FATAL MURINE TYPHUS INFECTION IN THE dba STRAIN OF MICE, WITH OBSERVATIONS ON STRAIN VARIATION IN SUSCEPTIBILITY

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Nicolle (1) in 1925 found that European (human) typhus produced only inapparent infection in white mice. The survival of infectivity for periods up to 8 days could be demonstrated by guinea pig inoculation of brain from inoculated mice, but attempts to transmit the infection serially in mice were unsuccessful. Laigret and Jadin (2) obtained similar results, and were likewise unable to transmit the infection serially in mice by either the intraperitoneal or the intracerebral route. Savoor and Velasco (3) and also Kligler, Aschner, and Levine (4) repeated the experiments of Laigret and Jadin, and obtained the same results. Liu, Snyder, and Enders (5) found that irradiated white mice developed a highly fatal rickettsial peritonitis when injected intraperitoneally with the Breinl strain of European typhus. Repeated attempts to infect normal white mice with transfer material from successive passages resulted, however, only in inapparent infection similar to that obtained by previous workers. A fatal rickettsial pneumonitis may be produced by the intranasal injection of large doses of European typhus rickettsiae (Durand and Sparrow (6)) (Durand and Giroud (7)).

With murine strains of typhus, conflicting results have been reported. Laigret and Jadin (2), using the intraperitoneal route of inoculation, and using brain tissue as the inoculum, carried out sixteen serial transfers in white mice. They also transmitted the infection serially for four generations by the intracerebral route of inoculation, again using brain tissue as the inoculum. None of their mice died or showed evidence of illness, the infection being purely of the inapparent type. Entirely similar results were obtained by Savoor and Velasco (3), and by Kligler, Aschner, and Levine (4). Other workers, however, have reported variable percentages of mortality in white mice injected with murine typhus. Wohlrab (8), for example, using brain tissue as the inoculum and injecting by the intraperitoneal route, obtained experimental disease in 76.5 per cent of his mice and death in 65 per cent. He carried the infection through 25 generations of white mice in this way. Castaneda (9) found that a highly fatal pneumonitis could be produced in white mice injected intranasally with murine typhus rickettsiae.

The experiments to be reported here may explain these conflicting results, since they show that murine typhus in mice, when the initial dosage is held

constant, may vary from purely inapparent infection to a disease with a mortality of 100 per cent, depending on (1) the strain of mouse used, and (2) relatively slight variations in the environmental temperature. This paper deals chiefly with strain variation in susceptibility, while the effect of environmental temperature is discussed in the following paper.

Material and Methods

The strain of typhus used was a typical murine strain originating in Mexico. Mice were originally injected either with a suspension of scrotal sac exudate from an infected guinea pig or with a suspension of infected yolk sac from fertile hens' eggs. Transfer from mouse to mouse was carried out with 10 per cent saline emulsions of brain, liver, or spleen from infected mice. Mice were injected intracranially with 0.03 cc. of inoculum and intraperitoneally with 0.5 cc.

The strains of mice used were the following: dba, brown agouti, Swiss, and A albino. The dba and A albino strains are inbred strains obtained from the Roscoe B. Jackson Memorial Laboratory at Bar Harbor, Maine. The brown agouti strain was developed by Dr. N. Wade in this School in 1939, through the crossing of a wild mouse and a Wistar albino. The Swiss mice were pen-mated.

Mice found dead or moribund were studied for the presence of rickettsiae by Giemsa-stained smears, and in some cases paraffin sections, in order to determine whether death was caused by typhus or by some other factor.

Since the primary purpose of this paper is to show the variable results obtained in different strains of mice, we have included only parallel experiments in which mice of different strains were injected with identical amounts of the same inoculum, and maintained simultaneously under the same environmental conditions.

Intracerebral Inoculation

In the initial experiment, 7 dba mice and 9 Swiss mice were inoculated intracerebrally with 0.03 cc. of a suspension of exudate from a guinea pig's tunica vaginalis. All of the dba mice suddenly developed experimental disease on the 4th or 5th day; 4 of them died of typhus (3 on the 5th day and 1 on the 6th day). Impression smears of the brains of moribund or dead mice showed large numbers of inflammatory cells in which neutrophiles were prominent. Numerous rickettsiae, both intra- and extracellular, were also seen. In some of the smears there were cells greatly distended with rickettsiae.

