CLOSTRIDIUM WELCHII FOOD POISONING

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PART I. EPIDEMIOLOGY

(1) *Introduction*

Though it is now well established that acute gastro-enteritis following some hours after a meal is usually due to the ingestion of food contaminated with certain bacteria, it is not always easy to isolate the infecting organism. Even if those incidents are excluded in which adequate bacteriological examination is impossible, many sporadic cases and outbreaks fail to yield organisms recognized as causes of food poisoning. There may be no organisms of the salmonella group, staphylococci, paracolon bacilli, *Proteus vulgaris* or *morgani*, and no large numbers of α-haemolytic streptococci (Moore, 1948*a*), enterococci (Buchbinder, Osler & Steffen,1948) or aerobic spore-bearing bacilli (e.g. *B. cereus*, Hauge, 1950), all of which have been proved or suspected to be causes of food poisoning. Indeed, an analysis of the

https://doi.org/10.1017/S0022172400015515 Published online by Cambridge University Press

2431 outbreaks of food poisoning recorded for 1949 shows that in no less than 36 % no adequate cause was found.

In this paper evidence is brought forward that a large proportion of food poisoning incidents hitherto unclassified may be due to heat-resistant *Clostridium welchii*. It is suggested that the occurrence of this type of food poisoning is linked with changes in the eating habits of the community, and methods for the prevention of such food poisoning are considered.

(2) *Previous records of food poisoning due to* Clostridia

Little attention was paid by the earlier workers to the possibility of food poisoning due to anaerobic bacteria other than *Cl. botulinum*. *Cl. welchii* is ubiquitous; Type A strains are frequently isolated from normal stools and from a wide variety of foodstuffs. It does not spore readily, and Headlee (1931) reported that its spores were killed in 30 min. at 90° C. and 5 min. or less at 100° C. These facts have weighed heavily against serious consideration of *Cl. welchii* as a cause of food poisoning. In some instances, however, failure to isolate organisms other than *Cl. welchii* in significant numbers has led authors to incriminate it. Klein (1895) isolated *Cl. welchii* from the stools of patients affected in two epidemics of diarrhoea involving 59 and 144 cases in a hospital; abdominal pain was a constant symptom, vomiting relatively uncommon; all patients recovered within 12 hr. Andrewes (1899) isolated *Cl. welchii* from the stools of patients suffering from mild but chronic diarrhoea in the same hospital. The investigations made in these two outbreaks were, however, not sufficient to establish with certainty the causative role of the organism.

Attention was again drawn to the possibility that anaerobic spore-bearing bacteria might be responsible for food poisoning in a report by Knox & MacDonald (1943), who described illnesses among school children after meals prepared at a central kitchen. Gravy made on the previous day was found on several occasions to be heavily contaminated with anaerobic spore-bearing bacilli. Duncan (1944) also described outbreaks of diarrhoea in residents of a wartime camp after the consumption of meat and potato pie containing large numbers of *Cl. bifermentans*.

McClung (1945) reported a series of four outbreaks of food poisoning from chicken dishes cooked the day before they were served. The symptoms appeared 8–12 hr. after the meal and included nausea, intestinal cramps and profuse diarrhoea lasting for about 12 hr.; vomiting was rare. The chickens had been steamed at low pressure for 3 hr.; they were then removed from the broth, which was saved for further use. Direct microscopic examination of all samples of broth showed large numbers of Gram-positive rods, proved by culture to be *Cl. welchii*. Attempts to demonstrate enterotoxin production in mice and guinea-pigs were unsuccessful; one human volunteer showed typical symptoms after eating a sample known to be contaminated with *Cl. welchii*.

From 1947 to 1949 a large number of cases of haemorrhagic enteritis (enteritis necroticans, 'Darmbrand') occurred in north Germany—the patients, after eating tinned meat, rabbit or fish paste, developed some hours later, severe lower abdominal pain and diarrhoea. In the worst cases the stools contained blood and

sloughed mucosa, and the patients died from dehydration and circulatory failure or, where the oedema of the intestinal mucosa was sufficiently severe, from intestinal obstruction. *Cl. welchii* was present in enormous numbers in the stools and in the lesions; rough colonies were numerous in culture. These strains of *Cl. welchii* were markedly heat-resistant, surviving 100° C. for 1–4 hr. (Zeissler & Rassfeld-Sternberg, 1949). Several strains examined by one of us (Oakley, 1949) produced *Cl. welchii* β-toxin. Though in the latter respect they resembled *Cl. welchii* Type C, Oakley, for various reasons, regarded them as more closely related to Type B; in view of their striking heat-resistance compared with these types, however, it is convenient for the present to call them Type F. Hain (1949) examined 108 stools from persons not suffering from enteritis necroticans and showed that about one-sixth of them contained heat-resistant *Cl. welchii*. He believed that the stools were a fair sample of those of the population from which they were obtained, and deduced that one-sixth of the inhabitants of Hamburg during the winter of 1947–8 must have carried this organism. It was not certain, however, that the strains isolated from normal persons were the same in all respects as those from cases of enteritis necroticans. They were heat-resistant, but far less pathogenic for animals.

(3) *The outbreaks*

(*a*) *General observations*

Our own observations on food poisoning suspected to be due to *Cl. welchii* were begun in June 1946, when one of the authors ate for lunch a portion of home-made pork pie prepared a few days before and kept in the larder. Abdominal pain and diarrhoea occurred during the night. An almost pure culture of *Cl. welchii* was isolated from the few remaining scraps of pie. In June 1947 an outbreak of gastro-enteritis in a factory canteen was investigated. The illnesses were short and, although milder than those usually seen with staphylococcal food poisoning, resembled the bacterial toxin type. Soon afterwards a number of similar outbreaks were studied, and it became possible to recognize a clinical and epidemiological pattern, which may be briefly described.

Clinical. After an incubation period of 8–22 hr., patients develop acute abdominal pain and diarrhoea; nausea and vomiting are rare. Pyrexia, shivering, headache and other signs of infection are seldom present. The illness is of short duration—one day or less, except in elderly debilitated patients amongst whom it is suspected that fatal cases may have occurred.

Medium of infection. This is almost invariably a cold or warmed-up meat dish made from meat, boiled, braised, steamed or stewed for 2–3 hr. on the day before it is required, and allowed to cool slowly overnight. Roast meat is seldom incriminated. Those who eat the contaminated meat dishes say that they are appetizing and make no complaint about their smell or taste. When received for examination, the meat varies in appearance and smell; some samples appear normal, others smell sour and show signs of gas formation.

Causal organism. Direct smears of suspected meats show Gram-positive bacilli resembling *Cl. welchii*. Sometimes they are present in small numbers only, but if other organisms are not numerous, this finding is considered suspicious. Direct

anaerobic cultures on blood agar usually show a profuse growth of non-haemolytic *Cl. welchii* of typical colonial appearance; aerobic plates often show scanty growth of saprophytic organisms only. If *Cl. welchii* is isolated only from enrichment and not from direct cultures some doubt is cast on the diagnosis of *Cl. welchii* food poisoning.

Heat-resistant *Cl. welchii* with colonial appearance and serological reactions similar to those of the strains obtained from food are isolated from a high proportion of faeces from patients and others at risk. In control groups of persons not at risk the proportion of faeces yielding heat-resistant *Cl. welchii* is low.

(b) A typical outbreak

In February 1950, mild food poisoning occurred in a school canteen, affecting about 275 of 475 children, 18 of 41 school staff and 10 of 11 canteen staff. The shortest incubation period recorded was 6 hr., and the longest 22 hr. Most of the patients complained of sudden diarrhoea and abdominal pain; some felt nausea, but few vomited; no one complained of headache; pyrexia was not recorded. Recovery was rapid and most of the children were back at school next day; no secondary cases occurred. Boiled salt beef eaten cold was thought to be responsible. The meat (Argentine), delivered in small joints each weighing 4–5 lb., was kept in the larder till midday, when it was placed in lukewarm water, which reached boiling-point in half an hour, and boiled for 3 hr. The joints were then removed from the boiler, put on enamel trays, covered with a tea-cloth and placed in the larder overnight; next day they were sliced and served with freshly made gravy. Two other schools had been supplied with the same batch of salt beef; at one the beef was eaten freshly cooked on the day of delivery without ill effect; no information was available from the other school.

