Clostridium welchii and *Bacillus cereus* infection and intoxication

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Summary

Clostridium welchii type A is a common agent of food poisoning when allowed to proliferate to large numbers in cooked foods, usually meat and poultry. The main factors of importance are survival of the spores, frequently found on raw products, through the cooking process, and possible contamination of cooked meats transferred to unclean containers; subsequent germination of spores and rapid multiplication of the vegetative cells during long slow cooling and non-refrigerated storage lead to heavy contamination. The toxin responsible is different from the soluble antigens, and its formation in the intestine is associated with sporulation.

Large numbers of *Cl. welchii* of the same serological types in food and faeces is the main diagnostic factor. Important preventive measures are rapid cooling and cold storage to prevent growth.

Bacillus cereus is an aerobic sporulating organism commonly found in cereals. Outbreaks described from Europe have a different aetiology with regard to food vehicles, incubation period and symptoms from those that have been reported recently in the U.K. from fried and boiled rice. The spores survive through cooking procedures and grow out to cells which sporulate readily in the cooked food and which are assumed to produce toxin in the food. Large numbers of *B. cereus* are found in foods causing illness and, as with *Cl. welchii*, the main preventive measure is inhibition of growth by quick cooling and cold storage of foods cooked ahead of requirements. A comparative table of the characteristics and clinical symptoms of *Cl. welchii* and *B. cereus* is given.

Clostridium welchii

There were early reports of intestinal disturbances due to *Cl. welchii* in 1895 (Klein, 1895) and 1899 (Andrewes, 1899). In 1943 Knox and MacDonald described outbreaks of food poisoning in children after eating school meals. Gravy prepared in bulk on the previous day was heavily contaminated with anaerobic sporulating bacilli including *Cl. welchii*. McClung (1945) in the U.S.A. was definite in his assertion that certain food poisoning outbreaks after meals of cooked poultry were due to *Cl. welchii*. In

1948, there was a large outbreak of intestinal disease described as enteritis necroticans in Hamburg, Germany; it was reported in a number of papers (Ernst, 1948; Schutz, 1948; Marcuse and König, 1950). The clinical and bacteriological findings were clarified in papers by Zeissler and Rassfeld-Sternberg (1949), Oakley (1949) and Hain (1949). These workers described a small outbreak of haemorrhagic enteritis in a family who had eaten tinned rabbit meat or fish; the outbreak was thought to be part of the much larger episode. The strain of *Cl. welchii* isolated from food and patients survived 100°C for 1-4 hr and produced much β -toxin. This strain was given the new type letter F; later it was described as a subgroup (heat-resistant) of type C (Brooks, Sterne and Warrack, 1957; Warrack, 1963; Sterne and Warrack, 1964). Hobbs *et al.* (1953) established *Cl. welchii* type A as a major cause of gastro-enteritis of a much milder nature than that described as enteritis necroticans by the German workers. The outbreaks reported in this paper were due to heat-resistant 'non- or α -haemolytic *Cl. welchii*'. Later McKillop (1959), Hall *et al.* (1963), Taylor and Coetzee (1966), and Sutton and Hobbs (1968) showed that the so-called 'heat-sensitive' strains, mostly β -haemolytic on horse blood agar, were also responsible for outbreaks. Although the spores of these strains are less resistant to heat, nevertheless they can survive cooking. Many other papers have been written on *Cl. welchii* food poisoning up to the present day, when the means by which the organism causes symptoms is described.

Of the five types of *Cl. welchii* A to E differentiated on the proportion of soluble antigens in each, types A, C and D are pathogenic for man and types B, C, D and E affect animals. Type A is further divisible into serological types; sera are obtained by inoculation of the whole organism into rabbits.

