SEROLOGICAL DETECTION OF ENTEROTOXIN FROM FOOD-POISONING STRAINS OF *STAPHYLOCOCCUS AUREUS*

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THE risk of poisoning from the ingestion of food containing enterotoxin from coagulase-positive staphylococci is well known. The occurrence of more than one immunological type of staphylococcal enterotoxin has been conclusively established by several investigators (Surgalla, Bergdoll and Dack, 1953; Bergdoll, Surgalla and Dack, 1959; Casman, 1960; Bergdoll, Borja and Avena, 1965; Casman *et al.*, 1967). Classification has been based on precipitin reactions with specific antibodies, and the enterotoxins A, B, C, and D are now recognised (Casman, Bergdoll and Robinson, 1963; Bergdoll *et al.*, 1965; Casman *et al.*, 1967).

Enterotoxin A is often implicated in food poisoning (Casman *et al.*, 1967). Enterotoxin B is seldom associated with food poisoning, but is produced along with enterotoxin A by staphylococci from hospital patients suffering from enterocolitis (Surgalla and Dack, 1955; Casman, 1960). Enterotoxin C is sometimes implicated in food poisoning and seems unique in that it is consistently emetic for the monkey but variably emetic for the cat. Enterotoxin D is also associated with food-poisoning strains of staphylococci, but many of these also produce enterotoxins A, B, or C (Casman *et al.*, 1967).

The purification of enterotoxin and the demonstration of antigenicity have made possible the serological detection of enterotoxin in culture supernatants (Casman and Bennett, 1963; Donnelly *et al.*, 1967) and in foods (Hall, Angelotti and Lewis, 1963; Casman and Bennett, 1965). The serological test is more sensitive than methods involving the feeding of monkeys or parenteral injection into kittens. There are several immunological methods for detecting enterotoxin, including haemagglutination inhibition (Robinson and Thatcher, 1965), immunofluorescence (Hopper, 1963; Friedman and White, 1965), but the most widely used techniques are those employing gel-diffusion (Crowle, 1961). Comprehensive reviews on the enterotoxins have been given by Bergdoll (1967), Casman (1967), and Angelotti (1969).

This report describes the use of the slide-gel double-diffusion technique (Crowle, 1958) for the identification of enterotoxins produced by strains of *Staphylococcus aureus* involved in food-poisoning incidents in Great Britain during the period 1961–69. Cultures of coagulase-positive staphylococci not associated with food poisoning but isolated during the routine bacteriological examination of foods were also studied. In addition, relation of the phage-group to enterotoxin production was studied, in view of reports that most staphylococci implicated in food-poisoning outbreaks are lysed by group-III

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phages (Allison, 1949; Williams, Rippon and Dowsett, 1953; Parker and Lapage, 1957).

MATERIALS AND METHODS

Cultures. Prototype strains of *Staph. aureus* known to produce enterotoxins A, B, C, D, and A+B (table I) were obtained from the National Collection of Type Cultures. Each strain was grown in nutrient broth and was subcultured daily for 5 days before being tested for enterotoxin production.

One hundred and twenty-three cultures were received from Public Health Laboratories and hospitals in Great Britain. They were obtained from 36 food-poisoning incidents during the years 1961-69. In each incident there was good clinical and bacteriological evidence that staphylococcal food poisoning had occurred; *Staph. aureus* had been isolated, usually in large numbers, from one or more foods. In many cases isolations had been made from faecal specimens and in some cases from the vomit of patients, from swabs of food equipment, and from the hands and nose of food handlers.

TABLE I

Sources of prototype strains of Staph. aureus used for enterotoxin production

American Type Culture Collection no.	National Collection of Type Cultures no.	Often reported in literature as strain:	Types of enterotoxin produced
13565	10652	196E	A
14458	10654	243	B
19095	10655	137	C
23235	10656	494	D
13566	10657	S-6	A+B

Thirty-six cultures isolated from a variety of foods (cooked meat and poultry, 21; continental sausage and salami, five; sea foods, cooked or canned, six; Cheddar cheese, three; cream, one) not associated with food poisoning but submitted for routine tests were also examined for enterotoxin production. All the strains examined were coagulase-positive.

