J. Hyg., Camb. (1980), **84**, 443
Printed in Great Britain

Application of serological typing to the investigation of outbreaks of *Clostridium perfringens* food poisoning, 1970–1978

By M. F. STRINGER, P. C. B. TURNBULL and R. J. GILBERT
Food Hygiene Laboratory, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT

SUMMARY

Serological typing was used as an epidemiological tool in the investigation of 524 outbreaks of *Clostridium perfringens* food poisoning in the United Kingdom and 37 outbreaks in other countries.

Five thousand five hundred and fifty-four (77%) of 7245 strains of *C. perfringens* associated with the 561 outbreaks were typable with the 75 Food Hygiene Laboratory antisera; in 354 (63%) of these outbreaks a specific serotype was established as being responsible for the outbreak.

An assessment is made of the ability of two additional sets of antisera, prepared against 34 American and 34 Japanese strains of *C. perfringens*, to increase the number of strains which can be typed. The extent of cross-reaction between the three sets of antisera was determined and the results are discussed in relation to the source and history of the type strains.

INTRODUCTION

A serological typing scheme for *Clostridium perfringens* type A was first developed in this laboratory when Hobbs *et al.* (1953) drew attention to *C. perfringens* as the causative agent in twenty-three outbreaks of food poisoning and prepared antisera against strains from eight of these incidents. The strains isolated from all of the outbreaks formed spores which survived heating at 100 °C for 1 h; these strains were subsequently referred to as heat-resistant strains (HR). Strains of *C. perfringens* whose spores could not survive this treatment were designated as heat-sensitive (HS). Sutton (1969) and other workers in this laboratory increased the number of antisera in the typing set to 42; 24 sera were prepared against HR strains and the remaining 18 against HS strains. During the next 10 years a further 40 antisera were prepared. usually against strains incriminated as the cause of outbreaks of food poisoning and consisting of a mixture of both HR and HS strains.

Antisera prepared against HR strains were denoted originally by Arabic numerals, and Roman numerals were used for antisera prepared against HS strains. However, following the reports by Taylor & Coetzee (1966) and Sutton & Hobbs (1968) that HS strains were also implicated in outbreaks of food poisoning, it was felt that this distinction was no longer justified. The use of Roman numerals to denote HS strains was then abandoned and the serotypes were renumbered

0022–1724/80/0094–1979 $01.00

Table 1. *Details of the Food Hygiene Laboratory type strains of* Clostridium perfringens *type A*

Culture reference number*	Old sero-type number	New sero-type number	Source and history†		Haemo-lysis‡	Heat resist-ance§
NCTC 8797	1	1	Salt beef	C	NH	+
NCTC 8238	2	2	Salt beef	C	NH	+
NCTC 8239	3	3	Salt beef	C	NH	+
NCTC 8247	4	4	Meat pasty	C	NH	+
NCTC 8678	5	5	Faeces	C	NH	+
NCTC 8679	6	6	Faeces	C	NH	+
NCTC 8449	7	7	Stewed lamb	C	NH	+
NCTC 8235	8	8	Stew	C	NH	+
NCTC 8798	9	9	Rissole	C	NH	+
NCTC 8799	10	10	Roast meat	C	NH	+
NCTC 9851	11	11	Braised heart	C	NH	+
NCTC 10239	12	12	Faeces	C	NH	+
NCTC 10240	13	13	Chicken	C	NH	+
NCTC 10611	14	14	Faeces	C	NH	+
NCTC 10612	15	37	Mincemeat	C	NH	+
NCTC 10613	16	15	Faeces	C	NH	+
NCTC 10614	17	16	Faeces	C	NH	+
F.6849/67	18	17	Faeces	C	NH	+
F.10486/67	19	18	Faeces	C	NH	+
F.1149/68	20	19	Faeces	C	NH	+
F.3697/67	21	E	Faeces	C	NH	+
F.3483/68	22	20	Faeces	C	NH	+
F.3746/68	23	21	Faeces	C	NH	+
F.3858/68	24	22	Minced kidney	C	NH	+
F.7869/66	i	23	Faeces	NC	NH	–
F.7870/66	ii	24	Faeces	C	H	–
F.8588/66	iii	25	Cream doughnut	NC	H	–
F.9191/66	iv	E	Faeces	C	NH	–
F.4062/70	v	25	Faeces	C	NH	–
F.1086/67	vi	26	Roast pork	C	H	–
F.1073/67	vii	38	Roast pork	C	H	–
F.7871/66	viii	27	Faeces	NC	H	–
F.1543/67	ix	28	Mincemeat	C	NH	–
F.1539/68	x	29	Faeces	C	H	–
F.9222/67	xi	39	Brisket	C	H	–
F.3795/67	xii	23	Tongue	C	H	–
F.4294/67	xiii	17	Faeces	NC	NH	–
F.2098/68	xiv	3/4	Faeces	NC	NH	+
F.3701/67	xv	9	Pork	NC	NH	–
F.2932/67	xvi	40	Roast beef	C	H	–
F.3794/67	xvii	30	Tongue	NC	H	–
F.3853/67	xviii	31	Tongue	C	H	–
F.3663/73	43	41	Pork	C	H	+
F.3911/73	44	32	Faeces	C	H	–
F.4436/73	45	33	Tongue	C	H	–
F.5337/73	46	34	Minced beef	C	H	–
F.50/72	47	35	Blancmange	NC	H	+
F.4552/72	48	36	Turkey	NC	H	–
F.2925/74	49	42	Faeces	NC	H	–
F.1922/74	50	43	Semolina	C	H	–