Cl. welchii type A may be isolated in small numbers from many foods both raw and cooked, and it forms a minor component of the faecal flora in most if not all healthy adults, occurring in numbers of about 10^4 /g of faeces. When it becomes established in the large gut, where toxin is produced, large

numbers appear in the faecal flora. Outbreaks of enteritis necroticans due to the type C strains are uncommon; they have been reported from New Guinea by Murrell *et al.* (1966a, b); Collee (1973) describes similar enterotoxaemic diseases produced by various types of *Cl. welchii* in animals; the type C strains in pigs show evidence of bacterial adhesion to intestinal epithelial cells, which may explain the more serious nature of the disease caused by type C strains in humans as well as in animals.

The annual statistics for *Cl. welchii* food poisoning in England and Wales record 47 outbreaks and approximately 1534 cases in 1969 and 32 outbreaks and 1263 cases in 1970. The symptoms of abdominal pain, nausea and acute diarrhoea occur 8–24 hr after eating food, such as cooked meat and poultry, which has become grossly contaminated with the organism during long slow cooling periods and storage at ambient temperatures. Roast, boiled, steamed, and stewed meats and poultry, and pies and gravies are the predominant vehicles of infection, whether eaten cold or reheated. When meat is cooked in bulk, the heat gain is slow and subsequent cooling is slow. The heat drives off dissolved oxygen and the meat maintains an anaerobic environment. The heat-shocked spores are activated to germinate and when the temperature drops to below 50°C the outgrowths of vegetative cells multiply rapidly. The so-called heat-sensitive strains of *Cl. welchii* type A, usually β -haemolytic on horse blood agar and more active in production of α , θ and κ toxins, require less heat shock for germination. Spores of these strains present on the inside of containers may grow in cooked meat foods poured into the containers.

The spores of the organisms *Cl. welchii*, *Cl. botulinum* and *B. cereus* can survive in food after cooking, they can be activated to germinate by the heat treatment and grow at various rates in slowly cooling food. Large numbers are assumed to be required to initiate toxin production in the intestine or to produce toxin in the foodstuff before consumption.

The characteristics of *Cl. welchii* are important in relation to the food poisoning. Temperature limits for the growth of *Cl. welchii* are about 25°C and 50°C and the optimum temperature for growth in cooked meat is between 43°C and 47°C (Collee, Knowlden and Hobbs, 1961). The generation time can be as short as 10–12 min (Mead, 1969). Spores of the classical strains (β -haemolytic) of *Cl. welchii* are more sensitive to heat (D_{90° 3–5 min)* than spores of the 'heat-resistant' strains (D_{90° 15–145 min)* using the same sporulation medium (Roberts, 1968). It was also reported by Roberts (1968) that 30–50% of spores of the classical strains grew without heating, and heat activation was not detected. In contrast

* (D_{90° = Decimal reduction time at 90°C).

less than 4% of spores of the more heat-resistant strains grew without heating, and activation was usually detected at 75–80°C. The heat resistance, heat activation and rapid generation time are the factors which predispose this organism to survival followed by rapid multiplication in slowly cooling masses of meat and poultry. The organism is anaerobic, but not to a marked degree, nevertheless, its growth depends on anaerogenic conditions within a mass of cooked food. Large numbers (millions per gram) of viable *Cl. welchii* cells in cooked foods is indicative of a health hazard.

The toxin of *Cl. welchii* responsible for the food poisoning symptoms is produced in the large intestine during sporulation. The potential for sporulation in cooked foodstuffs is poor and if the toxin is formed at all it is unlikely to be detected. In the intestine, spores are readily formed and the toxin also. Ingestion of a large dose of *Cl. welchii* and sporulation of the organism *in vivo* are important factors in the production and release of the enterotoxin. Ligated gut experiments in rabbits and lambs, those with monkeys (Duncan and Strong, 1969; Hauschild, Niilo and Dorward, 1970; Duncan and Strong, 1971) and volunteer experiments in man (Strong, Duncan and Perna, 1971) have confirmed the association between sporulation and enterotoxin production.