Phage-typing. The strains from food-poisoning incidents had been phage-typed in various laboratories, but for the sake of uniformity representative strains from each incident were retyped in the Cross-Infection Reference Laboratory at Colindale. Those isolated from routine food samples were also phage-typed at Colindale. The international basic set of 22 typing phages was used (Report, 1967).

Antibiotic sensitivity. Sensitivities to penicillin, streptomycin, tetracycline, erythromycin, chloramphenicol, novobiocin, and oleandomycin were determined by a disk-diffusion method with Oxoid "Multodisks" (code no. 11-14C). Undiluted broth cultures were used for the test; strains producing penicillinase were recognised either by the absence of an inhibition zone around the penicillin disk or by the presence of a characteristic small sharp-edged zone.

Sensitivity to neomycin was determined with a separate 5- μ g neomycin disk on four cultures lysed only by phage 85.

Control enterotoxins and antisera. Enterotoxins A, B, C, and D, all partially purified, and their specific antisera were kindly supplied by Professor M. S. Bergdoll (A, B, and C) and by Dr E. P. Casman (D). The enterotoxins and antisera of A, B, and C were diluted in 0.02M phosphate buffer in 0.85 per cent. saline, pH 7.3, containing 1 in 5000 thiomersal, and those of D were diluted in Difco Brain-Heart infusion (BHI) in 0.85 per cent. saline.

Dilutions of the enterotoxins and antisera were distributed into sterile ampoules and stored at -20°C. Reagents for daily use were stored at 4°C for not more than 7 days.

Production of enterotoxin. Five methods were compared for yield of enterotoxin. Brain-

Heart infusion (BHI) or BHI solidified with 1.0 per cent. agar (Davis) was used as the growth medium. The first and second methods were based on the Cellophane-sac culture techniques of Casman and Bennett (1963) and of Donnelly *et al.*; in the third method, plates containing solid media covered with Cellophane (Hallander, 1965) were used; in the fourth, semi-solid BHI plates were used (Casman and Bennett, 1963), and in the fifth, single-strength BHI in screw-capped 1-oz. (25-ml) universal containers.

Of the five methods used, only the Cellophane-sac cultures yielded large amounts of enterotoxin. The method of Donnelly *et al.* was chosen rather than that of Casman and Bennett (1963) because the sac-culture assemblies were easier to prepare and inoculate, and the crude enterotoxin produced was larger in volume and easier to recover. The sac-culture assembly was made from dialysis tubing, 2.7 cm wide. A 50–55-cm length of tubing was washed thoroughly in distilled water and tightly knotted at one end; 50 ml of double-strength BHI was put in the sac and the open end was knotted. The sac was then put in a 250 ml Erlenmeyer flask so that it rested on the bottom, and the flask was autoclaved at 121°C for 15 min.

Cultures were incubated on two nutrient-agar slopes at 35°C for 24 hr. The growth from the two slopes was harvested in 2 ml of buffered saline (0.02M phosphate buffer in 0.85 per cent. saline); this volume of suspension and another 8 ml of the same buffered saline were added to the flask to provide an inoculum of 10 ml outside the sac of medium. Several sac-culture assemblies were prepared and inoculated at one time and were incubated at 35°C for 48 hr. The liquid culture surrounding the sac was removed from the flask and was centrifuged at 4200*g* for 30 min. The supernatant fluid was preserved with 1 in 5000 thiomersal and stored at 4°C. This sac-culture method confined the staphylococcal cells and the enterotoxins to a small volume (10 ml) although the cells were nurtured by a large volume of medium (50 ml).

