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Commonwealth Scientific and Industrial Research Organization
Sydney, Australia

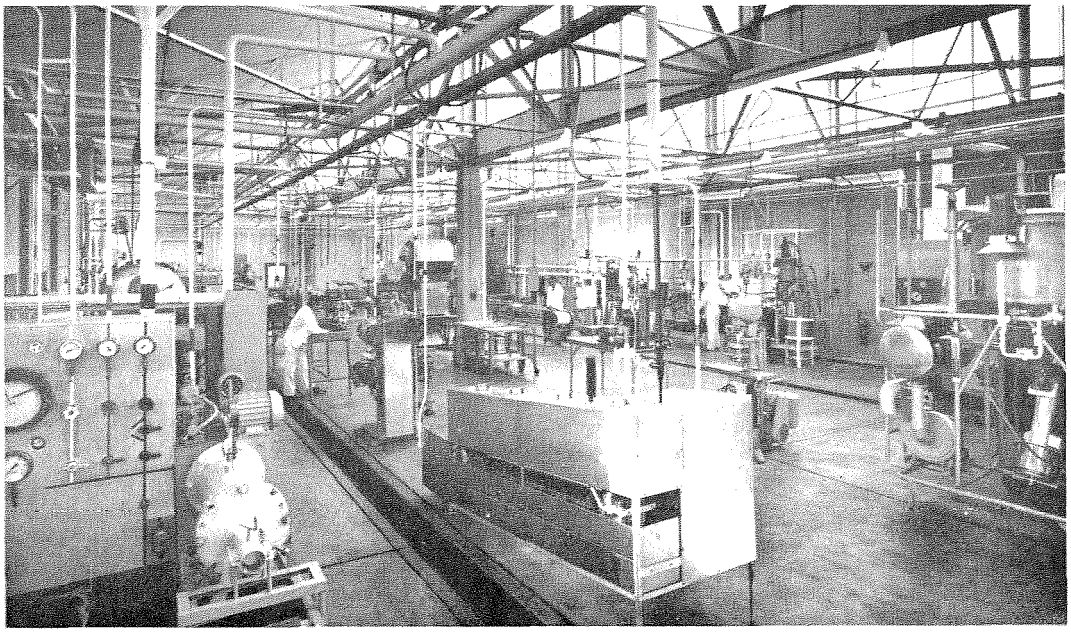
# Food Processing Facilities in the Division of Food Preservation

Among the facilities in the Ryde laboratories of the Division of Food Preservation is a Food Processing Building with an area of 7500 sq ft. In the five years since the Division moved to Ryde an attempt has been made to build up a comprehensive collection of pilot-scale food processing equipment. Substantial success in achieving this aim has been possible largely because of generous support from the food and ancillary industries in response to the Division's annual appeal for funds for special equipment. In addition, several organizations have provided pieces of equipment as gifts or as long-term loans.

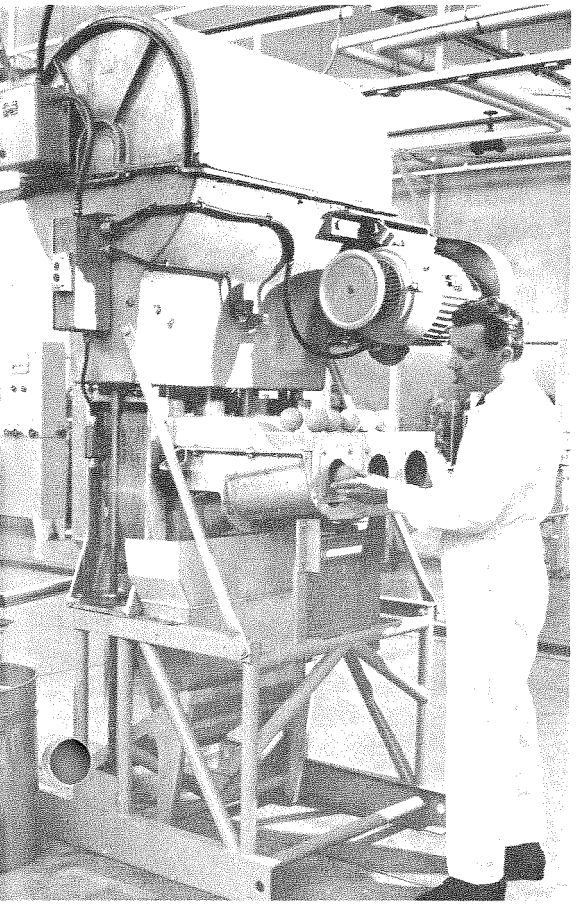
While all this equipment is essential to the Division's research programmes, individual items are used only intermittently. Accordingly, the Division wishes to invite the food industry to share in the facilities which they have assisted to set up. Already food manufacturers have found these facilities useful for pilot runs of new products, new processes, or new packages, and for the evaluation of new equipment on a pilot scale before purchase. Food machinery manufacturers have also used the area for demonstration of new equipment and assessment of operational characteristics. Inquiries from organizations wishing to make use of the food processing facilities will be welcomed by the Chief of the Division.

Organizations making use of the facilities would be expected to supply raw materials and containers which might be required, and also some skilled and unskilled labour according to the nature of the project.

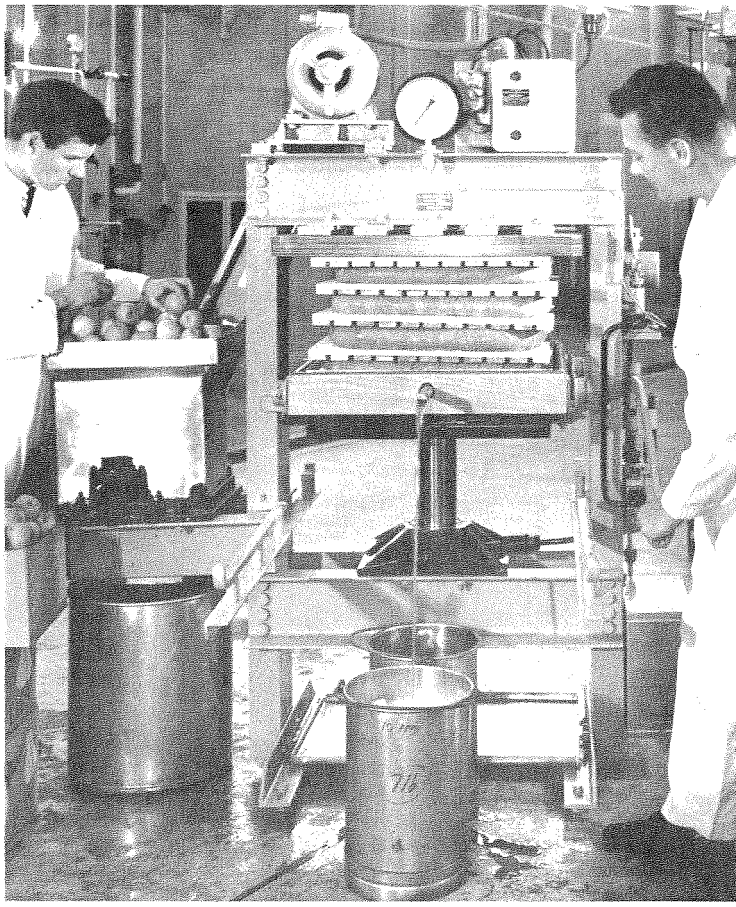
In general, the Food Processing Area is equipped to carry out the following unit operations:



General view in the Food Processing Building. *Left foreground,* pressure spin cooker. *Centre foreground,* atmospheric spin cooker.



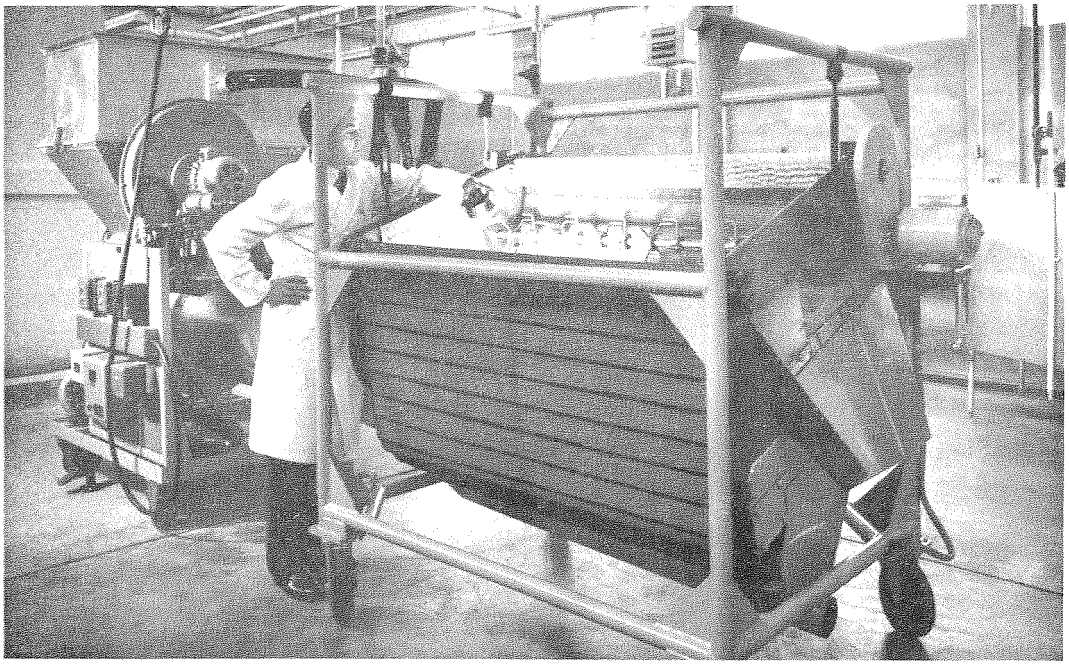
Citrus juice extractor with three extracting heads to suit different fruit sizes. Capacity 30 fruits/min/head.



Grater mill and rack-and-cloth press for extraction of fruit juices.

- *Food preparation.*—Washing, abrasive and lye peeling, slicing, dicing, shredding, grinding, comminuting, brush finishing, screening, and size grading.
- *Juice extraction.*—Hand and mechanical extraction of citrus juices, hydraulic pressing of apple and berry fruit juices, and centrifugal separation of fruit juices.
- *Packaging.*—Vacuum and steam-flow closing of cans, vacuum closing of glass containers, and heat sealing of flexible film packages.
- *Thermal processing.*—Stationary and agitated processing of cans and glass containers at atmospheric and higher pressures, continuous pasteurization of liquid foods.
- *Evaporation.*—Centrifugal and plate-type vacuum evaporators.
- *Drying.*—Cross-flow and through-flow tunnel drying, agitated-bed belt-trough drying, vacuum tumble drying, spray drying, atmospheric drum drying, freeze drying.
- *Freezing.*—Air-blast freezing with variable air flow.

Adjoining the Food Processing Area is a small laboratory for quality control and analytical purposes.



Belt-trough drier, which uses town-gas combustion products to heat the food material in an agitated bed. Evaporation capacity approximately 250 lb of water per hr.

Centrifugal vacuum evaporator, capable of giving a high degree of concentration during a single pass.

