HUMAN BOTULISM AND STAPHYLOCOCCAL FOOD POISONING :

A REVIEW¹⁾

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These two types of food poisoning have few features in common. They are both due to ingestion by man of specific toxins released through bacterial multiplication in foodstuffs before consumption. Also, these toxins act primarily upon the central nervous system, though on quite different areas of it, and with results which, in the case of enterotoxin (as its name implies) mostly affect the gastro-intestinal tract. Again, certain foodstuffs, notably hams and prepared meats, are prone to convey either of these types of bacterial intoxication. But these similarities are greatly outweighed by the contrasts between the two conditions, which will be emphasized in a brief review under various headings.

History

Towards the end of 1895, at the village of Ellezelles in Belgium, 34 members of a musical society attending a funeral ate some raw salted ham. Most of them fell ill 20-36 hours later, displaying a paralytic syndrome resembling the sausage poisoning (*botulismus*) first described in south Germany in 1735, and the fish poisoning (*ichthyosismus*) long recognized in Russia. Three of the musicians died, and 10 others were seriously ill, while those who ate small amounts of ham had milder illnesses. The ham, from an apparently healthy pig slaughtered 12 days before, had lain at the bottom of the brine barrel. From the rancid remnant of the ham, van Ermengem of Ghent isolated an anaerobic, spore-forming bacillus, whose culture filtrates produced characteristic and often fatal paralyses in many species of laboratory animals. In a report of classic thoroughness and undisputed observations, published 60 years ago, van Ermengem (1897) proved the Ellezelles tragedy was due to a neurotoxin released into the ham by this anaerobe; blamed the same mechanism for the traditional sausage and fish poisonings; and proposed that the causal micro-organism be named *Bacillus botulinus*.

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Within the next decade, confirmation of van Ermengem's work came from many parts of Europe, including a report by Madsen (1907), from Denmark, which alluded to the first bacteriologically verified fish-borne outbreak. Later, as industrial canning of vegetables and fruits expanded in the United States, especially in California in the 1920's, it became evident that such proteinaceous foods as hams and the notorious blood sausages, were not necessarily the most dangerous. Interest awakened in botulism across the Atlantic, and much intensive work was done there in this field. During the 10-year period 1919–29, *Bacillus botulinus* was classified into 4 types, A, B, C and D, with E following in 1936. Under natural conditions, *i.e.* when *ingested* by man or animals, the 5 types of toxins display different degrees of pathogenicity for various host species. Thus, types A and B may affect man, horses and chickens; C is associated with outbreaks among horses, cattle, wildfowl and some fur-bearing rodents, such as mink; D seems chiefly to affect cattle; while type E has been implicated so far only in human botulism. The explanation of this species-selectivity remains obscure, the more so since in the laboratory, when the different types of toxins are *injected* in sufficient dosage, qualitatively similar reactions are provoked in many kinds of animals, from monkeys, mink and mice to guinea pigs and goldfish. Despite these complexities, it has never been disputed that botulism, whether in man or animals, is due to the same pharmacological action upon host tissues of a group of closely related bacterial toxins. The paralytic characteristic of botulism apparently results from disruption of the mechanism of acetylcholine-release at the junctions of motor nerve endings and muscles. According to Brooks (1953), type A toxin blocks conduction in the terminal twigs of motor nerves, proximal to the site of acetylcholine-release.

By contrast, the site of action of staphylococcus enterotoxin remains largely unsettled; indeed, the very recognition of this substance has been, for many reasons, delayed and grudging. A year or so before the Elzevelles fatalities, van de Velde (1894) reported that culture filtrates of *Staphylococcus pyogenes* contained a "leucocidin" which caused marked degenerative changes in rabbit leucocytes *in vitro*, and led to production of circulating anti-leucocidin when injected into rabbits. Five years later, at the turn of the century, Kraus (1900) drew attention to the haemolytic action of certain staphylococcal cultures, while von Lingelsheim (1900) reported the necrotizing and lethal effects of what he first termed the "toxin" of staphylococci. But not until 1914, when Barber reported from the Philippines that visitors to a certain farm were liable to attacks of vomiting and diarrhoea, was it suggested that staphylococci might also produce a substance capable of inducing acute gastro-intestinal disturbances in man when taken by mouth. These attacks were traced to the consumption of milk from one particular cow, which persistently secreted a strain of *Staphylococcus albus* from the udder (Barber, 1914). Barber's pioneer paper caused little stir at the time, but came into the limelight about 15 years later, when Jordan, Dack and their associates used human volunteers to show that the