Gel immunodiffusion tests for enterotoxin. A slight modification of the slide-gel double-diffusion test (Crowle, 1958; Zehren and Zehren, 1968) was used for the detection of enterotoxin. A test area was prepared on clean agar-coated slides between two parallel double layers of white tape (Nopitape Ltd, St Peter's Road, Huntingdon) set 2 cm apart on the slide. This area was filled with a 10:7 mixture of 0.5 per cent. agarose (Koch-Light Labs. Ltd, Colnbrook, Bucks) in 0.85 per cent. saline and 0.04M phosphate buffer in 0.85 per cent. saline, pH 7.3, containing 1 in 5000 thiomersal, at 50°C, and immediately covered by a Perspex template (2.5 × 2.5 × 0.3 cm) with four funnel-shaped wells surrounding a fifth central well. The distance between the centres of the central and each peripheral well was 4 mm. When the agarose-buffer mixture had solidified, three of the outer wells were filled with dilutions of a culture supernatant, the fourth outer well with a reference enterotoxin, and the central well with reference antiserum of the corresponding type. About 0.025 ml was required to fill each well to convexity. At least six slides were prepared for each culture supernatant, i.e., two for each of the reference enterotoxins A, B, and C. There was a shortage of enterotoxin D and antiserum and it was possible to test only a few culture supernatants for D. The slides were incubated in moist chambers at room temperature for at least 48 hr, and were then stained and examined. The presence of one or more of the enterotoxins A, B, C, and D in an unknown culture supernatant was verified by the coalescence of its precipitation line with the reference line of the corresponding type. To enhance the lines of precipitation and so increase the sensitivity of the test, a staining procedure was used (Crowle, 1958; Casman *et al.*, 1969). Slides were consecutively immersed for 10 min. in: distilled water (twice), 0.1 per cent. thiazine red (G. T. Gurr, Ltd, London, S.W.6) in 1 per cent. glacial acetic acid, and three washes in 1 per cent. glacial acetic acid. Excess fluid was drained off and the slides were dried at 35°C.

The formation of an optimal line of precipitation, mid-way between the two reactant wells and visible to the naked eye, required 2.5 µg per ml of enterotoxins A, B, or C and a 1 in 25 dilution of antiserum A, or a 1 in 75 dilution of antisera to B or C respectively. The reaction for enterotoxin D was optimal at a 1 in 20 dilution of enterotoxin and a 1 in 20 dilution of antiserum. A faint but just visible line of precipitation was formed at a toxin concentration of 0.6 µg per ml for enterotoxins A and C and 0.3 µg for toxin B.

TABLE II

*Comparison of methods for the production of staphylococcal enterotoxin A**

Method	Approximate concentration of enterotoxin and the greatest dilution giving precipitin lines † after incubation at 35°C for					
	24 hr		48 hr		72 hr	
	µg per ml	dilution	µg per ml	dilution	µg per ml	dilution
Sac culture (Casman and Bennett, 1963)	48	1 in 80	60	1 in 100	48	1 in 80
Sac culture (Donnelly <i>et al.</i> , 1967)	48	1 in 80	60	1 in 100	36	1 in 60
Plate method (Hallander, 1965)	<0.6	not found	<0.6	not found	<0.6	not found
Plate method (Casman and Bennett, 1963)	1.2	1 in 2	0.6	undiluted	0.6	undiluted
Single-strength BHI in universal containers	1.2	1 in 2	0.6	undiluted	0.6	undiluted

* *Staph. aureus* used was strain no. 3 (table IV), isolated from chicken implicated in a food-poisoning incident.

† 0.6 µg per ml just detectable.

