chemicals will be tolerated only if we are satisfied that this will not be hazardous to health and if both need and efficacy of the chemical have been demonstrated. To provide an *extra* margin of safety, it is our policy to restrict tolerances to the lowest level compatible with effective use.

International cooperation

To ensure foods are safe and wholesome on a

world-wide basis, Canada participates fully with international commissions and agencies involved in food safety. Officers of the Health Protection Branch contribute substantially to the *Codex Alimentarius Commission* whose function it is to develop acceptable international standards for food specifications and food safety. Internationally acceptable specifications for food products will assist greatly in stimulating the international food trade and ensuring quality foods for all member states of the Codex Alimentarius Commission. The staff of the Health Protection Branch also is intimately involved in assisting the World Health Organization. We contribute to reports and status documents which define potential sources of hazard from chemicals in foods and recommend research programs or improved production procedures to assist in the elimination of such hazards. To further ensure that the foods we eat are safe, the Health Protection Branch keeps close contact with individual governments both in the Americas and Europe with the aim of obtaining, or providing, information regarding food safety or changes in the regulatory control of chemicals in food. For example, Canadian health officials maintain a close liaison with the EEC Commission in Brussels so that we are fully aware of changes in food legislation introduced by Common Market countries. Canada is a regular contributor to the Pan-American Health Organization and is involved in assisting certain nations in Latin America in the establishment of laboratory facilities and in the introduction of food legislation. Clearly, the role of the Health Protection Branch involves broad-based, constructive cooperation. Nonetheless, it is our legal duty to enforce the Food and Drugs Act, and we do not shrink from doing so.

We must not refrain from proper science-based enforcement action simply and solely because it might upset an ethnic group, for example; or disrupt a local economy; or increase production costs. On the other hand we must not imagine that our powers go further than they do, and, in effect persecute a company by bringing a bad case to court with its inevitable damage to the company's reputation. I will have more to say on that later in this paper.

The detail of the Food and Drugs Act and Regulations is the heart of what we do. I will try to explain briefly their major attributes so far as chemicals in food are concerned.

The Food and Drugs Act and Regulations

The Act provides authority (*a*) to establish standards for the composition and identity of foods; (*b*) to prohibit the sale of foods that are manufctured under unsanitary conditions or that are adulterated; (*c*) to prohibit the advertising, labelling, packaging, processing or sale of foods "in a manner that is false, misleading or deceptive or is likely to create an erroneous impression regarding its character, value, quantity, composition, merit or safety."

The practical enforcement of the Food and Drugs Act requires that certain sections be supplemented with regulations that interpret the meaning of the Act. Consequently, there has grown up over the years a considerable volume of regulations (now numbering several hundred pages) which provide standards, set forth requirements for labelling, and establish prohibitions or exemptions for certain substances or classes of substances within the scope of the Act.

Years of experience have established that there are very few occasions for which the Food and Drugs Act does not provide adequate powers to protect the health of the public in a satisfactory manner. On the other hand, the regulations frequently are changed, nearly every week or so, in one technical way or the other. New food commodities are introduced to the market each year and new processing methods are developed. These often require the prompt amendment of existing standards or the establishment of new standards. The use of certain chemicals as direct or incidental additives is under continual review. New data on the safety of a particular chemical may require regulatory amendments. Such changes can be made in the regulations with minimal delay by Order-in-Council.

Safety versus fraud

I must emphasize that at the Health Protection Branch our interest and responsibility in the food area deal with safety and not with fraud. There is of course, that difficult-to-define borderland between health and fraud that requires interaction between our department and other government agencies. For example, there might be excessive use of a filler in a meat product. The consumer might think he is getting top quality protein but all he is really getting is an inferior product. His diet is thus not what he thinks it is and he isn't getting value for his money. He suffers from actual fraud and potential health hazard. We work directly with the Department of Consumer and Corporate Affairs on problems of this nature. We develop regulations and coordinate programs that are of mutual concern. The two agencies do much together that ensures safety and guards against fraud.

The consumer

Consumer involvement in problems of food safety has many facets. In recent years consumers have become more knowledgeable about health, about medicine and medical services, about drugs, and about food. They are more concerned now about real or imaginary corporate ripoffs, more confused by simplistic media coverage of complex health issues, more suspicious of the motives behind press releases, whether from corporations or Government agencies. They are more concerned too about rising food costs and about hidden dangers to health associated with foods. They are more ready and able to speak up to Government, to the food industry and to the medical community about what they see as problems.

Today's consumer demands the right to be assured about the safety of foods on the market. There is a

reaction of a necessary food additive (nitrites) with natural components of foods (secondary amines). The nitrosamines generally are potent carcinogens. Work on this problem has been carried out on a worldwide basis for several years since the original demonstration by Magee of the carcinogenic potential of nitroso compounds.

Our initial efforts in this area were concerned with developing quantitative and qualitative analytical methods for nitrosamines in foods. Surveys, concentrating on cured meats but also including cheese and fish, were done to estimate the levels of nitrosamines in the Canadian food supply.

A major finding indicated that much of the nitrosamine content of certain specialty cured meats was due to formation of nitrosamines in mixtures of spices and curing salts used in production of the products. Elimination of the use of spice-curing salt premixes significantly reduced the incidence and levels of nitrosamines in these meat products. This is an example of "preventive toxicology".

In our laboratories it was shown that the levels of nitrosamines formed during the cooking of bacon were related to the levels of nitrite used in curing the bacon. Studies were undertaken to develop procedures to reduce or prevent the formation of nitrosamines during frying. To date, the addition of either of two antioxidants (ascorbyl palmitate or propyl gallate) to the frying bacon has greatly reduced the levels of nitrosamines in the cooked bacon.

I hope these research findings will eventually be adapted to the commercial situation. If so we will have provided another route for the application of preventative toxicology.

MICROORGANISMS IN FOODS

If preventive toxicology is a legitimate concern of a regulatory agency, preventive microbiology is perhaps of even more concern. Microorganisms are, of course, ubiquitous. In what is generally believed to be the shortest poem in the English language, fittingly entitled "Lines on the Antiquity of Microbes," Strickland Gililan summed up our knowledge about the pervasive occurrence of these little beasties when he wrote:" Adam had 'em." For the harmful microbes that are important in food poisoning, there are only three avenues of approach: (*a*) if they are not in the food, prevent their entrance; (*b*) if they are naturally in the food or get in from contact with the environment, destroy them; (*c*) if they cannot be destroyed, prevent their growth. It is only rarely that a few cells of a food-borne pathogen are able to cause a disease. Usually it takes a considerable number of microbes. However, unlike chemical contaminants, microorganisms grow readily given suitable nutrients and adequate moisture and temperature. Thus, the opportunities and challenges for preventative microbiology are very great indeed.

Permit me to illustrate by means of a case history concerned with *Salmonella.* We have experienced outbreaks of human salmonellosis in Canada from a wide variety of foods including powdered milk, chocolate, black pepper, and even watermelon. However, the main reservoir of *Salmonella* in Canada appears to be poultry. It is, therefore, pertinent that I use a poultry product, processed eggs, as my third case.

Until the late 1950's, *Salmonella thompson* was of little consequence as a cause of human disease in Canada—only 0.25% of all reported cases of salmonellosis in humans were due to this serotype. However, by 1961-62, this figure had jumped to 25%—a 100-fold increase. The cause of this dramatic increase was found to be associated with the use of powdered eggs contaminated with *Salmonella thompson* in commercial cake mixes. Although baking the cake would destroy *Salmonella,* the kitchens of the consumers were undoubtedly heavily contaminated and, undoubtedly, many children got more than they anticipated, when they licked the raw batter from the spoon. To correct this situation, a regulation was introduced under the Food and Drugs Act to prevent the sale of *Salmonella*-contaminated processed eggs and strong enforcement action was taken. This resulted in a marked decrease in salmonellosis due to *S. thompson,* but not, unfortunately, to the pre-existing level of 0.25%. From 1969-73, *S. thompson* was responsible for about 10% of all human salmonellosis. It still remains a cause of significant human salmonellosis and it is still one of the salmonellae most frequently isolated from poultry.