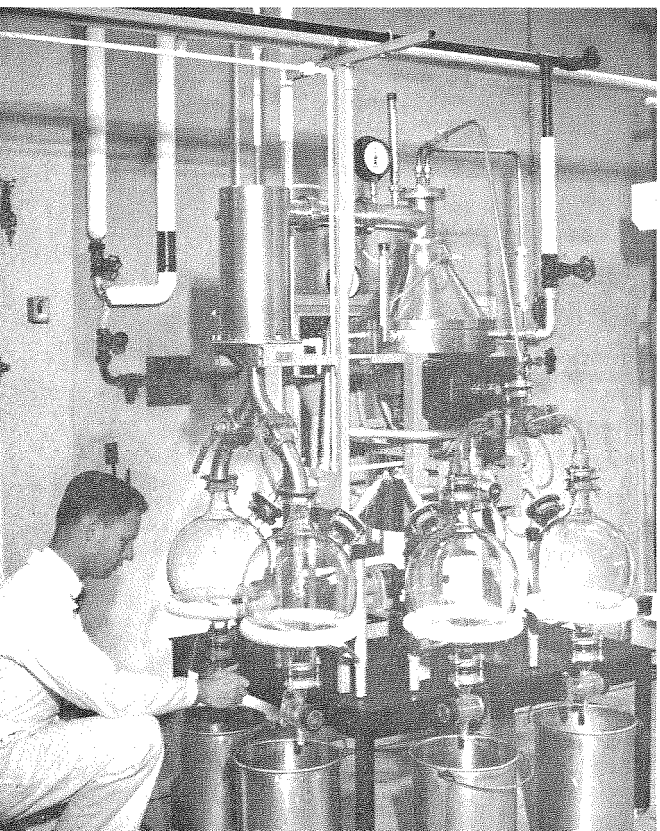
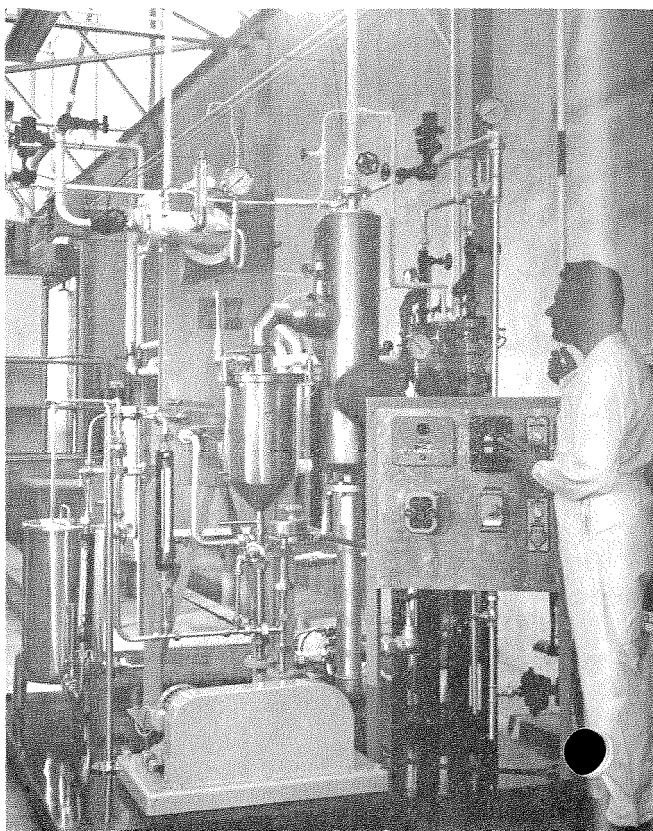
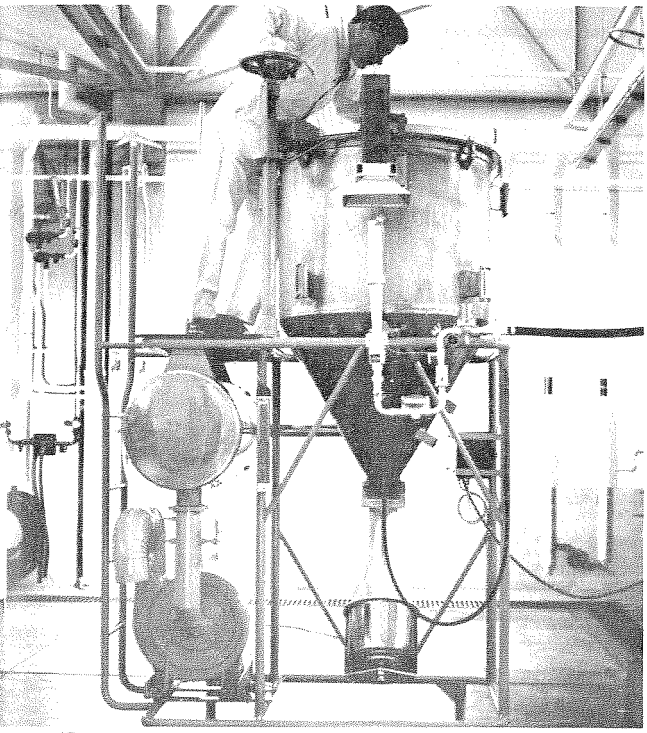
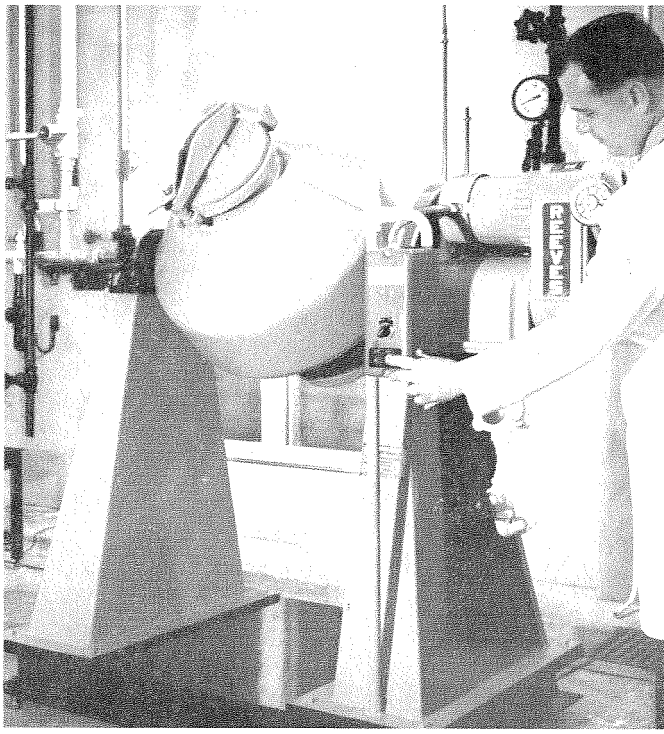


Plate evaporator, a single-effect unit capable of evaporating up to 100 lb of water per hr.



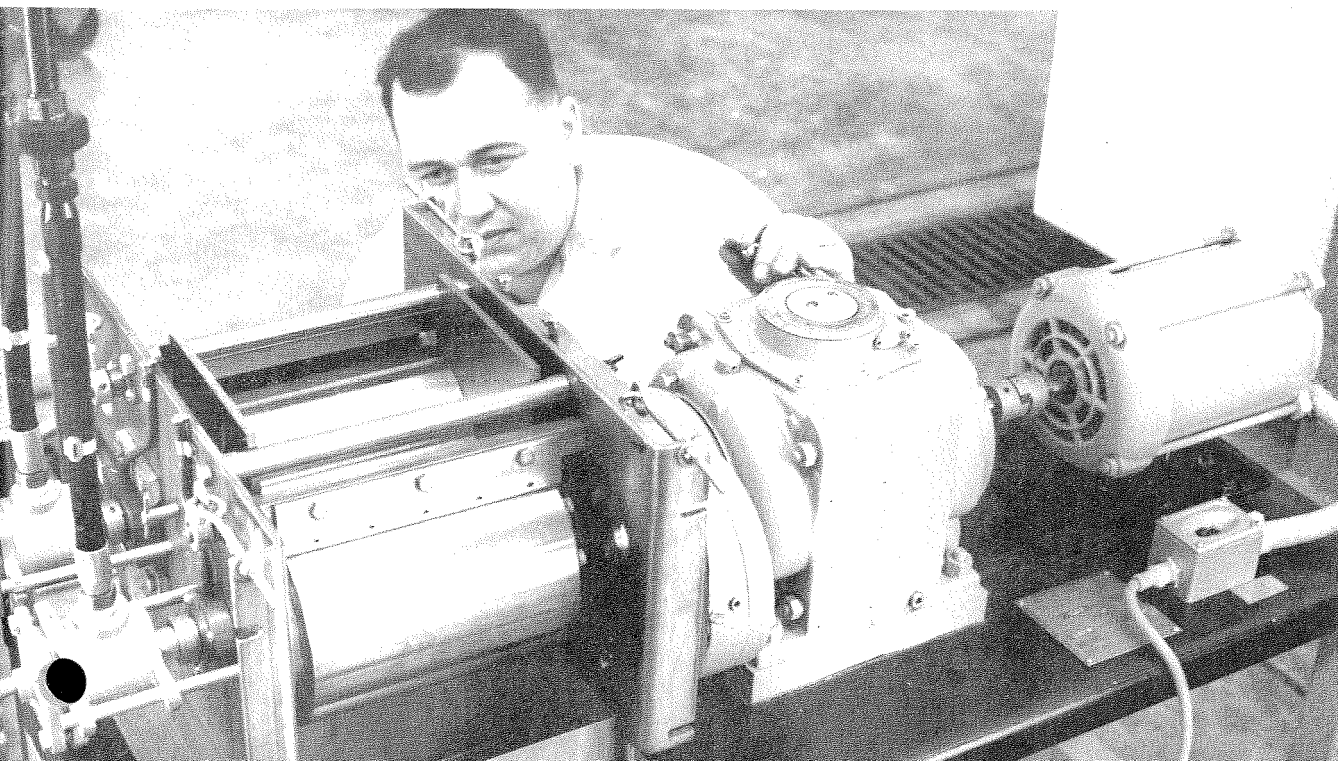


Spray drier with jet and centrifugal spray distributors. Feed capacity approximately 40 lb/hr.



Conical blender-drier, equipped for vacuum operation and used for vacuum tumble drying, vacuum steam blanching, and agitated freeze drying.

Laboratory atmospheric double-drum drier. Maximum working steam pressure 150 p.s.i. g.



# Botulism

## A Review of the Present Situation

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Interest in the problem of botulism involving commercially processed foods has been stimulated by outbreaks in North America within recent years. This article is based on a talk by Dr. W. G. Murrell at the 16th Annual Conference of the Institute of Food Technologists (Australian Regions) in May 1966.

BOTULISM, a neuroparalytic disease affecting man and animals, is relatively rare but is the deadliest type of food poisoning known. The comparatively low incidence of botulism can be accredited to the work of early researchers and to canners and other food processors. It has been said that we must keep a sense of proportion concerning the importance of botulism as a cause of death. At the same time, it must be borne in mind that botulism outbreaks are not necessarily confined to 3, 4, or even 12 victims. In the Soviet Union, for instance, at Dnepropetrovsk in 1933, intoxicated stuffed egg-plant relish caused 230 cases of type A botulism. Other large outbreaks on record include 180 cases in Leningrad in 1945, caused by salted sturgeon, and 100 cases in two Würrtemberg towns in 1869, caused by liver sausage. Such potentialities are a grim warning of what might conceivably happen if a toxic foodstuff were distributed massively and quickly throughout a country. Although the possibility is small, a set of conditions which might allow this to happen could still occur. The outbreaks from fishery products in the U.S.A. during 1960 and 1963 support this possibility.

This article discusses botulism in terms of its incidence and mortality rate, the nature and scope of the foodstuffs involved, and some of the principal factors governing the epidemiology and control of botulism.

### Causative Organism

Botulism is caused by the ingestion of toxin from *Clostridium botulinum*, a gram-positive, anaerobic, spore-forming, rod-shaped organism. When growth reaches a maximum it is followed rapidly by a period of cell degeneration and autolysis which results in the liberation of large quantities of toxin. The toxin, a heat-labile protein, is quite resistant to the proteolytic enzymes of the digestive tract, type E toxin being, in fact, activated by trypsin (Duff, Wright, and Yarinsky 1956). Botulinum toxin is also stable in acid but is readily inactivated by alkali or by heating at 80° for 30 minutes.

### Types of *Clostridium botulinum*

Six distinct types are now recognized. They are designated A, B, C, D, E, and F and are differentiated by the serological specificity of their toxins. Types A and B are readily distinguishable from type E by their heat resistance and minimum growth temperatures. Spores of types A and B will survive boiling for several hours, whereas type E spores are usually killed by heating to 80°C for 30 min or less. Moreover, types A and B grow slowly, if at all, at 10°C, while certain strains of type E have been observed to grow at temperatures as low as 3·3°C in beef stew medium (Schmidt, Lechowich, and Folinazzo 1961) and 5°C in cooked meat medium (Ohye and Scott 1957).