After an earlier outbreak at the same school, the kitchen staff had been instructed to keep small samples of each prepared foodstuff; a small specimen meal was therefore available for bacteriological examination. A direct microscopic smear of the salt beef showed occasional Gram-positive bacilli. A Yeastrel milk-agar pour plate incubated aerobically gave a colony count of about 1500 per gram.; a surface count on blood agar by the Miles and Misra technique showed anaerobically 130,000 colonies per gram. Direct aerobic cultures were almost sterile, whereas direct blood agar anaerobic cultures showed a pure growth of *Cl. welchii*. Small numbers of this organism were also isolated from enrichment cultures of a specimen of haricot beans in contact with the meat on the plate. The brine used to pickle the meat yielded a strain of *Cl. welchii* closely resembling that from the meat. The results of the bacteriological examination of the persons concerned are shown in Table 1. The details of the investigation are given in Part II. Nineteen samples of faeces received from patients connected with the outbreak all contained heat-resistant *Cl. welchii*. Of ten repeat samples taken after 13 days, six were still positive, of four taken after 26 days one was positive, of six further repeat samples taken after 38 days one was positive. The strains isolated from meat and faeces resembled each other closely in their morphology, cultural and serological characters and in their toxin production. Positive stools were also obtained from three persons who

had eaten the food without showing any symptoms (at risk, not ill). Five samples of faeces from school children who had not eaten the food were negative for *Cl. welchii*, and five samples from children from another school were also negative (persons not at risk).

Table 1. *Results of examination for heat-resistant* Clostridium welchii *of samples of food and faeces connected with school canteen outbreak of food poisoning*

Description of sample	No. of samples examined	No. positive for *Cl. welchii*	Properties of *Cl. welchii*: Provisional serological type*	Soluble antigens: α	$\beta, \delta, \epsilon, \theta$ and ι	κ	λ	μ	ν
Cold boiled beef	1	1	2	tr.	—	—	—	+ + + +	—
Haricot beans	1	1	2	.	.	.	.	.	.
Brine	1	1†	.	.	.	.	.	.	.
Faeces:									
Patients 1–3 days	19	19	2	tr. or —	—	— or tr.	—	+ or + + +	tr. or —
Repeat samples 13 days	10	6	2	tr.	—	—	—	+ + +	tr. or —
Repeat samples 26 days	4	1	2	tr.	—	—	—	+ +	+
Repeat samples 38 days	6	1	.	.	.	.	.	.	.
Persons at risk not ill	3	3	2	tr.	—	—	—	+ or + + +	tr. or —
Persons not at risk	10	—	.	.	.	.	.	.	.

* Tube agglutination to titre. † Strain died. tr. = trace.

In October 1948 an outbreak with the same characters had occurred at this school, due to salt beef that had been allowed to cool overnight in the liquid in which it had been cooked. At the same time joints from the same pickling tank, refrigerated after cooking, were eaten at two other schools without ill effect. This outbreak was doubtfully ascribed to *Proteus vulgaris*, though the strain isolated failed to affect two volunteers.

(c) *Survey of other outbreaks*

From September 1949 to February 1952 twenty-three outbreaks of food poisoning in the London area were investigated, in which the suspected agent was heat-resistant *Cl. welchii*. The medium was usually pre-cooked meat and the history of its preparation followed a uniform pattern. The incubation periods, symptoms, duration of illness, and bacteriology resembled those already described. Table 2 gives a list of some of these outbreaks, including a brief description of the meat dish and where possible the provisional serological type of the *Cl. welchii* strains isolated from food and faeces.

Attention is drawn to special features of some of these outbreaks. In outbreak 2, in a school canteen, the high proportion of victims among the persons at risk is to be noted. In outbreak 4, in a small factory, the food concerned was a pasty. Mince and vegetables, separately cooked the previous day, were mixed, covered with pastry and baked only sufficiently to cook the pastry. A count of 45 million *Cl. welchii* per gram was obtained from the remains of the meat and vegetable filling. The aerobic bacterial count was approximately 2 million per gram, the organisms being mainly coliform bacilli. In outbreaks 1, 2, 3 and 15 no faeces were received, but the epidemiological picture and the bacteriology of the food left little doubt that *Cl. welchii* was the causative agent. In outbreaks 7, 8, 11 and 13 no food was received, but the epidemiological picture, and the high percentage of

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Table 2. *Outbreaks of suspected* Clostridium welchii *food poisoning occurring in the London Area, September* 1949 *to January* 1952, *showing meat dishes responsible and some results of serological typing of strains from food and faeces*

					Food		Faeces			
							Patients			
Place of outbreak	Year	No. at risk	No. affected	Nature of sample	Heat-resistant *Cl. welchii*	Provisional serological type	No. examined	No. positive for *Cl. welchii*	Provisional serological type	Controls (not at risk)
1. Army camp	1949	80	70	Reheated lamb, boiled previous day	+	3	.	.	.	.
2. School canteen	1949	360	316	Reheated steak, stewed previous day	+	1	.	.	.	.
3. Factory canteen	1949	500	30	Lamb's tongues, boiled 3 hr. before	+	1	.	.	.	.
4. Factory canteen	1949	70	40	Beef and vegetable pasty. Beef received, minced and boiled previous day	+	3	11	10	.	.
5. Factory canteen	1949	20	20	Reheated beef, stewed previous day	+	Not 1–6	1	1	.	.
6. School canteen	1950	527	303	Cold salt beef, boiled previous day	+	2	19	19	2 (18), 3 (1)	10 all negative
7. School canteen	1950	400	52	Presumed stock from meat cooked previous day. Not received	.	.	10	8	3	3 all negative
8. Municipal canteen	1950	145	45	Steak pie meat stewed previous day. Not received	.	.	3	3	Not 1–6	4 all negative
9. Factory canteen	1950	128	100	Cold salt beef, boiled previous day	+	1	6	6	1	.
10. Municipal canteen	1950	76	26	Mutton pie, meat boiled previous day	+	.	1	1	.	.
11. School canteen	1950	35	22	Reheated beef, steamed previous day. Not received	.	.	7	6	.	8 all negative
12. School canteen	1950	300	177	Cold salt beef, boiled previous day	+	3	8	8	3	.
13. Municipal canteen	1951	145	32	Steak pudding, stewed previous day. Not received	.	.	20	18	Not 1–6 (12) 5 (6)	20 all negative except 1 (not 1–6)
14. R.A.F. canteen	1951	140	35	Cold salt beef, steamed three days before	+	3	4	4	Not 1–6 (1) 3 (3)	.
15. R.A.F. canteen	1951	94	90	Reheated steak, stewed previous day	+	8	.	.	.	.
16. Family	1951	4	4	Reheated beef, braised previous day	+	3	2	2	3	.
17. Municipal canteen	1951	100	30	Meat pudding, mince stewed previous day. Not received	.	.	12	11	6	.
18. School canteen	1952	200	50	Beef and pork rissole, meat roasted two days before	+	Not 1–6	3	3	Not 1–6	.

. = Not received or not examined.

positive faeces from patients when compared with control groups not at risk led to a fairly confident diagnosis of *Cl. welchii* food poisoning. Table 2 shows a comparison of the results obtained from the faeces of patients with those of small control groups of persons not at risk because they had not eaten the contaminated food. In school outbreak no. 6, when all of nineteen specimens of faeces from patients were positive, all ten control faeces (five from children in a neighbouring school) were negative. In school outbreak no. 7, where eight of ten faeces from patients were positive, three controls were negative. In school outbreak no. 11, of seven specimens of faeces from patients six were positive; all eight controls were negative. These outbreaks, 6, 7 and 11 with two others (8 and 13), yielded fifty-nine samples from individual patients, and of these fifty-four (91·6 %) were positive; whereas from forty-five controls only one positive sample was obtained (2·2 %).

During the investigation of various outbreaks, out of 129 samples of faeces from patients, 116 (89·9 %) were positive for heat-resistant *Cl. welchii*; of 385 samples from persons not at risk only twenty (5·2 %) were positive (see Table 3). Of thirty samples of faeces from persons who had eaten food contaminated with *Cl. welchii* but who had not been ill sixteen (53·3 %) were positive.

Table 3. *Heat-resistant* Clostridium welchii *from stool samples of various groups of persons*

Group	Description	No. examined	Positive	
			No.	%
1	Normal people	45	1	2·2
2	Miscellaneous cases of diarrhoea	142	3	2·1
3	Dysentery (Sonne outbreak)	80	3	3·8
4	Food poisoning other than *Cl. welchii*	65	5	7·7
5	Old people in hospital (healthy and with diarrhoea)	53	8	15·1
	Total	385	20	5·2
6	*Cl. welchii* food poisoning	129	116	89·9

It is clear that heat-resistant strains of *Cl. welchii* are found much more frequently in the faeces of persons who have eaten the contaminated food, specially if they have symptoms, than in persons not at risk. The bacteriology of the organisms is discussed fully in Part II in which supporting evidence for the causative role of the organism is produced, including the results of volunteer experiments. Of three volunteers who swallowed cooked meat cultures of strains isolated from incriminated food, one had a short acute illness resembling that described by victims of the outbreaks, another had a similar mild illness and the third had trivial symptoms that almost escaped notice.

Towards the end of 1951 there were two outbreaks, the general characters of which were similar to those of *Cl. welchii* food poisoning. The predominant organism isolated from food and faeces was, however, a strain of *Cl. welchii* producing β-haemolysis on blood agar. This was a characteristic Type A strain; it resisted boiling for 5 but not 15 min., and failed to agglutinate with sera against provisional Types 1–6 of heat-resistant *Cl. welchii* (see Part II).