TABLE III

*Comparison of methods for the production of staphylococcal enterotoxin B**

Method	Approximate concentration of enterotoxin and the greatest dilution giving precipitin lines † after incubation at 35°C for					
	24 hr		48 hr		72 hr	
	µg per ml	dilution	µg per ml	dilution	µg per ml	dilution
Sac culture (Casman and Bennett, 1963)	120	1 in 400	300	1 in 1000	240	1 in 800
Sac culture (Donnelly <i>et al.</i> , 1967)	240	1 in 800	300	1 in 1000	240	1 in 800
Plate method (Hallander, 1965)	0.6	1 in 2	0.6	1 in 2	0.6	1 in 2
Plate method (Casman and Bennett, 1963)	6	1 in 20	15	1 in 50	6	1 in 20
Single-strength BHI in universal containers	1.5	1 in 5	1.5	1 in 5	0.6	1 in 2

* *Staph. aureus* used was strain no. RJG/12, isolated from a patient in Sweden (Dornbusch, personal communication).

† 0.3 µg per ml just detectable.

RESULTS

Comparison of methods for the production of enterotoxin

Of the five methods used, only the Cellophane-sac-culture methods yielded large amounts of enterotoxin after incubation at 35°C for 24–72 hr. A strain producing enterotoxin A yielded 48–60 μg per ml of enterotoxin (table II), and a strain producing enterotoxin B yielded 120–300 μg per ml (table III). The optimal incubation period was 48 hr.

Examination of cultures from food-poisoning incidents

The supernatant fluids from sac-cultures of 123 strains of *Staph. aureus* isolated from 36 food-poisoning incidents were all tested for enterotoxins A, B, and C. Most isolates negative for enterotoxins A, B, and C were also tested for enterotoxin D.

Enterotoxins A, B, C, or D were produced by cultures from 33 of the 36 incidents (92 per cent.). Of the 123 cultures, 87 produced enterotoxin A, four produced D, three produced C, three produced C and D, and one produced A and B. An additional seven cultures came from incidents in which cultures of the same phage pattern were shown to produce enterotoxin D, but these were not tested because of the shortage of antiserum. Eight cultures not producing enterotoxin had phage-typing patterns different from other cultures from the same incident which produced enterotoxin. The remaining ten cultures were from three incidents and enterotoxins A, B, C, or D were not demonstrated.

On completion of the phage-typing and enterotoxin tests an analysis of the results indicated the strain most probably responsible for each incident. A single strain from the food apparently implicated was chosen to represent each of the 36 food-poisoning incidents. The details of the incidents and features of the representative strains are shown in table IV.

Enterotoxin A was produced by the representative strains from 25 of the 36 incidents and enterotoxins A and B by a strain from one other incident (table IV). Enterotoxin C was produced by two strains and enterotoxin D by three strains; two strains produced both enterotoxins C and D. All 26 of the strains that produced enterotoxin A were lysed by phages of group III, but eight of them were also lysed by one or more of the group-I phages (table V). The phage-typing patterns of strains producing enterotoxins C and D were less uniform. Only one was lysed exclusively by group-III phages, four were lysed by phages of groups I and III, one was lysed by phage 42D only and was thus a member of phage-group IV, and one, classified as "miscellaneous", was lysed by phage 187. It is unfortunate that more phage-group IV strains were not available for examination. This group is rarely isolated from human sources, but is common in cattle, and has been associated on several occasions with staphylococcal food poisoning attributable to dairy products (Allison; Epsom, 1964).

Enterotoxins A, B, C, and D were not produced by three of the representative

TABLE IV
Enterotoxin production, phage-typing results, and other information from 36 incidents of staphylococcal food poisoning in Great Britain