Table 1 (*cont.*)

Culture reference number*	Old serotype number	New serotype number	Source and history†		Haemolysis‡	Heat resistance§
F.2889/74	51	44	Beef	C	H	–
F.2890/74	52	45	Beef	C	NH	+
F.1774/74	53	46	Gravy	NC	NH	–
F.1185/74	54	47	Faeces	NC	H	–
F.3158A/74	55	48	Faeces	C	H	–
F.3158B/74	56	49	Faeces	C	NH	–
F.3565/74	57	50	Faeces	NC	H	–
F.3579/74	58	51	Faeces	NC	H	–
F.3799/74	59	52	Faeces	NC	H	–
F.4137/74	60	53	Lesion of necrotic enteritis	C	NH	–
F.4314/74	61	54	Faeces	C	NH	+
F.4930/74	62	55	Chicken pie	C	H	–
F.5049/74	63	56	Faeces	NC	NH	–
F.5069/74	64	57	Faeces	NC	H	–
F.2154/75	65	58	Turkey rissotto	C	H	–
F.1502/75	66	59	Faeces	C	H	–
F.1700/75	67	60	Faeces	C	NH	–
F.2227/75	68	61	Faeces	C	H	–
F.1066/75	69	62	Blood – neonatal meningitis	C	H	–
F.1078/75	70	63	Faeces	C	H	–
F.1149/75	71	64	Faeces	C	NH	+
F.2208/75	72	65	Faeces	NC	H	–
F.2724/75	73	66	Wound – gas gangrene	C	H	–
F.1998/75	74	67	Butter beans	NC	H	–
F.2177/75	75	68	Faeces	NC	H	–
F.2198/75	76	69	Faeces	NC	H	–
F.2980/75	77	70	Faeces	C	NH	+
F.5236/75	78	71	Faeces	C	H	+
F.5230/75	79	72	Chicken	NC	H	–
F.5744/75	80	73	Roast pork	NC	NH	–
F.485/75	81	74	Faeces	NC	H	–
F.478/75	82	75	Faeces	NC	H	–

E = these serotypes have been eliminated from the new set of antisera.
* NCTC = National Collection of Type Cultures. F = Food Hygiene Laboratory.
† C = causative of food poisoning outbreak (NC = not causative).
‡ H = haemolytic on Columbia base horse blood agar (NH = non haemolytic).
§ + = spores survive heating at 100 °C for 60 min. (heat-resistant). – = spores do not survive heating at 100 °C for 60 min. (heat-sensitive).

(Hughes, Turnbull & Stringer, 1976); later additions to the serotyping set were accorded the appropriate Arabic number (see Table 1). At the same time, where experience showed that agglutination occurred consistently with a combination of two antisera, for example, i and xii, 3 and xiv and 4 and xiv, these were pooled and given a single serotype number (Table 1 and Hughes *et al.* 1976).

Although outbreaks of *C. perfringens* food poisoning have been recorded in many countries throughout the world, for example Japan (Itoh, 1972), Sweden

(Fabiansson & Normark, 1976), Finland (Raevuori, 1976), Italy (Caroli *et al.* 1977), U.S.A. (Bryan, 1978) and Canada (Todd, 1978), only two countries (U.S.A. and Japan) other than England have, to our knowledge, developed a serological typing scheme for use as an epidemiological tool in the investigation of outbreaks.