COST VERSUS BENEFIT

As we have seen, there are substances, chemical or microbiological which are present in foods, that can cause human disease and that give rise to tremendous public concern. Our practical goal for these compounds is minimal hazard rather than perfect safety. It is clear that in the present and future state of the world wherever chemicals *genuinely* and irreplaceabley lead to more food production (or less waste, which comes to the same thing) there is a health benefit—even if it is in a distant hungry country rather than Canada—which can offset some of the risk to health from using the chemical. We must, therefore, balance increased food production against possible adverse effects in health from use of chemicals.

William Darby, in his thoughtful essay "The Conflict of Nutrition and Toxicology" noted that worldwide, *natural* toxins have produced greater known injury to man than have those in other categories of chemicals in food. Inadvertent or accidental contaminants have also made a substantial, though lesser, contribution to the total incidence of food-borne illness. On the other hand, agricultural chemicals, food additives, chemicals derived from packaging materials, and chemicals produced in processing of food are not known to have been responsible for adverse effects on human health when

such materials have been used in good agricultural and manufacturing practice. Of course, all of the data are not yet in, and we must continue vigilant surveillance. In this field, to paraphrase John Philpott Curran, the price of safety is eternal vigilance.

This problem of minimal hazard was considered at a recent tripartite meeting between Canada, the United States, and the United Kingdom. It was recognized that whether we like it or not, carcinogens like aflatoxin, dimethylnitrosamines, and vinyl chloride and organisms like *Clostridium botulinum* and *Salmonella* are woven into the very fabric of our environment. Nobody wants them there and eternal vigilance must be maintained to assure they are present at the lowest possible level—zero, if we can attain that goal. We advocate and promote a continuing effort to systematically reduce those existing levels. Often these efforts, to be most effective, must be international in scope. The potent carcinogenic mycotoxin, aflatoxin that occurs on some imported nut products is a case in point. For peanuts imported into Canada from the U.S., the raw peanuts are analyzed by the U.S. government and by the Canadian importer as well. If there is a dispute concerning aflatoxin analysis our Branch acts as an analytical referee. As a further safeguard we have a national monitoring program for aflatoxin in peanut butter. In addition, our research laboratories have a continuing program designed to improve methodology for aflatoxin detection. Given today's realities, to completely eliminate aflatoxin from the food supply effective tomorrow morning, would require a ban on the import of peanuts. This would result in the loss of an important source of dietary protein. The establishment of acceptable levels of potential harmful chemicals in the diet of man, therefore, requires that a benefit/cost evaluation be conducted. I would like to consider some general principles that must be included in the evaluation of benefit/cost. The following definitions of risk acceptability are involved; they apply generally to both food additives and contaminants like aflatoxin.

RISK is defined as the probability of occurrence of a deleterious effect in man. This depends upon: (*a*) The scientific validity of experimental data on the effects of the chemical. These data usually are obtained from studies in experimental animals or in some instances, such as with aflatoxin, a combination of epidemiological and toxicological data. (*b*) The level and frequency of human exposure to the chemical. Obviously, as level and frequency of human exposure increase, the risk increases.

LOSS is defined as the extent of human suffering and/or economic loss, and will depend on the number of people exposed to the particular chemical who as a result, may ultimately develop a toxic response. Factors involved in loss include the type of disease induced, age at onset, duration of illness, amenability to treatment or cure, and resultant incapacity or poor quality of life. From such factors, economic loss can be estimated, though imperfectly and somewhat subjectively, in universally understood units of money.

COST is defined as the risk multiplied by the loss. Cost would decrease with diminishing risk and diminishing loss.

BENEFIT includes factors such as convenience, economic gain, relief of suffering, improved nutritional status and enhanced quality of life. Some chemicals have important applications in industry and food production which augment national resources and provide substantial income and employment.

ACCEPTABILITY is defined as the beneft/cost ratio. When risk is balanced against benefit, the risk will be acceptable in some situations but not in others, depending on the many varied temporal and parochial, not to say political, concerns. Starr has examined the problem of societal criteria for evaluating benefits vs. costs. He noted that societal activities fall into two categories—voluntary and involuntary. Voluntary activities are controlled by the individual. Involuntary activities differ in that the criteria and options are determined by a controlling body, such as a government agency, a leadership group, or "opinion makers". Socio-economic, cultural, legal and philosophical considerations are often important modifiers that make decisions on the use of a particular chemical more or less restrictive. A good estimate of the acceptability of risk can only be obtained when the risk, possible loss, and the compensating benefits are adequately evaluated.

In Canada, attempts to establish maximal acceptable levels of dietary chemicals including carcinogens are made in this context. In addition, evaluations of risk acceptability are reviewed as further scientific information becomes available and original decisions may be changed if the new evidence supports such change.

Considerations of risk acceptability are related, in part, to our ability to determine the human exposure level or dose. With food components, food additives and environmental chemicals that come into contact with food, the dose range may be variable and often very difficult to determine because of variations in individual consumption patterns. This point is best illustrated by problems a few years ago associated with consumption of cobalt in beer. When arriving at an acceptable daily intake for cobalt in beer, estimates of intake by our agency were found to be low by a factor of about ten times. We simply did not know that some people drink 40 pints of beer a day. In terms of our ability to more accurately determine risks to man associated with the presence of chemicals in food, increased emphasis must be placed on techniques to acquire human data. Increased emphasis on epidemiological studies related to chemicals in foods conducted on certain population groups, specifically those known to consume large quantities of a chemical in the form of food, would be useful, not only in identifying possible toxic states, but in providing more accurate data on consumption patterns. I

think it a more fruitful approach in the long run than the current fashion of mega-mouse studies involving large numbers of experimental animals given doses of a test chemical which far exceed those to which humans ever would be exposed. The recently completed "Nutrition Canada Survey" will provide much needed information on food consumption patterns at highest risk regarding exposure to chemicals in food. Such studies, coupled with metabolic studies in man, would provide additional assurance of safety and would be of benefit to both industry and governments in arriving at safe levels of chemicals in food.

Finally, we have to keep the public better informed. It is increasingly hard to give consideration *only* to the arguments of those who urge maximum caution about every chemical irrespective of its utility to man. Nevertheless there is a need to let people know the degree and nature of hazard that exists to allay their concerns about food safety, or at least put them in proper perspective. In this way they can make informed judgment for ultimately, it is the people of this country—not the officials, in spite of their competence, who must make the decisions about the kind of society they want. If society wants a given degree of product control, so be it; if it wants to change what now is being done, it has the power to do so. At some point, the amount of risk a society is willing to assume to achieve a certain benefit becomes a matter for the public at large to decide. We intend to uphold our responsibility for food safety by defining these hazards and contributing effectively to their minimization.

ACKNOWLEDGMENT

Presented at the 62nd Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Toronto, Ontario, Canada, August 10-13, 1975.

Amendment to 3-A Sanitary Standards for Fittings Used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Milk and Milk Products

Number 08-16

Formulated by International Association of Milk, Food and Environmental Sanitarians
United States Public Health Service
The Dairy Industry Committee

The "3-A Sanitary Standards for Fittings Used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Milk and Milk Products, Number 08-09" is further amended as follows:

Replace subsection 1 and 2 of section A. SCOPE with the following:

A.1 These standards cover the sanitary aspects of gaskets, fittings, valves and glass tubing used on processing equipment and on equipment and lines which hold or convey milk or milk products.

A.2 In order to conform to these 3-A Sanitary Standards, gaskets, fittings, valves and glass tubing shall comply with the following design, material and fabrication criteria.

Replace subsection 1 of section B. DEFINITIONS with the following:

B.1 *Product:* Shall mean milk and milk products.

Delete subsection 9 of section D. FABRICATION.

Delete subsection 2 of section E. FABRICATION.

Replace the second sentence of subsection 3 of E. SPECIAL CONSIDERATIONS with the following:

The inside diameter of the butt welding ends shall be the same as that of the part to which it is to be welded.

This amendment is effective May 12, 1976.

Amendment to Rescind 3-A Sanitary Standards for Inlet and Outlet Leak Protector Plug Valves for Batch Pasteurizers

October 8, 1954, Serial #14-00, As Amended By #14-01

Number 14-02

Formulated by International Association of Milk, Food and Environmental Sanitarians
United States Public Health Service
The Dairy Industry Committee

In accordance with the action of the 3-A Sanitary Standards Committees, as recorded in Section VII of the minutes of October 9, 1975, the "3-A Sanitary Standards for Inlet and Outlet Leak Protector Plug Valves for Batch Pasteurizers," dated October 8, 1954, Serial #14-00, and Amendment #14-01, are hereby rescinded effective May 12, 1976, and become null and void.