Incident number	Year	Number of persons affected	Food responsible	Details of <i>Staph. aureus</i> strains isolated			
				Enterotoxin* produced	Phage group	Phage-typing pattern†	Antibiotic resistance pattern
1	1963	15	Tongue, corned beef, or both	A	III	6/42E/47/53/54/75/83A/85/+	P
2	1967	61	Ham	A	III	6/42E/47/53/54/75/83A/81	S
3	1968	18	Chicken and ham	A	III	6/42E/47/53/54/75/83A/81	P
4	1968	6	Hollandaise sauce	A	III	6/42E/47/53/54/75/83A/81	P
5	1968	37	Creamed potato	A	III	6/42E/47/53/54/85/+	P
6	1968	83	Corned beef	A	III	6/42E/47/53/54/75/83A/85/81/+	P
7	1969	60	Cheese salad	A	III	42E/47/53/54/83A	P
8	1969	2	Pork pie	A	III	6/42E/47/53/54/75/85/81	S
9	1969	4	Ham	A	III	6/42E/54/+	P
10	1969	30	Chicken§	A	III	6/42E/47/53/54/75/83A/81/+	P
11	1969	15	Pork pie	A	III	42E/47/53/75/85/+	P
12	1969	25	Chicken, custard, or both	A	III	6/42E/47/53/75/77/83A/85	P
13	1969	87	Peas (canned)	A	III	6/42E/47/53/54/85/81	P
14	1969	50	Tongue	A	III	42E/47/53/75/85/+	P
15	1969	3	Ham	A	III	85	M
16	1969	4	Pork pie	A	III	85	P
17	1969	6	Tongue, ham, or both	A	III	85	M
18	1962	9	Custard pastry	A	I/III	29/6/42E/47/53/54/75/83A/+	P
19	1967	6	Ham	A	I/III	29/52/52A/80/6/42E/47/53/54/75/83A/85/81/+	S
20	1968	2	Ham	A	I/III	29/79/6/42E/47/53/54/75/85/+	P
21	1968	5	Chicken	A	I/III	29/52/52A/79/80/42E/53/54/75/77/84/81	P
22	1968	63	Trifle	A	I/III	29/53/83A/85/+	P
23	1968	50	Chicken	A	I/III	29/52A/80/6/42E/47/53/54/75/77/83A/84/85/81/+	P

TABLE IV—continued

Incident number	Year	Number of persons affected	Food responsible	Details of <i>Staph. aureus</i> strains isolated			
				Enterotoxin* produced	Phage group	Phage-typing pattern†	Antibiotic resistance pattern
24	1969	3	Brawn	A	I/III	79/42E/53/83A/85	S
25	1969	38	Tongues, beef and ham	A	I/III	29/80/6/42E/47/53/54/75/83A/81/+	P
26	1969	1	Prawns (canned)§	A and B	III	6/42E/47/54/+	P
27	1968	2	Corned beef	C	I/III	NT (29/77)	P
28	1968	2	Sardines (canned)	C	Misc.	187	P
29	1969	63	Chicken and ham	D	I/III	29/6/42E/47/53/54/75/83A/81/+	S
30	1969	3	Pork luncheon meat	D	I/III	29/52A/79/80/53/77/83A/84/85/+	S
31	1961	135	Cheese (imported Cheddar)	D	IV	42D	P
32	1962	5	Ham	C and D† (very weak)	III	53/85	S
33	1962	14	Chicken	C and D†	I/III	NT (29/52A/80/42E/47/53/54/75/81/+)	P
34	1967	3	Corned beef	not found	III	42E/53/54/84/+	P
35	1969	43	Prawns (frozen)	not found	III	85	P
36	1969	21	Chicken; ham or sausage rolls or both	not found	I/III/IV	52A/79/80/42E/47/53/54/75/77/83A/84/85/81/42D	P

* Tests for enterotoxin D were not made on all strains.

† Phage-typing pattern at routine test dilution $\times 1000$ given in brackets; NT = non-typable.

‡ Dr E. P. Casman (personal communication).

§ Enterotoxin detected in the food (Gilbert and Šimkovičová, in preparation).

|| P = Produces penicillinase but sensitive to antibiotics other than penicillin tested; M = produces penicillinase and resistant to streptomycin, tetracycline, erythromycin, and neomycin; S = sensitive to all antibiotics tested: penicillin, streptomycin, tetracycline, erythromycin, chloramphenicol, novobiocin, and oleandomycin.

strains. Two strains belonged to phage-group III and one to phage-groups I/III/IV.