At the 10th International Congress of Microbiology in Mexico in 1970 an informal meeting of microbiologists from Australia, Canada, England, U.S.A. and Japan discussed the possibility of an international serotyping scheme. At that time, serotyping was carried out by all of these countries apart from Canada. The range of antisera used in England and Australia was identical, whereas the sera in the U.S.A. and Japan differed in both numbers and source from each other and those in England. It was agreed that the Food Hygiene Laboratory should receive the type strains from the U.S.A. and Japan and, having assessed any overlap, combine them into a single scheme.

This paper describes the results of the application of serological typing to the investigation of outbreaks of *C. perfringens* food poisoning and discusses the use of an enlarged set of antisera derived from strains isolated in three countries.

MATERIALS AND METHODS

Type strains

Details of the 82 Food Hygiene Laboratory type strains of *Clostridium perfringens* are shown in Table 1; both the old and the new serotype designations are given together with the source and history of the strain. The history and other relevant data on the 56 type strains from Japan were described by Itoh (1972). All the Japanese strains were heat-resistant strains isolated from the faeces of healthy people. Eighty-five of the 91 type strains from the U.S.A. were received. These strains had been isolated from a wide variety of sources – food poisoning outbreaks, clinical infections, and from foods and faeces not associated with illness.

Strains from food poisoning outbreaks

Strains of *C. perfringens* in cooked meat medium were received from Public Health Laboratory Service and hospital laboratories throughout the United Kingdom. Wherever possible full details relating to each outbreak were obtained through the use of a specific questionnaire. Strains originating from outbreaks overseas were generally received as freeze-dried cultures, although occasionally cooked meat cultures were sent. All strains were plated onto Columbia base blood agar with added neomycin (0·06 ml of a 1 % solution spread over the surface of the plate) and incubated anaerobically at 37 °C for 18–24 h. The degree of haemolysis was recorded and typical colonies were subcultured onto plates of nutrient egg yolk medium (McClung & Toabe, 1947) of which half the plate had been spread with specific *C. perfringens* type A antitoxin (Wellcome Laboratories, Beckenham, Kent). The Nagler reaction (Nagler, 1939) was recorded after 18–24 h incubation at 37 °C.

Confirmation of type strains as C. perfringens

Strains were inoculated into nitrate-motility and lactose-gelatin agars according to Hauschild & Hilsheimer (1974). Guinea pig skin neutralisation tests were carried out with 6–8 h culture filtrates of strains grown in cooked meat medium + 1% starch (Smith, 1965) to confirm that the strains belonged to toxigenic type A (Oakley & Warrack, 1953).

Preparation of antisera

Antisera were prepared according to the method described by Hughes *et al.* (1976).

Serological typing

Slide agglutination. Serotyping was carried out with the 75 antisera used routinely at the Food Hygiene Laboratory in the investigation of outbreaks of food poisoning. The antisera were arranged in a system of 8 primary pools and each pool contained equal volumes of 9 or 10 individual antisera. Each primary pool was also divided into 3 secondary pools containing 3 or 4 antisera. Individual antisera were used at a dilution of 1 in 5. Bacterial growth (from nutrient egg yolk medium) was emulsified in a loopful of 0·85% physiological saline on a glass slide, mixed with a loopful of antiserum and observed for agglutination. Some strains agglutinated with more than one antiserum, but for such strains these were consistent properties and the strain was accordingly designated as being a multiple serotype (see Table 6). Type 3/4 for example was a common strain designation for many food poisoning strains.

Tube agglutination. The degree of cross-reaction between the American (34), English (75) and Japanese (34) type strains was examined by tube agglutination. Initially, each antiserum was tested at a dilution of 1/20 against suspensions of all 143 type strains, any positive agglutinations were then titrated. Homologous and cross-reacting titres were determined by making doubling dilutions of antisera in 0·85% physiological saline, to give a final volume of 0·5 ml in the wells of an agglutination tray. Two drops (0·04 ml) of antigen suspension were added to each well and the tray then incubated at 37 °C for 18 h. The titre was taken as the reciprocal of the highest dilution at which strong agglutination was observed.

RESULTS

Table 2 shows the number of American and Japanese strains that were submitted to the Food Hygiene Laboratory (FHL) for serological studies. Initially, all strains were tested for agglutination with the 47 FHL antisera available at that time by slide agglutination tests. Thirty-five American and 11 Japanese strains showed positive agglutination with one or more of the English antisera. Twenty of the 141 strains were unsuitable for antiserum production because they could not be obtained in the smooth capsulated form and a further seven strains did not belong to toxigenic type A. This left a total of 68 strains (34 American and 34 Japanese)

Table 2. *American and Japanese type strains of* Clostridium perfringens *received by the Food Hygiene Laboratory (FHL)*

Country	Source of strain	Number of serotypes submitted	Number agglutinating with the English antisera	Number unsuitable for for antiserum production†	Number against which antiserum prepared
U.S.A.	Food poisoning, clinical infections and foods and faeces not associated with illness	85	35*	16‡	34
Japan	Faeces from healthy people	56	11	11	34

* This number includes 13 'Hobbs-type strains' which were already in the English typing set.