[Note: The substantive content of the 3-A standard for leak protector valves #14-00 and amendment #14-01 has been incorporated in the 3-A standard for sanitary fittings #08-17, and criteria for leak protector plug valves remain in effect henceforth under standard #08-17.]

Notice of this rescinding amendment is published in the Journal of Milk and Food Technology in accordance with the provisions of the 3-A Standard Operating Procedure.

Holders of 3-A Symbol Council Authorizations on February 20, 1976

"Questions or statements concerning any of the holders of authorizations listed below, or the equipment fabricated, should be addressed to Earl O. Wright, Sec'y.-Treas., 413 Kellogg Ave., P.O. Box 701, Ames, Iowa 50010."

01-06 Storage Tanks for Milk and Milk Products

28 Cherry-Burrell Corporation (10/ 3/56)
575 E. Mill St., Little Falls, N.Y. 13365

102 Chester-Jensen Company, Inc. (6/ 6/58)
5th & Tilgham Streets, Chester, Pennsylvania 19013

2 CREPACO, Inc. (5/ 1/56)
100 C. P. Ave., Lake Mills, Wisconsin 53551

117 Dairy Craft, Inc. (10/28/59)
St. Cloud Industrial Park
St. Cloud, Minn. 56301

76 Damrow Company (10/31/57)
196 Western Avenue, Fond du Lac, Wisconsin 54935

115 DeLaval Company, Ltd. (9/28/59)
113 Park Street, So., Peterborough, Ont., Canada

109 Girton Manufacturing Company (9/30/58)
Millville, Pennsylvania 17846

114 C. E. Howard Corporation (9/21/59)
9001 Rayo Avenue, South Gate, California 90280

127 Paul Mueller Company (6/29/60)
P.O. Box 828, Springfield, Missouri 65801

31 Walker Stainless Equipment Co. (10/ 4/56)
Elroy, Wisconsin 53929

02-06 Pumps for Milk and Milk Products

214R Ben H. Anderson Manufacturers (5/20/70)
Morrisonville, Wis. 53571

212R Babson Bros. Co. (2/20/70)
2100 S. York Rd., Oak Brook, Ill. 60621

29R Cherry-Burrell Corporation (10/ 3/56)
2400 Sixth St., S. W., Cedar Rapids, Iowa 52406

63R CREPACO, Inc. (4/29/57)
100 C. P. Ave., Lake Mills, Wisconsin 53551

205R Dairy Equipment Co. (5/22/69)
1919 So. Stoughton Road, Madison, Wis. 53716

65R G & H Products, Inc. (5/22/57)
5718 52nd Street, Kenosha, Wisconsin 53140

145R ITT Jabsco, Incorporated (11/20/63)
1485 Dale Way, Costa Mesa, Calif. 92626

26R Ladish Co., Tri-Clover Division (9/29/56)
9201 Wilmot Road, Kenosha, Wisconsin 53140

236 Megator Corporation (5/ 2/72)
125 Gamma Drive, Pittsburgh, Pa. 15238

241 Purity S. A. (9/12/72)
Alfredo Noble #39, Industrial Pte. de Vigas
Tlalnepantla, Mexico

148 Robbins & Myers, Inc. (4/22/64)
Moyno Pump Division
1345 Lagonda Ave., Springfield, Ohio 45501

163R Sta-Rite Industries, Inc. (5/ 5/65)
P.O. Box 622, Delavan, Wisconsin 53115

72R L. C. Thomsen & Sons, Inc. (8/15/57)
1303 53rd Street, Kenosha, Wisconsin 53140

219 Tri-Canada Cherry-Burrell Ltd. (2/15/71)
6500 Northwest Drive, Mississauga, Ont., Canada L4V 1K4

175R Universal Milking Machine Div. (10/26/65)
National Cooperatives, Inc.
First Avenue at College, Albert Lea, Minn. 56007

52R Viking Pump Div.
Houdaille Industries, Inc. (12/31/56)
406 State Street, Cedar Falls, Iowa 50613

5R Waukesha Foundry Company (7/ 6/56)
Waukesha, Wisconsin 53186

04-03 Homogenizers and High Pressure Pumps of the Plunger Type

247 Bran and Lubbe, Inc. (4/14/73)
2508 Gross Point Road, Evanston, Illinois 60201

87 Cherry-Burrell Company (12/20/57)
2400 Sixth Street, S.W., Cedar Rapids, Iowa 52404

37 CREPACO, Inc. (10/19/56)
100 C.P. Ave., Lake Mills, Wis. 53538

75 Gaulin, Inc. (9/26/57)
44 Garden Street, Everett, Massachusetts 02149

237 Graco Inc. (6/ 3/72)
60-Eleventh Ave., N.E., Minneapolis, Minn. 55413

256 Hercules, Inc. (1/23/74)
2285 University Ave., St. Paul, Minnesota 55114

05-13 Stainless Steel Automotive Milk Transportation Tanks for Bulk Delivery and/or Farm Pick-up Service

131R Almont Welding Works, Inc. (9/ 3/60)
4091 Van Dyke Road, Almont, Michigan 48003

98R Beseler Steel Products, Inc. (3/24/58)
417 East 29th, Marshfield, Wisconsin 54449

70R Brenner Tank, Inc. (8/ 5/57)
450 Arlington, Fond du Lac, Wisconsin 54935

40 Butler Manufacturing Co. (10/20/56)
900 Sixth Ave., S.E., Minneapolis, Minn. 55114

66 Dairy Equipment Company (5/29/57)
1818 So. Stoughton Road, Madison, Wisconsin 53716

45 The Heil Company (10/26/56)
3000 W. Montana Street, Milwaukee, Wisconsin 53235

201 Paul Krohnert Mfg., Ltd. (4/ 1/68)
811 Steeles Ave., Milton, Ontario, Canada L9T 2Y3

80 Paul Mueller (Canada), Ltd. (11/24/57)
84 Wellington Street, So., St. Marys, Ont., Canada

85 Polar Manufacturing Company (12/20/57)
Holdingford, Minn. 56340

71 Progress Industries, Inc. (8/ 8/57)
400 E. Progress Street, Arthur, Illinois 61911

121 Technova Inc. Gosselin Division (12/ 9/59)
1450 Hebert c. p. 758
Drummondville, Quebec, Canada

47 Trailmobile, Div. of Pullman, Inc. (11/ 2/56)
701 East 16th Ave., North Kansas City, Mo. 64116

189 A. & L. Tougas, Ltée (10/ 3/66)
1 Tougas St., Iberville, Quebec, Canada

25 Walker Stainless Equipment Co. (9/28/56)
New Lisbon, Wisconsin 53950

08-09 Fittings Used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Milk and Milk Products

79R Alloy Products Corporation (11/23/57)
1045 Perkins Avenue, Waukesha, Wisconsin 53186

138R APV-CREPACO of Canada Limited (12/17/62)
1250 Ormont Dr., Weston, Ont., M9L 2V4

245 Babson Brothers Company (2/12/73)
2100 S. York Road, Oak Brook, Illinois 60521

82R Cherry-Burrell Company (12/11/57)
2400 Sixth Street, S.W., Cedar Rapids, Iowa 52406

260 CREPACO, INC. (5/22/74)
100 CP Ave., Lake Mills, Wis. 53551

124R DeLaval Company, Ltd. (2/18/60)
113 Park Street, South, Peterborough, Ont., Canada

255 The Duriron Company (1/18/74)
45 North Findlay Street, Dayton, Ohio 45401

67R G & H Products, Inc. (6/10/57)
5718 52nd Street, Kenosha, Wisconsin 53140

199R Graco, Inc. (12/ 8/67)
60 Eleventh Ave., N.E., Minneapolis, Minn. 55413

203R Grinnell Company (11/ 7/68)
260 W. Exchange St., Providence, R. I. 02901

218 Highland Corporation (2/12/71)
74-10 88th St., Glendale, N.Y. 11227

34R Ladish Co., Tri-Clover Division (10/15/56)
2809 60th St., Kenosha, Wisconsin 53140

