MOUSE-VIRULENCE OF STRAINS OF *SALMONELLA TYPHI* FROM A MILD AND A SEVERE OUTBREAK OF TYPHOID FEVER

By H. T. FINDLAY (formerly in charge of the Army Vaccine Laboratory)

Intraperitoneal inoculation of the mouse has been employed for many years as a means of assaying the virulence of different strains of *Salmonella typhi* or of detecting alterations in virulence in the same strain occurring from time to time in the laboratory. Such changes are usually in the nature of loss of virulence resulting from antigenic change. Occasionally, however, there may be increase of virulence, either as a result of mouse passage or simply from a more strict observance of nutritional requirements and improvements in the method of cultivation. Where cultures of typhoid organisms are to be used for vaccine production the detection of these antigenic changes and the associated loss of virulence is obviously of the greatest practical importance.

Any change in the Vi- and O-antigen content of the cultures of a degree sufficient to cause diminution of mouse-virulence is detectable by a number of *in vitro* tests, i.e. alteration in O-inagglutinability and Vi-agglutinability (Felix & Pitt, 1934; Bensted, 1937), susceptibility to bactericidal and phagocytic action of the blood of a variety of animal species (Felix & Bhatnagar, 1935) and to the action of Vi bacteriophage. Nevertheless, the fact that the mouse is not susceptible to typhoid infection in the normal manner, that is by mouth, must raise legitimate doubt whether estimations of mouse-virulence obtained by intraperitoneal inoculation bear any relationship to virulence or clinical severity in man. Further, because of the important part that virulence of the challenge organism plays in active and passive protection tests in the mouse, the value of these tests in denoting the most suitable protective antigen for man is open to question. The demonstration of correlation between virulence of the typhoid bacillus for man and for the mouse would not resolve all doubts on the value of mouse-protection tests as a guide to protection in man, but it would enhance the significance of the results of well-designed mouse-protection tests.

It is, therefore, worth recording a short series of virulence tests denoting a relationship between virulence for mice and man, particularly as Felix & Anderson (1951) have recently demonstrated that the infecting strain in an unusually mild outbreak of typhoid fever was well below the level of mouse-virulence of several of their laboratory cultures representing strains of average virulence.

The cultures of the two typhoid strains that were compared for mouse-virulence were isolated during two small outbreaks of typhoid fever, each involving about twenty cases, that occurred during the summer of 1943. In the outbreak at Richmond (Surrey) a characteristic of all the cases was their mildness and there

was no fatal case. In the outbreak at Truro (Cornwall) nearly all the cases were severe and five of the patients died. The cultures isolated from all the patients in the two outbreaks were examined by Dr A. Felix, at the Central Enteric Reference Laboratory of the Emergency Public Health Laboratory Service, and were found to belong to two hitherto unknown Vi-phage types, provisionally labelled Type Richmond and Type Truro. Subsequently, Type Richmond was designated as Vi-phage Type N and Type Truro as Vi-phage Type T, according to the internationally adopted typing scheme of Craigie & Felix (1947).

Five cultures of *Salm. typhi* isolated in June and July 1943 from cases in Richmond, and five cultures isolated from those in Truro in June–August 1943 were received from Dr Felix on 28 August 1943 and were used in the mouse-virulence tests detailed below during the first week of September 1943. The cultures had been maintained on Dorset egg-medium from the time of receipt at the Central Enteric Reference Laboratory till the day of test. All cultures were colonially smooth and stable in hypertonic salt solution (2·5 and 5 % NaCl).

Table 1. *Comparison of the mouse-virulence of* Salmonella typhi *strains from two epidemics of different clinical severity*

Strain	Isolated from	Culture received at the Central Enteric Reference Laboratory	Dose in millions of organisms	Death rates among mice: Ratio of* deaths to numbers challenged	Death rates among mice: Percentage
Epidemic origin: **Richmond**					
Harris	Faeces	30. vi. 43.	125	$\frac{0}{20}$	0
Reid	Blood	30. vi. 43.	125	$\frac{1}{20}$	5
Pini	Blood	30. vi. 43.	125	$\frac{2}{100}$	2
Reed	Urine	30. vi. 43.	150	$\frac{0}{10}$	0
Hutchinson	Blood	3. viii. 43.	150	$\frac{1}{10}$	10
Epidemic origin: **Truro**					
Col. Bridge	Blood	29. vi. 43.	125	$\frac{8}{20}$	40
Pearce	Faeces	5. vii. 43.	125	$\frac{8}{20}$	40
Pilgrim	Faeces	13. vii. 43.	125	$\frac{53}{100}$	53
Mrs Bridge	Faeces	24. vii. 43.	150	$\frac{10}{10}$	100
Etches (Carrier responsible for the outbreak)	Faeces	13. vii. 43.	100	$\frac{7}{10}$	70

* Numerator = number of mice that died; denominator = number of mice inoculated.

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METHOD OF TEST

Comparative tests were carried out on successive days in pairs—one Richmond strain being matched with a Truro strain. Three or four perfectly smooth colonies were inoculated together into nutrient broth and incubated at 37° C. for 17 hr. The centrifuged deposits were resuspended in saline, matched to the required count and against each other, by opacity. Large Swiss mice of 25 g. weight were inoculated intraperitoneally with the test dose in 0·5 ml. of normal saline. Male and female mice were distributed in equal numbers between the two groups. After inoculation, mice were kept under observation for 5 days.

From a preliminary trial the approximate LD 50 for the Truro strain was estimated at 125 million organisms and that of the Richmond strain as being greater. 125 million organisms were employed as the test dose on the first 3 days; it was raised to 150 million organisms on the fourth day. On the last test, 150 million organisms of the Richmond strain were inoculated as against 100 million of the Truro strain.

It will be seen from Table 1 that each of the strains from the clinically severe Truro outbreak was more mouse-virulent than any of the strains from the mild outbreak at Richmond.

Taken in conjunction with the observations of Felix & Anderson (1951) there are good grounds for believing that virulence of *Salm. typhi* for mice runs *pari passu* with virulence for man.

REFERENCES

Bensted, H. J. (1937). *J. R. Army med. Cps*, **68**, 1.
Craigie, J. & Felix, A. (1947). *Lancet*, **1**, 823.
Felix, A. & Anderson, E. S. (1951). *J. Hyg., Camb.* (in the press).
Felix, A. & Bhatnagar, S. S. (1935). *Brit. J. exp. Path.* **16**, 422.
Felix, A. & Pitt, R. M. (1934). *J. Path. Bact.* **38**, 409.

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