staphylococcal food poisoning substance was filterable, and producible by growing appropriate cultures in fluid nutrient media (Jordan, 1930; Dack *et al.*, 1930). Yet clinical accounts of the condition existed in the European medical literature at least 100 years before the work of these University of Chicago bacteriologists.

Consider, for example, this report about a man who dined on ham pie, which had been purchased from a pastry-cook in Paris. Three hours later, he "was seized with general uneasiness, followed by cold sweats, shivering, violent pain in the stomach, and frequent vomiting; then with burning thirst, extreme tenderness of the belly, so that the weight of the bed-clothes could scarcely be borne, profuse purging, and colic of extreme violence. His daughter, 27 years of age, and a child 9 years old, were similarly attacked. . . . In a few days all the three individuals recovered. . . . About the same period several accidents of the like nature occurred among the customers of this pastry-cook; and in consequence a judicial investigation was ordered. The shop being properly inspected it was found that every operation was conducted with due attention to cleanliness. . ." This excellent description of staphylococcal food intoxication is taken from an English medical journal, nearly 1½ centuries ago (Lancet, 1830).

For some years after most bacteriologists in the United States and Canada were becoming convinced that staphylococcal food poisoning was a distinctive, widespread and common entity, in the United Kingdom a sceptical attitude prevailed, based on a somewhat circuitous argument; staphylococci were too ubiquitous for their presence in large numbers in a foodstuff to carry any special significance; and, if these organisms did cause food poisoning so frequently, they would surely have been incriminated before. The situation became more confused as suitable laboratory animals were sought for detecting the enterotoxic substance in place of human volunteers, for very few animal species reacted to this agent given by mouth, and when it was injected, the chief effects were often due to other staphylococcal toxins.

Incidence and Distribution

Nowadays, most bacteriologists agree that the enterotoxin is a single entity, of uncertain composition, and responsible for the commonest of all types of bacterial food poisoning. The short duration, with generally rapid and complete recovery, have tended to postpone recognition of this world-wide disease, whose incidence seems comparable with that of the common cold. The condition tends to be more prevalent in hot climates, where staphylococcal multiplication is encouraged in exposed foods: but of course in a cold climate, storing a foodstuff near a warm kitchen stove will serve the same end.

Fortunately, the incidence of human botulism is at the other extreme. Nowhere is it a common disease, and in some countries it is very rare or else unidentified. Moreover, the geographic distribution of botulism is irregular. For instance, in the United States over the 50-year period 1899-1949, there

were 477 outbreaks of botulism, involving 1281 persons, with 829 deaths, a fatality rate of 65 per cent (Meyer, 1950). Altogether, 409 cases, or 32 per cent, were proven due to type A toxin, and only 9 to type E toxin. In Canada the incidence of botulism is lower in proportion to population, and the type pattern is different. During the 40-year period, 1917–1957, in 17 recorded outbreaks, 77 persons were affected, with 45 deaths, a case fatality rate of 58 per cent. Only 3 outbreaks, involving 6 persons (under 8 per cent of the total affected), were of type A, whereas 6 outbreaks, involving 20 persons, were of type E. At the other side of the Pacific Ocean, in Japan, this predilection for type E botulism is carried further; for of the 23 outbreaks identified in the past 6 years, affecting 110 persons, with 45 (40 per cent) fatalities, all have proven of this type. Or across the Atlantic Ocean, in France, some 500 episodes of type B botulism were reported there during the occupation of 1940–44, with over 1,000 persons affected, and a case fatality rate of only 2 per cent (Legroux *et al.*, 1947). Yet in the British Isles, only one proven outbreak of botulism is on record, *viz.* the type A outbreak in 1922 at Loch Maree, in the Western Highlands, where 8 holiday-makers died after a picnic meal which included potted duck paste sandwiches (Leighton, 1923).