239 LUMACO (6/30/72)
Box 688, Teaneck, N.J. 07666

200R Paul Mueller Co. (3/ 5/68)
P.O. Mox 828, Springfield, Mo. 65801

242 Purity, S.A. (9/12/72)
Alfredo Nobel #39 Industrial Pte. de Vigas, Tlalnepantla, Mexico

149R Q Controls (5/18/64)
Occidental, California 95465

89R Sta-Rite Industries, Inc. (12/23/68)
P.O. Box 622, Delavan, Wis. 53155

73R L. C. Thomsen & Sons, Inc. (8/31/57)
1303 43rd Street, Kenosha, Wisconsin 53140

191R Tri-Canada Cherry-Burrell, Ltd. (11/23/66)
6500 Northwest Drive, Mississauga, Ontario, Canada L4V 1K4

250 Universal Milking Machine Division (6/11/73)
Universal Cooperatives, Inc.
408 First Ave. S.
Albert Lea, Mn. 56007

86R Waukesha Specialty Company, Inc. (12/20/57)
Darien, Wisconsin 53114

266 Condor Manufacturing Company (8/ 1/75)
418 W. Magnolia Avenue
Glendale, California

09-07 Instrument Fittings and Connections Used on Milk and Milk Products Equipment

32 Taylor Instrument Process Control, Div. Sybron Corp. (10/ 4/56)
95 Ames Street, Rochester, New York 14601

206 The Foxboro Company (8/11/69)
Neponset Ave., Foxboro, Mass. 02035

246 United Electric Controls (3/24/73)
85 School Street, Watertown, Massachusetts 02172

269 Babson Bros. Company (1/23/76)
2100 South York Road
Oak Brook, Illinois 60521

10-00 Milk and Milk Products Filters Using Disposable Filter Media, As Amended

35 Ladish Co., Tri-Clover Division (10/15/56)
2809 60th Street, Kenosha, Wisconsin 53140

11-03 Plate-Type Heat Exchangers for Milk and Milk Products

20 A.P.V. Company, Inc. (9/ 4/56)
137 Arthur Street, Buffalo, New York 14207

30 Cherry-Burrell Corporation (10/ 1/56)
2400 Sixth Street, S.W., Cedar Rapids, Iowa 52404

14 Chester-Jensen Co., Inc. (8/15/56)
5th & Tilgham Streets, Chester, Pennsylvania 19013

38 CREPACO, Inc. (10/19/56)
100 CP Avenue, Lake Mills, Wisconsin 53551

120 DeLaval Company, Ltd. (12/ 3/59)
113 Park Street, South Peterborough, Ont., Canada

17 The DeLaval Separator Company (8/30/56)
Dutchess Turnpike, Poughkeepsie, N.Y. 12602

15 Kusel Dairy Equipment Company (8/15/56)
100 W. Milwaukee Street, Watertown, Wisconsin 53094

267 De Danske Mejeriers Maskinfabrik (10/15/75)
The Danish Dairies' Machine Factory
P.O. Box 66, 6000 Kolding, Denmark

12-04 Internal Return Tubular Heat Exchangers, for Milk and Milk Products

248 Allegheny Bradford Corporation (4/16/73)
P.O. Box 264, Bradford, Pa. 16701

243 Babson Brothers Company (10/31/72)
2100 S. York Road, Oak Brook, Illinois 60521

103 Chester-Jensen Company, Inc. (6/ 6/58)
5th & Tilgham Street, Chester, Pennsylvania 19013

152 The DeLaval Separator Co. (11/18/69)
350 Dutchess Turnpike, Poughkeepsie, N.Y. 12602

217 Girton Manufacturing Co. (1/23/71)
Millville, Pa. 17846

252 Ernest Laffranchi (12/27/73)
P.O. Box 455, Ferndale, Calif. 95536

238 Paul Mueller Company (6/28/72)
P.O. Box 828, Springfield, Missouri 65801

96 C. E. Rogers Company (3/31/64)
P.O. Box 118, Mora, Minnesota 55051

13-06 Farm Milk Cooling and Holding Tanks

240 Babson Brothers Company (9/ 5/72)
2100 S. York Road, Oak Brook, Illinois 60521

11R CREPACO, Inc. (7/25/56)
100 C. P. Ave.
Lake Mills, Wisconsin 53551

119R Dairy Craft, Inc. (10/28/59)
St. Cloud Industrial Park, St. Cloud, Minn. 56301

4R Dairy Equipment Company (6/15/56)
1919 S. Staughton Road, Madison, Wisconsin 53716

92R DeLaval Company, Ltd. (12/27/57)
113 Park Street, South Peterborough, Ontario, Canada

49R The DeLaval Separator Company (12/ 5/56)
Dutchess Turnpike, Poughkeepsie, N.Y. 12602

10R Girton Manufacturing Company (7/25/56)
Millville, Pennsylvania 17846

95R Globe Fabricators, Inc. (3/14/58)
3350 North Gilman Rd., El Monte, California 91732

179R Heavy Duty Products (Preston), Ltd. (3/ 8/66)
1261 Industrial Road, Preston, Preston, Ont., Canada

12R Paul Mueller Company (7/31/56)
P.O. Box 828, Springfield, Missouri 65801

249 Sunset Equipment Co. (4/16/73)
3765 North Dunlap Street
St. Paul, Minnesota 55112

42R VanVetter, Inc. (10/22/56)
2130 Harbor Avenue S.W., Seattle, Washington 98126

16R Zero Manufacturing Company (8/27/56)
Washington, Missouri 63090

14-00 Inlet and Outlet Leak Protector Plug Valves for Batch Pasteurizers, As Amended

122R Cherry-Burrell Company (12/11/59)
2400 Sixth St., S.W., Cedar Rapids, Iowa 52406

69 G & H Products Corporation (6/10/57)
5718 52nd Street, Kenosha, Wisconsin 53140

27 Ladish Co. - Tri-Clover Division (9/29/56)
2809 60th Street, Kenosha, Wisconsin 53140

78 L. C. Thomsen & Sons, Inc. (11/20/57)
1303 43rd Street, Kenosha, Wisconsin 53140

16-04 Evaporators and Vacuum Pans for Milk and Milk Products

254 Anhydro, Inc. (1/ 7/74)
130 S. Washington St., North Attleboro, Mass. 02760

132R A.P.V. Company, Inc. (10/26/60)
137 Arthur Street, Buffalo, New York 14207

164R Anderson IBEC (4/25/65)
19609 Progress Drive
Strongsville, Ohio 44136

263 C. E. Howard Corporation (12/21/74)
9001 Rayo Avenue
South Gate, California 90280

107R C. E. Rogers Company (8/ 1/58)
P. O. Box 118, Mora, Minnesota 55051

186R Marriott Walker Corporation (9/ 6/66)
925 East Maple Road, Birmingham, Mich. 48010

259 Pollution Control, Inc. (4/ 5/74)
P.O. Box 208, Wilson Place, South Barre, Vt. 05670

17-04 Fillers and Sealers of Single Service Containers For Milk and Milk Products

192 Cherry-Burrell Corporation (1/ 3/67)
2400 Sixth St., S.W., Cedar Rapids, Iowa 52404

137 Ex-Cell-O Corporation (10/17/62)
2855 Coolidge, Troy, Michigan 48084

220 Hercules, Inc., Package Equipment Division (4/24/71)
2285 University Ave., St. Paul, Minnesota 55114

211 Twinpack, Inc. (2/ 4/70)
2225 Hymus Blvd., Dorval 740 P.Q.

19-00 Batch and Continuous Freezers, For Ice Cream, Ices and Similarly Frozen Dairy Foods, As Amended