In the antibiotic-sensitivity tests, 29 of the 36 representative strains gave results characteristic of penicillinase production (table IV). In 12 of these the small zones obtained were typical of cultures producing relatively small quantities of the enzyme. Penicillinase production has previously been found characteristic of many phage-group III strains associated with food-poisoning outbreaks, even when isolated before 1941 (Parker and Lapage). Only two of our strains showed resistance to antibiotics other than penicillin; these were lysed by phage 85 only and showed resistance to streptomycin, tetracycline,

TABLE V
Relation between phage group and enterotoxin production of strains of Staph. aureus from food poisoning incidents in Great Britain

Phage group	Number* of strains	Number producing enterotoxin	Number of strains producing enterotoxin					
			A	B	C	D†	A+B	C+D
III	21	19	17	0	0	0	1	1
I/III	12	12	8	0	1	2	0	1
IV	1	1	0	0	0	1	0	0
I/III/IV	1	0	0	0	0	0	0	0
Misc.	1	1	0	0	1	0	0	0
Totals	36	33	25	0	2	3	1	2

* One representative strain from each incident.

† Only a few strains were tested for enterotoxin D.

erythromycin, and neomycin in addition to penicillinase production. Cultures with the phage-typing pattern 85 when encountered in hospitals also typically show multiple antibiotic resistance (Jevons, John and Parker, 1966). In neither of the incidents from which these two strains came was there any apparent direct contact with hospitals.

Examination of cultures from foods not associated with food poisoning

The supernatant fluids from sac-cultures of 36 strains of *Staph. aureus* isolated from 36 foods not associated with food poisoning were tested for enterotoxins A, B, and C. Of these, 13 produced enterotoxin (table VI); six produced enterotoxin A, five produced enterotoxin B, and two produced enterotoxin C. The six cultures producing enterotoxin A were lysed by phages of group III and with two cultures reactions were also obtained with phages of group I. Of the five cultures producing enterotoxin B, two were lysed by phages of group III, one was lysed by phages of group II, and two were non-typable with the phages used. Of the two cultures producing enterotoxin C, one was lysed by phages of group II and one was non-typable. Several cultures that were lysed by phages of group III did not produce enterotoxins A, B, or C.

DISCUSSION

In England and Wales, staphylococcal food poisoning is less common than salmonella and *Clostridium welchii* food poisoning. However, in the USA it continues to be the most common type of food poisoning reported, and in 1968 accounted for nearly one-quarter of all incidents and one-quarter of all cases (World Health Organization, 1969).

Not all strains of coagulase-positive staphylococci produce enterotoxin. Nearly all enterotoxigenic strains are coagulase-positive, but apparently there is no correlation between enterotoxigenicity of food-poisoning strains and production of other toxins or virulence factors (Angelotti, 1969).

TABLE VI

Relation between phage group and enterotoxin production of Staph. aureus from food not associated with food poisoning

Phage group	Number of strains	Number producing enterotoxin	Number of strains producing enterotoxin		
			A	B	C
I	3	0	0	0	0
II	3	2	0	1	1
III	9	6	4	2	0
I/III	12	2	2	0	0
III/IV	1	0	0	0	0
Non-typable	8	3	0	2	1
Totals	36	13	6	5	2

One of the aims of the present work was to determine whether the detection of enterotoxins produced by strains of *Staph. aureus* from food-poisoning outbreaks was sufficiently rewarding to justify its use as a routine test in some Public Health Laboratories. The sac-culture method for producing enterotoxin and the slide gel-diffusion method for identification were found to be useful for this purpose.