Sections of the brain, fixed in Regaud's fluid and stained by the Giemsa method, revealed a picture of meningoencephalitis, with an inflammatory exudate on the meningeal surface composed mainly of neutrophiles and mononuclear phagocytes. The same type of exudate was present in the choroid plexus, in the ependyma, and in perivascular spaces in the substance of the brain. Great numbers of rickettsiae were found in the cells of the meningeal exudate, in the ependymal lining cells, and in some of the cells in perivascular spaces.

One or 2 of the Swiss mice presented slight experimental disease, manifested by weakness and trembling, but none of them died.

The infection was passed from brain to brain in the dba strain of mice for eleven serial transfers, always reproducing the experimental disease. The transfer dose was 0.03 cc. of a 10 per cent suspension of dba mouse brain.

Of 46 dba mice injected intracerebrally in this way, 15 died with typhus, 9 were sacrificed when moribund (for transfer or study), and 22 recovered after an experimental illness. Impression smears of the brains of dead or sacrificed mice showed in every case large numbers of rickettsiae. Sections of brain from several of the dead mice also revealed large numbers of rickettsiae, and the picture of meningoencephalitis already mentioned.

Of 24 Swiss mice injected in parallel series with the dba mice, an occasional animal presented mild experimental illness; but none was severely ill, and no deaths occurred. Two Swiss mice showing mild illness were sacrificed in order to compare the brain smears with those of the dba mice; in both cases the smears showed no definite rickettsiae.

TABLE I

Mice Injected Intracranially

Room temperature range 60–80°F. (approximate)

Strain	Mice injected	Mice showing illness	Mice dying	Time of death
dba	53	53	28	5–6 days
Swiss	33	Approximately 10 per cent mild illness	0	
A albino	3	0	0	

In view of the lesser susceptibility of the Swiss mice we always used dba mice for transfer. All transfers were made between the 5th and 9th days. At different intervals during the serial transfers we inoculated guinea pigs with the transfer material; in every case these animals developed a typical temperature curve and scrotal reaction. Our series was discontinued voluntarily after eleven serial transfers, and at that time the strain was still fully virulent for guinea pigs by intraperitoneal inoculation. On the eleventh transfer, 3 A albino mice were injected intracerebrally. These mice did not develop experimental illness, while of the 4 simultaneously injected dba mice, all became seriously ill, but none died.

The results of these experiments are summarized in Table I, and it will be seen that the Swiss mice showed a morbidity (mild symptoms) of about 10 per cent as compared with 100 per cent morbidity for the dba strain, and a mortality of zero, as compared with 53 per cent for the dba strain. Because of limitation of the supply, only 3 of the A albino strain were used. The absence of morbidity or mortality in all 3, however, suggests that mice of this strain have a natural immunity, under these conditions, similar to that seen in the Swiss strain.

Intraperitoneal Inoculation

Five dba mice and 5 Swiss mice were injected intraperitoneally with 0.5 cc. of a suspension of exudate from guinea pig tunica vaginalis, and maintained at a room temperature varying between 70° and 80°F. All of the dba mice became sick and died (3 on the 4th day, 2 on the 5th day). The Swiss mice did not develop experimental disease. Peritoneal smears from all the dba mice showed large numbers of extracellular rickettsiae, many large peritoneal lining cells completely packed with rickettsiae (Mooser cells), and some neutrophiles containing moderate numbers of rickettsiae. One of the Swiss mice was sacrificed on the 5th day and showed a small number of extracellular rickettsiae and a few rickettsiae in an occasional neutrophile, but no Mooser cells were found.

