SHORT ARTICLES

VIRULENCE OF *STAPHYLOCOCCUS AUREUS* TESTED BY INTRACEREBRAL INOCULATION INTO MICE

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In preceding papers (Torres Pereira, 1961, 1965, 1967) attention has been drawn to the nearly general occurrence of the phenomenon of antigen-loss variation in recently isolated strains of *Staphylococcus aureus* and to the correlation between the antigenic structure and the virulence of the strains. It was suggested that antigen-loss variation is accompanied by a weakening of other biological characters and that the different changes are collectively responsible for the diminution of virulence.

Elek (1959) reviewed the animal-inoculation techniques used since the beginning of the century to demonstrate virulence in strains of *Staphylococcus*. Inoculation has been done by the intrapleural, intradermal, intra-articular and intravenous routes in rabbits and guinea-pigs, and by the intravenous and intramuscular routes in mice. Although Dumas (1914) considered mice the best and the cheapest animal model, Elek regarded the use of mice for testing virulence as no more than a makeshift.

In tests of virulence we have injected strains into mice by the intracerebral and intraperitoneal routes. The present contribution describes an assessment of the value of intracerebral inoculation as a method for testing virulence.

MATERIAL AND METHODS

Strains of Staphylococcus aureus. Strain 25542, which was coagulase-positive and had been isolated from pus, was selected for intracerebral inoculation into mice. The form of the strain as originally isolated contained antigen 17, but not antigen 1, as determined by agglutination tests with type-specific sera (Torres Pereira, 1961), i.e., it was 17+ 1-. Variant 17- 1+, which lacked antigen 17 but contained antigen 1, was obtained after 30 daily subcultures on nutrient agar slants; it was cloned once by plating and subculturing from a single colony and at the time of the intracerebral inoculations a total of at least 150 subcultures had been made. The 17+ 1- culture, which had been preserved by refrigeration, and its 17- 1+ variant were tested in parallel for virulence in mice. In addition 100 recently isolated coagulase-positive strains from purulent lesions, sputum, nose and throat were also tested, each strain in a group of six mice.

Mice. Albino 3-mth-old mice of either sex, weighing about 22 g, were used.

Intracerebral virulence test. The staphylococci were cultured on nutrient agar for 18 hr at 37°C. The cocci were suspended in saline and, after turbidimetric measurement, a dilution of the suspension was plated for viable counting and used for inoculation of mice. The experiment was discarded whenever the number of viable cocci (colony-forming units) was not close to 10⁹ per ml. The volume injected intracerebrally was $\frac{1}{32}$ ml of the diluted suspension and contained about 30 × 10⁶ colony-forming units. In some experiments the animals were observed hourly for the first 24 hr after inoculation, but normally deaths were recorded after 24 and 48 hr. Many animals were given a further two, three or more intracerebral injections at intervals ranging from 5 to 15 days.

Preparation and assay of alpha-haemolysin. Alpha-haemolysin was prepared by centrifugation of staphylococcus cultures grown for 3 days in Todd-Hewitt broth in an atmosphere

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containing 30 per cent. CO₂. The supernates were titrated for haemolytic activity against a 2 per cent. v/v suspension of rabbit erythrocytes.

TABLE I

Lethality of antigenic variants 17⁺ 1⁻ and 17⁻ 1⁺ of Staphylococcus aureus strain 25542 for mice given an intracerebral inoculation of 30 × 10⁶ colony-forming units. (Cumulative results of ten experiments)

Antigenic variant of <i>Staph. aureus</i> injected intracerebrally	Number of mice	
	given the injection	dead within 48 hr
17 ⁺ 1 ⁻	172	141
17 ⁻ 1 ⁺	62	15

$$\chi^2 = 68 \text{ for 1 d.f.; } P < 0.01.$$

RESULTS

Intracerebral inoculation in mice. Table I shows the results obtained with the strain 25542 before and after antigenic-loss variation had taken place. The variant containing antigen 17 showed considerably greater virulence than the variant that lacked it and contained instead antigen 1.

Conditions under which the infected mice died. Mice that did not die within 48 hr generally survived. When this phenomenon was analysed it was found that mice never died in less than 7½ hr. Table II shows the time to death in mice given injections of strain 25542. It

TABLE II

Time of death in mice given an intracerebral inoculation of Staph. aureus strain 25542 (17⁺ 1⁻). (Cumulative results of four experiments)

Period (hr) after intracerebral inoculation	Number of mice dying in period
0-6½	0
6½-7½	14
7½-8½	7
8½-9½	11
9½-10½	2
10½-11½	8
11½-12½	1
12½-13½	1
13½-20	3
20-25	2
Total (0-25)	49

will be seen that 42 out of a total of 49 mice died in the 4-hr period between 7½ and 11½ hr after inoculation.

Measurement of virulence of 100 recent isolates of Staphylococcus aureus. Each of 100 recently isolated strains of *Staph. aureus* was injected in doses of 36 × 10⁶ colony-forming

units into groups of six mice, and deaths were recorded at 48 hr. Alpha-haemolysin was also prepared from each strain. The results are shown in table III. A considerable proportion of the strains isolated from the nose or throat failed to kill any mice, whereas none of the strains from pus failed to do so. Moreover, none of the strains from nose or pharynx killed 6 out of 6 mice, whereas three strains from pus killed 6 out of 6.