Type C includes two subtypes, C$\alpha$ and C$\beta$, which differ in their effects on animal species. Types C and D have only rarely been implicated in outbreaks of human botulism, but they have caused huge losses in wild and domestic animals. Type F, first recognized in 1958, has been involved in only one known outbreak of botulism, caused by home-prepared liver paste in Denmark (Moller and Schiebel 1960). Since then, however, the toxin of type F has been demonstrated in cultures from two marine sediments taken off the coasts of Oregon and California (Eklund

and Poysky 1965) and the organism has been isolated from a salmon taken from the Columbia River (Craig and Pilcher 1966).

Grimenez and Ciccarelli (1966) have reported the isolation of a new type of *Clostridium botulinum* from an Argentine soil sample. The toxin of this new type is not neutralized by types A, B, C, D, E, or F antitoxins, but the strain resembled type A in biochemical reactions. The toxin was heat-labile, being completely inactivated by heating for 2 min at 75°C, and the spores withstood heating to 100°C. The rate of neutralization of homologous toxin and type A toxin by serum prepared from the new strain was similar. The strain has been tentatively called type A$\beta$.

Types A, B, and E have been responsible for the majority of the known outbreaks of botulism among humans. Cultures of type E show much lower toxicities than types A and B when injected into mice, but the potency of type E cultures can be increased 10- to 100-fold by treating with trypsin. Toxins of types A and B are not usually activated by trypsin, because these organisms usually produce their own proteolytic enzymes which presumably perform the same function as trypsin.

Similar symptoms are produced by all the types and include vomiting, constipation, double vision, thirst, and difficulty in swallowing. Temperature is often subnormal. The symptoms of eight patients suffering from type E botulism were recently reported in some detail by Rogers, Koenig, and Spickard (1964). With certain exceptions they resemble those observed in outbreaks of type A. Severe nausea and vomiting occur within 12–24 hours, followed by weakness and dizziness. A common symptom was marked dryness of the mouth and tongue. Within 12–60 hours neurological symptoms appeared, including blurred vision and widely dilated pupils that did not react to light or near objects. The frequency with which the various symptoms were experienced by the eight patients is shown in Table 1.

Although nausea, vomiting, and urinary retention have not been notable features in type A outbreaks, the occurrence of dilated pupils, dry mucous membranes, progressive respiratory paralysis, abdominal distention, and constipation are common to all types of botulism. These symptoms are a logical sequence of the cholinergic block produced by the botulinum toxin. In fatal cases, death is caused by asphyxiation, usually within 3–6 days. It may take several months for complete recovery in non-fatal cases.

The toxin, consisting of quite large protein molecules (molecular weight 900,000), passes from the upper part of the intestinal tract into the lymphatic system. Having gained access to the circulatory system, the toxin acts in a well-defined manner, blocking cholinergic junctions by preventing the presynaptic release of acetylcholine. This results in the failure of nerve impulses to be transmitted across nerve fibre junctions. About $1 \times 10^{-10}$ g (approximately $10^8$ molecules) kills a mouse and 1 oz could kill 200 million people.

Botulism cases have been treated with specific antitoxin but, despite the availability of antitoxin, botulism therapy is still unsatisfactory. Equine antitoxin, although proved effective in many cases, has the disadvantage of causing serum sickness. The use of humans as antitoxin producers has proved both effective and practical. Not only is the problem of serum sickness overcome, but antibodies produced in one animal species are more effective therapeutically in that

**Table 1**
**Symptoms of Type E Botulism in 8 Patients**
Data from Rogers, Koenig, and Spickard (1964)

| Symptoms | | Frequency |
|---|---|---|
| General | Nausea, vomiting | 7 |
| | Weakness, lassitude | 7 |
| | Dry mouth | 7 |
| | Pharyngeal pain | 3 |
| Neuromuscular | | |
| Ocular | Blurred vision | 5 |
| | Diplopia (double vision) | 4 |
| Pharyngeal | Dysphonia (confused speech) | 5 |
| Laryngeal | Dysphagia (difficulty in swallowing) | 5 |
| Respiratory | Respiratory distress | 7 |
| Other | Other muscle weakness | 3 |
| | Abdominal muscle weakness | 3 |
| | Constipation | 3 |
| | Urinary retention | 3 |

**Table 2**
**Main Features of the Different Types of *Cl. botulinum***
Data from Dolman (1964)

| Type | Species Mainly Affected | Commonest Vehicles | Highest Geographic Incidence |
|---|---|---|---|
| A | Man; chickens ('limberneck') | Home-canned vegetables and fruits; meat and fish | Western U.S.A., Soviet Ukraine |
| B | Man; horses; cattle | Prepared meats, especially pork products | France, Norway, eastern U.S.A. |
| Cα | Aquatic wild birds ('western duck sickness') | Fly larvae (*Lucilia caesar*); rotting vegetation of alkaline ponds | Western U.S.A. and Canada, South America, South Africa, Australia |
| Cβ | Cattle ('Midland cattle disease'); horses ('forage poisoning'); mink | Toxic forage; carrion, pork liver | Australia, South Africa, Europe, North America |
| D | Cattle ('lamziekte') | Carrion | South Africa, Australia |
| E | Man | Uncooked products of fish and marine mammals | Northern Japan, British Columbia, Labrador, Alaska, Great Lakes region, Sweden, Denmark, U.S.S.R. |
| F | Man | Home-made liver paste | Denmark |

species than in another. Polyvalent antitoxins have been used, particularly combinations of types A and B. Recently Frieman *et al.* (1966) produced a highly active quadrivalent antibotulinal serum by a complex immunization of horses with antigens of types A, B, C, and E. However, successful treatment with antitoxin depends on a quick diagnosis of botulism and the administration of antitoxin before the neurological symptoms become too advanced.

## Geographical Distribution

Table 2 summarizes some features of the different types of botulism and lists the commonest vehicles for infection of different animals.

Case fatality rates and distribution of botulinum types show marked variation between the United States, the Soviet Union, Germany, and France (Table 3). It seems plausible to correlate this range of fatality rates with the nature of the foodstuff and the type of *Cl. botulinum* chiefly involved. In the United States, home-canned vegetables and fruits have been the most common vehicles, and type A toxin most often implicated. In the Soviet Union, type A was again predominant, but frequently the vehicles were big fish in which the toxin was probably unevenly distributed, thus tending to lower the mortality. In Germany the fatality rate is further reduced because the less lethal type B strains predominate and the most common vehicle, the large sausage, provides opportunities for irregular formation and uneven distribution of toxin. A unique combination of factors is believed to account for the astonishingly low death rate in France during the wartime occupation. The organisms involved were almost exclusively type B; the botulogenic foods were, as a rule, briefly stored products of clandestinely slaughtered pigs—this meat was heated if possible in a frying-pan before eating, so that a portion of any preformed toxin was destroyed; and physicians in the affected localities became extremely adept at diagnosing the typical syndromes of botulism.

An examination of Table 3(*a*) suggests that either type A or type B spores predominate over large areas of North America, Europe, and Russia. The reason for this remains an ecological mystery, although it has

been observed that in the U.S.A., type A predominates in the western States where virgin forest soils are common, while type B, possibly an environmentally induced variant of type A, occurs mainly in the Mississippi valley and the Great Lakes region in cultivated areas. However, any explanation must be reconciled with the reverse kind of phenomenon illustrated in Table 3(*b*), which indicates the numbers and types of human botulism outbreaks reported in several countries in the northern hemisphere. Of those which were bacteriologically verified, 76% of occurrences were due to type E, the British Isles alone having so far failed to report any outbreaks of this type. Perhaps even more puzzling are the heterogeneous patterns of botulinic types in the neighbouring countries of Denmark, Norway, and Sweden, despite the similar dietetic habits of their people.

It seems remarkable that there have not been more outbreaks of type E botulism in the Scandinavian countries. Johanssen (1963) has reported a very high incidence of type E spores in the soil of the catchment area of the Baltic, and in Sweden, 33–84% of cultured sedimentary samples from watercourses, lakes, and coast produced type E toxin. Similarly, in the Soviet Union, where type E spores are known to be indigenous and fish is a dietetic staple for a very large population, the number of type E outbreaks reported so far has been extremely small. Such statistics indicate that reasonably satisfactory sanitary and processing standards are maintained throughout the fishing industries in these countries.

The view that type E strains are of terrestrial rather than marine origin has become increasingly popular in recent years, and Johanssen's (1963) finding that potato peelings yielded a very high incidence of this organism provides further evidence for the theory. Dolman (1964) has postulated that healthy animals may be carriers of organisms of those types to whose toxins they were themselves immune, and serve as reservoirs, thereby replenishing and possibly scattering the spores and even vegetative forms of the botulinum organism. Many years ago the larvae of the blowfly *Lucilia* were shown to be reservoirs for type C organisms (Bengston 1920). Insect and animal carriers may partly account for irregularities in the distribution of the various spore types. Perhaps the attention that is now being focused on fish as intestinal carriers of type E organisms may reactivate interest in the similar potentialities of land animals.

The occurrence of type E organisms throughout the world is illustrated in Figure 1. Eklund and Poysky (1966) reported type E on the Pacific coast of the United States in marine mud samples from 49° to 36° N. latitude. Only types A, B, and F were found south of 36° N. However, type E has been found in the Gulf of Mexico on the coast of Texas at a latitude of 30° N. Recently type E organisms have been isolated from

**Table 3**
**Incidence, Fatality Rates, and Types of Botulism**
Data from Dolman (1964) and Meyer and Eddie (1965)

| | Country | Outbreaks | Cases | Deaths | Fatality Rate (%) | Type | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | A | B | E | F | Untyped |
| (*a*) | United States (1899–1964) | 651 | 1670 | 1008 | 60·3 | 166 | 42 | 32 | | 328 |
| | Soviet Union (1818–1939) | 163 | 1283 | 459 | 35·8 | (A mostly) | | 1 | | ? |
| | Germany (1898–1948) | 434 | 1294 | 179 | 13·8 | (Mostly B) | | — | | ? |
| | France (1940–48) | 500 | >1000 | 15 | 1·5 | 3 | 202 | — | | 195 |
| (*b*) | Japan (1930–64) | 62 | 347 | 97 | 28·0 | — | 1 | 46 | — | 15 |
| | Canada (1919–64) | 36 | 110 | 62 | 56·4 | 6 | 3 | 12 | — | 15 |
| | Denmark (1901–64) | 12 | 34 | 14 | 41·2 | 1 | — | 3 | 1 | 7 |
| | Norway (1934–64) | 13 | 63 | 1 | 1·6 | — | 8 | 1 | — | 4 |
| | Sweden (1932–64) | 7 | 16 | 2 | 12·5 | — | — | 3 | — | 4 |
| | Britain (1922–64) | 11 | 21 | 16 | 76·2 | 3 | 1 | — | — | 7 |

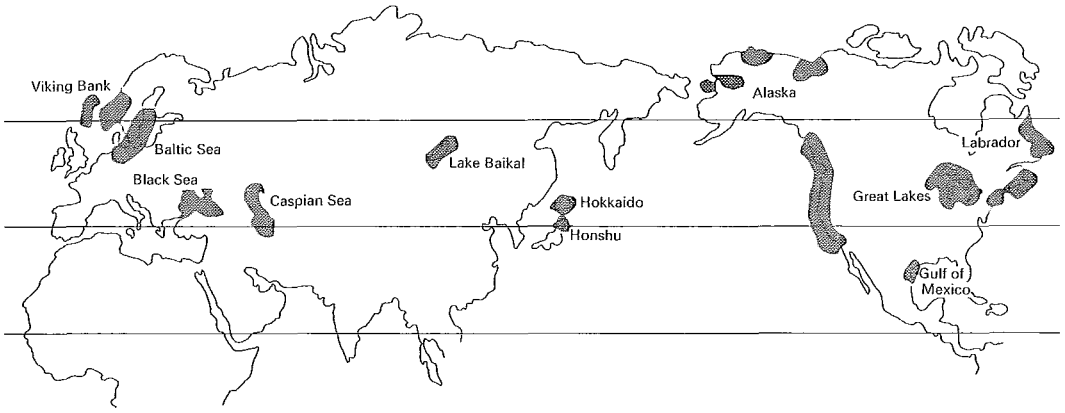


Fig. 1.—Known incidence of *Cl. botulinum* type E.

fish taken from Lake Cayuga, a freshwater lake in New York State, 383 ft above sea level (Chapman and Naylor 1966). Eklund and Poysky (1965) found type F along the coastal areas of Oregon and California in marine sediments from 34° to 43° N. latitude. Matveev, Nefedjeva, and Bulatova (1966), reporting on the epidemiology of botulism in the Soviet Union, say that outbreaks of botulism were generally associated with fish caught privately and home-processed, or bought in markets. Outbreaks from bony fish can be divided into two geographical groups, one near Lake Baikal, where the most frequent vehicle of botulism, Baikal omul, is usually eaten slightly salted, and the other around the Caspian Sea, Black Sea, and the Sea of Azov, where various fishes were involved.