Hauschild (1973) refers to the toxin as enterotoxin, and he gives an excellent summary of the properties of the substance in his review. After precipitation with ammonium sulphate the toxin may be purified by gel filtration and ion exchange chromatography. The purified preparation is free of nucleic acids, fatty acids, phosphatides and reducing sugars. The u.v. absorption spectrum is maximum at 278–280 nm and minimum at 250 nm. By hydrolysis, 19 amino-acids have been demonstrated, amongst which aspartic acid, serine, leucine and glutamic acid are predominant. The substance is probably a protein of mol. wt. 36,000 $\pm$ 4000 with an iso-electric point of 4.3. The toxin is inactivated by pronase and *B. subtilis* protease but not by trypsin, chymotrypsin or papain. It is heat labile with a D_{60° of 4 min. The MLD for mice (i.v.) is 2000/mgN and lethal doses of toxin caused death within 20 min (Hauschild and Hilsheimer, 1971). The enterotoxin may be isolated from the supernatant fluids of the contents of ligated intestinal loops in lambs and also from extracts of cells. Intradermal injection of the enterotoxin into guinea-pigs and rabbits causes erythema around the injection site within 1–2 hr. By relating the diameter of the erythema to toxin concentration, the skin can be used for rapid and accurate assay of the enterotoxin; this test is said to be more sensitive than that of the ligated intestinal loop. Niilo (1971) showed that the erythema reaction in a guinea-pig skin was

preceded by increased permeability of the blood capillaries around the injection site, and he suggested that the accumulation of fluid in the intestinal lumen might result from increased permeability of the blood capillaries in the intestinal wall.

Whereas the toxin ingested by volunteers or introduced into the stomachs of rhesus monkeys produces both emesis and diarrhoea, the natural illness in man is characterized by diarrhoea only from toxin produced in the intestine. The reaction from the ingestion of toxin by man and monkey can be neutralized with antiserum prepared against the enterotoxin.

Hauschild (1973) lists factors of similarity between the toxins of *Cl. welchii* and *Vibrio cholerae*; they are both produced *in vivo*, induce fluid accumulation in ligated intestinal loops, inactivated by heat, inactivated by pronase but not by trypsin and are free of lipids and reducing sugars. The mode of action may be similar and it is described as enhanced adenocyclase activity and inhibited phosphodiesterase activity with a chloride and sodium imbalance.

The sequence of events in *Cl. welchii* food poisoning is described neatly by Hauschild (1973) as follows: '(a) ingestion of food with about 10^6 – 10^7 vegetative cells of *Cl. welchii* per gram; (b) multiplication and sporulation of *Cl. welchii* in the small intestine; (c) production of enterotoxin in the sporulating cells; (d) release of toxin by cell lysis; (e) accumulation of fluid in the cell lumen caused by the enterotoxin whose mode of action remains to be investigated; and diarrhoea resulting from the excess fluid accumulation'. Thus symptoms are initiated by live microbial cells rather than preformed toxin and the causative enterotoxin is produced *in vivo*. It must be emphasized that the assay of enterotoxin in food is not required, what is needed is the quantitative estimation of live *Cl. welchii* cells in incriminated foods as well as in the stools of patients.

It was pointed out by Sutton and Hobbs (1971) that a large dose of the organism is required not only to initiate growth in the intestine but also to permit survival of a fair number of organisms through the acid regions of the stomach. It appears that the metabolic changes set up by the sudden influx in the intestine of enormous numbers of *Cl. welchii* from food encourages the indigenous *Cl. welchii* strains to grow also.

Bacillus cereus

B. cereus as a food poisoning agent was described by Hauge in 1950 and 1955; cornflour sauce pre-cooked to eat with fruit at picnics was responsible for outbreaks occurring in Norway. Christiansen, Koch and Madelung (1951) described an outbreak due to cornflour pudding. Clarenburg and Kampelmacher (1957) in Holland, Nikodemusz (1958) in Hungary,

Midura *et al.* (1970) in the U.S.A., Pivovarov *et al.* (1970) in the U.S.S.R., von Ludwig (1971) in Germany and Fredette and Planté (1971) in Canada, all described outbreaks of food poisoning due to *B. cereus* in which the incubation time and symptoms were similar to those for *Cl. welchii* food poisoning. Recent reviews include those of Goepfert, Spira and Kim (1972) and Goepfert *et al.* (1973). A wide variety of foods have been implicated in these outbreaks including soup, cooked meat, cooked potatoes and vegetables, vanilla sauce, cornflour pudding, fried rice and soya sauce.