In one outbreak cold roast beef cooked the day before it was eaten gave an

anaerobic surface colony count of 4·5 million β-haemolytic *Cl. welchii* per gram; the aerobic colony count was 190,000 per gram. A similar strain of *Cl. welchii* was isolated in large numbers by direct blood agar culture from all of eleven patients' faeces. Similar cultures from fifteen persons not at risk yielded four strains of β-haemolytic *Cl. welchii*. In the second outbreak braised beef cooked 3 days before it was used gave an anaerobic surface colony count of 18 million β-haemolytic *Cl. welchii* per gram and an aerobic colony count of 35,000 per gram. A similar strain of *Cl. welchii* was obtained from all of eight faecal samples from patients. As the strains were only feebly heat-resistant—in no instance were the organisms isolated from faeces steamed for 1 hr.—it is thought likely that the meat was contaminated after cooking. Food and faeces from both outbreaks failed to yield any of the usual food-poisoning organisms.

(4) *The source of infection*

The reservoirs of heat-resistant *Cl. welchii* are not yet fully known. Meat may be contaminated before cooking from at least two different sources: (1) human, from the hands of cooks, or butchers and others handling carcases in the slaughter-house, the meat market and the retail shop; (2) animal, from the invasion of muscles by intestinal organisms during life and after death, from faecal matter on the floor of the slaughter-house, or from rats, mice or other animals. When opportunities arose, therefore, faeces were examined from groups of persons and from various animals. A few batches of flies were also examined.

Human. The carrier rate of heat-resistant *Cl. welchii* amongst the normal population, and amongst those who are suffering from diarrhoeal diseases not associated with *Cl. welchii* appears to vary slightly with different groups.

Table 3 gives a summary of the results obtained from the examination for heat-resistant *Cl. welchii* of stool samples from persons presumed not at risk from *Cl. welchii* food poisoning.

The first group of samples was collected from forty-five healthy children and adults not at risk in schools and factories where outbreaks of suspected *Cl. welchii* food poisoning had occurred; 2·2% of samples were positive.

The 142 samples of faeces in the second group, of which 2·1% were positive, came from a public health routine laboratory. They had been submitted for investigation from cases of diarrhoea diagnosed as dysentery, 'pyrexia of unknown origin', or one of the enteric diseases, but not suspected food poisoning. The eighty samples in the third group, showing 3·8% of positives, were all from cases of Sonne dysentery. The fourth group of sixty-five samples, in which 7·7% were positive, were related to persons suffering from food poisoning not thought to be due to *Cl. welchii*. In view of the higher percentage of positives in this group compared with the other three groups of persons not at risk it seems likely that a few of the cases of food poisoning may have in fact been caused by *Cl. welchii*.

After an outbreak of suspected *Cl. welchii* food poisoning among old people in a hospital the fifth group of faeces were examined from fifty-three old people in neighbouring hospitals said to be free from food poisoning. From one hospital two of fourteen samples from healthy persons and from the other three of twenty-one

samples from healthy persons and three of eighteen samples from those with diarrhoea were found to be positive. Therefore eight out of fifty-three (15·1 %) samples of faeces in this group were positive. This figure, higher than that of the other groups, may indicate that heat-resistant *Cl. welchii* is particularly prevalent in elderly persons in hospital, specially those with diarrhoea. It was not possible to say whether the presence of the organism was related to the onset of diarrhoea.

The excretion of *Cl. welchii* usually stops 1–2 weeks after the contaminated food has been eaten; it is known, however, that the organism may persist for considerably longer periods. In one instance a cook produced positive stools for some months. Further investigations are needed along these lines.

Sewage. Sewage swabs, as designed by Moore (1948*b*), each sampling the sewage of seventy houses, were examined for *Cl. welchii*. These swabs, taken primarily as part of another investigation, were obtained from various parts of the country. The results are shown in Table 4.

Table 4. *Results of examination for heat-resistant* Clostridium welchii *of batches of sewage swabs*

		Sewage swabs			
			Positive for *Cl. welchii*		Provisional serological type
Batch	Origin	No. examined	No.	%	
1	Colne; Wilts	15	6	40·0	Not 1–6
2	Wallingford, Berks	29	18	62·1	3–5 (5) Not 1–6 (13)
3	Wantage, Berks	29	15	51·7	2 (1) 2 and 3 (1) 3 (1) Not 1–6 (12)
4	Bodmin, Cornwall	13	10	76·9	1 (2) 6 (4) Not 1–6 (4)
5	Marlow, Berks and Eastern, Berks	15	7	46·6	Not 1–6
6	Beccles, Suffolk	10	0	0	—
7	Hoole, Cheshire	6	6	100·0	6 (1) Not 1–6 (5)
8	Prudhoe, Northumberland	8	8	100·0	3 (1) 6 (2) Not 1–6 (5)
	Total	125	70	56·0	

Figures in brackets indicate number of strains.

Animals. The carrier rate among live animals, including rats and mice, and in live and recently slaughtered cattle was investigated. The results are given in Table 5.

Cattle faeces were obtained from a farm and two slaughter-houses. Of the seventy-six samples of pig faeces, fifty-two were obtained from the feeding passages serving pens under conditions in which it was not possible to be sure that contamination had not spread from pen to pen. Of twelve samples taken from slaughter-house pens four were positive and of twelve samples from individual pigs none was positive.

https://doi.org/10.1017/S0022172400015515 Published online by Cambridge University Press

The rat faeces were collected by local authority officers during the process of extermination.

Table 5. *Heat-resistant* Clostridium welchii *from animals*

Samples	No. examined	Positive No.	Positive %
Faeces			
Pigs	76	14	18·4
Rats and mice	41	6	14·6
Cattle	113	2	1·7
Raw meat			
Pork	55	11	20·0
Beef	54	13	24·1
Veal	7	1	14·3
Lamb	17	0	0

To investigate the occurrence of heat-resistant *Cl. welchii* in raw meat, small samples were collected from various sources. After direct anaerobic cultures were made on blood agar, the portions of meat were shaken with broth in screw-capped jars and steamed for 1 hr. The lids were tightened and the jars cooled and incubated aerobically overnight; next day these meat broths were plated out aerobically and anaerobically. The results were grouped according to the origin of the sample and they are shown in Table 5. Some of the groups were small, unfortunately, because it was difficult to obtain rationed meat.

It will be seen that the highest percentage of positives was found in samples of beef and pork. The fourteen samples of lamb were all negative.

Flies. Heat-resistant *Cl. welchii* was isolated from batches of blowflies collected from different sources, a hospital, a butcher's shop, a fried-fish shop, a slaughter-house and a refuse sorting depot. The majority of flies were of the genus *Lucilia* (greenbottles) but some *Calliphora* and *Phormia* (bluebottles) were also included; they were removed from the traps some hours before examination.

Two collections were made from each site, the flies being attracted to the traps by liver bait. In the first collection each batch consisted of several hundred flies some of which had touched the liver bait which might possibly have been the source of infection. The second collection was therefore made using sterilized traps in such a way that the flies could not come in contact with the bait. Only small catches were obtained, ranging from two to fifteen flies, but, nevertheless, *Cl. welchii* was isolated from every batch. None was isolated from a sample of liver bait and it seems reasonable to assume that the organism was carried by the flies in the wild state.

The strains of *Cl. welchii* were isolated by treating a suspension of ground flies in Ringer solution by the method used for faeces. Some strains failed to agglutinate with the serological typing sera 1–8; others appeared to belong to the provisional types 3 and 7.

It seems likely that the blowfly may play an important role in the spread of *Cl. welchii* especially as it breeds and feeds on meat and other animal products.

(5) *Comment*

The outbreaks of food poisoning described above were almost all caused by meat that had been cooked and allowed to cool slowly overnight, conditions very favourable for the multiplication of surviving anaerobes. From 8 to 20 hr. after the food was eaten, symptoms appeared and a large proportion of the persons at risk were usually affected. The same mild brief type of illness, characterized by colic and diarrhoea without vomiting, was observed in all the outbreaks. Heat-resistant strains of *Cl. welchii* predominated in the food and were frequently present in large numbers. An organism with the same characters was isolated from the faeces of about 90% of patients and other persons at risk and was found very much less frequently (average 5·2%) in the faeces of normal persons or persons suffering from diarrhoeal illnesses of different origin. Salmonella organisms were not isolated and the other recognized bacterial causes of food poisoning were virtually excluded. Heat-resistant *Cl. welchii* occurs in the faeces of pigs and cattle, and of rats and mice; it was also found in various market samples of pork, beef and veal, and in flies. These are possible sources of infection, and it is clear that the methods of preparation of the foods afforded ample opportunity for bacterial multiplication, once contamination had occurred. The epidemiological evidence is thus compatible with the view that these outbreaks of food poisoning were caused by heat-resistant *Cl. welchii*.