Type E organisms have been isolated in Japan north of 38° N. latitude and at various points around the coast of Alaska. Although it has been shown that the incidence of type E is high in the Scandinavian coastal waters, no type E organisms have been isolated from the British Isles region. Type E has been found throughout the Great Lakes area in the U.S.A., with the highest incidence in Green Bay, Lake Michigan.

The preponderance of type E botulism in Canada is exceeded only in Japan, although in terms of national population, Canada has suffered the greatest incidence of such outbreaks, with a remarkably high case fatality rate of 63%, as compared with 39% in the United States and 26% in Japan.

Although types A, B, C, and D occur in Australia, type E has not yet been isolated.

## Types of Food Affected

The range of foods that have been involved in botulism outbreaks is very extensive. Table 4 indicates the foods responsible for outbreaks in the U.S.A. and Canada over the past 65 years. Types A and B caused almost all the outbreaks in vegetables and meats, whereas the outbreaks in fish or fish products resulted mainly from type E. The vast majority of cases in the U.S.A. have involved home-canned vegetables, while in Europe, pork products are the major vehicles and salted or pickled fish are frequently incriminated. A pickled relish called 'izushi', which is made of raw fish, rice, and diced vegetables, has caused over 90% of the outbreaks in Japan. The first recorded outbreaks of botulism in Australia occurred in 1942 in north Queensland and the Northern Territory. Although specific bacteriological and serological evidence is lacking, a conclusive chain of circumstantial evidence points to canned beetroot as the vehicle (Gray 1948).

Milk and milk products are listed in Table 4 as having caused eight outbreaks in the United States and Canada. There have, however, been no recorded cases due to surface-ripened cheese. Grecz, Wagenaar, and Dack (1959*a*, 1959*b*) have shown that the growth of *Cl. botulinum* is inhibited in aged surface-ripened cheese although *Cl. botulinum* spores survive in cheese for 5–6

years. Possible explanations are that fatty acids in cheese increase during the normal ripening process; rancid fatty acids have been shown to inhibit the germination of *Cl. botulinum* spores; inhibition may also be of an antibiotic nature. Grecz, Wagenaar, and Dack (1965) demonstrated toxin production in cheese by *Cl. botulinum*. After initiating growth from detoxified spores, regular assay during subsequent storage of samples at 2–4°C revealed a characteristic two- to five-fold increase in toxin titre.

U.S.A. led many workers to wonder if the organism had recently been introduced into the country.

The first type E outbreak ever recorded in the U.S.A. occurred in New York State in 1932, the vehicle being smoked salmon from Labrador (Dolman and Iida 1963). Then in 1934, canned sprats from Germany caused a further outbreak in New York State. Mushrooms from Yugoslavia canned in San Francisco led to another outbreak in California in 1941, the only recorded example of

**Table 4**
**Foods involved in Outbreaks of Botulism in the U.S.A. and Canada, 1899–1964**
Data from Meyer and Eddie (1965)

| | Preparation | | Outbreaks | Cases | Deaths | Type | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Home Canned or Preserved | Commercially Canned | | | | A | B | C | E | A and/or B |
| Vegetables | 365 | 18 | 383 | 1037 | 652 | 127 | 28 | 0 | 1 | 5 |
| Fruits* | 38 | 13 | 51 | 171 | 106 | 20 | 3 | 0 | 0 | 1 |
| Meats† | 37 | 8 | 45 | 110 | 65 | 7 | 4 | 0 | 0 | 0 |
| Fish and sea foods‡ | 52 | 22 | 74 | 184 | 96 | 5 | 4 | 0 | 31 | 0 |
| Milk and milk products§ | 5 | 3 | 8 | 24 | 9 | 2 | 2 | 0 | 0 | 0 |
| Other foods | 7 | 1 | 8 | 30 | 15 | 5 | 0 | 0 | 0 | 0 |
| Unknown | — | — | 82 | 114 | 65 | 0 | 1 | 1 | 0 | 0 |
| Total | 504 | 65 | 651 | 1670 | 1008 | 166 | 42 | 1 | 32 | 6 |

* Apple sauce, apricots, avocado sauce, cactus, figs, mangoes, peaches, pears, persimmons, pimentos.

† Beef, blood sausage, bologna, buffalo meat, chicken, ham, lamb stew, liver sausage, liver paste, pork and beans, pickled tongue.

‡ Clam, crab, fish eggs, herring, salmon, sardines, seal flippers, seal liver, smoked ciscoes, sprats, trout, tuna.

§ Cottage cheese, milk.

Commercially processed ham has never been implicated in a botulism outbreak either. The main reason for this is probably the low incidence of spores present initially in the product.

## Recent Outbreaks in U.S.A.

The 1963 outbreaks of botulism in the United States aroused new interest in the food-borne disease, principally because they involved commercially prepared foods. Three of the outbreaks were caused by type E organisms in fishery products, including smoked fish from the Great Lakes. This sudden awareness of type E botulism in the type E not due to piscine or marine mammalian products. The first outbreak of type E caused by a native United States food occurred in Minneapolis in 1960, and resulted from the consumption of smoked ciscoes from Lake Superior (Anon. 1964). Since then, type E organisms have been found to be common in the Great Lakes area (Fig. 1).

Table 5 summarizes the outbreaks that occurred in 1960 and 1963 in North America. Chief causes of spoilage in the products were faulty processing or refrigeration, and defective can seams coupled with contaminated cooling water.

**Table 5**
**Recent Botulism Outbreaks in North America**
Data from Meyer and Eddie (1965)

| Year | Location | Type | Outbreaks | Cases | Deaths | Food | Cause |
|---|---|---|---|---|---|---|---|
| 1960 | Minneapolis | E | 1 | 2 | 2 | Smoked whitefish, vacuum packed | Inadequate salting, moisture control, heat treatment, or refrigeration |
| 1963 | New York | A | 1 | 2 | 0 | Liver paste | |
| | Canada | B | 1 | 2 | 1 | Liver paste | |
| | Tennessee, Alabama, Kentucky, Michigan, Minnesota | E | 7 | 20 | 7 | Smoked ciscoes, vacuum packed | As above |
| | Michigan | E | 1 | 3 | 2 | Tuna fish | Defective can seams |

## Occurrence and Growth of Organisms and Toxin Production

The geographical distribution of the various botulinum types is probably related to temperature, soil type, and various ecological factors of which little is known at present. Some of the factors influencing outgrowth of spores of *Cl. botulinum* are temperature, salt or sugar concentrations (or water availability), pH, and substrate.

Ohye and Scott (1953) reported a minimum growth temperature of 10°C and a maximum of 45°C for types A and B. They found that growth proceeded from spore inocula at temperatures from 15 to 42·5°C but not at 12·5 or 45°C. The minimum growth temperature for type E spores is considerably lower, growth proceeding consistently from spore inocula at temperatures between 5 and 37·5°C (Ohye and Scott 1957). Ohye and Scott (1957) found growth initiation in 3 to 4 weeks at 5°C, with toxin detectable at 8 weeks. Under the same conditions, no evidence of growth was found after 22 weeks at 2·5°C. Schmidt, Lechowich, and Folinazzo (1961) reported outgrowth and toxin formation by mildly heat-shocked spore inocula of four type E strains at 3·3°C in a beef stew medium. The time for outgrowth as judged by gas formation was 31 to 45 days. No outgrowth or toxin formation by these strains could be detected at 1·2 or 2·5°C after 104 days of incubation. Graikoski and Kempe (quoted by Schmidt 1964) have confirmed outgrowth and toxin formation from type E spore inocula at 3·3°C. Although growth has been demonstrated at 3·3°C, it may not always be found and may depend on the substrate.

It is generally considered that 50% sugar or 10% salt in the aqueous phase completely inhibits outgrowth of spores of types A and B. These concentrations correspond to a water activity ($a_\omega$) of 0·935, so that the actual effective inhibiting factor may be a critical $a_\omega$ level limiting outgrowth. Table 6 summarizes the growth characteristics of types A, B, and E. Ohye, Christian, and Scott (1966) have shown that over the range of temperatures likely in unrefrigerated foods, the water requirement for growth and toxin production by type E is increased appreciably by a 15 degC reduction in temperature. Further, combinations of unfavourable $a_\omega$, pH, and temperature tend to restrict growth to much narrower limits than those given in Table 6 (Ohye and Christian 1966).

**Table 6**
**Physical Conditions governing Growth of Food-poisoning Types of *Cl. botulinum***
Data from Schmidt (1964)

| Type | pH | Temperature (°C) | | Limiting Solute Concentrations | | |
|---|---|---|---|---|---|---|
| | | Range | Optimum | Sugar (%) | Salt (%) | $a_w$ |
| A, B | 4·6–8·0 | 10–55 | 37–40 | 50 | 10 | 0·935 |
| E | 4·8–8·0 | 3·3–40 | 35 | | 5–6 | 0·965 |

Recent studies with type F reveal that growth occurs in the range 4–10°C, the amount of growth being dependent on the strain used, the size of the inoculum, the physiological state of the organisms, and the medium in which it is grown (Walls 1966). Toxin is produced at temperatures as low as 4°C and treatment with trypsin effects an increase in toxicity.

In recent years considerable attention has been given to the effect of some of these physiological conditions on growth and toxin production in various food products.

**Table 7**

**Spoilage and Toxicity of Smoked Fish inoculated with Type E Spores and Vegetative Cells and incubated in Sealed Plastic Wraps***

Data from Kautter (1964)

| Temp. (°C) | Incubation (days) | No. of Fish Toxic | Organoleptic Analysis | | |
|---|---|---|---|---|---|
| | | | Putrefaction & Rancidity | Obvious Growth | Edible Appearance |
| 5 | 18 | 0/10 | − | − | + |
| 10 | 5 | 2/15 | − | − | + |
| | 18 | 8/15 | − | − | + |
| | 38 | 12/20 | + | + | − |
| 15 | 18 | 11/20 | ± | − | ± |
| 30 | 11 | 10/10 | + | + | − |

*Inoculum 80% spores, 20% vegetative cells, $7 \cdot 5 \times 10^4$ viable spores per plastic pack.

*Oxygen.*—It is well known that the absence of air is not necessary for the growth and toxin production of such strict anaerobes as *Cl. botulinum*. Oxidation-reduction potentials low enough to permit growth of anaerobes can exist in food masses exposed to air. Kautter (1964) inoculated smoked fish with type E spores and vegetative cells and incubated them in different atmospheric environments. Within 11 days incubation at 30°C, toxin had been produced in all groups of smoked fish. The amount of toxin formed was approximately equal in fish injected with the organisms and incubated under either aerobic or anaerobic conditions. However, the amounts of toxin developing in surface-inoculated fish incubated aerobically were less than those for the other groups. Table 8 also shows toxin production by type A in the presence of air.

*Temperature.*—Kautter (1964) studied the effect of temperature on toxin production in smoked fish packed in sealed plastic wrappers. He found that type E toxin was formed in smoked fish at a temperature approaching that of household refrigeration (10°C) in a period of 5 days without any obvious sign of quality reduction. Moreover, at 10°C the proportion of toxic fish increased substantially, with no indication of spoilage until after 18 days (Table 7).