TABLE II

Mice Injected Intraperitoneally

Room temperature range 70–80°F. (approximate)

Inoculum	No. and variety of mice	Mice showing illness	Mice dying	Time of death
Exudate from guinea pig tunica vaginalis (moderate dosage)	5 dba	5	5	4–5 days
	5 Swiss	0	0	1 killed on 5th day
Yolk sac suspension (massive dosage)	5 dba	5	5	3–4 days
	5 brown agouti	5	5	3–4 days
	5 Swiss	4	3	3–4 days
	5 A albino	2	0	

In a 2nd experiment, we injected 5 dba, 5 Swiss, 5 A albino, and 5 brown agouti mice intraperitoneally with 0.5 cc. of a 10 per cent suspension of yolk sac very rich in rickettsiae. All the dba and all the brown agouti mice died on the 3rd or 4th day; 3 of the Swiss mice also died, but none of the A albinos died. Smears from the peritoneal cavity of all the dead mice showed large numbers of rickettsiae and many Mooser cells. The smears from the dead Swiss mice did not show as many rickettsiae as those from the dba and brown agouti mice.

We carried the infection by intraperitoneal injection through five serial passages in dba mice, using as transfer material liver and spleen, liver alone, or brain. In all cases these mice developed experimental illness. High environmental temperatures, ranging from 85–96°F. (approximate) greatly reduced the mortality (see following paper) but mice of the dba strain injected intraperitoneally and kept at temperatures between 70° and 80°F. (approximate) invariably became ill and died between the 3rd and 7th days after injection, the time depending on the dosage inoculated.

The results of these experiments are seen in Table II. All dba mice after

intraperitoneal inoculation with heavy or massive doses died when the environmental temperature range was between 70–80°F. (approximate). The brown agouti strain with massive dosage appeared to react much like the dba strain. Three mice of the A albino strain, injected with massive doses showed no evidence of illness. In the Swiss strain no deaths occurred in 5 mice given a moderately heavy dosage, while 3 out of 5 animals died after injection with massive doses.

Further data on strain variation were obtained from a series of experiments in which mice were injected intraperitoneally with 0.5 cc. of a 10 per cent emulsion of brain from dba mice dying after intraperitoneal injection. (Such brain tissue contains fewer rickettsiae than brain tissue from mice injected intracranially.) Of 42 dba mice injected with this material, however, all died when kept at a room temperature of 65–73°F. Of 16 Swiss mice under identical

TABLE III

Mice Injected Intraperitoneally

Room temperature 65–73°F.

Inoculum	No. and variety of mice	Mice showing illness	Mice dying	Time of death
Brain tissue from intraperitoneally injected dba mouse (light dosage)	42 dba	42	42	6–7 days
	16 Swiss	10	10	6–11 days
	9 A albino	6	6	7–11 days
	5 brown agouti	3	3	6–8 days

conditions and with identical inoculum 10 died, while of 9 A albino mice 6 died, and of 5 brown agouti 3 died.

The results of these experiments are shown in Table III. Here again, it will be seen that the mortality in the dba mice was 100 per cent, while a considerable number (about one-third) of the Swiss, A albino, and brown agouti mice survived.

DISCUSSION

The reason for the strain variation in susceptibility to murine typhus which is shown in the above experiments is not known.

The occurrence of serially transmissible meningoencephalitis by intracranial injection has not previously been reported in the rickettsial diseases. This observation is of some interest in that it further emphasizes the biological similarity of rickettsial and viral diseases.

SUMMARY AND CONCLUSIONS

Injected intracranially in the dba strain of mice, murine typhus rickettsiae caused a serially transmissible meningoencephalitis similar to that produced

by psittacosis and certain other viruses. All injected mice of this strain suddenly became ill and approximately half of these animals died on the 5th and 6th days after injection. Swiss mice injected in parallel series showed illness in only a few animals, and no deaths occurred. These experiments were carried out at room temperatures ranging from 60–80°F.

After intraperitoneal injection, a uniformly fatal rickettsial peritonitis developed in all dba mice kept at environmental temperatures of 65–73°F. or 70–80°F. Death occurred between the 3rd and 7th days after injection, depending somewhat on the dosage used. Among Swiss, brown agouti, and A albino mice injected in parallel series, the mortality was less than 60 per cent.

These experiments indicate that mice of the dba strain are much more susceptible to murine typhus than are mice of the other three strains studied.

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