† Strains were colonially rough or autoagglutinated in saline.

‡ This number includes 7 strains which did not belong to toxigenic type A.

Table 3. *Summary of cross-agglutination reactions between English, American and Japanese type strains of* Clostridium perfringens

Country	Number of type strains	Number of type strains cross-agglutinating with antisera to strains from: England	U.S.A.	Japan
England	75	27 (36)*	9 (12)	2 (3)
U.S.A.	34	17 (50)	11 (32)	5 (15)
Japan	34	5 (15)	3 (9)	1 (3)

* Percentages in brackets.

against which antisera were raised. Titres of specific antisera varied between 100 and 6400. Table 3 summarizes the extent of serological cross-agglutination between the 143 type strains of *C. perfringens*. Approximately a third (36%) of the English type strains were found to agglutinate with one or more antisera in addition to that of the homologous type, whereas only 12 and 3% respectively of the American and Japanese strains 'cross-reacted' with the English antisera. A relatively high proportion of strains from all three countries cross-reacted with the American antisera and in contrast, a low porportion of strains cross-reacted with the Japanese antisera. For epidemiological purposes, however, it has never been found necessary to absorb out cross-reacting antigens in order to make monospecific sera. The ability of some strains to agglutinate with more than one antiserum acted as a means of further subdivision.

Serological typing results using the English, American and Japanese antisera to type strains of *C. perfringens* isolated from outbreaks occurring in the United Kingdom from 1970 to 1978 are shown in Table 4. During this time there was an increase in the number of outbreaks investigated and this led to an increase in the number of English typing sera from 42 in 1970 to the present total of 75. The

Table 4. *Use of the English, American and Japanese sets of antisera to serotype strains of* Clostridium perfringens *isolated from food poisoning outbreaks in the United Kingdom* (1970–1978)

	Outbreaks of food poisoning					Number of isolates of *C. perfringens*				
		Number in which causative serotype established using antisera from					Typable with antisera from			
Year	Number investigated	England	U.S.A.	Japan	Total*	Examined	England	U.S.A.	Japan	Total
1970	38	22 (58)†	—	—	22 (58)	348	254 (73)	—	2	256 (73)
1971	45	18 (40)	—	—	18 (40)	480	256 (53)	2	2	260 (54)
1972	36	18 (50)	—	—	18 (50)	421	229 (54)	1	1	231 (55)
1973	37	24 (65)	—	—	24 (65)	520	395 (76)	—	2	397 (76)
1974	48	26 (54)	—	—	26 (54)	654	500 (76)	4	4	508 (77)
1975	76	50 (66)	1	3	54 (71)	1165	937 (80)	20	13	970 (83)
1976	96	73 (76)	1	3	77 (80)	1289	1056 (82)	19	30	1105 (86)
1977	94	67 (71)	3	2	72 (75)	1241	1087 (87)	20	22	1129 (91)
1978	54	34 (63)	1	2	37 (68)	799	599 (76)	22	16	637 (80)
9 years	524	332 (63)	6	10	348 (66)	6917	5313 (77)	88	92	5493 (79)

* Results using the 34 American, 34 Japanese and 75 English antisera.

† Percentage in brackets.

Table 5. *Distribution of the English serotypes of* Clostridium perfringens *from 524 outbreaks of food poisoning in the United Kingdom, 1970–1978*

Serotype	Number of outbreaks*		Serotype	Number of outbreaks
1	25		30	2
1/26	1		30/67	1
3	8		31	1
4	33	105	31/68	1
3/4	64		32	2
4/25	1		33	2
5	6		34	3
6	4		35	3
7	9		36	1
7/10/21	1		38	6
7/10/21/36	1		39	1
7/11/13	3		39/57	1
7/13/20	2		40	2
8	1		41	31
9	3		43	2
10	6		44	1
10/55	1		45	1
11	9	17	46	1
11/13	8		47	1
12	4		48	2
13	3		49	1
14	3		50	1
15	4		51	4
16	2		52	3
17	6		53	1
17/52/63	1		54	5
18	1		55	2
19	1		56	1
20	2		58	2
21	1		59	1
23	6		60	1
24	2		61	1
24/62	1		62	2
25	10		63	4
25/27/31/68	8		64	3
25/68	1		65/69	3
27/31/68	1		68	5
27	1		70	1
28	4		71	4
29	28		75	1
			Not determined†	192

* The total number of oubtreaks caused by all serotypes is greater than 332 because in some outbreaks more than one serotype was involved.