**Table 8**

**Effect of Spore Load, Atmosphere, and Packaging on Toxinogenesis by *Cl. botulinum* in Sliced Cooked Ham**

Data from Pivnick and Bird (1965)

| Type | Temp. (°C) | Incubation (days) | Spores per g | Packaging | | |
|---|---|---|---|---|---|---|
| | | | | Polyethylene in Hydrogen | Air | P811* in Air |
| A | 30 | 3 | 0 | 0/2† | 0/2 | 0/2 |
| | | | 7 | 0/2 | 0/2 | 0/2 |
| | | | 700 | 0/2 | 0/2 | 0/2 |
| | | | 7000 | 1/2 | 1/2 | 1/2 |
| | | 6 | 0 | 0/2 | 0/2 | 0/2 |
| | | | 7 | 2/2 | 2/2 | 1/2 |
| | | | 700 | 2/2 | 2/2 | 1/2 |
| | | | 7000 | 2/2 | 2/2 | 2/2 |
| | | | | | | Vacuum-packed |
| A | 15 | 30 | 100 | | | 0/5 |
| | | | 10,000 | | | 1/5 |
| | | 60 | 100 | | | 0/5 |
| | | | 10,000 | | | 1/5 |
| A+B | 15 | 30 | 100 | | | 0/5 |
| | | | 10,000 | | | 3/5 |
| | | 60 | 100 | | | 0/5 |
| | | | 10,000 | | | 2/5 |

*Saran-coated (0·00006 in.) cellulose (0·001 in.) laminated to low-density polyethylene (0·002 in.). P811 has a very low permeability to $O_2$, being about 1000 times less permeable than polyethylene.

†Number of samples toxic.

*Contamination level.*—In many foods which receive a mild heat process during preparation, contamination will be largely confined to spores. It is also now apparent that heat-damaged spores or spores heated in the

presence of curing ingredients are much more sensitive to unfavourable conditions of pH and salt concentration, and to curing ingredients (Roberts and Ingram 1966), than unheated spores. Further, the number of spores present has a very important effect on whether outgrowth from a spore inoculum will succeed. These factors largely explain why mild heat processing treatments with cured products have resulted in successful preservation, but all these factors may be outweighed if the spore load is increased (Table 8; Fig. 2). Within 6 days at 30°C, an inoculum of 7 spores/gramme resulted in toxin production in sliced cooked ham in an atmosphere of hydrogen or in air (Table 8).

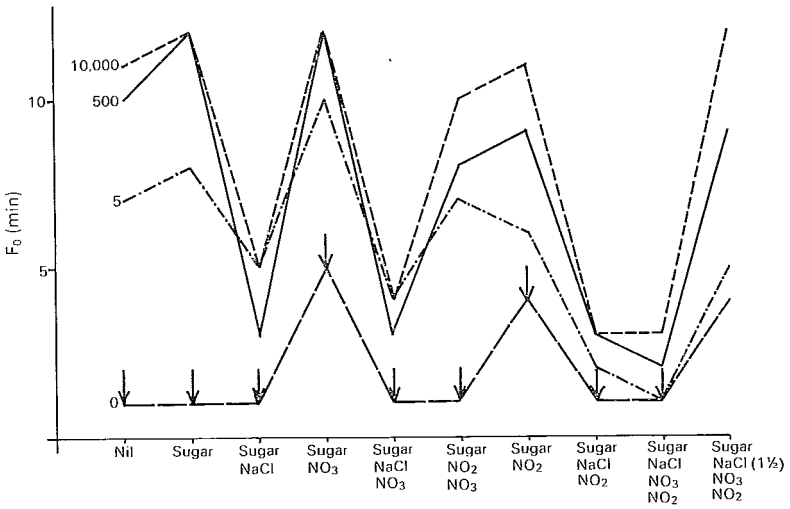


Fig. 2.—Effect of spore load, heat process, and curing ingredients on the spoilage of cured meat by *Cl. sporogenes*. Meat treated 24 hr with curing ingredients before processing. Incubated 1 yr at 28°C. NaCl, 3½ lb; sugar, 1 lb; $NaNO_3$, 2¾ oz; $NaNO_2$, ¼ oz, per 100 lb meat. Data from Stumbo, Gross, and Vinton (1945).

At 15°, 100 spores/g did not succeed in producing toxin in 2 months, whereas 10,000 spores/g did so within 1 month. Figure 2 illustrates well the effect of heat process and spore load in the presence of different mixtures of curing ingredients. In this case the test organism was *Cl. sporogenes*, which is very similar to *Cl. botulinum* in its growth requirements.

The above results indicate clearly that spoilage and toxin production can occur at low temperatures and that, if the level of contamination is high enough, spoilage and toxin production will result in cured products processed with a non-botulinum cook, i.e. $F_0 < 2{\cdot}78$ min. ($F_0$ is the time in minutes at 250°F that is equivalent in lethality to the thermal process.) Under these conditions, however, toxin production is usually accompanied by putrefaction, which provides a very useful safety factor.

A primary reason for the excellent public health record of canned ham is undoubtedly the low level of all putrefactive, anaerobic, spore-forming bacteria indigenous to it. Riemann (1963) found none in 1 g samples of raw pork luncheon meat from seven Danish packing plants, except in one sample which contained 15/g. Aerobic spore counts averaged 82/g. In 80 samples of pork trimmings from four plants counts of less than 3 putrefactive anaerobic spores occurred in 92% of the samples; three samples contained more than 8 spores/g with a maximum of 51/g. Similar figures have been reported from the U.S.A.

It is difficult to explain why the spore load is so low, but many conditions exist which are likely to raise the load considerably. Naturally it is of prime importance to maintain this low level of contamination, especially as spoilage and toxin production can occur so readily if the spore load gets too high. This situation has arisen in Europe, where outbreaks of botulism have resulted from the consumption of home-pickled hams.

## Warning Signals

It is extremely fortunate that spoilage of food caused by *Cl. botulinum* is generally accompanied by gas production and putrid odours. However, we have seen that at low temperatures (5–15°C) toxin production may occur in smoked fish that are apparently organoleptically acceptable (Table 7). Salt concentrations of 6·25% and higher produce a similar effect (Table 9). Below 6% salt concentration, spoilage results in obvious organoleptic changes, so that as the 6% brine level is exceeded the built-in safety factor is negated (Greenberg, Silliker, and Fatta 1959). Although *Cl. botulinum* toxin production cannot be prevented by organoleptically acceptable levels of sodium chloride, the tendency has been to demand increasing amounts of salt because of its delaying effect on the appearance of spoilage. Under these high salt conditions, inhibition of *Cl. botulinum* is more apparent than real, resulting in a potentially dangerous situation.

**Table 9**

**Effect of Salt Concentration on *Cl. botulinum* Toxin Formation and Organoleptic Condition of Perishable Cured Meat**

Data from Greenberg, Silliker, and Fatta (1959)

| Salt Concn. (% NaCl in brine) | Cans Examined | Organoleptic condition | | | | | Cans Toxic |
|---|---|---|---|---|---|---|---|
| | | Normal | Putrid | Decomposed | Cheesy | Softened | |
| 3·0 | 205 | | | 205 | | | 38/40 |
| 5·1 | 101 | | | 101 | | | 14/20 |
| 6·2 | 132 | | | 118 | | 14 | 9/14 |
| 7·1 | 207 | | 6 | | | | 3/6 |
| | | 201 | | | | | 27/201 |
| 9·0 | 103 | 103 | | | | | 0/103 |
| 11·5 | 102 | 102 | | | | | 0/102 |

## Methods of Control

The foods incriminated in botulism outbreaks almost invariably are given an inadequate preliminary treatment such as heating, salting, smoking, drying, or pickling, allowed to stand at a temperature that will permit the growth of *Cl. botulinum*, and eaten without cooking. Indeed, preservation processes for food often make growth and toxin development easier than in unprocessed food. The removal of natural microbial competitors, the removal of air by heating, and the destruction of cellular tissues with the consequent release of cellular fluids are influences favouring growth of clostridia. Canned and preserved foods are more often involved in botulism outbreaks because so often they are kept for long periods. Control procedures differ somewhat, depending on the method of preservation. The type of preservation, product, and appropriate control procedures are summarized in Table 10.

The 'botulinum cook' for low-acid, uncured canned foods has an $F_0$ value of 2·78 min and ensures a high degree of safety. To prevent spoilage by organisms more resistant to heat, a higher $F_0$ value than 2·78 is used, particularly in products that contain considerable amounts of fats or carbohydrates. Adequately processed cans are potentially hazardous if can leaks are coupled with contaminated cooling water. The control of cooling water contamination is therefore of great importance, not only to reduce spoilage, but as a health safeguard. Potable water supplies should be used where possible, and if the water is re-used, or comes directly from dams or rivers, it should be properly chlorinated.

Unfortunately, the 'botulinum cook' adversely affects the organoleptic qualities of many foods. Canned, cured meats belong to a group of low-acid, commercially sterile foods which are not always given the heat process considered adequate to maintain protection against botulinum spores. The pH of these products is always above 5 and $F_0$ values as low as 0·2–0·6 min are reported to be in use. This process is considered to be adequate for luncheon meat, corned beef, and bully beef canned with curing ingredients, provided ambient storage temperatures are low. In Australian experience much higher $F_0$ values must be used to prevent spoilage where storage is often at high ambient temperatures.

**Table 10**
**Methods of Preventing Botulism**

| Method of Preservation | Products | Control Procedures |
|---|---|---|
| Canning | Low-acid foods receiving a botulinum cook | Adequate heat processing ($F_0 \geqslant 2 \cdot 78$ min)<br>Prevention of defective seams<br>Rigid control of cooling water<br>Food hygiene and good sanitation to reduce spore load |
| | Cured foods receiving less than the botulinum cook, e.g. luncheon meat, corned meat, 'sterile' ham packs | Adequate sodium chloride (6% in aqueous phase), nitrite, and nitrate<br>Process $F_0 > c.0 \cdot 6$<br>Good food hygiene and plant sanitation<br>Hygienic killing conditions<br>Refrigeration during precuring and curing |
| | Pasteurized cured products. Semi-conserves labelled 'Keep under refrigeration', e.g. hams | Process giving centre temperature 80°C (176°F)<br>Adequate sodium chloride, nitrite, and nitrate<br>Refrigeration during precuring and curing<br>Good food hygiene and plant sanitation<br>Storage at 3°C (38°F) or less |
| Non-canned | Processed sausages, cheese | Adequate processing, smoking, etc.<br>Refrigeration<br>Good food hygiene and plant sanitation |
| Refrigerated products | Processed products not adequately heated before eating, e.g. marine products, smoked fish, TV. dinners | Continuous refrigeration at 3°C or less with continuous temperature control<br>Good food hygiene and plant sanitation |

Cured hams receive a heat treatment to give a centre temperature of 165–175°F (75–80°C). Such a product is regarded as a semi-conserve and requires storage under refrigeration. This precaution is often disregarded, however, even though storage directions are printed on the container.

The production of 'sterile' canned hams requires a process with an $F_0$ value of 0·4–1·0 min. At low ambient temperatures, an $F_0$ of 0·4 min may be adequate but to prevent spoilage by the more numerous *Bacillus* spores during transport and during storage in tropical areas, an $F_0$ of 1 to 2 min is probably necessary.

To ensure absence of spoilage in cured products, particularly in sterile ham packs, several precautions must be observed. Standards of hygiene and conditions of killing should be rigidly controlled; the meat should be adequately refrigerated during precuring and curing stages; sterile spices or spice extracts and good-quality or sterile curing ingredients only should be used; additives such as starch, sugar, milk powder, or gelatin should have a low spore count and be free of putrefactive anaerobes. It is advisable to use sodium chloride at 6% in the aqueous or brine phase and sodium nitrate and sodium nitrite at maximum permissible limits. Although commercially canned hams usually have extremely low numbers of clostridial spores, if for some reason the numbers happened to be high, a large percentage of spores would survive a process having an $F_0$ of 0·6 min.

Smoked sausages and smoked or salted fish require similar rigidly controlled procedures during production. Processes, ingredients, and heat treatments need to be standardized and refrigeration needs continuous temperature control. Contamination levels should be checked and minimum spore loads maintained by observing adequate standards of hygiene.