Evidently, neighbouring countries of comparable hygienic standards and medical skills, may display very dissimilar incidences and types of human botulism. Of the many variables which help to determine such regional differences, the most important appear to be the density of dissemination of botulinus spores in the local soils or off-shore waters, and the extent to which prevailing methods of preserving foods render them subject to pollution by viable type A, B or E spores, which can then germinate and multiply to form toxin within the food. The mere presence of such spores in the soil of a region will not *per se* give rise to endemic botulism. For example, types A and B spores have been demonstrated in the soil of several populated parts of Canada, where human botulism has never been identified. Again, only a few months before the unique Loch Maree (type A) disaster in Scotland, 5 out of 64 soil samples from various parts of England had yielded type B cultures. Conversely, if botulinus spores are to all intents absent from a regional soil, such unhygienic but traditional habits as the burial of duck eggs, meats and cheeses, may be followed with relative impunity. Meyer and Eddie (1951) refer to a family outbreak of type A botulism in California, due to cheese, which a woman had prepared from her own cows' milk, placed in a large crock, covered with a canvas cloth, and buried in the garden. Near Christmas time, the crock was unearthed and the cheese tasted, with 3 cases of botulism, one of them fatal, resulting. This woman's ancestral land was Armenia, where the custom of burying milk products to mature in the dry desert soil was not considered hazardous.

Symptomatology

There is little need to describe the distinctive symptomatology of these two conditions in their acute forms but some less well-known characteristics may

be mentioned. First, while the most striking features of botulism are neuro-paralytic, and those of the enterotoxin syndrome are gastro-intestinal, some degree of overlapping may occur. In a severe attack of botulism, gastro-intestinal disturbances, including vomiting, diarrhoea and abdominal cramps, often precede the onset of obvious paralyses. Sometimes, but not always, where the botulogenic food is highly contaminated, such disturbances are explicable in terms of the coincident presence of the enterotoxin. A diagnosis of botulism should not be ruled out solely because of prodromata resembling staphylococcal food intoxication. Conversely, when large amounts of the enterotoxin are absorbed, the resulting vertigo, prostration and shock may simulate botulinic paralysis to a casual examiner.

Secondly, these conditions customarily have quite different incubation periods. In botulism seldom less than 10 hours elapse before the onset of symptoms, whereas in staphylococcal food intoxication it is rarely more than 4 hours. But in our laboratory, human volunteers have reacted to the ingestion of enterotoxin after intervals ranging from less than 1 hour to roughly 36 hours. Two volunteers who had become partially immune as a result of several injections of toxoid prepared from an enterotoxic strain of *Staphylococcus*, ingested a challenge dose of a potent filtrate. One had a single vomiting episode about 24 hours later, and the other suffered a typical gastro-intestinal disturbance after nearly 36 hours.

Thirdly, mild atypical manifestations of these diseases need further clinical study. The comparatively low fatality rates for botulism recorded in Europe in recent years, such as only 2 per cent in France (Legroux *et al.*, 1947), signifies the inclusion of many ambulatory syndromes which pass unrecognized elsewhere. In countries where botulism is specially endemic, physicians should be on the alert for vague or unusual complaints, ranging from unexplained general weakness to oesophageal paralysis, and for oddly associated symptoms, such as constipation with poor visual accommodation.

Finally, staphylococcal enterotoxaemias which are not food-borne could arise from proliferation of suitable strains carried in the bowel. Some years before the antibiotic era, we noted that some children with chronic or subacute dysenteric symptoms, whose stool cultures were repeatedly negative for *Salmonella-Shigella* organisms, were excreting enterotoxigenic staphylococci. Since the use and abuse of intestinal antibiotics became fashionable, acute staphylococcal entero-colitis has caused much concern. This acute and often rapidly fatal toxæmia may be due mainly to absorption from the bowel of other staphylococcal toxins, but the enterotoxin could be contributory. The lethal potentialities of this agent have been demonstrated by a few instances of fatal staphylococcal food intoxication.