DISCUSSION

The results show that a strain of *Staphylococcus aureus* containing antigen 17 was more virulent for a mouse into which it was injected intracerebrally than a strain lacking antigen 17

TABLE III

Classification of 100 recently isolated strains of Staph. aureus according to the number of deaths they caused on intracerebral inoculation in a group of six mice, their source, and the titre of alpha-haemolysin they produced after 3 days' culture in Todd-Hewitt broth

Number of mice dying in group of six given inoculation	Number of strains (out of 100) causing stated number of deaths										
	Total	and having been isolated from					and giving alpha-haemolysin titre of				
		pus	sputum	nose	throat	other source	80	40	20	10	<10
6	4	3	0	0	0	1	1	2	1	0	0
5	13	2	1	7	1	2	8	5	0	0	0
4	17	1	3	9	3	1	7	7	2	1	0
3	17	1	1	11	3	1	6	4	2	2	3
2	20	3	3	13	0	1	3	7	6	3	1
1	11	1	0	7	3	0	0	1	2	2	6
0	18	0	1	15	2	0	0	2	2	4	10

after antigen-loss variation. Although it is possible that antigen 17 functions as a phagocytosis-inhibiting antigen, the short time, $7\frac{1}{2}$ – $11\frac{1}{2}$ hr, between inoculation and death suggests that death is caused by a lethal toxin, and it has been suggested that strains with antigen 17 produce larger amounts of lethal toxin than strains with antigen 1 (Torres Pereira, 1967).

Gorrill (1951), Selbie and Simon (1952), Smith and Dubos (1956), Noble (1966) and others have tried to assess virulence for the mouse by very different criteria. We believe that our experiments indicate that a standardised technique of intracerebral inoculation is a useful test of virulence in mice, though the relevance of the pathogenic factors measured to human infection is uncertain. In the experiment shown in table III only 18 out of 100 strains failed to kill any mice and were classified as avirulent. The number of mice dying in each group of six mice challenged by injection of a strain indicates the degree of virulence of the strain. The most highly virulent strains, four out of the 100 studied, killed 6 out of 6 mice.

The distribution of the strains according to their clinical source seemed to show that the degree of virulence was related to the source, since none of the strains from pus failed to kill some of the mice, whereas a number of strains from the nose and throat did fail to do so. There appeared also to be a correlation between the number of mice killed by a strain and the titre of alpha-haemolysin it produced in broth culture. Virulence is not an absolute attribute of any strain, and fully virulent strains may not cause staphylococcal disease unless the factors responsible for specific and non-specific resistance have been depressed.

Basic studies on the biology of staphylococci are indispensable, but they tend to focus attention to an undue extent on the micro-organismal aspects of the host-parasite relation.

SUMMARY

When tested by intracerebral injection in mice, a strain of *Staphylococcus aureus* containing antigen 17 was found to be much more virulent than a variant that lost this antigen and contained antigen 1 in its place. Since 85.7 per cent. of the mice challenged died between 7½ and 11½ hr after inoculation it is suggested that a toxin was the cause of death.

A standardised test by intracerebral inoculation is considered to be a useful test of virulence in mice. Out of 100 recently isolated coagulase-positive strains assessed by this method, only 18 were considered to be avirulent. The degree of virulence was correlated with the clinical source of the strains.

REFERENCES

- DUMAS, M. J. 1914. *Annls Inst. Pasteur, Paris*, **24**, 213.
 ELEK, S. D. 1959. *Staphylococcus pyogenes*, and its relation to disease, London, pp. 333-343.
 GORRILL, R. H. 1951. *Br. J. Exp. Path.*, **32**, 151.
 NOBLE, W. C. 1966. *J. Path. Bact.*, **91**, 181.
 SELBIE, F. R., AND SIMON, ROSEMARY D. 1952. *Br. J. Exp. Path.*, **33**, 315.
 SMITH, J. M., AND DUBOS, R. J. . . . 1956. *J. Exp. Med.*, **103**, 87.
 TORRES PEREIRA, A. 1961. *J. Path. Bact.*, **81**, 151.
 „ 1965. *Int. Bull. Nomencl. Taxon.*, **15**, 117.
 „ 1967. *Int. J. System. Bact.*, **17**, 395.

ENDOCARDITIS DUE TO *HAEMOPHILUS PARAINFLUENZAE*

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ALTHOUGH a variety of micro-organisms may be implicated in infective endocarditis, viridans streptococci remain responsible for more than 50 per cent. of all cases and are the aetiological agent in 70-80 per cent. of cases of subacute bacterial endocarditis (Cates and Christie, 1951; Wedgewood, 1955; Morgan and Bland, 1959; Friedberg, Goldman and Field, 1961; Wilson, 1963; Lerner and Weinstein, 1966). Bacterial endocarditis due to Gram-negative bacilli of the *Haemophilus* group occurs relatively infrequently. In an extensive review covering the period from 1935 to 1948, Jones (1950) found only 25 cases caused by *Haemophilus* species, and these organisms were responsible for only nine of 1324 cases recorded by Keith and Lyon (1963).

Insufficient cases of haemophilus endocarditis have been reported in the antibiotic era to allow the proper formulation of optimum treatment of this type of infection (Garrod and O'Grady, 1968). We describe in this paper a case of bacterial endocarditis due to *Haemophilus parainfluenzae*.

CASE REPORT

A young man of 17 yr was rejected for service in the Royal Air Force because he had a heart murmur. He had no undue breathlessness, and remained in excellent health for 3 yr when he developed fever, sweating, and pain in his limbs. He received penicillin with temporary improvement, but later obtained no benefit from tetracycline or salicylate. After 3 wk he was referred for hospital treatment.

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