Irradiation will produce commercial sterility in foods, but irradiation pasteurization has both negative and positive aspects concerning botulism: negative because natural competitors of *Cl. botulinum* are often killed; positive because vegetative cells of *Cl. botulinum* are also killed and the numbers of

such spores are reduced. It must also be remembered that radiation pasteurization does not inactivate any preformed botulinum toxin, whereas heat pasteurization does. Combined heat, irradiation, and chemical sterilization treatments have been proposed. Irradiation and heat used sequentially produce a synergistic killing action that reduces the amounts of both heat and irradiation required for sterilization. Kempe (1964) inoculated cans of raw beef with $5 \times 10^6$ spores of *Cl. botulinum* per can and found that when no irradiation was used, an $F_0$ of 1 min was needed to sterilize the meat. Where no heat was used, the irradiation required to produce sterility was about 3 megarads. On the other hand, when 1·2 megarads of irradiation were used with heat, the $F_0$ required to sterilize the meat was reduced by 75%.

According to Erdman, Pontefract, and Thatcher (1966) the control of botulism in processed foods requires four distinct activities: (1) use of a process carefully defined in relation to each specific food formulation; (2) use of effective methods for detection of *Cl. botulinum* and its toxin in suspect foods; (3) regulatory enactments to encourage education in effective practices for processing, food handling, and storage; (4) effective control by manufacturers and regulatory agencies.

While the food regulations of most countries cover processing, they are usually indefinite. The onus for ensuring that food receives an adequate process is usually placed on the processor. For instance, in Australia no $F_0$ values or process schedules are prescribed, but canned meats are required to be processed 'by heat to ensure preservation', and vegetables to be 'sterilized by heat'.

Only three Australian States have regulations requiring that canned hams and other semi-conserve forms of canned meat product shall be labelled 'keep under refrigeration below 40°F'.

While most meat processors are fully aware of the need to store 'semi-conserves' at temperatures below 40°F, it is very doubtful whether much awareness exists amongst retailers. Labelling alone is not an effective method of control; regulations are needed to cover handling and storage at all stages from production to retail sale.

In conclusion, perhaps the remarks of some eminent authorities speaking at the Canning Safety Conference of the Royal Society of Health in London, January 1965, are apt. T. G. Gillespy said that although food canned in metal containers has never been the cause of an outbreak of botulism in the United Kingdom, he warned against allowing laxity to develop in the principle of minimum processes. In raising the question whether the safety margin is enough, J. W. Howie stated that some modification of the curing process may take away a necessary inhibiting influence. He also pointed out that cans of pasteurized meats bear, in small print, instructions that they should be kept at low temperatures, but asked how many housewives read the small print? Professor Semple raised the question of imported pasteurized meats which are often brought into Britain in non-refrigerated ships, virtually the only storage instructions being 'Keep away from engines'. The safety margin may not always be enough to guarantee the safety of pasteurized foods if such casual handling and storage are allowed to continue.

It is obvious from this discussion that it is impossible to formulate for all types of food a general rule to prevent botulism. However, if recommended procedures embodying correct principles of food hygiene, processing, storage, and handling are observed, control should pose no problems.

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# Bubble Formation
## in Polystyrene Containers filled with Jam

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The formation of gas bubbles on the inside surface of rigid polystyrene containers filled with jam has been investigated in the laboratories of the Division of Food Preservation. The following account of the investigation of this unusual problem may be of interest to manufacturers of food and food containers.

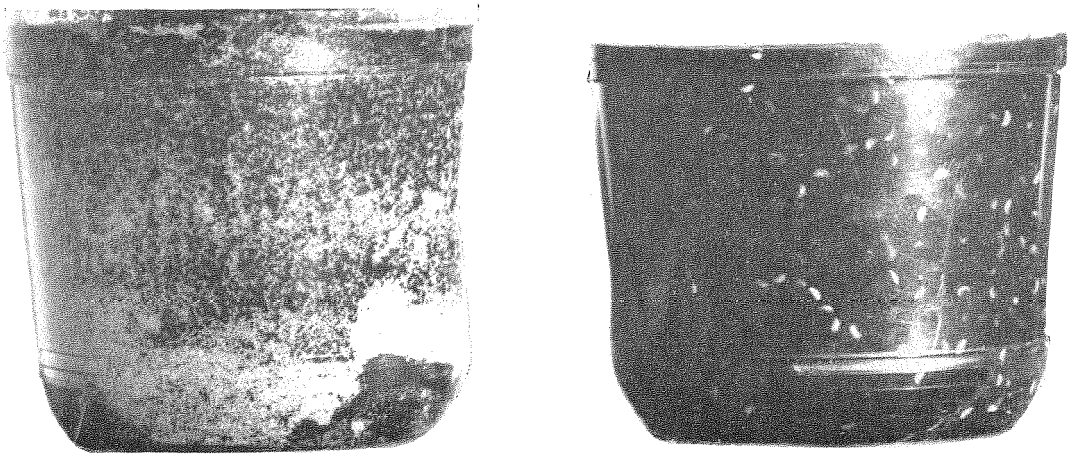
THE permeation of gases and vapours through plastic and cellulose materials used for the fabrication of food containers is a well-known phenomenon. For the retention of quality, some foods require containers made from materials whose permeability permits high rates of exchange of gases and vapours between the atmospheres inside and outside the sealed containers, whereas with other foods low rates of exchange are desirable. Thus permeability is of fundamental importance in the choice of container materials for the packaging of specific foods.

### Nature of the Problem

A manufacturer reported that small bubbles appeared on the inside surface of clear rigid polystyrene containers of jam a few hours after the product had been filled at about 180°F into the containers, which were then closed with a gasketed metal closure and cooled in air. The bubbles first occurred in small patches which increased in area until most of the internal surface was covered. Although there was no evidence that the quality of the jam was affected adversely, the bubbles imparted an objectionable whitish appearance to the pack, particularly with dark jam. The general appearance of packs with and without bubbles is illustrated in the photographs.

It seemed likely that the bubbles were formed by one or more of the following mechanisms:

- Coalescence of atmospheric gases adsorbed on the container surfaces.
- Evolution of atmospheric gases dissolved in the container material.
- Atmospheric gases permeating through the container walls under the influence of a pressure difference.



Polystyrene containers packed with youngberry jam—*(left)* bubbles present and *(right)* no bubbles.

Table 1

**Effect of Type of Closure, Fill-in Temperature, and Closer Vacuum on Bubble Formation in Containers filled with Plum Jam**

| Treatment Code | Type of Closure | Temp. (°F) | Closer Vacuum (inHg) | Bubbles Observed |
|---|---|---|---|---|
| A | Polyethylene | 185 | 0 | Absent |
| B | " | 185 | 5 | " |
| C | " | 140 | 0 | " |
| D | " | 140 | 10 | " |
| E | Tinplate | 185 | 0 | Present |
| F | " | 140 | 0 | Trace |

## Laboratory Investigations

To determine the cause of bubble formation, test packs were made with several batches of 8-oz rigid polystyrene container samples from normal production runs. The test products selected were red plum or youngberry jams, made from fresh fruit. Two types of press-on closure were used: lacquered tinplate with a flowed-in rubber gasket, and white-pigmented polyethylene with no gasket.

The containers were filled with jam at 140 and 185°F leaving a headspace of approximately $\frac{1}{4}$ in., and closed in a Heine Model 71D vacuum can closer. The filled containers, with closures loosely fitted, were placed on the lower movable chuck which was then raised manually so that the closure was pressed into position by the top chuck. Closing was carried out at atmospheric pressure and under known levels of vacuum. The sealed packs were allowed to cool at room temperature and were examined for bubbles 24–48 hours after processing.

## Results and Discussion

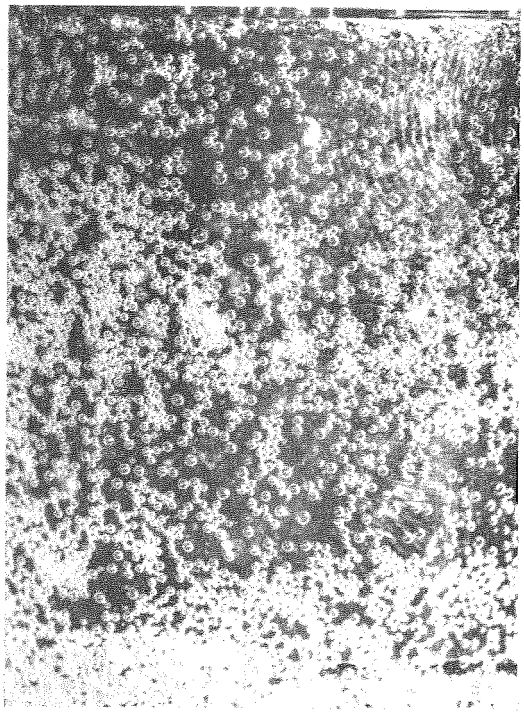
The first series of experiments, made with plum jam, was designed to study the influence of packaging procedures and two types of closure on bubble formation. The results are summarized in Table 1.

No bubbles were observed in containers with polyethylene closures over the range of fill-in temperatures and initial vacuum levels (treatments A, B, C, and D) investigated. Containers with tinplate closures developed bubbles when filled with jam at 185°F (treatment E), but only traces of bubbles when filled at 140°F (treatment F).

Further inspection of the packs from all treatments in Table 1 showed that the polyethylene closures were slack and often slightly convex, and these containers therefore had no internal vacuum. The containers with tinplate closures had some vacuum since the closures were concave and there was an audible entry of air on opening.

The polyethylene closures on the containers in treatments A, B, C, and D, which showed no bubbles, were replaced by tinplate closures, and the packs were resealed under a vacuum of 10 inHg. Half of these packs were stored immediately in a desiccator evacuated to 9 inHg and the remaining containers were stored at room conditions. Bubbles subsequently appeared only in those packs stored under room conditions.

A second series of experiments was made to study the effect of various container pretreatments on bubble formation in containers filled with youngberry jam and closed with tinplate closures. The results are summarized in Table 2.



Close-up appearance of bubbles on inside surface of polystyrene container packed with jam.

**Table 2**
**Effect of Container Pretreatment on Bubble Formation in Containers filled with Youngberry Jam at Two Temperatures**

| Treatment | Container Pretreatment | Filling Temp. (°F) | Bubbles Observed |
|---|---|---|---|
| G | Nil | 185 | Present (many small bubbles) |
| H | 1 Min in live steam | 185 | Present (few large bubbles) |
| J | 1 Min in 0·1% Teepol at 185°F and 1 min rinse in water at 185°F | 185 | Present (few large bubbles) |
| K | Nil | 140 | Trace |
| L | 1 Min in live steam | 140 | Absent |
| M | 1 Min in 0·1% Teepol at 185°F and 1 min rinse in water at 185°F | 140 | Absent |

Bubbles were observed in all treatments (G, H, J) filled with jam at 185°F. However, pretreatment of the containers in live steam or in aqueous 0·1% Teepol followed by a hot-water rinse produced a marked difference in the appearance of the bubbles. The bubbles in the untreated containers (G) were very numerous and small and gave the typical whitish appearance to the pack, whereas in the treated containers (H, J) the bubbles were much larger and less numerous and the overall appearance was less objectionable. None of the treatments (K, L, M) made with jam at 140°F showed bubble formation, apart from a trace in treatment K.

These observations show that the bubbles are caused by the permeation of air through the walls of containers under the influence of a pressure difference. The absence of bubbles in containers with polyethylene closures may be accounted for by leakage at the closure seals with resultant loss of internal vacuum. Similarly, the absence of bubbles in packs with tinplate closures filled at 140°F and stored at room conditions, or evacuated to 10 inHg and stored under a vacuum of 9 inHg, may also be attributed to the low pressure difference across the container surfaces.

Pretreatment of containers in either live steam or hot detergent solutions might be expected to remove adsorbed or dissolved gases from the container material. These treatments, however, failed to eliminate bubbles, although the character of the bubbles was altered, probably because changes in the surface properties of the container walls permitted small bubbles to coalesce.