141 CREPACO, Inc. (4/15/63)
100 C. P. Avenue, Lake Mills, Wisconsin 53551

146 Cherry-Burrell Company (12/10/63)
2400 Sixth Street, S. W., Cedar Rapids, Iowa 52404

22-03 Silo-Type Storage Tanks for Milk and Milk Products

168 Cherry-Burrell Corporation (6/16/65)
575 E. Mill St., Little Falls, N.Y. 13365

154 CREPACO, Inc. (2/10/65)
100 C.P. Ave., Lake Mills, Wisconsin 53551

160 Dairy Craft, Inc. (4/ 5/65)
St. Cloud Industrial Park
St. Cloud, Minn. 56301

181 Damrow Company, Division of DEC International, Inc. (5/18/66)
196 Western Ave., Fond du Lac, Wisconsin 54935

262 De Laval Company Limited (11/11/74)
113 Park Street South' Peterburough, Ontario

156 C. E. Howard Corporation (3/ 9/65)
9001 Rayo Avenue, South Gate, California 90280

155 Paul Mueller Co. (2/10/65)
P.O. Box 828, Springfield, Missouri 65801

195 Paul Mueller (Canada), Ltd. (7/ 6/67)
84 Wellington St., So., St. Marys, Ont., Canada

165 Walker Stainless Equipment Co. (4/26/65)
Elroy, Wisconsin 53929

23-00 Equipment for Packaging Frozen Desserts, Cottage Cheese and Milk Products Similar to Cottage Cheese in Single Service Containers

174 Anderson Bros. Mfg. Co. (9/28/65)
1303 Samuelson Road, Rockford, Illinois 61109

209 Doboy Packaging Machinery (7/23/69)
Domain Industries, Inc.
869 S. Knowles Ave., New Richmond, Wis. 54017

258 Hercules, Inc. (2/ 8/74)
2285 University Ave., St. Paul, Minnesota 55114

222 Maryland Cup Corporation (11/15/71)
Owings Mills, Maryland 21117

193 Triangle Package Machinery Co. (1/31/67)
6655 West Diversey Ave., Chicago, Illinois 60635

24-00 Non-Coil Type Batch Pasteurizers

161 Cherry-Burrell Corporation (4/ 5/65)
575 E. Mill St., Little Falls, N.Y. 13365

158 CREPACO, Inc. (3/24/65)
100 C. P. Avenue, Lake Mills, Wisconsin 53551

187 Dairy Craft, Inc. (9/26/66)
St. Cloud Industrial Park
St. Cloud, Minn. 56301

177 Girton Manufacturing Co. (2/18/66)
Millville, Pennsylvania 17846

166 Paul Mueller Co. (4/26/65)
P.O. Box 828, Springfield, Mo. 65601

25-00 Non-Coil Type Batch Processors for Milk and Milk Products

162 Cherry-Burrell Corporation (4/ 5/65)
575 E. Mill St., Little Falls, N.Y. 13365

159 CREPACO, Inc. (3/24/65)
100 C.P. Avenue, Lake Mills, Wisconsin 53551

188 Dairy Craft, Inc. (9/26/66)
St. Cloud Industrial Park
St. Cloud, Minn. 56301

167 Paul Mueller Co. (4/26/65)
Box 828, Springfield, Mo. 65801

196 Paul Mueller (Canada), Ltd. (7/ 6/67)
84 Wellington St., So., St. Marys, Ont., Canada

202 Walker Stainless Equipment Co. (9/24/68)
New Lisbon, Wis. 53950

26-00 Sifters for Dry Milk and Dry Milk Products

228 Day Mixing, Div. LeBlond, Inc. (2/28/72)
4932 Beech Street, Cincinnati, Ohio 45202

229 Russell Finex Inc. (3/15/72)
156 W. Sandford Boulevard, Mt. Vernon, N.Y. 10550

173 B. F. Gump Division (9/20/65)
Blaw-Knox Food & Chem. Equip. Inc.
750 E. Ferry St., P.O. Box 1041
Buffalo, New York 14240

185 Rotex, Inc. (8/10/66)
1230 Knowlton St., Cincinnati, Ohio 45223

176 Koppers Company, Inc., (1/ 4/66)
Metal Products Division
Sprout-Waldron Operation
Munsy, Pennsylvania 17756

172 SWECO, Inc. (9/ 1/65)
6111 E. Bandini Blvd., Los Angeles, California 90022

28-00 Flow Meters for Milk and Liquid Milk Products

253 Badger Meter, Inc. (1/ 2/74)
4545 W. Brown Deer Road, Milwaukee, Wis. 53223

223 C-E IN-VAL-CO, a division of Combustion Engineering, Inc. (11/15/71)
P.O. Box 556, 3102 Charles Page Blvd., Tulsa Oklahoma 74101

231 The DeLaval Separator Company (3/27/72)
350 Dutchess Turnpike
Poughkeepsie, New York 12603

265 Electronic Flo-Meters, Inc. (3/10/75)
12115 Self Plaza, Dallas, Texas 75218

226 Fischer & Porter Company 12/ 9/71)
County Line Road, Warminster, Pa. 18974

261 Foss America, Inc. (11/ 5/74)
Route 82
Fishkill, N.Y. 12524

224 The Foxboro Company (11/16/71)
Foxboro, Massachusetts 02035

270 Taylor Instrument Process Control (2/ 9/76)
Sybron Corporation
95 Ames Street
Rochester, N.Y. 14601

29-00 Air Eliminators for Milk and Fluid Milk Products

30-00 Farm Milk Storage Tanks

257 Babson Bros. Co. (2/ 7/74)
2100 S. York Road, Oak Brook, Illinois 60521

32-00 Uninsulated Tanks for Milk and Milk Products

264 Cherry-Burrell Company, Division (1/27/75)
of Paxall, Inc.
575 E. Mill St., Little Falls, N.Y. 13365

268 Dairy Craft, Inc. (11/21/75)
P.O. Box 1227
St. Cloud, Minnesota 56301

Letter to the Editor

DEAR SIR:

Whether molds used to ripen Camembert and Roquefort cheese are detrimental to human health or not has been a matter of concern in mycotoxin research for some years. In the Federal Republic of Germany investigations have been done for about 3 years to find out whether such molds may produce mycotoxins which may eventually exhibit carcinogenic effect. These studies were prompted by recent publications of Gibel et al. (*1*), Kanota (*2*), and Ichinoe et al. (*3*).

I would like to inform you now of initial results that were published recently and that may not be accessible to your readers because they were published in German (*4*).

Rainbow trout fingerlings (*Salmo gairdneri*) were fed pelletized fungal mycelium and commerical feed ad libitum for 100 days. The portion of fungus in this feed mixture was about 30% of dry matter, and the intake of fungus varied between 5 and 6.2 g/kg of fish/day. Three different molds used for Camembert cheese and one used for Roquefort were fed.

The weight gain of the fish receiving the diet with the fungus was equal or superior to the controls. The feed quotient was approximately 2, the animals were of good appearance, and there were no abnormal findings after slaughtering. The livers appeared healthy and were red-brown in color. There were no differences in taste between the test fish and controls. In view of the large portion of mycelium in the feed it may be concluded that the tested strains of *Penicillium caseicolum* and *Penicillium roqueforti* did not cause any damage in rainbow trouts.

I hope that these results which are important for consumers in Europe will also be of interest in the United States even though per capita consumption of Camembert and Blue cheese is much lower there than in Europe.

H. K. FRANK
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für Ernährung
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D-75 Karlsruhe 1
West Germany

1. Gibel, W., K. Wegner, and G. P. Wildner. 1971. Experimentelle Untersuchungen zur Frage einer kanzerogenen Wirkung von *Penicillium camemberti* var. *candidum*. Arch. Geschwulstforsch. 38:1-6.
2. Kanota, K. 1970. Studies on toxic metabolites of *Penicillium roqueforti*. p. 129. In: M. Herzberg (ed.) Proceedings of the first U.S.-Japan conference on toxic microorganisms, mycotoxins, botulism. Honolulu, Hawaii, 1968. U.S. Department of the Interior, Washington D.C.
3. Ichinoe, M., S.-I. Udagawa, M. Tazawa, and H. Kurata. 1970. Some considerations on a biological method for the detection of mycotoxins in Japanese foods. p. 191. In: M. Herzberg (ed.) Proceedings of the first U.S.-Japan conference on toxic microorganisms, mycotoxins, botulism. Honolulu, Hawaii, 1968. U.S. Department of the Interior, Washington D.C.
4. Frank, H. K., R. Orth, G. Reichle, and W. Wunder. 1975. Fütterungsversuche an Forellen mit Camembert- und Roquefort-Kulturen. Milchwissenschaft 30:594-597.

News and Events

American Dry Milk and Whey Products Institute to Meet

The 51st Annual Meeting of the American Dry Milk Institute and the 5th Annual Meeting of the Whey Products Institute will be held jointly at the Marriott Motor Hotel, Chicago, on April 28, 29 and 30, 1976, it has been announced by J. T. Walsh, Executive Director of both organizations.