PART II BACTERIOLOGY

(1) *General characters of the heat-resistant strains of* Clostridium welchii

(*a*) *Morphology*

The morphology of the organism in direct smears, apart from slight variations in size, is typical of *Cl. welchii* as described in any text-book. Spores have rarely been seen in smears from foodstuffs, although structures resembling spores are seen occasionally in direct smears from colonies. Further work on the sporulation of *Cl. welchii* will be described later.

(*b*) *Colonial appearance*

The colonies on blood agar differ in appearance and haemolysis from those usually given by *Cl. welchii* Type A. On primary isolation they are non-haemolytic; after a few days a faint haemolysis may develop. The colonies are approximately 2–5 mm. in diameter after 18 hr. They are usually smooth with an entire edge, but they may be opaque and slightly raised or umbonate in the centre with a flat translucent periphery showing radial striations. On first isolation they may be small, rough, and flat with an irregular edge resembling a vine leaf.

The organisms isolated from the suspected foodstuffs in the first outbreak to be investigated were all of the rough type but these have rarely been seen since. It is possible that some difference in the blood agar medium used at that time was responsible for the rough colonies. Zeissler & Rassfeld-Sternberg (1949) described the appearance of rough colonies of *Cl. welchii* Type F and noted how they differed from the normal smooth colonies of Type A strains. A further variation in the colonial appearance will be described later in the section dealing with induced sporulation.

(*c*) *Biochemical reactions*

The fermentation reactions of these strains are similar to those of any other type of *Cl. welchii*. Acid and gas are produced from glucose, maltose, lactose and saccharose but not from mannitol or salicin. Acid and clot are usually formed in litmus milk. Of many hundreds of strains examined all but two gave a positive Nagler reaction inhibited by Type A antitoxin.

(*d*) *Heat-resistance and sporulation*

It was known that some of the foodstuffs examined from suspected outbreaks and from which we isolated heat-resistant *Cl. welchii* had been boiled for 2–3 hr. or even longer before being left overnight in the kitchen or larder. If it is assumed that the organism was already present on the meat before it was cooked, then its development after cooking must depend on the survival of spores.

The powers of resistance to heat of *Cl. welchii* vary from 1 min. at 98° C. (Dunham, 1897), 1½ hr. at 100° C. (Rodella, 1910; von Hibler, 1906), to 1–4 hr. at 100° C. (Zeissler & Rassfeld-Sternberg, 1949).

One of the strains isolated during the present work was grown in a large volume of cooked meat for 48 hr. at 37° C., then gently boiled to imitate the stewing of meat in a canteen. It survived this treatment for 5 hr. but not for 6 hr. In another experiment cultures sealed in ampoules survived 4 hr. but not 5 hr. submersion in a boiling-water bath. An overnight cooked meat culture of another strain survived steaming for 1 hr. but not autoclaving at 10 lb. (117° C.) for 10 min.

It was observed that some strains retained their heat-resistance through several subcultures and storage in the refrigerator for many months. With others it was impossible to demonstrate heat-resistance even after the first subculture in cooked meat medium.

It has been noted that heat-resistant *Cl. welchii* could be readily isolated from samples of faeces which had been steamed for 1 hr., whereas it was rarely possible to isolate them from foodstuffs by the same method.

Wild (1898) observed that spores in stools were more resistant to heat than those in cultures. Simonds (1915) observed that the sporulation of *Cl. welchii* in suspensions of faeces had an important bearing on the relationship of this organism to intestinal disturbances. He commented on the variation in the readiness with which different strains of *Cl. welchii* formed spores, and suggested that most important among the numerous factors that influenced sporulation were the reaction of the medium in the presence or absence of fermentable carbohydrate and the temperature. He observed that sporulation occurred promptly and abundantly in pure cultures and in sterilized alkaline and neutral, but not acid, suspensions of faeces. All the evidence suggests that heat-resistance is dependent on the formation of spores.

In order to find out the approximate proportion of spores to vegetative forms and the effect of heat on meat broth cultures of *Cl. welchii*, strain 2985 (Lab. no.), incubated for 5, 24 and 48 hr. at 37° C., the supernatant fluid was divided into several parts. Each part was heated for 10 min. at various temperatures ranging from 50 to 100° C. Counts were made on an unheated control and on each heated

sample immediately after removal from the water bath. The results are shown in Table 6; they indicated that in young cultures of 5 hr. the vegetative cells were killed at 50–60° C. and that no spores were formed. In 24 and 48 hr. cultures the vegetative cells were destroyed at 60–70° C. Of three 24 hr. cultures two showed approximate proportions of spores to vegetative forms of 1 to 37,000 and 1 to 8800, while in the third culture no spores were demonstrated. The ability of *Cl. welchii*, strain 2985, to produce spores in cooked meat medium after 24 hr.

Table 6. *Effect of heat on* 5, 24 *and* 48 *hr. cultures of* Clostridium welchii *and the approximate proportion of spores to vegetative forms in these cultures*

Age of culture (hr.)	Surface plate count (anaerobic) per ml. of culture							Approximate proportion of spores to vegetative forms
	Unheated control	After heating for 10 min. at various temperatures (° C.)						
		50	60	70	80	90	100	
5	$2{\cdot}2 \times 10^6$	7×10^6	< 500	< 500	< 500	< 500	< 500	Nil
24 (1)	40×10^6	22×10^6	8,500	< 500	< 500	< 500	< 500	Nil
24 (2)	130×10^6	—	$3{\cdot}8 \times 10^6$	—	—	—	3,500	1/37,000
24 (3)	105×10^6	—	—	—	—	—	12,000	1/8,800
48 (1)	70×10^6	5×10^6	—	25,000	15,000	38,000	110,000	1/640
48 (2)	68×10^6	—	46,000	—	—	—	15,000	1/4,500

appears to be variable. In the two 48 hr. cultures, spores were present in the proportions of 1 to 640 and 1 to 4500 vegetative forms.

These few experiments suggested the reason for variation in the survival of heat-resistant *Cl. welchii*. They also demonstrated the presence of spores in cultures which appeared to be free from spores when stained films were examined.

Various methods were used in an attempt to increase the formation of spores, mostly without success. No spores could be seen in films from cultures in cooked meat medium with 0·005 % manganese sulphate added or in Brewer's thioglycollate medium. A slide cell technique using a sloppy nutrient agar containing 0·1 % thioglycollate and also plain nutrient agar gave promising but unreproducible results. The medium was melted, allowed to cool, and inoculated with a 48 hr. culture of heat-resistant *Cl. welchii*; a small amount was pipetted on to a sterile slide and covered with a sterile cover-slip, and the culture incubated aerobically in a Petri dish. On two occasions, at least, numerous spores were demonstrated which presented an unforgettable picture under a phase-contrast microscope. Many of the spores appeared unusually large and some were contained in grotesque and swollen vegetative cells. A very high proportion of the bacterial cells were arranged in palisades and contained large oval subterminal or almost central spores.

The only method which has so far consistently encouraged the sporulation of this organism was evolved from the following procedure. 100 ml. of a 48 hr. nutrient broth culture of *Cl. welchii*, strain 2985, were centrifuged and the deposit was washed twice and resuspended in distilled water. This suspension was left on the bench for 6 weeks; during this time no spores were seen in stained smears made at weekly intervals. A few ml. of the 6-week suspension were incubated in cooked meat medium and failed to grow in 24 hr.; 0·1 % of sterile starch solution was added and the culture re-incubated. After 5 hr. the formation of gas was noticed, and a stained smear of the culture showed occasional spores. The following day,

a blood agar plate inoculated from this 5 hr. culture and incubated anaerobically showed approximately 40 % of normal colonies; the remainder were either small and 'poached egg' or 'pin-point' in appearance. Films were made from these three types of colony. Those with a typical appearance showed no spores while the others, 'poached egg' and 'pin point', consisted almost entirely of free spores and sporing bacilli; bacilli from these atypical colonies were agglutinated by the anti-serum to the parent strain. There was a great variation in the size of the central and subterminal spores; some were large, oval and thicker than the bacilli, others were small. These colonies showed involution forms, long filaments, club-shaped and short coccoid forms, and some large swollen bacilli staining feebly. After two or three subcultures through cooked meat medium or on blood agar no spores were found; the atypical colonies reverted to the normal appearance and showed non-sporing bacilli only. When the original starch cooked meat culture was incubated for 24 hr. before subculture no atypical colonies were found.

This method of obtaining spores from *Cl. welchii*, strain 2985, was repeated several times with similar results; the method has not yet been used for other strains.

The germination time of the spores varied a great deal. The addition of 0·1 % starch to the cooked meat medium speeded up the growth, which was noticeable after 5–24 hr.; without starch growth did not occur for 48 hr. Once germination had commenced the spores grew rapidly into non-sporing bacilli. The cooked meat culture was therefore examined frequently and plated out as soon as growth was observed.