The polystyrene containers used had a wall thickness of approximately 0·035 in. Assuming that the gas permeability of polystyrene is inversely proportional to thickness up to this level, calculations indicate that approximately 0·2 c.c. air may permeate into polystyrene containers having a surface area of 145 $cm^2$ and an internal vacuum of 10 inHg over a period of 24 hr. These calculations are based on values reported by Myers *et al.* (1957)* for the oxygen and nitrogen permeabilities of 0·001 in. polystyrene film at 30°C (2850 and 750 c.c. (s.t.p.)/$m^2$/24 hr/76 cmHg respectively). This volume (0·2 c.c.) of air, dispersed as a film of small bubbles between the container wall and the jam, is probably sufficient to impart the typical whitish appearance to the pack.

Commercially acceptable remedial measures to eliminate the formation of bubbles were not studied, but the use of an organic coating designed to reduce the air permeability of the containers would probably provide a practical solution. Reduction of the internal vacua in the containers by filling with jam at low temperatures of approximately 140°F or by using closures which leak avoids the bubble problem, but these procedures cannot be recommended because they may lead to microbial spoilage of the jam.

* Myers, A. W., Rogers, C. E., Stannett, V., and Szwarc, M. (1957).—Permeability of polyethylene to gases and vapors. *Mod. Plastics* **34**(9), 157–8, 160, 162, 164–5.

# Sulphur Dioxide Levels
## for Sulphuring Tree Fruits before Drying

By D. McG. McBean,* M. W. Miller,† A. A. Johnson,* and J. I. Pitt*

Sulphur dioxide is almost universally accepted as a permissible additive to dried tree fruits such as apricots, peaches, nectarines, and pears. The main purpose of this preservative is to retard certain chemical reactions which lead to the formation of brown to black pigments from compounds occurring naturally in the fruit and ultimately make the fruit inedible. This article discusses present commercial methods for treating fruit with sulphur dioxide before drying, their efficiency, and possible improvement.

DURING the period 1945–50, investigations in the Division of Food Preservation indicated that dried tree fruits needed 3000 p.p.m. of sulphur dioxide ($SO_2$) initially, if they were to remain acceptable for usefully long periods when kept at room temperatures under Australian climatic conditions. As a result of these studies, health authorities throughout Australia laid down 3000 p.p.m. as the maximum level of $SO_2$ in dried tree fruits. It is therefore important that orchardists should be able to control, with reasonable accuracy, the amount of $SO_2$ that they add to their fruit during the sulphuring process. With present practices wide variations in $SO_2$ level occur in dried fruits. In some instances, concentrations as low as 1000 p.p.m. have been found, providing little protection to the fruit, while on other occasions, levels as high as 7000 p.p.m. have made fruit unsalable. This variation is not surprising in view of the multiplicity of techniques and equipment used by orchardists to apply $SO_2$ to fruit before drying.

For sulphuring, halved fruits are placed on wooden trays which are stacked one above the other in an enclosure that can be sealed. Sulphur, in a metal pan, is burnt in an adjoining pit or box and the resulting hot, gaseous $SO_2$ moves by convection into the enclosure through a short duct. Small vent holes are generally provided near the top of the enclosure to assist the convective movement of the hot gas. Efforts are made to control the rate at which the sulphur burns by regulating the air supply to it, and most orchardists are aware that fluctuating wind strength and direction affect the rate of burning.

The level of $SO_2$ in dried fruits depends mainly on two factors, the amount of $SO_2$ taken up by the fruit during sulphuring and the degree of retention of such absorbed $SO_2$ during sun-drying. Orchardists have little control over the second factor, as retention is largely dependent upon weather conditions while drying is in progress. McBean, Johnson, and Pitt (1964) showed that of the many factors influencing the uptake of $SO_2$, two of the most important were the time of exposure to $SO_2$ and the concentration of the gas. Time of exposure is usually standardized by each grower—although it differs widely between them—but the concentration of $SO_2$ within commercial sulphuring equipment is unknown.

This paper reports studies made in 1962 and 1965 in which, for the first time in Australia, measurements were made of the $SO_2$ levels within commercial sulphuring equipment. In 1962, measurements were taken to give an indication of the range of $SO_2$ concentration over which basic absorption studies should be conducted in laboratory experiments. Those in 1965 were done to provide additional information on which to base specifications for a mechanical sulphur burner. The only previous data on levels of $SO_2$ during commercial sulphuring were reported by Long, Mrak, and Fisher (1940) following their comprehensive survey of the process as used in California.

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## Method of Measuring Sulphur Dioxide

Long, Mrak, and Fisher determined $SO_2$ by bubbling the mixture of air and $SO_2$ removed by suction from the enclosure through an iodine solution of known strength to which starch was added. The volume of gas mixture required to reduce the iodine completely, as indicated by the disappearance of the blue starch–iodine colour, was measured, allowing the percentage of $SO_2$ to be calculated. The authors stated that the probable error was about 5% but as the rate of bubbling of the gas mixture through the iodine solution had to be slow, the method was rather time-consuming.

Sulphur dioxide concentration was measured in the present studies using an interferometer–refractometer. This small, battery-operated, optical instrument is widely used as a gas analyser in the petroleum and mining industries. It is eminently suited for measuring $SO_2$ in air because of the high refractive index of $SO_2$ relative to air. The interference fringe was adjusted to read zero with air in the test cell. On introducing a mixture of air and $SO_2$ into the cell by means of a rubber aspirator, the shift of the fringe on the optical scale was noted. This was referred to a calibration chart to give the percentage of $SO_2$. Individual readings took only about 30 sec and concentration could be measured with an accuracy of 0·05% over the range 0–4% $SO_2$ in air.

Polyvinyl chloride tubes of $\frac{1}{4}$ in. bore were fixed to trays at four points within the enclosure. Preliminary trials with 12 points showed that four were enough to obtain an accurate assessment of the average $SO_2$ concentration. The plastic tubes were carried to a central external position where the interferometer could be connected to each in turn. Two sampling points were at the rear and two at the front of the stacks of trays; at each end, one tube was installed 9 in. from the top of the stack, which was about 5 ft high, and the other 9 in. from ground level.

## Types of Sulphuring Equipment

Sulphur dioxide concentration was measured in the following four types of equipment. The first three are in common use, while the fourth was sufficiently unusual to be considered worth testing also.

*PVC tent.*—This plastic cover, 0·008 in. thick, is fashioned so that it slips snugly over two stacks of trays (3 ft×2 ft). These units may be set up on an earth floor, in which case the base is sealed by distributing dry soil on the skirt of the tent, or they may be placed on a concrete apron and sealed by placing heavy planks on the bottom of the tents. Most frequently, the pan containing burning sulphur is placed under a galvanized iron tunnel shaped in the form of an inverted V, one end being closed and the other open. A small flap is cut in the closed end and by altering its position, some control over the entry of air to the burning sulphur and thus over burning rate is effected. The open end leads under the skirt of the tent and directs the $SO_2$ into the enclosure. Two eyeleted holes about $\frac{3}{4}$ in. in diameter in the top of the tent permit a small amount of $SO_2$ to escape, thus assisting the convective flow of gas from the burning sulphur and up through the trays of fruit. Where a tent is set on a concrete floor, the sulphur is often burnt in a nearby pit which is connected to the interior of the enclosure by a short, underground duct made from 4-in. or 6-in. glazed earthenware pipes. A lid over the pit allows some control over burning rate.

Plastic tents have the advantages of being relatively inexpensive, leak-proof, and free from corrosion problems. Their chief disadvantages are that they can easily be torn on sharp projections, or holed by contact with a hot surface such as the pan in which the sulphur is burnt.

*Wood-framed house covered with asbestos cement sheeting.*—These chambers have a large framed door which should be gasketed and fitted with clamps to permit close sealing. Sulphur is burnt in a small brick or concrete box at the rear of the house, $SO_2$ passing into the enclosure via a short duct. A lid on the box provides some control over its burning rate. This type of house often has caulked joints to reduce leakage and is satisfactory if kept in sound order. However, warped or ungasketed doors, cracked sheets of asbestos cement, and flaking due to the corrosive action of $SO_2$ are often encountered.

*Concrete block chamber.*—Houses of this type have poured concrete roofs and wood-framed doors. They are built singly or in

multiples of two, placed back to back. For single units, sulphur is generally burnt in boxes similar to those described in the previous paragraph. When arranged back to back, a trench about 9 in. deep and 12 in. wide is located under each door and at right angles to it. Part of the trench is therefore external to the chamber and part internal. The external part has a lid under which the sulphur is burnt. The rest of the trench acts as a channel for the influx of $SO_2$ to the chamber. Vent holes are provided in the roof. Structures of this type are usually free from leakage if the doors are sealed as with the wood-framed houses.

*Concrete block tunnel.*—This is the only equipment in which a semi-continuous operation is used. The tunnel holds three trucks of fruit which are moved progressively through it, and has a wood-framed door at each end. Sulphur is burnt in pans placed inside the door at each or either end of the tunnel. The burning rate is controlled by adjusting the clearance at the base of the doors. Concrete is not immune from corrosive attack by $SO_2$ and flaking of the surface may occur.

## Test Material and Procedures

Sulphur dioxide levels were measured in 16 separate sulphuring runs, 5 of which were in plastic tents, 4 in asbestos cement houses, 4 in concrete block chambers, and 3 in the concrete block tunnel. Five runs were done in 1962 and 11 in 1965.

Apricots were sulphured in all tests except one, when nectarines were treated. All orchardists were requested to follow their normal sulphuring procedure. In every trial, a load consisted of about 500 lb of fruit and charges of sulphur were between 3 and 4 lb in weight. Some orchardists used a hessian wick to assist ignition but others burnt the sulphur alone.

## Gaseous Sulphur Dioxide Levels

*Uniformity within enclosures.*—$SO_2$ concentration was fairly uniform within sealed enclosures, readings taken in rapid succession from the four sampling points differing by 0·1 to 0·3%. Occasionally, greater variations (up to 0·5%) were observed, but such spatial differences occurred only when gusty winds affected the sulphur burning rate or when a house was inadequately sealed. Although $SO_2$ always entered at the base of an enclosure and the gas is heavier than air, apparently heat from the combustion of the sulphur caused enough convective movement to keep $SO_2$ concentration reasonably uniform within the enclosures.

Measurements with thermocouples showed that temperatures inside the enclosures reached about 20°F above the external shade temperature. In adequately sealed chambers, temperature was also reasonably uniform, indicating considerable convection.

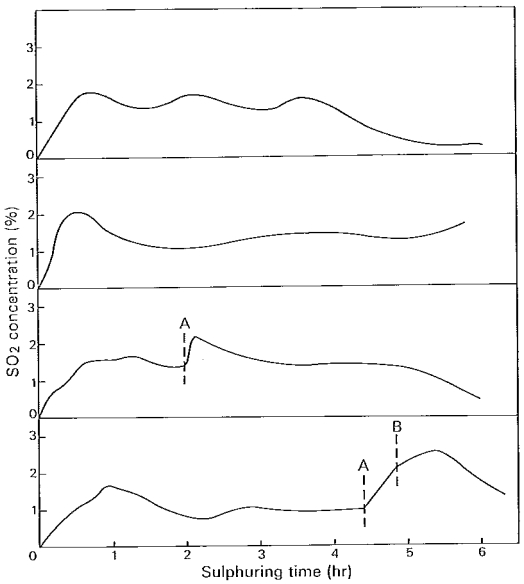


Fig. 1.—Variation in the average concentration of gaseous sulphur dioxide during 4 tests using a sulphuring chamber made of concrete blocks. *A*, stirring of molten sulphur to remove surface slag. *B*, admission of additional air to burning sulphur.

*Average levels.*—Average $SO_2$ concentration (the mean of readings at the four sampling points) fluctuated widely during each run. However, in spite of the wide differences in design of enclosures and of the many minor variations in procedure employed by the orchardists, approximately 80% of values fell within the range 1–2% $SO_2$. Higher levels occurred when winds were blowing strongly, the maximum observed being 3%. This is the same maximum $SO_2$ level observed by Long, Mrak, and

Fisher. Gas concentrations below 1% were obtained when the sulphur burned sluggishly through lack of oxygen or the formation of slag on its surface.