† Usually because insufficient cultures were sent for examination, or a wide range of serotypes were found and no particular type could be incriminated.

Table 6. *American and Japanese serotypes of* Clostridium perfringens *causing outbreaks of food poisoning in the United Kingdom,* 1970–1978

Year	Number of out-breaks	Causative serotype	Community involved in outbreak	Number of persons		Incriminated food
				Ill	At risk	
1970	1	TW 9*	NK	NK	NK	Roast beef
1975	4	PS 73	Hospital	30	NK	Minced pork
		TW 17	Factory	26	75	Lamb chops
		TW 18	Old peoples home	11	55	Roast pork
		TW 43	Factory	20	157	Chicken
1976	4	PS 40	NK	NK	NK	NK
		TW 9	Hospital	24	240	Chicken
		TW 22	Hospital	12	25	Minced beef
		TW 23	Hospital	NK	NK	Chicken
1977	5	PS 71	Factory	100	300	Roast beef
		PS 73	Hospital	36	62	Chicken
		PS 79	Old peoples home	12	48	Pork
		TW 5	School	50	NK	Stewed steak
		TW 8	Hotel	NK	270	Turkey
1978	5	PS 30	Hospital	NK	NK	NK
		PS 68†	Conference party	133	250	Chicken
		PS 68‡	Hospital	8	NK	Pork
		TW 22	Old peoples home	12	NK	Beef
		TW 24	School	20	50	Beef stew

* This serotype was involved jointly with English serotype 52; in Table 4, the associated outbreak was included in those typable with the English antisera. Likewise, type PS.68† was involved with English type 1 and PS.68‡ with English type 41.

NK = not known; TW = Japanese type; PS = American type.

increase in the numbers of strains examined reflected not only an increase in the number of investigated outbreaks, but an increase in the number of cultures sent for typing from any one outbreak. In 1978, an average of 14·8 strains per outbreak were received compared with 9·1 in 1970.

To assess the increased typing capability of the full set of 75 English antisera together with the 68 American and Japanese antisera, 554 strains from 111 'not-typable outbreaks' (i.e. outbreaks in which the majority of the strains failed to agglutinate with the English antisera available at the time) occurring during 1970-1978, were tested for agglutination with the complete range of antisera. Many strains (291) from several of the 111 outbreaks (predominantly those in 1970–1974) failed to survive storage. Three hundred and ninety (70%) of the 554 strains agglutinated with one or more of the additional antisera and in 25 of the 111 outbreaks, a causative serotype was established. Nine outbreaks were caused by one of the more recent English types and 6 and 10 outbreaks were caused by American and Japanese serotypes respectively. A causative serotype was established in 348 (66%) of the 524 outbreaks. In terms of isolations, 5313 (77%) of 6917 strains examined were typable with the 75 English antisera and a further 180 strains with the American and Japanese antisera, raising the percentage typability to 79%.

Table 7. *Use of the English antisera to type strains of* Clostridium perfringens *from food poisoning outbreaks occurring in countries other than the United Kingdom, 1970–1978*

Country	Number of outbreaks: Investigated	Number of outbreaks: Causative serotype established	Serotypes involved	Number of isolates of *C. perfringens*: Examined	Number of isolates of *C. perfringens*: Typable
U.S.A.	22	11 (50)*	1 (×2), 18 (×2), 29, 56, 11, 3, 25/31/68, 24/62, 65/69	199	143 (72)
Israel	6	6(100)	1 (×3), 1 and 6, 5, 3/4	66	56 (85)
Canada	4	2	27 and 71, 43	18	14 (78)
Chile	1	0	—	8	1
Netherlands	1	1	1	4	4
Norway	1	0	—	3	0
W. Germany	1	1	1 and 4	25	18 (72)
Yugoslavia	1	1	10/55	5	5
8 Countries	37	22 (60)		328	241 (73)

* Percentages in brackets.