In order to determine the growth rate of spores of heat-resistant *Cl. welchii* at different temperatures in a medium of cooked meat, three lots of 100 ml. of cooked meat were each inoculated with 3 ml. of a spore suspension (strain 2985) in distilled water and steamed for half an hour to destroy any vegetative forms. After cooling for half an hour the inoculated meat media were incubated one each at 37 and 22° C. and the third was placed in the refrigerator. Viable counts on blood agar incubated anaerobically were carried out after 2, 4, 6, 24 and 28 hr. With an inoculum of less than fifty spores per ml. there was a colony count of 45,000 per ml. after 6 hr., and 48 million per ml. after 24 hr. at 37° C. The count at 22°C. was 25,000 per ml. after 24 hr. In the refrigerator there was no growth in 48 hr. It may be assumed, therefore, that a few spores surviving in cooked meats will be sufficient to produce a growth of bacilli if the meat is left overnight in a warm room.

(2) *Method of isolation*

The organism is strictly anaerobic and grows rapidly in broth at 37° C.; on blood agar, colonies are well developed after 18 hr. It may be isolated from foodstuffs by direct overnight anaerobic culture on blood agar.

A profuse, almost pure, culture from foods is easily recognizable, but amongst a heavy growth of facultative anaerobic organisms the colony of non-haemolytic *Cl. welchii* is easily missed or mistaken for some other organism. Isolation is simplified if after incubation the anaerobic plate is left on the bench for 24 hr., when the aerobic organisms will develop and the anaerobes can be picked off more easily. *Cl. welchii* may also be isolated after enrichment overnight through cooked meat

medium or merely by incubation of the suspected meat in quarter-strength Ringer solution. If this method is used, however, aerobic organisms may outgrow the anaerobes, and moreover the presence of *Cl. welchii* in enrichment cultures only is not regarded as of the same significance as growth of the organisms on direct plates. An anaerobic surface colony count on blood agar carried out by means of the Miles and Misra technique will give a useful estimation of the proportion of anaerobes present in the foodstuff; if the food is heavily contaminated with aerobic organisms the value of such a count may be doubtful. Attempts to make use of heat-resistance for the isolation of the organism from foods by steaming in broth for 1 hr. followed by anaerobic incubation and subculture the next day have usually failed. This suggests that the anaerobes in pre-cooked meat may be recently germinated spores, that is, actively multiplying young organisms which have not yet formed spores. To isolate the organism from stools, however, good use may be made of its heat-resistance (Hain, 1949). A small portion of the stool, about the size of a pea, is steamed in broth for 1 hr., the heated suspension is incubated anaerobically overnight and plated on to blood agar; from positive samples an almost pure growth of the organism may be obtained. The regularity with which heat-resistant *Cl. welchii* may be isolated from stool samples in this way suggests that the organism spores readily during its residence in the intestine. Direct plating of faeces on to blood agar without preliminary heat treatment was not satisfactory for the isolation of non-haemolytic *Cl. welchii*, as its presence may be obscured by a heavy growth of coliform bacilli. For the isolation of the β-haemolytic Type A strains from faeces direct plating must be used because they do not appear to survive heat-treatment. On Wilson and Blair medium as used for the isolation of enteric pathogens two strains of *Cl. welchii* from food failed to grow, whereas they grew on the same medium adapted for the isolation of *Cl. welchii* from water. *Proteus* and some other organisms also grow on this medium; it was therefore considered to be of little value for the direct culture of *Cl. welchii* from faeces.

A comparison of the isolation of heat-resistant *Cl. welchii* from stools and from rectal swabs has shown that stool samples give a higher percentage of positive results. In one hospital outbreak of food poisoning among elderly persons 75 % of twenty faeces samples were positive, but only 49 % of sixty-one comparable rectal swabs.

(3) *Toxicology*

Toxin production. Organisms obtained in pure culture were grown for 5 hr. in 1 % glucose broth previously steamed for $\frac{1}{2}$ hr. and cooled; comparison with other media showed that the one used was adequate for estimation of toxigenicity. The cultures were then filtered through Seitz-sterilizing pads and the filtrates examined for toxins as soon as possible by a method published elsewhere (Oakley & Warrack, 1953).

Results. No new toxins were detected. In general the toxigenic power of food-poisoning strains of *Cl. welchii* appeared to be slight. Traces of α-toxins were almost invariably produced, but the concentration in filtrates never exceeded the equivalent of one α-unit, and was usually obviously far less. Almost all strains examined produced some deoxyribonuclease, as do almost all strains of *Cl. welchii* of all types

so far examined. No β-, δ-, ϵ-, or ι-toxin was detected in any filtrates, nor did any heat-resistant strains produce θ-toxin, even though the medium specially favoured its production; other types of *Cl. welchii* including strains that were not heat-resistant readily produced θ-toxin in this medium. The strains varied greatly among themselves in κ (collagenase) and μ (hyaluronidase) production. A fair number produced κ in quantities sufficient to attack collagen paper and for screening tests to be possible; no strain produced λ-antigen. Many strains affected azocoll, but insufficiently for screening tests. If a strain produced hyaluronidase at all it usually produced a great deal; the results showed either hyaluronidase activity at a dilution of 1 in 512, or no activity at all. Repeat filtrates from the same strain showed little or no variation in antigenic make-up. The general pattern of toxin production was therefore:

α	β	δ	ϵ	θ	ι	κ	λ	μ	ν
+	−	−	−	−	−	+ or −	−	+ + + or −	+

and this gave some assistance in comparing organisms isolated during an epidemic. Thus in one severe epidemic of food poisoning in an institution, stool samples were obtained from many persons who had eaten the suspected food; all yielded heat-resistant *Cl. welchii* producing large amounts of hyaluronidase; samples from another person who had not eaten the food yielded a heat-resistant *Cl. welchii*, but this strain did not produce hyaluronidase. Sometimes, as in the epidemic referred to in Table 1, organisms agreeing in all respects, cultural, toxicological and serological, were isolated from food, from sick persons and from persons at risk who were not ill, whereas no such organism could be isolated from those who had not eaten the food.

Classification of heat-resistant Cl. welchii. With the exception of *Cl. welchii* Type F, *Cl. welchii* types are differentiated from one another by their ability to produce various toxins (Wilsdon, 1931, 1932–3; Glenny, Barr, Llewellyn-Jones, Dalling & Ross, 1933; Oakley, 1949). In practice, the lethal toxins are given more weight than the others in classification, so that five of the six types are determined by their capacity to produce one or more of these—α, β, ϵ, ι. Thus, if a strain produces α as its only lethal toxin it is grouped in Type A; if it produces only α and β, or β only, in Type C; if α and ϵ, or ϵ only, in Type D; if it produces α, β and ϵ, or β and ϵ only, it is placed in Type B; Type E produces α and ι. Type F, which produces β and traces of α, would be classed as Type C on this basis, but differs from this type in the marked heat-resistance of its spores (Zeissler & Rassfeld-Sternberg, 1949). This method works fairly well on the whole, especially when it is applied to recently isolated strains, but will clearly break down if the strain undergoes any degree of degradation. Loss of capacity to produce β or ϵ is known in Type B and loss of β production in Type C strains; loss of capacity to produce ϵ is suspected in Type D strains. Examination of strains for subsidiary antigens, e.g. δ, κ, λ, μ, may give useful indications of the type from which degraded forms have been derived.

The strains described in this paper agree very well with Type A strains, from which they differ only in their heat-resistance. Their feeble α-toxigenicity, their

failure to produce θ, and their variable hyaluronidase production are no bar to their inclusion in Type A, for Evans (1945) has shown that typical A strains are highly variable in all these respects. Five out of thirty such strains studied by him produced only the equivalent of 0·02 unit α-antitoxin per ml. Ten out of thirty produced no detectable θ; twenty-three out of thirty no hyaluronidase. It is clearly convenient to retain the food-poisoning strains, except for Type F, in Type A until further research makes it possible to subdivide Type A on consistent groupings of characters.

Antitoxins in the sera of convalescents. In one epidemic we were able to examine sera from a number of patients and staff. These sera were examined by conventional methods for *Cl. welchii* α, β and ϵ-antitoxins. In no case was β- or ϵ-antitoxin detected, but a surprisingly large number of patients (seventeen out of thirty-two) and staff (six out of eleven) possessed detectable amounts of *Cl. welchii* α-antitoxin (more than 0·005 unit). The distribution contrasts markedly with the only other comparable set of observations, that of Glenny, (unpublished), who found only one person with detectable α-antitoxin in 200 examined. (Bower, Mengle & Paxson (1938) used a method which precluded the estimation of less than 1 unit *Cl. welchii* α-antitoxin per ml. All of Glenny's samples, and all of ours, were far below this level.) As *Cl. welchii* α-toxin is a poor antigen, it seems at least likely that the patients and staff of this institution had had considerable experience of it.

(4) *Serology*

Since strains of *Cl. welchii* from various sources cannot be satisfactorily differentiated for epidemiological purposes by their toxicological and biochemical properties, the possibility of differentiation by serological methods was investigated.