Different $SO_2$ concentration patterns were obtained during different runs in the same equipment. Figure 1 shows the levels during four tests in a chamber built from concrete blocks. The initial rate of rise in $SO_2$ concentration varied, probably due to differences in the area of the surface of sulphur which was burning when it was introduced into the system. There was always a tendency for $SO_2$ concentration to rise early to an above-average value before declining slightly. The reason for this is not known.

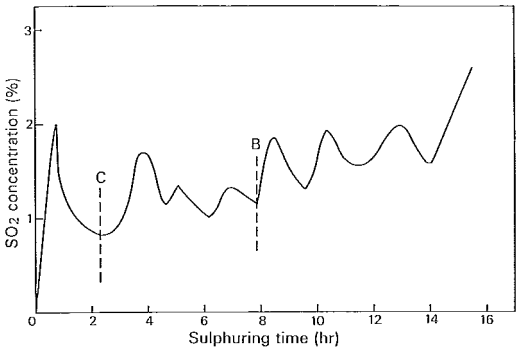


Fig. 2.—Fluctuation in the average concentration of gaseous sulphur dioxide during sulphuring in a plastic tent. *B*, admission of additional air to burning sulphur. *C*, relighting of sulphur after it was extinguished.

## Causes of Fluctuating Sulphur Dioxide Levels

During these trials, the shortest time for a 4-lb charge of sulphur to burn completely was 3·5 hr, and the longest 15 hr. The commonest cause of fluctuations in $SO_2$ level was uneven burning of sulphur. A sudden directional change or a drop in wind strength caused the sulphur to burn more slowly and this was reflected in a lower gas level about 5–10 min later. An increase in wind speed resulted in higher $SO_2$ levels soon after. When air conditions were particularly calm, burning was sluggish even if the vent to the box containing the pan was well open. Increases in wind strength are indicated at *B* in Figures 1 and 2.

A second cause of sluggish burning was the formation of a scum or slag on the surface of the molten sulphur, due to small amounts of organic impurities. This slag restricts oxygen supply for combustion and may extinguish the flame in severe cases. Stirring of the sulphur may break up the slag and overcome the problem. Figure 1 shows a rise in $SO_2$ concentration following stirring (indicated at *A*) of a slowly burning pan of sulphur. The formation of slag is a long-standing problem in the industry but it has been largely overcome in recent years since suppliers agreed to carry out burning tests and supply only free-burning material to fruit dryers.

Inadequate sealing of the sulphur house is another source of fluctuating $SO_2$ level. As mentioned earlier, this can cause marked spatial variation in $SO_2$ concentration as well as generally lowering levels. This problem is associated chiefly with gaskets and methods of sealing the door, but cracks in sheets of asbestos cement or tears in plastic are other sources of trouble.

Under calm conditions, plastic tents had very uniform gas concentrations, but wind caused them to act as bellows pushing large volumes of air and $SO_2$ into the atmosphere through the vent holes. Even slight breezes can cause sharp variations in $SO_2$ levels within plastic tents. Figure 2 shows marked fluctuations during a run caused by mild night breezes. This defect can be overcome by roping the tent securely to the stacks of enclosed trays. Under strong, gusty conditions ties should be no more than 12 in. apart.

## Sulphur Dioxide Content of Dried Fruit

Sulphur dioxide contents of dried fruits arising from the sulphuring trials were determined using the modified Monier–Williams method developed by Shipton (1954). These values are listed in Table 1, with significant data about the sulphuring procedures.

During the 1962 trials, weather conditions were generally hot and dry, thereby favouring high retention of $SO_2$ during drying. In contrast, cooler weather during the 1965 season resulted in lower retentions. Apricots required 8–9 hr exposure to $SO_2$ in 1965 to contain 3000 p.p.m. when dry, whereas in 1962 fruit sulphured for only 6 hr con-

**Table 1**
**Sulphur Dioxide Contents of Dried Fruits produced during Field Sulphuring Trials**
Apricots were used in the trials except those marked *, in which nectarines were sulphured

| Year | Type of Sulphuring Equipment | Exposure Time (hr) | Sulphuring Data | $SO_2$ Content (p.p.m.) |
|---|---|---|---|---|
| 1962 | Asbestos cement | 6 | Windy. 2 × 4 lb charges of S | 3670 |
| | | 8·5 | Windy. 2 × 4 lb charges of S | 3950 |
| | Plastic tent* | 8·5 | Windy. Up to 2·5% $SO_2$ | 3150 |
| | | 14 | 1–1·5% $SO_2$, 0·4% at end | 4400 |
| | | 15·5 | 1–2% $SO_2$ | 4470 |
| 1965 | Concrete chamber | 6 | $SO_2$ levels plotted in Fig. 1 | 2370 |
| | | 6 | | 2530 |
| | | 6 | | 2470 |
| | | 6 | | 2840 |
| | Concrete tunnel | 7 | Windy, cool. 1% $SO_2$ | 2310 |
| | | 7 | Windy, cool. 1% $SO_2$ | 2420 |
| | | 14 | <1% $SO_2$ for part of time | 2910 |
| | Asbestos cement | 8 | 1·5–1·75% $SO_2$ | 2900 |
| | | 9 | 3 hr above 2% $SO_2$ | 3150 |
| | Plastic tent | 15·5 | $SO_2$ levels in Fig. 2 | 5080 |
| | | 16 | Similar $SO_2$% to Fig. 2 | 5200 |

tained more than that figure. Opposed to this general trend, it can be seen from Table 1 that apricots sulphured overnight in plastic tents in 1962 had lower $SO_2$ levels when dry than those handled in a similar manner in 1965. The former were dried during a two-day period when humidity was very high whereas the latter were dried during the only period of the 1965 tests when temperatures exceeded 100°F. Apricots sulphured overnight generally had $SO_2$ contents in excess of the statutory level of 3000 p.p.m. when dried.

## Conclusions

During the exposure of cut tree fruits to the fumes from burning sulphur, considerable fluctuations were observed in the average $SO_2$ concentration within the various enclosures commonly in use. These fluctuations depended mainly on the rate at which sulphur burnt, which in turn was influenced by the air supply to the burner. Present methods of controlling burning rate are only partly effective and depend largely on the strength and direction of the wind.

Observed $SO_2$ levels were most commonly between 1 and 2% but exceeded this when wind conditions were turbulent and fell below it when sulphur burnt sluggishly. Freedom from leakage is essential in all sulphuring enclosures since loosely fitting doors, and cracks and holes in walls or roofs, cause not only lower average concentrations but also wide spatial variation of $SO_2$ within the houses. Special attention should be given to ensure that plastic tents cannot flap in the wind, because in so doing they act as bellows and discharge large amounts of $SO_2$ into the atmosphere.

The $SO_2$ contents of dried fruits produced during these trials ranged from 2300 to 5200 p.p.m. The highest values were obtained when fruit was sulphured overnight (14–16 hr). This may be a convenient procedure but it obviously results in $SO_2$ levels far in excess of the statutory 3000 p.p.m. The even wider range of $SO_2$ contents which occurs in practice suggests that some orchardists are using sulphuring methods very different from those which should be used.

The weather conditions during sun-drying influence the $SO_2$ content of dried fruits by affecting the retention of the preservative. However, if uniformity of sulphuring could be attained, orchardists could vary exposure time to compensate for this factor. From these trials, apricots should contain the target figure of about 3000 p.p.m. of $SO_2$ if

they are sulphured for 5–6 hr during a hot and arid season, whereas 8–9 hr are needed if the weather remains persistently cool and humid.

The tests showed that there is need for a mechanical sulphur burner capable of maintaining a concentration of 1·5–2% $SO_2$ within sulphuring enclosures. Such a burner could be operated in conjunction with many of the present sulphur houses, because they are fairly uniform in volume and in the weight of prepared fruit they hold per charge. As old houses are replaced or new units installed, further standardization in size and design could be introduced.


### Acknowledgments

Grateful acknowledgment is made to Mr. W. B. Harris, Department of Agriculture, South Australia, who, as manager of the experimental orchard at Berri in 1962, helped to organize the trials in that year. In 1965, similar help was received from members of the Research Liaison Subcommittee of the Australian Dried Fruits Association. We also wish to thank those orchardists who cooperated in the trials.


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# Conferences, 1967

The Twelfth International Congress of Refrigeration is to be held in Madrid from August 30 to September 6, under the auspices of the International Institute of Refrigeration. Registration and hotel reservation forms may be obtained from the National Correspondent of Australia for the Congress, Dr. J. R. Vickery, CSIRO Division of Food Preservation, Box 43, P.O., Ryde, N.S.W.

The Fifth International Congress on Canned Foods will take place from October 2 to 7 in Vienna. The convening body is Fachverband der Nahrungs- und Genussmittel-Industrie, Zaunergasse 1-3, Vienna III, Austria.

# Book Review

Practical Guide to Refrigerated Storage. International Institute of Refrigeration, Paris. 240 pp. F40 or £3 stg.

This Guide, which was first conceived in the 1950/55 session of Commission 5 of the International Institute of Refrigeration, analyses the problems to be faced in building a cold store or improving an existing one.

It is written in English and French, and its five chapters deal with design and construction, handling of merchandise in the store, conditions for storage, legal and commercial aspects, personnel, and safety.

The text is easy to read and contains information on all matters likely to arise in the course of planning a cold storage facility. The problems are posed rather than solved, since solutions will be influenced by local conditions. Nevertheless, sufficient detail is included to indicate possible solutions.

The authors point out that commercial stores are intended to run at a profit, and that they will not continue to do so if they do not succeed in retaining quality in the product. A cool storage facility is a warehouse, though it is run at low temperature, and its mode of operation will often be based on a compromise between economic and technical considerations.

The English version shows some slight indication of being a translation. 'Forced draft cooler' is referred to as 'air conditioner-diffuser', and 'fully automatic equipment' is said to posses 'integral automation'. It is a pity that some of the titles in the English bibliography are not fully translated, and a footnote to the effect that some modern insulants are completely impermeable to water vapour appears to require amplification.

Much of the text might be considered appropriate to the larger public or cooperative type of store, but the principles enumerated and the advice given apply to all stores and will repay study by anyone concerned with cold storage.

The comprehensive bibliography at the end of the Guide will enable the reader to obtain further information on special aspects. A folder containing plans and photographs of actual installations in various countries is a useful supplement to the volume.

R.A.

# NEWS FROM THE DIVISION OF FOOD PRESERVATION

## Meat Research

After being accommodated in quarters at the Queensland Meat Industry Board's abattoir at Cannon Hill, a suburb of Brisbane, since 1932, the Division's meat research team moved into fine new laboratories during January 1967.

The steel and masonry buildings, which have a floor area of 25,000 sq ft, have been erected on 14 acres of land in Wynnum Road, Cannon Hill, at a cost of $580,000. Finance for the project was provided by the Australian Cattle and Beef Research Committee (now the Australian Meat Research Committee) and by CSIRO.

The laboratories, which will be in charge of Dr. W. J. Scott, Assistant Chief of the Division of Food Preservation, will provide facilities for a considerable expansion in meat research. They will accommodate a staff of about 50 including some 25 scientists with special knowledge of such subjects as engineering, physics, microbiology, physiology, and biochemistry. As the greater part of the funds came from a levy on cattle slaughtered for human consumption, most of the research will be concerned with problems on the preparation and preservation of beef. However, plans are being prepared for a second stage in the development of the laboratory, to provide for research on mutton and lamb.

A CSIRO Meat Industry Research Advisory Committee has been established to aid communication between the research team and the meat industry. The Committee includes representatives from various producer and trade organizations, the Australian Meat Board, and the Commonwealth Department of Primary Industry, together with three representatives from CSIRO. The Committee provides a channel through which the meat industry can refer its technical problems to CSIRO for investigation and advice and through which industry representatives are kept informed of the progress of research.

## Overseas Travel

Dr. W. J. Scott, Assistant Chief of the Division, was absent from Australia from September 23 to November 24, 1966, during which period he visited England, Denmark, Germany, Canada, and the United States. The purpose of his visit was to study developments in meat science and technology and it involved discussions at a large number of research institutions and some meat processing plants. Dr. Scott also attended the forty-fifth meeting of the Society for General Microbiology at Bristol, and devoted time to recruiting research staff for the Division's Meat Research Laboratory at Cannon Hill.

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