The distribution of the English serotypes of *C. perfringens* responsible for 332 outbreaks of food poisoning during 1970–1978 is shown in Table 5. Fifty-one of the outbreaks involved two (or more) serotypes, therefore the total number of outbreaks exceeds 332 – the total given in Table 4. It can be seen that some types are encountered far more frequently than others as the cause of outbreaks; types 1, 3, 4 and 3/4, 11 and 11/13, 29 and 41 were involved in 206 (62%) of the 332 outbreaks in which a causative type was established. During this period types 3, 4 and 3/4 accounted for 105 (32%) of the typable outbreaks. Table 6 gives details of the 19 outbreaks in which American and Japanese serotypes were shown to be involved as the causative organism, in some cases along with English serotypes.

Table 7 summarizes the results of the application of the English antisera to type strains associated with outbreaks occurring in countries other than the United Kingdom. A causative serotype was established in 22 (60%) of the 37 outbreaks investigated. The serotypes encountered most frequently as the cause of the United Kingdom outbreaks were found in 12 (54%) of the 22 outbreaks; serotype 1 was the most common type, found in 8 (36%) of the 22 typable outbreaks. In

terms of strains isolated, 241 (73 %) of the 328 strains examined agglutinated with the English antisera. All the serotypes found in the 'overseas outbreaks' had been found previously as the cause of outbreaks in this country during 1970–1978.

DISCUSSION

Although *Clostridium perfringens* is one of the most important causes of food poisoning throughout the world, the investigation of many outbreaks is often inadequate and incomplete. Hauschild (1975) described four laboratory criteria which may be used to implicate *C. perfringens* as the agent responsible for an outbreak; two of these criteria involved demonstrating the same serological type of *C. perfringens* in the incriminated food and/or the faeces of those ill. The findings of Zen-Yoji *et al.* (1970), Hughes *et al.* (1976) and Schiemann (1977) together with the results of the present study on the application of serological typing to the investigation of 561 outbreaks of food poisoning clearly indicate the value of the typing scheme in the epidemiological study of food-borne disease caused by *C. perfringens*. It is unfortunate therefore, that although serological typing is widely recognized as an important epidemiological tool, it is not employed more extensively as a routine procedure in the investigation of outbreaks (Stringer, Shah & Gilbert, 1979).

Alternative methods to serology for typing or sub-dividing strains of *C. perfringens* have been reported. Paine & Cherniak (1975) suggested the possibility of a classification scheme based on the analysis of the capsular polysaccharides using gas liquid chromatography. A relatively simple procedure utilizing the susceptibility to a range of bacteriocins was described by Mahony (1974). However, neither of these methods have been used to type strains isolated from faeces and/or food involved in suspected outbreaks of food poisoning or assessed as a possible alternative to conventional serotyping. One method that has been compared with serology involved measuring the levels of *C. perfringens* enterotoxin in the faeces of ill patients; it was concluded that serology was a useful technique and that the detection of enterotoxin could be used as a complementary test (Dowell *et al.* 1975).

It is well established that the type-specific antigens on which the serology of *C. perfringens* is based are located in the capsular polysaccharides, and it has been suggested that cross reactions between strains may be due to the presence of group-specific polysaccharides (Cherniak & Henderson, 1972). After the production of antisera against the 68 Japanese and American strains which were not typable with the 47 English antisera available at the time, some of the strains were subsequently shown to cross-agglutinate with the English antisera with which they were originally not-typable. For example, PS 20 agglutinated with English type 1 (titre 40), PS 80 agglutinated with type 40 (titre 160) and TW 13 agglutinated with type 13 (titre 40). The titres of most of these cross-reactions ranged between 40 and 640 and were generally much lower than the titre with the homologous strain. It is likely that one of two explanations may account for this finding: (1) when strains are grown in 1 % glucose broth for antiserum preparation, antigens

are produced which may not exist (or not in large amounts) with cultures of the same organism grown on solid media; (2) when a strain of given antigenic character is injected into a rabbit, certain minor polysaccharide components may be better antigens than the type-specific antigens and hence the titre with the cross-reacting antigens may be disproportionately higher than that with the type-specific substance. This phenomenon has also been observed with the English antisera, i.e. when new antisera were prepared from strains that were implicated as the cause of outbreaks and found to be not-typable with the existing antisera, minor reactions were subsequently found with these new sera and the existing type strains. Although several of the cross-agglutination reactions were 'two-way', for example antiserum 10 agglutinated type 62 and the antiserum prepared from type 62 agglutinated type 10, 'one-way' cross-reactions were more common. However, the fact that a type strain exhibited a given set of cross-reactions does not necessarily mean that other strains belonging to that serotype will have the same set of cross-reactions. Two-way cross reactions have been observed with other organisms, for example klebsiellas (Edmondson & Cooke, 1979) and *Bacteroides fragilis* (Elhag, Bettelheim & Tabaqchali, 1977). The type specific antigens of klebsiellas are also located in the capsule polysaccharides and in studying the serology of this organism, Henriksen (1954) concluded that the most important cause of differences in cross-reaction was antigenic variation. Edmondson & Cooke (1979) also studied the antigens of klebsiellas and reported that when antisera produced in their own laboratory were compared with that produced in three other laboratories against the same type strains, few antisera were found to cross-react with the same strains. On comparing the cross-reactions within the Japanese set of type strains reported by Itoh (1972), with those found with the antisera prepared against the Japanese strains in the present study it was found that this observation also applied to *C. perfringens*.