From 1971 onwards U.K. reports have described several incidents of food poisoning associated with fried rice from Chinese restaurants. However, the incubation period and symptoms are different from those described hitherto. Vomiting occurs about 1–6 hr following a meal, sometimes there is diarrhoea a few hours later. Fried or boiled rice is the only food common to those affected. Samples of cooked rice showed high counts of 10^7 – 10^8 /g of *B. cereus*. Neither *Staphylococcus aureus* nor staphylococcal enterotoxin were detected in samples of food examined carefully because of the similarity of incubation time and symptoms with staphylococcal enterotoxin food poisoning.

B. cereus is widely distributed in cereals and other dried foods, and in dairy products it is a common spoilage organism. Spores are readily formed in the vegetative cells; they may survive cooking procedures and grow out in the rice. The common fault is to allow large quantities of boiled rice to drain overnight at ambient kitchen temperature. The following day the rice may be fried quickly with beaten up egg which, if not freshly prepared, may also be highly contaminated with a miscellaneous collection of bacteria. Often the fried rice is kept warm until customers arrive in the shop to take away portions. The large numbers of *B. cereus* isolated from samples of fried rice indicate that neither the organism nor the toxin are destroyed in the quick passage through hot fat described as frying.

A few years ago Nygren (1962) suggested that the symptoms of both *Cl. welchii* and *B. cereus* were due to the liberation of phosphorylcholine from lecithin by the phospholipase C enzyme of these organisms, but Stark and Duncan (1972) and Hauschild (1973) have shown that *Cl. welchii* food poisoning is due to an enterotoxin separate and distinct from phosphorylcholine.

Similar data described by Goepfert and summarized in 1973 indicate that *B. cereus* also produces an enterotoxic substance that can be readily differentiated from lecithinase. Goepfert (1973) describes this toxic substance as 'protein in nature actively synthesized and secreted during logarithmic growth, causing fluid to accumulate in ligated ileal segments

in the rabbit and inducing increased intestinal motility and overt diarrhoea upon oral administration to rhesus monkeys'. The toxin he says is 'thermo-labile, susceptible to various proteolytic enzymes and antigenic'; approximately 80% of the *B. cereus* strains tested by him, presumably mostly from European sources, have shown evidence of producing this enterotoxic substance. The work in Goepfert's laboratory suggests that there are two antigenically distinct enterotoxins (not produced by the same strain of *B. cereus*).

Among other *Bacillus* species tested by Goepfert *et al.* (1973), *B. thuringiensis* was observed to give similar pathological symptoms in laboratory animal models. They think it is unlikely that the enterotoxin factors are related to the parasporal crystal inclusion body in the insect pathogen. The enterotoxic activity of a culture filtrate of *B. thuringiensis* was neutralized by antiserum produced against a culture filtrate of *B. cereus*.

The work on *B. cereus* is in its early days. Explanation is needed for the differences in incubation time and clinical symptoms between outbreaks in Eastern Europe and other places and outbreaks recently associated with cooked rice dishes in the United Kingdom (Public Health Laboratory Service, 1972, 1973), Australia (J. Taplin, personal communication), and Canada (Lefebvre *et al.*, 1973). Furthermore, volunteer experiments in man are inevitable as final proof of the role of the organism in food poisoning and to fulfil Koch's postulates. The isolation of the

organism from faecal samples of patients is somewhat irregular and may depend on the freshness of the stool sample.

Work to be published by Gilbert, Stringer and Peace (in press) indicates an optimum temperature for growth of *B. cereus* between 30 and 37°C. The maximum temperature for growth was thought to be between 43 and 48°C, and the minimum temperature was found to fall between 10 and 15°C.