All dry milk and whey product manufacturers, allied industry friends interested in processing and marketing of these products, and representatives from government and universities are cordially invited to attend the meetings.

The General Sessions program will present knowledgeable speakers from industry, government, education and the Institute's staff, who will discuss topics of current interest to manufacturers and users of dry milk and whey products.

An interesting program also has been planned for ladies attending this joint annual meeting.

Sanitation Training Programs for Food Service Industry

National Educational Media, Inc., producer/distributor of internationally-used training motion pictures, has available a complete series of Sanitation Training Programs to help the Food Service Industry cope with increasing surveillance by government agencies.

The series includes five color/sound motion pictures, each approximately ten minutes in length. Throughout the series, the importance of personal cleanliness and good housekeeping practices are continually emphasized. The food service worker's personal responsibility to the public in maintaining sanitary eating conditions is a central theme.

NEM's training programs are used throughout the world. Film titles are available in English and Spanish.

For further information contact National Educational Media, Inc., 15760 Ventura Blvd., Encino, CA 91436. (213) 990-2125.

New Dietary Standard for Canada

National Health and Welfare Minister Marc Lalonde today made public a new Dietary Standard for Canada replacing that published in 1964.

The Dietary Standard for Canada is a statement, based on scientific data, of the daily amounts of energy and essential nutrients considered adequate to meet the needs of practically all healthy Canadians. These recommended quantities exceed the minimum requirements for most individuals because they must take into account variation in human needs for specific nutrients.

News and Events

Food Service Sanitation Brochure Offered for Bicentennial Use

From small picnics to church suppers all the way up to stadium-type mass-feeding events, food service management is becoming a key concern of civic and patriotic organizations in communities all over the country as they plan their part in the nation's Bicentennial celebrations. How do organization leaders, who often have little experience, work under difficult circumstances and depend on volunteer help, make sure of safety and sanitation for their members and guests?

A brochure especially designed to help such organizations insure food service sanitation at Bicentennial functions is now being offered through public health departments and environmental agencies to groups in their communities or jurisdictions. The brochure is entitled "Have a Safe and Sanitary Celebration: Some Tips on Serving Food at Bicentennial Affairs." Quantity copies can be obtained without charge on request from the Single Service Institute (250 Park Avenue, New York, New York 10017), the national trade association of manufacturers of disposable food service and packaging products.

The brochure points out that "existing food facilities in many communities are going to be severely strained" during the Bicentennial period. It notes that many civic and patriotic organizations will be serving food in settings not originally designed for meal service. Under these adverse conditions, the brochure continues, "the potential for human illnesses caused by food contamination or infection is likely to increase during the Bicentennial celebrations—unless extra precautions are taken to offset the special sanitation problems that will prevail."

After first urging organizations to check with local public health authorities for information and guidance on food preparation and service, the brochure spells out the key sanitary precautions that should be observed in order to prevent outbreaks of food-borne illness. These suggestions for hygiene and cleanliness apply in four areas: personnel, food preparation, food service, and cleaning and disposal.

The brochure points out that, under the difficult and often makeshift conditions likely to mark many Bicentennial affairs, adequate dishwashing facilities will often not be available. For such occasions especially, the brochure suggests, single-use items—paper and plastic plates, cups and utensils—may well be the most sanitary as well as the most practical.

The brochure concludes by describing the benefits of single service products and providing tips on their selection, storing, dispensing, use and disposal to insure that maximum benefits are gained.

Although prepared by the Single Service Institute, the brochure provides space for the imprint of the local health or environmental agency.

30th Annual Meeting

R&D Associates for Military Food and Packaging Systems, Inc. will hold its 30th Annual Meeting April 21-22, 1976 at the Drake Hotel, Chicago.

The theme is: "Quick Service Restaurants: The Future of Military Feeding?" The sessions are as follows:

Session I—An overview of all QSR Problems to include QSR Packaging Technology, QSR Equipment Technology, QSR Product Development, and Marketing.
Session II—QSR Distribution
Session III—QSR Impact on the Military
Session IV—QSR Impact on Health and Nutrition

This is a good opportunity for those in food, food packaging, feeding and feeding equipment to be brought up-to-date on current requirements, problems and progress in the Armed Forces.

For further information contact: Col. Merton Singer, USA (Ret) Exec. Sec., R&D Associates, Rm. 1315, 90 Church St., New York, N.Y. 10007. (212)264-7612.

Evaporated Milk Association Officers Elected at Annual Meeting

Bob L. Hall, General Manager, O-At-Ka Milk Products Cooperative, Inc., Batavia, New York was elected to serve a third term as President of the Evaporated Milk Association at its annual meeting in San Francisco, January 22, 1976.

Elected to serve a third term as Vice President was Ray Morris, President, Grocery Products Division, Pet Incorporated, St. Louis. Joseph M. Carson Jr., President, United Dairy, Inc., Martins Ferry, Ohio was reelected Treasurer.

Reelected to serve with the officers as the Association's Board of Directors were: Henry C. Arnest, Carnation Company, Los Angeles, John Campbell, Westerville Creamery, Covington, Ohio, and William A. Diehl, The Defiance Milk Products Company, Defiance, Ohio.

Organized in 1923, the Evaporated Milk Association is based in Washington, DC. J. C. Flake, PhD, is the Association's Executive Vice President.

The Evaporated Milk Association is supported by all processors of evaporated milk in the United States, and a number of associate members.

News and Events

Canadian Position on the Food Color Amaranth (Red No. 2)

National Health and Welfare Minister Marc Lalonde announced on February 2, 1976 that after a careful evaluation of all available evidence, and discussions with health experts in other countries, the Health Protection Branch (HPB) has concluded there is insufficient evidence available at this time to justify removal of the food color Amaranth from foods sold in Canada.

The recent decision by the Food and Drug Administration (FDA) of the Unites States to propose a ban on the use of Amaranth in that country raised legitimate concern among Canadian consumers over its use in this country. This concern, however, is not substantiated by the available scientific evidence.

It should be noted that Amaranth has received repeated approval for use by the Joint Expert Committee of the Food and Agriculture Organization (FAO) and of the World Health Organization (WHO). Canada was in agreement with the Committee's assessment and the FDA study has not altered our view.

The American studies

The U.S. decision to ban Amaranth resulted primarily from a study conducted in the FDA's own laboratories. In the FDA study, rats of both sexes were fed Amaranth at 3.0, 0.3, 0.03 or 0.003% in the diet for approximately 2½ years, with a control group of rats, also of both sexes, receiving no Amaranth in their diet. A variety of benign and malignant tumors was observed. A mix-up in the dosing of some of the animals of the low dose groups for part of the experiment precludes the possibility of establishing a dose response relationship. However, it was apparently possible to compare the group fed 3% Amaranth with the control group. Female rats, but not males, fed 3% Amaranth showed a statistically significant increase in the number of animals with malignant tumors. From the data available, HPB scientists consider this increase in tumors of no biological significance for the following reasons.

(*a*) The effect was not organ specific. Experts in the cancer field consider that for a study to have biological significance, it is necessary to demonstrate the presence of a number of tumors of an unusual tumor type for the particular species and strain of animal or an increase in the number of tumors for a particular organ. In both instances the increase would have to be significantly greater than in control animals. The tumors found in the FDA study were similar in number and type to those previously encountered in rats of the same strain and age, reared and housed under similar environmental conditions and fed diets free of the color.

(*b*) Amaranth has a chemical structure similar to other dyes that are non-carcinogenic and different from those that are carcinogenic.

(*c*) The tumor increase was confined to female rats and except for the mammary tumors the cancers were not sex related. This is very unusual, particularly since the number of malignant tumors in control male rats was greater than in male rats given 3% Amaranth in the diet. This latter observation could, by the same reasoning apparently used by the FDA, be used as evidence that in male rats, Amaranth actually prevents the production of cancer.

(*d*) Preliminary mutagenicity screening tests conducted by HPB indicate that Amaranth is not mutagenic and hence not likely to be carcinogenic. Almost all substances which are known to be mutagenic are also carcinogenic.