Smith (1965) commented that the heat-sensitive strains of *C. perfringens* are 'much more diverse in their surface antigens than the heat-resistant strains'; the findings of this paper support his view. There were considerably more cross-reactions between the English heat-sensitive strains and the American strains (the majority of which are believed to be heat-sensitive) than with the heat-resistant Japanese strains.

The use of the additional 68 Japanese and American antisera resulted in only a 3% increase in the percentage of outbreaks which could be serologically typed. This rather small increase, for such a large number of antisera, seems to confirm the value of continually selecting strains for antiserum production which are strongly incriminated as the cause of outbreaks. Among the American strains from which antisera were not prepared (because they agglutinated with English sera) were serotypes which agglutinated with seven of the eight English serotypes commonly causing outbreaks. Assuming that the serotypes causing outbreaks in the U.S.A. do so at a similar frequency to those in the United Kingdom, then the existing American set of antisera should type a reasonably high percentage of outbreak strains. C. L. Hatheway (personal communication) expressed the opinion that reagents should not be eliminated from a serotyping set even if they cross-

react with other strains, the 'goal' being to identify serologically every strain of *C. perfringens* encountered. Although in principle we agree with this comment, a compromise must be made in practice to ensure that the typing set contains a 'manageable' number of antisera. Originally, it was anticipated that an enlarged set of serotypes would be established incorporating a number of Japanese and American strains enabling laboratories to serotype an increased percentage of outbreak strains (Stringer *et al.* 1976). However, at the time, it was not expected that the English set of antisera would itself be capable of typing such a high percentage of strains.

In an attempt to estimate the demand for an internationally standardized serotyping scheme, a questionnaire was sent to 120 laboratories throughout the world to (i) assess the interest in *C. perfringens* food poisoning, (ii) gather information concerning the use of existing serological typing facilities, and (iii) plan for future developments. The response of over 70 laboratories from 11 countries clearly indicated that there was considerable interest in such a scheme. The general desire was for national (or in the larger countries, regional) typing centres, holding antisera to all the known types.

The authors are grateful to Dr V. R. Dowell and Dr C. L. Hatheway, Center for Disease Control, Atlanta, Georgia, U.S.A., Dr T. Itoh, Tokyo Metropolitan Research Laboratory, Tokyo, Japan and the many Directors of Public Health Laboratories and Hospital Consultant Bacteriologists both in this country and overseas for sending the cultures that have made this study possible.

REFERENCES

Bryan, F. L. (1978). Factors that contribute to outbreaks of foodborne disease. *Journal of Food Protection* **41**, 816.

Caroli, G., Armani, G., Sciacca, A., Bargagna, M. & Levré, E. (1977). Considerazioni epidemiologiche su due episodi di tossinfezione alimentare da *Clostridium perfringens*. *Annali Sclavo* **19**, 494.

Cherniak, R. & Henderson, B. G. (1972). Immunochemistry of the capsular polysaccharides from *Clostridium perfringens*: selected Hobbs strains 1, 5, 9 and 10. *Infection and Immunity* **6**, 32.

Dowell, V. R., Torres-Anjel, M. J., Riemann, H. P., Merson, M., Whaley, D. & Darland, G. (1975). A new criterion for implicating *Clostridium perfringens* as the cause of food poisoning. *Review of Latin-American Microbiology* **17**, 137.

Edmondson, A. S. & Cooke, E. M. (1979). The production of antisera to the Klebsiella capsular antigens. *Journal of Applied Bacteriology* **46**, 579.

Elhag, K. M., Bettelheim, K. A. & Tabaqchali, S. (1977). Serological studies of *Bacteroides fragilis*. *Journal of Hygiene* **79**, 233.

Fabiansson, S. & Normark, C. (1976). *Clostridium perfringens* son matförgiftningsframkallare. *Svensk Veterinärtidning* **28**, 687.