The results from the comparison of methods for the production of enterotoxin showed that relatively large amounts of enterotoxin A were produced when a sac-culture technique was used with a strain from one of the food-poisoning incidents. Although no attempt was made to obtain quantitative data for the production of enterotoxin by other food-poisoning strains, clear lines of precipitation were obtained in the slide gel-diffusion tests when dilutions of all the culture supernatants from enterotoxigenic strains were used.

Bacteriological evidence such as the isolation of large numbers of *Staph. aureus* from suspected food, faeces, or vomit, is essential for confirmation of outbreaks of staphylococcal food poisoning (Parker, 1953). Phage-typing enables strains of *Staph. aureus* from suspected food, victims of food-poisoning outbreaks, and suspected food-handlers to be correlated. The present work shows that the enterotoxigenicity of strains can also be determined. It is

possible to detect enterotoxin in foods causing outbreaks (Casman and Bennett, 1965; Zehren and Zehren, 1968), but the technique is tedious and involves the extraction, purification, and concentration of a small amount of enterotoxin, approximately 1–10 μ g, from 100 g of food. Preliminary attempts to do this have been rewarding (Gilbert and Šimkovičová, in preparation), enterotoxin A being detected in two foods from separate incidents and enterotoxins A and B in canned prawns from another incident (table IV). The detection of enterotoxin in food is especially important when cooking or other processing treatment has killed the organisms but left the enterotoxin still active.

All the food-poisoning strains examined were obtained from outbreaks in Britain that had been carefully investigated. Enterotoxins A, B, C, or D were produced by strains from 33 of 36 incidents (92 per cent.); the same enterotoxins were produced by strains from 77 of 80 incidents (96 per cent.) in the USA (Casman *et al.*, 1967). Enterotoxin A is the predominant type causing food poisoning in both countries. In view of the findings of Casman *et al.* (1967) that many food-poisoning strains produced enterotoxins A and D, it seems probable that many of the strains yielding enterotoxin A in Britain also produce enterotoxin D. Enterotoxin B was found only once in supernatants from the food-poisoning strains.

Of 36 cultures isolated from a variety of routine food samples, 13 produced enterotoxins A, B, or C. With one exception, the enterotoxigenic staphylococci were few (<500 per g) and were isolated only from enrichment cultures. A marked reduction in the number of staphylococci may occur in cheddar cheese during storage and curing (Takahashi and Johns, 1959; Jezeski *et al.*, 1961), and the number found in retail cheese samples may represent only a small fraction of those present when the cheese was made. It was disturbing, therefore, to find that one sample of imported Cheddar cheese contained 550,000 *Staph. aureus* per g. Cultures from this cheese produced enterotoxin B.

The results show that a staphylococcus incriminated in food poisoning is most likely to be lysed by phages of group III. However, enterotoxin studies on staphylococci from foods not associated with food poisoning indicate that it is impossible to predict whether a particular strain belonging to phage-group III is capable of producing enterotoxin. In both series of cultures, from food-poisoning incidents and from food not associated with food poisoning, producers of enterotoxin A were invariably lysed by phages of group III, but some were also lysed by group-I phages. On the other hand, some producers of other enterotoxins were found among members of phage-groups II and IV, in the "miscellaneous" group, and among untypable strains.

SUMMARY

Cultures of *Staphylococcus aureus* from 36 food-poisoning incidents in Great Britain during the period 1961–69 were examined for their ability to produce enterotoxins A, B, C and, in some tests, enterotoxin D. Enterotoxin was produced by means of a sac-culture technique and detected serologically by the slide gel double-diffusion method.

Enterotoxins A, B, C, or D were demonstrated from strains from 33 of 36 incidents (92 per cent.) and in some instances two enterotoxins were produced by one strain; enterotoxin A was the predominant type. Strains from 34 of 36 (94 per cent.) were lysed by phages of group III and in 13 of these incidents reactions were also obtained with phages of group I.

Of 36 cultures of *Staph. aureus* isolated from a range of routine food samples not associated with food-poisoning incidents, 13 produced enterotoxins A, B, or C.

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