Henderson (1940) found a wide diversity in the specificity of the somatic antigens of the classical Type A strains of *Cl. welchii* which appeared to be strain specific; antigens of Types B, C and D, however, possessed similarities. Type B strains with one exception shared an identical O-antigen, whereas Type D strains showed differences in specificity and thirteen strains were placed in seven groups. Cross O reactions between the various types were negligible or absent. In addition to the heat-stable O-antigen Henderson demonstrated a heat-labile L somatic component. He was not able, however, to detect L antigen in *Cl. welchii* Type A or C strains, but only in those types (B and D) that produced ϵ-toxin; even here it was not present in all strains.

Bacterial suspensions were prepared according to the methods described by Henderson (1940). They consisted of cultures grown overnight at 37° C. in glucose broth (steamed and cooled before inoculation). The organisms were twice washed and resuspended in distilled water. For use, suspensions were steamed for 1 hr. on 3 successive days (O suspensions) or treated with 0·4 % formalin for 2 days.

Antisera were prepared by the intravenous inoculation of rabbits with six successive increasing doses of bacterial suspension at intervals of 2–3 days. Formolized suspensions were found to produce a better agglutinin response than heated suspensions, in which, moreover, some antigenic constituent might be damaged.

They were, therefore, used throughout the work, although it was realized that the full antigenic relation between the strains might not be revealed. The immediate aim was to obtain a clear-cut method of type differentiation for epidemiological purposes only. No differences between formolized and heated suspensions were shown by absorption tests. Similar sera, although of lower titre, have been produced by intravenous injection of strains into horses.

Tube-agglutination tests were made by the standard technique for *Salmonella typhi* Vi agglutination tests. First readings were made after 4 hr. in the water bath at 43–45° C. and final readings after the tubes had been kept for 18 hr. in the cold room and 2 hr. at room temperature.

Slide-agglutination tests which proved useful for rapid routine work, were done with sera suitably diluted, usually, within the range 1/5 to 1/25. Positive strains agglutinated immediately. Strains not reacting in slide tests were never positive in tube tests, and non-specific agglutination reactions on the slide were not observed.

Table 7. *Comparison of the serology and toxicology of heat-resistant* Clostridium welchii *isolated from food and faeces connected with different outbreaks of food poisoning and used for the production of serological typing sera*

Lab. no.	Source	Provisional serological type	Soluble antigens					
			α	$\beta, \delta, \epsilon, \theta,$ and ι	κ	λ	μ	ν
3702/49	Stewed steak	1	tr.	–	–	–	+ + +	sl.tr.
2985/50	Boiled salt beef	1	tr.	–	–	–	–	sl. tr.
281/50	Boiled salt beef	2	tr.	–	–	–	+ + +	–
3958/50	Faeces	3	+	–	+	–	–	+
3653/50	Boiled salt beef	3	f. tr.	–	–	–	+ +	tr.
1690/50	Faeces	4	tr.	–	–	–	+ + +	sl. tr.
4322/49	Meat pasty	4	tr.	–	+	–	+ +	+
166/51	Faeces	5	tr.	–	–	–	–	tr.
3756/50	Faeces	6	v. sl. tr.	–	–	–	–	sl. tr.
1826/51	Steamed lamb	7	v. f. tr.	–	–	–	–	f. tr.
2204/51	Stew	8	f. tr.	–	–	–	+ + +	+

tr. = trace. f. = faint. v. sl. = very slight.

Results. Tables 7 and 8 show the origin of the heat-resistant strains of *Cl. welchii* selected for serological study, their toxicological properties and antigenic cross-reactions. Of the eight types so far identified and provisionally designated 1–8, Types 1, 2 and 6–8 show no serological cross-reactions with other types; Types 3–5 form a small group with some antigenic overlap but were shown by cross-absorption tests not to be identical.

The relation between these heat-resistant strains of *Cl. welchii* and other types of the organism was investigated by tube-agglutination tests. Of seventy-three strains of Type A, tested against antisera of Types 1–6, five were agglutinated by Type 2 and two by Type 3; all of those tested against antisera of Types 7 and 8 gave negative results.

Five sera prepared from Type A strains and kindly supplied by Dr Henderson did not agglutinate any of the eight typed strains of heat-resistant *Cl. welchii*. One

recently isolated strain, however, has been shown to agglutinate with one of the five type A antisera. These results indicate that antigenic constituents may be shared by heat-resistant and other Type A strains of *Cl. welchii*.

Of six strains of *Cl. welchii* Type F connected with the outbreak reported by Zeissler & Rassfeld-Sternberg (1949), none agglutinated with the provisional typing sera 1–6.

Table 8. *Tube agglutination of the provisional serological type strains of heat-resistant* Clostridium welchii *(unabsorbed sera)*

	Antisera							
Suspensions	1	2	3	4	5	6	7	8
1	1/3200	—	—	—	—	—	—	—
2	—	1/1600	—	—	—	—	—	—
3	—	—	1/3200	1/400	1/1600	—	—	—
4	—	—	1/400	1/400	1/100	—	—	—
5	—	—	1/800	—*	1/1600	—	—	—
6	—	—	—	—	—	1/400	—	—
7	—	—	—	—	—	—	1/1600	—
8	—	—	—	—	—	—	—	1/1600

— = No agglutination either with a 1/50 dilution of serum in the tube or with undiluted serum on the slide.

—* = Tube agglutination with serum diluted 1/20, and agglutinated with undiluted but not 1/5 serum on the slide.

Of eleven strains also obtained from Germany and said to be heat-resistant strains isolated from normal persons three agglutinated with the Type 3 antisera and two with Type 1 antiserum. None of them produced β-toxin. It is possible that at least some of these strains were similar to the heat-resistant types of *Cl. welchii* described in this paper.

Single strains of *Cl. welchii* Types B–E from the National Collection of Type Cultures and from the Wellcome Culture Collection failed to agglutinate with antisera 1–6.

Table 9 shows the serological type and toxicological properties of some strains isolated from food and faeces in some of the outbreaks of food poisoning. It will be seen that in any one outbreak the organisms isolated from the food and from the faeces of the victims belonged to the same serological and toxicological type, although serologically identical strains isolated from different epidemics varied in their hyaluronidase production.

(5) *Attempts to demonstrate pathogenicity*

(*a*) *Volunteer experiments*

The strain isolated from minced beef in the first outbreak studied in June 1947, was grown overnight in cooked meat medium; 3 ml. of the supernatant fluid were taken in milk by a volunteer without ill effect.

The strain (Lab. no. 3702) isolated from the steak in outbreak 2 (Table 2) was grown in meat broth and given to two monkeys in their food. One monkey ate a large amount and produced one loose stool some hours later; the other ate little

and appeared unaffected. A 24 hr. cooked meat culture (20 ml.) was then taken by a volunteer, who had a slight attack of pain and diarrhoea 15 hr. later; a further spasm occurred $2\frac{1}{2}$ hr. later. This mild reaction in one subject might be regarded by some as a psychological response only.

Further volunteer experiments were made with the same strain. As a preliminary precaution large volumes of the fluid portion of cooked meat cultures were mixed with potato and offered to two monkeys, one of which ate a large amount, the other much less. The animals showed no signs of illness, but a profuse growth of heat-resistant *Cl. welchii*, persisting for 8 days in the monkey that had eaten most food, was obtained from rectal swabs. Swabs taken from both monkeys before the experiment were negative for *Cl. welchii*.

Table 9. *Comparison of serology and toxin production of heat-resistant* Clostridium welchii *isolated from six outbreaks of food poisoning*

Source	Provisional serological type	Heat-resistant *Cl. welchii* Soluble antigens					
		α	β, δ, ϵ, θ, and ι	κ	λ	μ	ν
Boiled salt beef	2	tr.	–	–	–	+ + +	–
Faeces (19/19 positive)	2	tr.	–	–	–	+ + +	–
Boiled salt beef	3	tr.	–	–	–	+ + +	–
Faeces (8/8 positive)	3	tr.	–	–	–	+ + +	–
Faeces (13/13 positive)	1	tr.	–	–	–	+ + +	–
Boiled salt beef	1	tr.	–	– *	–	–	tr.
Faeces (6/6 positive)	1	tr.	–	– *	–	–	tr.
Faeces (12/14 positive)	6	tr.	–	–	–	–	tr.
Faeces (8/10 positive)	3	tr.	–	–	–	–	tr.

tr. = trace. – * affected azocoll, too weak to test.

Four persons, two of each sex, volunteered to take part in the experiment. Two 18–20 hr. cultures in cooked meat (10–15 ml.) were used, and two similar uninoculated tubes of the same medium. An independent observer allocated the bottles so that one woman (*A*) and one man (*C*) received the cultures, the others (*B* and *D*) the control tubes. The shaken-up tubes looked alike and it was afterwards agreed that none of the volunteers knew whether culture or sterile medium had been taken. All the supernatant broth and most of the meat were swallowed at 5 p.m. one afternoon.