Hauschild, A. H. W. (1975). Criteria and procedures for implicating *C. perfringens* in foodborne outbreaks. *Canadian Journal of Public Health* **66**, 388.

Hauschild, A. H. W. & Hilsheimer, R. (1974). Evaluation and modifications of media for emuneration of *Clostridium perfringens*. *Applied Microbiology* **27**, 78.

Henriksen, S. D. (1954). Studies on the Klebsiella group (Kauffmann) VI. Conclusions. *Acta Pathologica et Microbiologica Scandinavica* **34**, 281.

Hobbs, B. C., Smith, M. E., Oakley, C. L., Warrack, G. H. & Cruickshank, J. C. (1953). *Clostridium welchii* food poisoning. *Journal of Hygiene* **51**, 75.

HUGHES, J. A., TURNBULL, P. C. B. & STRINGER, M. F. (1976). A serotyping system for *Clostridium welchii* (*C. perfringens*) Type A, and studies on the type-specific antigens. *Journal of Medical Microbiology* **9**, 475.

ITOH, T. (1972). Incidence of heat resistant *Clostridium perfringens* in faeces of healthy subjects, serotyping of isolates, and food poisoning caused by new serotype organisms. *Annual Report of Tokyo Metropolitan Research Laboratory of Public Health*, no. 34. pp. 7.

MAHONY, D. E. (1974). Bacteriocin susceptibility of *Clostridium perfringens:* a provisional typing schema. *Applied Microbiology* **28**, 172.

MCCLUNG, L. S. & TOABE, R. (1947). The egg yolk plate reaction for the presumptive diagnosis of *Clostridium sporogenes* and certain species of the gangrene and botulinum groups. *Journal of Bacteriology* **53**, 139.

NAGLER, F. P. O. (1939). Observations on a reaction between the lethal toxin of *Cl. welchii* (type A) and human serum. *British Journal of Experimental Pathology* **20**, 473.

OAKLEY, C. L. & WARRACK, G. H. (1953). Routine typing of *Clostridium welchii*. *Journal of Hygiene* **51**, 102.

PAINE, C. M. & CHERNIAK, R. (1975). Composition of the capsular polysaccharides of *Clostridium perfringens* as a basis for their classification by chemotypes. *Canadian Journal of Microbiology* **21**, 181.

RAEVUORI, M. (1976). The occurrence of food poisoning outbreaks in Finland. *Suomen Elaeinlaeackaerilehti* **82**, 501.

SCHIEMANN, D. A. (1977). Laboratory confirmation of an outbreak of *Clostridium perfringens* food poisoning. *Health Laboratory Science* **14**, 35.

SMITH, L. D. (1965). *The Pathogenic Anaerobic Bacteria*. Springfield, Illinois: Charles C. Thomas Publ.

STRINGER, M. F., SHAH, N. & GILBERT, R. J. (1979). Serological typing of *Clostridium perfringens* and its epidemiological significance in the investigation of food poisoning outbreaks In *Proceedings of the Xth International Symposium on Food Microbiology and Hygiene, Szczecin, Poland*, p. 189. Academy of Agriculture, Szczecin.

STRINGER, M. F., TURNBULL, P. C. B., HUGHES, J. A. & HOBBS, B. C. (1976). An international serotyping system for *Clostridium perfringens* (*welchii*) Type A in the near future. *Developments in Biological Standardisation* **32**, 85.

SUTTON, R. G. A. (1969). The pathogenesis and epidemiology of *Clostridium welchii* food poisoning. Ph.D. thesis, University of London.

SUTTON, R. G. A. & HOBBS, B. C. (1968). Food poisoning caused by heat-sensitive *Clostridium welchii*. A report of five recent outbreaks. *Journal of Hygiene* **66**, 135.

TAYLOR, C. E. D. & COETZEE, E. F. C. (1966). Range of heat resistance of *Clostridium welchii* associated with suspected food poisoning. *Monthly Bulletin of the Ministry Health and the Public Health Laboratory Service* **25**, 142.

TODD, E. C. D. (1978). Foodborne disease in six countries – a comparison. *Journal of Food Protection* **41**, 559.

ZEN-YOJI, H. SAKAI, S., ITOH, T., MARUYAMA, T. & KUDOH, Y. (1970). Epidemiological and bacteriological examination on nine outbreaks of food poisoning due to heat-resistant *Clostridium perfringens* in Tokyo. *Journal of Food Hygienic Society of Japan* **11**, 282.