Epidemiology

The natural habitats of *Cl. botulinum*, and the modes of conveyance to human food, will be discussed later. It seems superfluous to review the many familiar

sources of staphylococcal food contamination, from cow's milk to a food handler's nasal mucosa or infected finger. These were summarized adequately many years ago (Dolman, 1943). Only two epidemiological phenomena will be considered briefly here, *viz.* the broadening range of foodstuffs revealed as potentially hazardous, and the apparent insusceptibility shown by some exposed persons.

Certain foodstuffs, such as inadequately cooked pork products, raw fish, and home-preserved vegetables and fruits, are well established as potentially botulogenic. But lately the remarkable adaptability of *Cl. botulinum* has been illustrated in the isolation of type E cultures from some 20 "izushi"—borne outbreaks in Japan; of types A and B cultures from several samples of soft ripened cheese spread sold in the United States, including one Liederkranz cheese which caused a fatal case of type B botulism (Meyer and Eddie, 1951); and of a type A culture from bread in England (Ingram and Robinson, 1951). In view of such versatility, undue reliance must not be placed on new methods of processing foods, which either fail to ensure destruction of botulinus spores, as in mild-cured canned hams (Scott, 1955), or may even help to preserve these spores for possible later germination and toxin production, as in deep frozen and dehydrated foods.

Staphylococci can also survive and proliferate in a great diversity of foods, besides the favoured milk products and manufactured meats. Moreover, the comparative heat-stability of enterotoxin may prove a snare. Several outbreaks of staphylococcal food intoxication due to spray-dried milk have been reported. In addition to the extensive outbreak involving nearly 2,000 schoolchildren in Tokyo in 1955, several smaller outbreaks occurred in England 2 or 3 years ago (Anderson and Stone, 1955), and 19 separate outbreaks, affecting 775 school children, have just been reported from Puerto Rico (Armijo *et al.*, 1957).

In many outbreaks of botulism, a few persons at risk escape symptoms altogether. For instance, in the Japanese series, 110 cases developed among at least 232 exposed persons (Iida; Yamamoto). Since there is no evidence that man naturally acquires specific immunity, more likely alternative explanations are a smaller intake (lesser amounts, or atoxic portions, of food ingested) and delayed absorption, with inactivation of the toxin by other agents in the stomach and intestines, such as alcohol or proteolytic enzymes.

In staphylococcal outbreaks, attack rates characteristically range from 40 to 80 per cent. Differences in susceptibility can be demonstrated also on human volunteers. Some 15 years ago, we reported that among a group of 7 laboratory associates, 4 consistently gave a marked reaction after swallowing 1 cc of a strongly enterotoxic filtrate; one required 2 cc, and another 3 cc, of the same filtrate, to produce a reaction; and the remaining volunteer was unaffected by 5 cc (Dolman, 1943). Whether or not such natural variations in susceptibility are due to different levels of specific antibody remains unsettled.

Control

The guiding principles in the control of both forms of bacterial food intoxication must be:—(i) To minimize the chances of causal agents gaining access

to the food. (ii) By proper temperature control, to achieve a 3-fold objective—to kill toxigenic organisms which have got into the food despite all precautions; to inhibit toxin production by any surviving organisms; and to destroy toxin which nevertheless may have been elaborated.

No attempt will be made here to discuss, or even to list, the techniques available for implementing these principles. Let it suffice to emphasize that the first principle entails applying sound knowledge of the respective habitats and ecologies of *Cl. botulinum* and of enterotoxigenic staphylococci, through enlightened personal hygiene and sanitary regulations. In these connections, health departments should naturally seek advice from bacteriologists. The second principle, proper temperature control, again involves a programme of public health education, particularly for the housewife, coupled with wider availability of such devices as pressure cookers and refrigerators. Close cooperation between the canning industry and bacteriologists is also essential; for only the latter are able to assess and investigate such findings as the remarkably high and low thermostabilities of types A and E botulinus spores, or the greater heat resistance of the enterotoxin than of botulinus toxins.