Volunteer *A*, who had swallowed a culture, woke up between 4.30 and 5 a.m. next morning with pain in the upper abdomen; her temperature was normal. At 5.15 a.m. the pain was very severe but intermittent, gradually diminishing in intensity. Abdominal discomfort with slight pain, headache and nausea without vomiting continued with severe diarrhoea for about 2 hr. She got up feeling fairly well and came to the laboratory, but began to feel ill again with a further attack of diarrhoea. She returned home at 10 a.m. and improved during the day, recovering by the evening. There were no further symptoms the following day.

Volunteer B who had been mildly affected in the previous experiment was given a control bottle of cooked meat; she experienced no symptoms whatsoever.

https://doi.org/10.1017/S0022172400015515 Published online by Cambridge University Press

Volunteer *C* who had swallowed the second culture was a heavy sleeper. He woke up at 7.30 a.m. with slight pain resembling colic, but failing to recall the experiment he thought it was 'wind', and after drinking a cup of tea went to sleep again. It was his free Saturday morning and he slept until midday. When visited at 2 p.m. he was well and surprised to learn that he had taken one of the cultures.

Volunteer *D* who had been given the second sterile bottle of cooked meat, was unaffected.

Stools from all four volunteers taken before the experiment gave no growth of *Cl. welchii* after steaming for 1 hr. Further samples were taken on the day following the experiment and again after 1- or 2-day intervals. Heat-resistant *Cl. welchii* was recovered from the faeces of volunteers *A* and *C*. Stools from volunteer *B* were negative; no samples were examined from volunteer *D*. Volunteer *A* continued to excrete the organism for 3 days; no further specimens were taken.

To investigate the possibility that symptoms were due to an exotoxin, volunteer *B* drank a Seitz filtrate of a duplicate culture grown at the same time as the previous cultures and held for 3 days in the refrigerator. No ill effects were observed.

Two months later, when it was hoped that any acquired immunity would have waned, further experiments were made by volunteers *A* and *B* with *Cl. welchii*, strain 3702. A broth culture without meat, and a washed suspension of organisms from a similar culture, produced no ill-effects. This strain had been repeatedly subcultured, so another strain, Lab. no. 281, freshly isolated from incriminated meat, was used. Washed cells from cooked meat cultures produced no symptoms in one volunteer but caused slight colic in the other. The centrifuged supernatant fluid of a cooked meat culture taken by one volunteer, and a similar fluid filtered through a gradocol membrane and taken by the other, produced slight nausea and abdominal discomfort. The inconclusive results of these experiments may have been due to the development of some immunity or, perhaps, to the removal of meat from the cultures.

In general the volunteer experiments indicated that *Cl. welchii*, strain 3702, growing actively in cooked meat broth produced in volunteers swallowing the whole culture symptoms resembling those observed in the outbreaks. Experiments with filtrates were inconclusive. A toxic substance in the filtrate may be destroyed by the gastric juice, but the whole organism, particularly if enclosed in meat, may survive passage through the stomach and produce toxin in the intestine.

(*b*) *Animal experiments*

In addition to the feeding tests on monkeys already described, cultures were introduced through a stomach tube into the stomach of monkeys anaesthetized with ether. No signs other than an occasional loose stool were noted, but the organism was isolated from the faeces. Cultures given by the mouth to guinea-pigs produced no effect.

Two frogs, *A* and *B*, were given approximately 0·5 ml. by mouth of an overnight culture of *Cl. welchii*, strain 281; two, *C* and *D*, were given approximately 0·5 ml. of broth from uninoculated cooked meat. Frogs *C* and *D* remained quiet all day, whereas frog *B* became very restless after 2 hr., continually moving about and

sitting up on its hind legs; it did not vomit and after a further 3 hr. became quiet and remained so for the rest of the day.

Attempts were made to demonstrate pathogenicity or production of enterotoxin in mice by parental injection and by oral administration of cultures. The strains of *Cl. welchii* used showed typical morphological and biochemical characters, and gave a positive Nagler reaction, inhibited by Type A antitoxin. They had been isolated from meat considered to have been the cause of the outbreaks or, from faeces of victims, as follows: outbreaks of October 1949 (four strains), February 1950 (seven strains), June 1950 (seven strains), and later outbreaks in 1950 and 1951 (six strains). The material consisted of the fluid portion of cultures in Robertson's cooked meat incubated for 24 hr. at 37° C. The results appear in Table 10.

Table 10. *Tests for enterotoxin production of* Clostridium welchii *strains in mice and guinea-pigs*

		Mice. 24 hr. cultures			Guinea-pigs. Intraduodenal injection	
Lab. no.	Source	Intra-muscular	Intra-peritoneal	Oral	5 hr. culture	24 hr. culture
3702/49	Stewed steak	D 8 days	D 18 hr.	S	S (3)	S
3790/49	Boiled beef	S	—	S	—	—
3864/49	Boiled tongue	S	—	S	—	—
3954/49	Boiled lamb	S	—	S	—	—
281/50	Boiled salt beef	S	S	S	S	S
293/50	Faeces	S	—	S	—	—
294/50	Faeces	S	—	S	—	—
298/50	Faeces	S	—	S	—	—
322/50	Faeces	S	—	S	—	—
323/50	Faeces	S	—	S	—	—
324/50	Faeces	S	—	S	—	—
1465/50	Faeces	S	—	S	—	—
1466/50	Faeces	S	—	S	—	—
1467/50	Faeces	S	—	S	—	—
1468/50	Faeces	S	—	S	—	—
1469/50	Faeces	S	—	S	—	—
1470/50	Faeces	S	—	S	—	—
1471/50	Faeces	S	—	S	—	—
1690/50	Faeces	—	S (2)	S	S	S
2985/50	Boiled salt beef	—	S	S	S (3)	S
3653/50	Boiled salt beef	—	S	S	S (2)	S
3756/50	Faeces	—	D 18 hr.	S	S (2)	S
166/51	Faeces	—	D 18 hr. S (1)	S	S (3)	S
914/51	Faeces	—	S	S	S (3)	S
C.N. 2076	Type F	—	D 48 hr.	D 4 days	S (2)	S (2)
C.N. 1990	Type B	—	D 6 day	D 10 days	D 18 hr. (2) S (1)	D 18 hr. (2)

D = died. S = survived. — = not tested.
Where more than one animal was used, the number is indicated in brackets.

Of the eighteen mice injected intramuscularly with 0·3 ml. of culture, only one died, on the eighth day after injection. Of ten mice injected intraperitoneally with 0·5 ml., three died, all within 18 hr. Of twenty-four mice given 0·5 ml. of culture through a fine catheter directly into the stomach all survived. No symptoms were observed in the surviving mice, which were kept under observation for 14 days.

The tests made in guinea-pigs were based on the observations of previous workers (Thomson, personal communication) who had demonstrated an enterotoxin in lamb dysentery (Type B) strains of *Cl. welchii* by injection directly into the duodenum. As a preliminary step, these observations were confirmed with a Type B strain (CN 1990) obtained from Mr A. Thomson. Guinea-pigs inoculated intraduodenally with 0·5 ml. of 5 hr. cooked meat cultures of this strain died within 18 hr. with acute enteritis; the lower half of the small intestine was intensely congested, deep purple in colour and dilated with fluid. It was also confirmed that, for an unknown reason, some guinea pigs receiving the same material survived without showing any symptoms. It is advisable, therefore, to use several animals for each test.

The present experiments were made with eight strains of *Cl. welchii* from a number of different outbreaks. The sources of the strains, and the numbers of guinea-pigs injected, are shown in Table 10. In view of the possibility that a labile enterotoxin may be produced in young cultures only, tests were made with cooked meat cultures grown at 37° C. for 5 and for 24 hr. Young guinea-pigs were anaesthetized by the intraperitoneal injection of hexabarbitone or Cyclonal, the abdominal cavity was opened by a vertical incision and the duodenum exposed; 0·5 ml. of culture was injected through a fine needle and the abdomen closed with a few sutures. Of the twenty-six guinea-pigs injected all survived, and none showed any symptoms after recovery from the anaesthetic. Observations were continued for at least 14 days.

The experiments on mice and guinea-pigs thus failed to demonstrate enterotoxin. The occasional deaths of mice after parenteral injection were probably due to the traces of α-toxin known to be produced by these strains; repeated tests with the same strains gave irregular results so that neutralization tests were not practicable.