(*e*) As indicated above, in the FDA study, there was a mix-up in the animal numbers and in the diets fed to certain groups of animals. Many tissues were in a state of advanced decomposition making microscopic examination extremely difficult to carry out properly. This would appear to indicate inadequate experimental control, and makes it well-nigh impossible to adequately assess the U.S. study.

Other research in the U.S., not evidently considered by the FDA in its decision-making process, included teratogenic studies conducted by Dr. J. Verrett, an FDA staff-member. Dr. Verrett expressed particular concern about possible adverse reproductive effects from Amaranth on the CBC program *Market Place,* January 25, 1976. She stated that Amaranth caused deaths and birth defects in chick embryos. Dr. Verrett's observations are not considered relevant in terms of the safety of Amaranth to humans. The chick embryo is not considered to be a suitable test species by teratologists generally. One reason for this unreliabliltiy is its unusual sensitivity to a wide variety of compounds. Common agents such as salt, sucrose, and sand cause teratogenic effects in the chick embryo but not in man or other animals. During its embryonic growth the avian embryo remains isolated from its mother and thus the metabolizing and detoxifying mechanisms which protect the mammalian embryo do not operate in the avian embryo. This, and the ultrasensitivity of the avian embryo, have led most laboratories to discontinue its use for chemical safety evaluation. Dr. A. C. Kolbye, a senior official of the FDA has stated that "while the test can be useful for detecting toxicity of compounds, further evaluation of this test is needed before its usefulness in predicting birth defects in animals can be determined." The chick embryo technique was replaced in 1966 at HPB by more reliable techniques that employ mammalian species. HPB deplores the fact that CBC—*Market Place* did not check on the reliablility of the chick embryo test.

In the FDA cancer study on Amaranth, the rats which purportedly developed tumors as a result of receiving Amaranth, were given the color at 3% in the diet, equivalent to approximately 1500 mg/kg body weight/day. This means that every day a person would have to eat more than 1600 pounds of food containing approximately 100 parts per million (ppm) of Amaranth to be exposed to an equivalent amount to that purported to cause an increase in total malignant tumors in female rats. (Although the maximum permitted level of Amaranth in Canada is 300 ppm, the actual level of use averages 100 ppm.)

It is important to point out that HPB has discussed the validity of the FDA rat study with a number of University-based toxicologists and cancer experts in the United States. All those contacted agree with the Canadian assessment of the FDA experiment.

Status of Amaranth in other countries

Amaranth is permitted as a food color in the nine countries of the European Economic Community (EEC), and in Sweden, Denmark, West Germany, Japan, and many other countries. Its use is not permitted in Russia.

The view of international health agencies

In 1964 the FAO/WHO Joint Expert Committee on Food Additives evaluated the safety of Amaranth on the basis of all the relevant data available at that time. This Committee of international experts concluded that adequate data were available to rule out carcinogenicity of this color in rats and mice. The Committee established a level of Amaranth (termed the Acceptable Daily Intake or a.d.i.) which if consumed every day throughout the entire life-span would be safe.

In 1972 this Committee reevaluated the safety of Amaranth in the light of new data that had become available. The new data included reports from Russia suggesting carcinogenic effects and fetotoxic effects in rats. Difficulty in interpreting the results of the Russian studies and the fact that further relevant studies were then in progress caused the Committee to defer a final reevaluation. However, in the interest of prudence, the previous A.D.I. was lowered by 50% and given a temporary status pending results of the work in progress.

After the Russian reports were published, extensive studies were undertaken by HPB scientists and in at least three laboratories in the U.S.A. HPB studies initiated in 1973 and completed in 1974 on the teratogenic and reproductive effects of Amaranth in cats and rats showed the color to be without adverse effects. Furthermore, none of the U.S. studies produced conclusive evidence that Amaranth caused adverse effects on fetal development. In November 1975 the National Toxicology

News and Events

Canadian Position—*Continued . . .*

Advisory Committee in the U.S. reached the conclusion that Amaranth is not fetotoxic.

The FAO/WHO Expert Committee on Food Additives considered Amaranth for a third time in 1975. The Committee confirmed the A.D.I. of Amaranth arrived at in 1972.

Amaranth research at HPB

As new technololgy is developed the safety of Amaranth and other food colors is continually reevaluated by HPB scientists. In studies conducted in our laboratories in the late 1950's, the cancer producing potential of a number of food colors was assessed. Most, including Amaranth, were found to be safe. One of the colors, Ponceau 3R, was found to be carcinogenic and was removed from the Canadian market. This pioneering Canadian research led to the banning of Ponceau 3R throughout the world.

New techniques for assessing the mutagenic effects of chemicals have been developed in recent years and these techniques now are being applied by HPB to the study of Amaranth. To date, all results from these mutagen testing procedures have been negative, giving the strong indication that Amaranth does not induce mutations. Recently a supply of radioactive Amaranth has been obtained by HPB. This has enabled more detailed studies of the metabolism of the color to be undertaken. These studies, which will be completed in a few months time, will assist greatly in the assessment of species differences in the metabolism of Amaranth and in the interpretation of other experimental data on the safety of Amaranth for humans.

In addition to data developed in our own research laboratories, HPB scientists also have access to published and unpublished reports on Amaranth and an HPB scientist serves on the WHO/FAO Expert Committee mentioned previously. Each time a concern has been raised over some aspect of potential hazard of Amaranth as a food color, the evidence has been evaluated and, if necessary, additional scientific evidence has been collected.

Canadian use of food colors

Canada's list of permitted synthetic food colors is shorter than in most countries. In addition to the natural colors, nine synthetic dyes are available for food use in this country. Seven of these may be used in foods in general, if the coloring of that particular food is provided for by regulation under the Food and Drugs Act. The other two synthetic colors are used specifically for the coloring of the skins of whole oranges with Citrus Red No. 2 and of fruit peel, glace fruits, and maraschino cherries with Ponceau SX.

The seven synthetic colors for general food use are Amaranth, Erythrosine, Indigotine, Sunset Yellow FCF, Tartrazine, Brilliant Blue FCF, and Fast Green FCF. In some industrialized countries, as many as 20 or more synthetic colors are permitted in foods.

History of department regulation of food colors

Many changes have occured in the regulation of food colors in Canada over the last 20 years. Some colors have been withdrawn from use because of adverse effects found in our studies or in tests carried out in other laboratories. Other colors have been removed from our permitted list because they had limited usage and could be replaced by combinations of other colors. The chronological list of Canadian regulatory action on food colors is as follows:

1965: Orange 1, Orange S.S., and Oil Red XO were withdrawn from use in foods sold in Canada.

1959: Oil Yellow AB and Oil Yellow OB were withdrawn from use. Citrus Red No. 2 permitted on skins of whole oranges at a maximum use level of 2 ppm.

1960: Ponceau 3R withdrawn from use.

1965: Naphthol Yellow S, Light Green SF Yellowish, and Guinea Green B withdrawn from use.

1966: Use of Amaranth, Erythrosine, Tartrazine, Sunset, Yellow FCF, and Indigotine set at a maximum of 300 ppm singly or in any combination.
Use of Fast Green FCF, Brilliant Blue FCF and Benzyl Violet 4B set at a maximum of 100 ppm singly or in combination. Use of Ponceau SX restricted to fruit peel, glace fruits, and maraschino cherries at a level up to 150 ppm.

1973: Benzyl Violet 4B withdrawn from use.

A permitted food color is always subject to reevaluation if new data become available. New food colors are carefuly reviewed by HPB scientists and data provided by manufacturers are thoroughly evaluated before these new colors are permitted for use in food products. For example, FD & C Red #40, a red food color known in Canada as allura red, is permitted for use in foods in the United States. It is not permitted in Canada because HPB scientists concluded 2 years ago that evidence submitted by the manufacturer with respect to the safety of the product was inadequate.

James B. Mickle, 1927-1976

Dr. James B. Mickle, 48, nationally recognized dairy foods scientist at Oklahoma State University, died January 27, 1976, of complications of a heart ailment. Burial was at Fairlawn Cemetery, Stillwater.

Born and reared at Lincoln, Nebraska, Mickle received B.S. and M.S. degrees from the University of Nebraska and the Ph.D. degree from Michigan State University. He had been on the faculty at OSU since 1953.