The heat resistance of *B. cereus* spores during the process of boiling is of practical concern in survival of the organism during cooking. Studies carried out in 1972 (Gilbert, Stringer and Peace, personal communication) suggest that whereas 30% of the spores of one strain of *B. cereus* were capable of surviving at 95°C in aqueous suspension after 20–25 min, only 0.0001% of the spores of another strain were able to germinate under the same conditions; there was a similar pattern of survival in rice allowed to stand after boiling. The growth rate of the two strains tested was also different. There was a rapid fall in counts after frying but the subsequent increase in numbers after storage at room temperature was further evidence of the marked heat resistance and survival properties of the *B. cereus* spores. Spores of both strains of *B. cereus* tested showed a wide range of heat resistance and when germinated large numbers of spores rapidly appeared in the vegetative cells. In a similar way to *Cl. welchii* the significance of *B. cereus* in cooked foods depends on the numbers present. Colony counts below 10⁶/g are

TABLE 1. Characteristics in relation to food poisoning

	<i>Clostridium welchii</i>	<i>Bacillus cereus</i>
Prevalence	Common Food, faeces, soil, dust	Common Cereals, soil, dust
Growth	Anaerobic growth in meat and poultry	Aerobic growth in rice and other cereals
Generation time	10–12 min	27 min
Optimum temperature	43–47°C	30°C
Minimum	15–20°C	10–12°C
Maximum	50°C	48°C
Sporulation	Poor in food Good in intestine	Good in food
Heat resistance D ₁₀₀	Less than 1 min to 17 min	1 min to 7.5 min
Mechanism	Toxin liberated in intestine	Toxin produced in food
Incubation	12–18 hr	2 hr (15 hr)
Symptoms	Diarrhoea and pain (vomiting)	Vomiting (diarrhoea and pain)
Dose required	Large numbers of organisms	Large numbers of organisms plus toxin
Stool	Large numbers of organisms	Variable number of organisms
Prevention	Stop growth in food	Stop growth in food

(From Hobbs, 1973.)

probably of little significance but counts above $10^6/g$ in cooked foods stored at ambient temperatures may indicate that food poisoning could occur.

The foods responsible for outbreaks both of *Cl. welchii* and *B. cereus* food poisoning are invariably those which have been prepared ahead of requirements. Their bulk and the general lack of proper facilities for cooling and refrigeration enable and encourage the organisms to grow. Sutton, Kendall and Hobbs (1972) demonstrated that spores of *Cl. welchii* survived in large and small portions of beef cooked in conventional and moist air Rapidaire ovens, whereas vegetative cells were destroyed. When the cooked meats were cooled at room temperature growth occurred, and some multiplication took place in the centre of large roasts even when they were cooled under refrigeration. Dehydrated foods frequently contain bacterial spores which may be added to foods at a late stage of cooking, for example, spices and pepper in curries and other so-called hot foods. Thus even small numbers of *Cl. welchii* spores in dehydrated foods may be a hazard when these foods are used as ingredients.

Food hygiene education should stress the facts that raw foods are frequently contaminated with food poisoning organisms before they even reach the kitchen, that bacterial spores are often present and that they can survive boiling and other methods of cooking with the exception of steam under pressure. The outgrowth of *Cl. welchii* spores occurs in the absence of air inside rolled joints, poultry carcasses, pastry casings and at the bottom of stews; *B. cereus* spores will grow out under aerobic conditions in masses of food such as rice, cooked and left wet for some hours at ambient temperature. Long, slow cooling and non-refrigerated storage provide ideal conditions for bacterial multiplication. A better understanding of these facts, provision by architects of facilities for rapid cooling of foods and sufficient cold storage for large bulks of food would help to prevent both *Cl. welchii* and *B. cereus* food poisoning. Table 1 is quoted from Hobbs (1973) and shows the relevant characteristics of the organisms *Cl. welchii* and *B. cereus*.

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