(6) *Comment*

The strains of *Cl. welchii* isolated from suspected foodstuffs and from the faeces of victims in the reported outbreaks of food poisoning have some distinctive characters. They form non-haemolytic colonies, which may later show a faint haemolytic zone. They are heat-resistant, usually withstanding steaming for 1 hr.; some strains were shown to survive boiling for several hours. Although spores were not readily demonstrable under natural conditions, certain strains have been induced to spore freely in the laboratory. They produce a Nagler reaction inhibited by *Cl. welchii* Type A antitoxin. In their toxicological reactions they resemble Type A strains; no new toxins have been identified. Eight serological types have so far been recognized by agglutination tests; they cross-react serologically with some other Type A strains, but no relation to Types B, C, D, E or F has been shown. In any one outbreak, organisms of the same type were isolated. Attempts to demonstrate pathogenicity in laboratory animals were unsuccessful, but cooked meat cultures swallowed by volunteers produced gastro-enteritis resembling that observed in the outbreaks. It is considered that the epidemiological and bacteriological findings justify the view that heat-resistant strains of *Cl. welchii* are the cause of the reported outbreaks of food poisoning.

GENERAL DISCUSSION

Cl. welchii has long been suspected as a cause of food poisoning, but the occurrence of a particular type capable of producing spores which would survive prolonged boiling was not recorded before the work of Zeissler and his colleagues. The difficulties involved in anaerobic work and the fact that this type of *Cl. welchii* is non-haemolytic and not easy to pick out in the presence of other bacteria may partly explain why it has not received more attention in relation to food poisoning. It seems probable, however, that as a result of changes in social habits the mild type of food poisoning due to *Cl. welchii* is becoming more common.

Before 1939 most persons ate at home, or in small restaurants serving freshly cooked food. As treatment of food at home was highly individual, outbreaks of food poisoning would affect only a few persons and, if they were as mild as most of those due to *Cl. welchii*, would have occasioned little but temporary annoyance. Again the clientele of restaurants, though working in a limited area, would in all probability live farther afield; illness occurring as a result of such meals, therefore, might not be associated in the mind of the victim with the restaurant; moreover, the restaurant would probably offer some choice of dishes so that not all persons eating there on a particular day would be affected.

The 1939–45 war changed all this. Canteens became common, in factories, in schools and as municipal ventures; existing canteens were enlarged to supply many more meals, or were used on a larger scale without alteration. Communal feeding became usual, so that large numbers of people would eat the same meal. The consequent increase in canteen staff led to the employment as food handlers of many persons untrained in the elements of food hygiene, and the shortage of equipment, refrigerators, washing facilities, hot water, towels and overalls made adequate hygienic precautions difficult. Above all, the shortage of meat made economy in its use essential. Not only were 'left-overs' hoarded and re-used if possible, but the practice of cooking meat on the day before it was to be eaten became widespread; meat is carved more economically when cold. No doubt some canteen staff were genuinely convinced that meat would not keep overnight unless it were cooked. Everything favoured the development of heat-resistant *Cl. welchii*. In a stew, for instance, all aerobes and non-heat-resistant spore-bearers would be killed off, the oxygen would be boiled out of the liquid, while glutathione and other reducing substances were extracted from the meat. Heat-resistant *Cl. welchii* if present would then germinate and grow freely as the stew cooled slowly in the kitchen; such perfect anaerobic conditions could hardly be improved by laboratory technique.

The frequency with which heat-resistant *Cl. welchii* was isolated from the suspected food and from the faeces of patients and persons at risk, the general similarity of the strains cultured from food and patients, and the very low carrier rate in normal persons makes it fairly certain that the patients acquired their heat-resistant *Cl. welchii* from the contaminated food. It is by no means so easy to show that the food poisoning is due to heat-resistant *Cl. welchii*. The organisms are only feebly toxigenic; they do not produce β-toxin at all; and no enterotoxin has been demonstrated by animal experiment. Experiments on volunteers, however, suggest

that a mild form of food poisoning can be produced in man by ingestion of cooked meat cultures of heat-resistant *Cl. welchii* obtained from foods.

The type differentiation of heat-resistant strains of *Cl. welchii* is important for the investigation of food poisoning outbreaks; it is essential for the correlation of organisms isolated from the suspected meat, cooked and uncooked, from patients' faeces and from carriers amongst the kitchen staff. Even if the suspected food were not available, a high percentage of stools showing heat-resistant *Cl. welchii* of the same serological type would be a strong indication that the outbreak was in fact due to *Cl. welchii*.

Meat may be contaminated with *Cl. welchii* in various ways—by organisms in dust, or by faecal organisms of human or animal origin; and it is clear that this contamination can occur in slaughter-houses, in transit and in canteens. To prevent the transfer of *Cl. welchii* from the human intestine to foodstuff via the hands depends, as usual, on the personal hygiene of the food handler. It cannot be too strongly emphasized that the prevention of this type of food poisoning lies not in the control of carriers by treatment with anti-bacterial substances nor by their removal from the kitchen; but in attention to clean hands, care in food manipulation and, most important of all, correct food preparation and storage.

Almost all our outbreaks have been associated with the consumption of meat cooked the previous day and allowed to cool for long periods. It is evident that most of them would have been prevented if the meat had been eaten hot on the day on which it was cooked. It might be worth while in future to consider the installation of pressure cookers in canteens; these would enable the cooking to be done in very much less time than is now possible. Whatever the future policy, however, the practical results of eating meat on the day after it is cooked are clear enough. In certain boroughs where many of the first outbreaks of *Cl. welchii* food poisoning were investigated, the principal canteens in schools and factories have ceased to cook their meat a day ahead of requirements, preferring to use it freshly cooked. This improvement in technique, encouraged by the officers of the local Public Health Departments, has resulted in freedom from *Cl. welchii* food poisoning in these areas.

If this ideal solution is not practicable, cooked meats intended to be eaten on the following day must be cooled quickly and refrigerated; if any further heating is necessary, it must be done just before the meat is eaten. Furthermore, the heat penetration during cooking will be more effective for small joints than for large ones and the subsequent cooling will be more rapid. Therefore, it seems advisable to use a number of small joints in preference to one large one. Cooked meat should be kept either too cold or too hot for bacterial multiplication; it should never be allowed to remain warm for more than the shortest possible time. Cooling arrangements for this purpose undoubtedly raise economic difficulties.

It is suggested in the Report of the Manufactured Meat Products Working Party that the best arrangement for rapid cooling under hygienic conditions is a cool air-conditioned room. Facilities may not allow this and the wooden cabinet with shelves, to which is attached an electric fan and an air filter, referred to in the Report may solve the difficulty.

The household type of refrigerator could be adapted to take a series of shallow trays so that stews and similar fluid dishes can be divided into smaller quantities of large surface area. There may be other simpler devices which are as effective. Education in methods of food preparation and storage is an essential factor in the prevention of food poisoning.

SUMMARY

1. Outbreaks of mild food poisoning have been investigated in which heat-resistant *Cl. welchii* appeared to be the causative organism. The outbreaks were characterized by colic and diarrhoea without vomiting, commencing 8–20 hr. after ingestion of the contaminated food.

2. The strains of *Cl. welchii** concerned are only feebly toxigenic, and apart from the heat-resistance of their spores, and some colonial characters, fit well into *Cl. welchii* Type A. The toxin production and the serology of the strains is uniform within an outbreak.

3. Mild food poisoning similar to that seen in natural epidemics has been produced in volunteers by ingestion of cultures of heat-resistant *Cl. welchii* isolated from contaminated meat.

4. Infection is almost invariably due to meat which has been boiled, steamed, braised, stewed or insufficiently roasted, allowed to cool slowly, and eaten the next day, either cold or reheated.

5. Outbreaks of this kind should be prevented by cooking meat immediately before consumption; or, if this is impossible, by cooling the cooked meat rapidly and keeping it refrigerated until it is required for use.

We wish to thank many persons for their help. First, those who have provided facilities and materials for the investigation of outbreaks of *Cl. welchii* food poisoning in the London Area, in particular, the Medical Officers of Health and Sanitary Inspectors of Public Health Departments at Southgate, Wembley, Uxbridge, Edmonton, Islington, Barnet, Potters Bar, Ilford, Enfield, Finsbury, St Pancras, Shoreditch, Brentwood and Kensington. Secondly, the directors and staff of laboratories of the Public Health Laboratory Service who have provided details of outbreaks and many hundreds of cultures, in particular those at Edmonton and Coppetts Road (London), Manchester, Newport, Brighton, Leicester, Epsom, Newcastle and Salisbury.

We are also most grateful for the generosity and kindly co-operation in the supply of meat and faeces samples, of Messrs Aplin and Barrett Ltd., Yeovil, and the Public Health Departments of Uxbridge, Watford, Wembley and Birkenhead and also the Lister Institute, Elstree. The flies were provided through the kindness of Mr A. A. Green, Pest Infestation Laboratory, Slough.

Our thanks are also given to Dr A. G. Signy and Dr Edith Straker of St Mary Abbots Hospital for much information and for specimens connected with a particular outbreak, to Mr J. Smiles of the Medical Research Council, Mill Hill, for the preparation and microscopy of slide cells showing spores of *Cl. welchii*, and to the staff of the Immunology and Experimental Pathology Department, Welcome Research Laboratories, for invaluable technical assistance.

* These strains of *Cl. welchii* are in the National Collection of Type Cultures.

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(*MS. received for publication* 30. VII. 52.)