Nature of Staphylococcus Enterotoxin

Whereas types A and B botulinus toxins have been crystallized, all efforts to purify the enterotoxin have failed so far. Indeed, little is known of its chemical nature and pharmacological activity, while there is disagreement regarding its antigenicity. Most authorities now agree that the enterotoxin is a distinct entity, which only a relatively small percentage of strains can produce in detectable amounts, in either foodstuffs or laboratory media. By means of human volunteers, we clearly showed that the enterotoxin differed from the α - and β -toxins (Dolman, 1934; 1943). Enterotoxigenic strains generally produce some α -toxin, while a few produce both α - and β -toxins. We have also encountered an α -toxigenic food poisoning strain, which gave rise to mutant colonies. These simultaneously acquired β -toxigenicity and lost the original enterotoxigenicity (Dolman, 1943).

Again, an individual's circulating α - and β -antitoxin titres are unrelated to his degree of susceptibility to the enterotoxin; but resistance may be increased about 5-fold by a series of injections of formalinized filtrate obtained from an enterotoxigenic culture grown in a crude nutrient broth medium (Dolman, 1944). When prepared in synthetic media, enterotoxin may show little or no antigenicity.

Laboratory Diagnosis of Staphylococcal Food Poisoning

The greatest handicap to progress in this field of research has been the lack of a simple and unequivocal animal test, or of methods for *in vitro* detection and assay. The complete absence of vomiting or diarrhoeal reactions shown by all the common laboratory rodents to enterotoxin administered by any route, is presumably due in part to their gastro-intestinal mechanisms and bowel

contents differing from those of the known susceptible species—man, monkey, cat and dog. But there must be other factors to account for the especially high human sensitivity to this agent. For example, 1 cc of a potent filtrate taken orally, which suffices to cause acute gastro-intestinal disturbances in most experimental subjects, may evoke only a borderline or even negative reaction, if administered intraperitoneally to a kitten, or intravenously to a large cat. Yet in practised hands, the kitten or cat test developed in our laboratory over 21 years ago (Dolman *et al.*, 1936), with subsequent modifications (Dolman, 1943), is believed more reliable, and is of course far cheaper, than monkey feeding methods. Alternative animal tests have been recommended, involving the administration of enterotoxic material to piglets, chicks, suckling mice, decerebrated frogs, and isolated loops of rabbit intestine; but all seem to have fallen by the wayside. Perhaps the cat test has some inherent imperfections of specificity and sensitivity; but according to a recent appraisal by Matheson and Thatcher (1955), this remains the method of choice for enterotoxin research.

Proper performance of the cat test involves prior elimination of all α - and β -toxins from the filtrate, by means of specific antitoxic sera, incubation with formalin, or boiling for 30 mins.—all of which carry certain disadvantages. A simpler method is to grow suspected cultures on soft agar medium for 3½ days in air at 20–23°C., under which conditions potent enterotoxin may develop, without accompanying α - or β -toxins. The resulting filtrates may be injected directly into cats (Dolman, 1944).

None of the numerous *in vitro* methods or media yet advocated for the detection of food poisoning strains appears reliable; but 'phage typing may provide useful, though not infallible, epidemiological clues. Meanwhile, the routine laboratory diagnosis of staphylococcal food intoxication must rest usually upon demonstrating the presence of large numbers of staphylococci (of the order 0.5×10^6 or more per g) in the implicated foodstuff. Some confirmation would derive from a positive enterotoxic reaction induced in a cat by a filtrate of this strain. On the other hand, a negative cat reaction can be misleading, since some strains of *Staphylococcus* may quickly cease to be enterotoxigenic in laboratory media. Nor is it essential that large numbers of staphylococci should be demonstrable in the suspected food. As we showed many years ago (Dolman, 1943), by inoculating a sausage with a known food poisoning strain of *Staphylococcus*, and 24 hrs. later with a harmless culture of *Proteus vulgaris*, it is possible for one contaminating culture to overtake or mask another. Spread plates of the sausage showed *Proteus* growth almost exclusively. However, this did not interfere with the development of enterotoxin in the sausage, as was proved by 2 volunteers, who suffered violent gastrointestinal disturbances after sharing it.

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