Mickle was nationally respected for his research on methods of processing milk to maintain and improve quality. Much of his work focused on the influence of emulsifiers in milk and foods made from dairy products. In recent years he had developed a high protein snack food, called Cowboy Wheyfers, made from whey, a waste product of cheese making. He also had developed nutritious custard and ice cream products tailored specifically for the needs of elderly persons.

Mickle was a member of numerous professional and honorary organizations and had published more than 45 professional journal articles, some of which appeared in the *Journal of Milk and Food Technology.* He was a past adviser of the national student branch of the American Daily Science Association.

Survivors include his wife, Lois at home; two daughters, Mary Ann, a student at North Texas State University, and Laurie at home; a son, Robin, a student at the U.S. Naval Academy; and his stepmother, Mrs. Loretta Mickle, Lincoln, Nebraska.

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Planning for the 63rd Annual Meetingof IAMFES are, from left to right, Dr. Henry Atherton, IAMFES Program Chairman; Dale Termunde; Dave Fry, First Vice-President and Charles Price, Chairman of the Local Arrangements Committee.

1976 - A Star Spangled Meeting

The 63rd Annual International Milk, Food and Environmental Sanitarians Meeting will be held August 8-11, 1976, at the Arlington Park Hilton Hotel, Arlington Park, Illinois.

This meeting will be developed with a bicentennial theme. The facilities are classified as one of the most complete convention facilities in the world. A partial listing of these features include: lighted golf course, lighted tennis courts, indoor and outdoor pools, saunas and health clubs, theater in the round, restaurants, cocktail lounges, its own race track (the horses will be running) — five minutes from the world's largest indoor shopping center.

The Wednesday evening banquet and dinner dance will include a performance by the Brothers & Sisters, a nationally known group of singers and dancers.

Plan to attend this bicentennial meeting August 8-11, 1976, and bring your wife and family. The 63rd Annual meeting is going to be a Star Spangled Affair.

Affiliate Annual Meetings

Florida—March 16-18, 1976, Langford Hotel, Winter-Park
Iowa—March 22, 1976, Ramada Inn, Ames
Michigan—March 1976
South Dakota—May 11-14, 1976, Holiday Inn, Aberdeen
Virginia—March 8-10, 1976, Donaldson, Brown Center, Blacksburg

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NSF Gets High Marks from Health Community in 1975 Standards Use Survey

Last year we conducted a national survey among public health officials to determine how many endorse and enforce NSF standards in their jurisdictions.

More than 400 officials found time to reply to our questionnaire. As expected, the oldest standards got the highest marks.

These standards:

1. *Soda Fountain & Luncheonette Equipment*
2. *Food Service Equipment*
3. *Spray-type Dishwashers*

have each been in effect for more than 20 years and have had numerous revisions. Among our survey respondents, *nine* out of *ten* replied that NSF Standards 1, 2 and 3 are required in their jurisdictions.

Fourteen other NSF food service equipment standards covered by the survey are in use by a majority of these public health officials.

Send for the NSF Publications List and choose the publications you need. They will be sent free to any public official requesting them on his letterhead.

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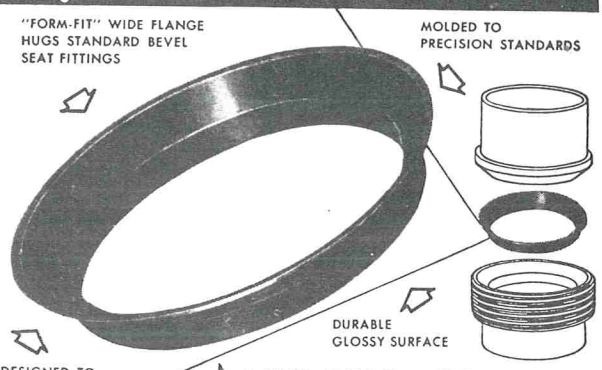

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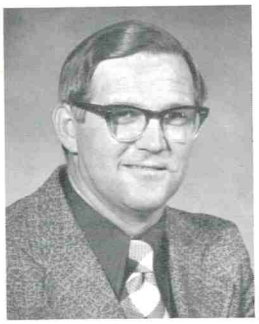
Dairy authorities speak out on better cow milking



Dr. Robert D. Appleman
Professor of Animal Science
University of Minnesota

Automatic take-off milking units: They can save and protect.

There are two primary reasons why an investment in more mechanized milking is being considered by many dairymen. One is to reduce labor. The other is to improve udder health and maintain production of high quality milk.

LABOR TAKES A BIG BITE

Labor accounts for 15 to 30 percent of all costs in a dairy operation. About 55 percent of this labor is expended in the milking operation. In general, the total labor cost to produce 100 pounds of milk in a herd averaging 12,000 pounds per cow annually when labor* is valued at $3.00 per hour approaches $2.50 per cwt. in 30-cow herds; $2.10 in 50-cow herds; $1.68 in 100-cow herds; $1.13 in 250-cow herds; and $.91 in 500-cow herds**.

With an investment to modernize milking parlors, including unit take-off, it is not unusual to substantially lower the labor costs of producing milk.

Many of the milking chores are repetitious and result in drudgery. According to our studies, 5 to 10 percent of the milker's chore time is spent removing the milking unit. On top of that, the typical milker spends from 12 to 30 percent of his time machine-stripping cows.

THE OPERATOR IS A BUSY MAN

Proper stimulation of cows in a milking parlor is important to good milk letdown. Recent New Zealand work shows there is a loss of up to 1,000 pounds of milk per cow yearly when cows are not properly stimulated. In many barns the milker cannot effectively handle as many milking units as today's economy demands. Frequently, washing and stimulation time is limited to less than 15 seconds per cow because the milker is too "busy" with machine stripping or handling other units. The result is slow milking combined with considerable overmilking. Automatic unit take-off should improve this situation. Addition of automated prep stalls will help even more, provided they function properly.

SOME RESEARCH RESULTS

Research studies comparing automatic take-off and conventional milking units involving 550 cows in a Louisiana herd resulted in these conclusions:

1. Automatic unit take-off significantly reduced the number of quarters infected with mastitis.
2. Automatic unit take-off reduced udder irritation as evidenced by lower CMT scores.
3. The men operating the automatic take-off units reduced their walking distance in the parlor by more than 25%.

Dr. Nelson Philpot, leader of this study, says one should not expect miracles. Automatic take-off units do not make a poor operator better. They do, however, allow a conscientious operator to do an even better job on more cows.

According to dairymen using this equipment, proper maintenance and proper operation of equipment is even more important with non-automated systems. The ability, cooperative attitude and location of your local serviceman should become a primary factor in deciding whether to install this more sophisticated and expensive equipment.

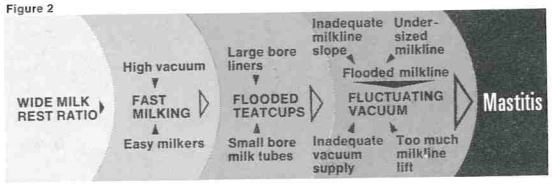
MASTITIS PREVENTION NOT SIMPLE

Dairymen should not necessarily expect a reduction in the number of cows requiring treatment for clinical mastitis (gargot). In a marginal system, more cases may result because the significance of a single variable is not the same in every milking system or in every situation.

Frank Smith, California milking system specialist, illustrates this point well. He indicates that too many researchers and educators have attempted to over-simplify the cause of mastitis. In turn, they have over-simplified its prevention. *The concept of a direct, independent relationship shown in Figure 1 is incorrect.* Figure 2 arranges these same variables in a manner which is sequential, additive, and interdependent.

Figure 1

Figure 2



As mentioned earlier, installing automatic unit take-off may allow one to milk cows faster and reduce overmilking. However, if such a change resulted in flooded milk lines and fluctuating vacuum, the incidence of mastitis might increase rather than decrease. Providing all other deficiencies in the system were corrected, automatic take-off would prove highly beneficial.

AUTOMATIC TAKE-OFFS A COMMON SIGHT?

Where cost of this mechanization is not excessive and such installations prove to be reasonably trouble-free over time, I'm sure that automatic take-off units will become an increasingly more common sight on dairy farms.

*For our purposes, the labor figures include all dairy chore labor, feeding labor, and the raising of offspring. Field labor isn't included.

**In 250-cow and 500-cow herds, we assume the existence of a parlor and a free-stall barn with mechanized feeding and waste handling.