TYPHUS FEVER

I. Comparative Study of European and American Typhus in Laboratory Animals

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American strains of typhus (Mooser (1), Maxcy (2)) differ from European strains (Nicolle (3), Wolbach (4)) chiefly in the fact that the former produce a severe acute inflammation of the scrotal sac in laboratory animals.

In a previous paper (5), attention was called to the occurrence of a periodic scrotal sac exudate in guinea pigs inoculated with the Wolbach strain of European typhus and to the presence of *Rickettsiae*-filled cells in this exudate. It therefore seemed probable that the differences between European and American typhus are unimportant and that the two diseases are essentially identical. More detailed clinical, pathological and morphological studies have since been made on four strains of typhus—two of European origin and two of American origin. The object of this paper is to record certain interesting features which were brought out in this comparative study.

The origin of these four strains[1] was as follows:

European Strain 1 (Wolbach). Established in guinea pigs in Warsaw, Poland (1919). Maintained in this laboratory up to the present time.

European Strain 2 (Breinl). Established in guinea pigs in Prague (1928). Received in this laboratory in Oct., 1929, and maintained until Sept., 1930.

Mexican Strain (Mooser). Established in guinea pigs in Mexico (1928). Received in this laboratory in Oct., 1928, and maintained until Jan., 1930.

North Carolina (U. S. A.) Strain (Maxcy). Established in guinea pigs in Wilmington, N. C. (1928). Received in this laboratory in Sept., 1929, and maintained up to the present time.

[1] I am indebted to the originators of the various strains for the privilege of making these studies.

The differences between the four strains, and the more important points of similarity are brought out concisely in Table I.

In all four strains the characteristic brain lesions were demonstrated. In the two American strains, however, brain lesions were practically absent in male guinea pigs which had been inoculated intraperitoneally and in which the characteristic acute inflammation of the scrotal sac had occurred. In female guinea pigs (in which no marked local reaction takes place) or in male guinea pigs inoculated subcutaneously, brain lesions were usually found fairly easily, although they were not as numerous as in the European strains. In six male guinea pigs inoculated subcutaneously with a minute amount of infectious material (scrotal sac exudate, Maxcy strain) brain lesions were fully as numerous as in the European strains. In order to obtain numerous brain lesions in the American strains it would seem that we must prevent the occurrence of a severe local reaction.

In all four strains, *Rickettsiae*-laden cells were found in the scrotal sac exudate. In the two American strains (which were alike in all respects as far as could be learned) there was always a copious exudate in the scrotal sac, and *Rickettsiae*-filled cells were found easily in every case. In the Wolbach European strain, the exudate was usually slight in amount and a careful search was necessary in order to find the specific organisms. During a period of intensification of the scrotal reaction, however, infected cells were found practically as easily as in the American strains. In the Breinl strain, the exudate in the scrotal sac was always scanty, and a large number of attempts were made before the characteristic picture was found. When found, however, the appearance of the intracellular organisms did not differ from that seen in the other strains.

These two facts—the presence of characteristic brain lesions in all strains and the presence of *Rickettsiae*-filled cells in the scrotal sac in all strains, leave no doubt concerning the essential similarity of the four strains studied. As far as can be learned, all strains established from endemic sources have shown this marked local reaction in the scrotal sac of the guinea pig, while all strains established from epidemics of high mortality have, when first established, shown a mild inconspicuous scrotal reaction and more numerous brain lesions. It therefore seems justifiable to conclude that variations in the intensity of the

TABLE I

Comparative Study of Four Strains of Typhus

Strain.....	European No. 2 (Breinl)	European No. 1 (Wolbach)	Mexican (Mooser) Wilmington (Maxcy)
Incubation period	5–7 days up to 21 days with very small dose	7–10 days, up to 21 days. Reduced to 5 days with scrotal sac exudate	2–19 days depending on dose
Swelling of scrotum after intraperitoneal inoculation	Never observed definitely	Rare. Periodic. Transient (6–24 hrs.). May be induced by passage through rats	Practically constant
Scrotal sac exudate after intraperitoneal inoculation	Frequent at end of incubation period. Never copious	Practically always at end of incubation period. Often copious	Always copious
Scrotal sac adhesions	Temporary, rare. Permanent, never	Temporary, often. Permanent, rare	Usually permanent
Rickettsiae-filled cells in scrotal sac exudate	Found only in comparatively small numbers	Few to many, depending on amount of exudate present	Numerous in every case
Exudate on spleen	Frequently	In about 60% of cases	Occasionally
Rickettsiae-filled cells in exudate on spleen	Found in two preparations after long search	Rare. One or two cells found after long search	As in other strains.
Brain lesions	Constant and usually numerous	Constant and usually numerous	Frequent in female pigs. In male pigs, only after subcutaneous inoculation

scrotal reactions are correlated with strain variations in virulence similar to those shown by various members of the streptococcus group.

The behavior of the Wolbach strain of European typhus during its 12 year residence in the guinea pig is worthy of special comment. During the first 2 years after this strain was established, a large number of guinea pigs were autopsied and their scrotal sacs carefully examined. Involvement of the testes was confined to an occasional small area of superficial hemorrhage and no obvious exudate was ever found. During the next 7 years autopsies were made from time to time but a mild scrotal sac exudate might have escaped notice. No obvious involvement of the scrotal sac was noted during this period. 3 years ago, when the scrotal reaction was first noticed, as it was mild, transient, of rare occurrence and not visible externally During the past 3 years the scrotal reaction has become more pronounced and more frequent (although it still occurs only periodically). Recently the reaction has been entirely comparable to that seen in American typhus in four successive transfers, but it practically disappeared again in the fifth transfer. At present it has been practically absent for six generations (about 2 months). This European strain has therefore been definitely altered by its long residence in guinea pigs and now occupies a position intermediate between that of a recently isolated European strain and the American strains (see Table I).

The scrotal reaction in the Wolbach strain was intensified during quiescent periods, in the following way: A rat was inoculated intraperitoneally with 1 cc. of brain emulsion from a typhus-infected pig (without scrotal reaction). 14 days later the spleen and testicles of the rat were ground in a mortar and two guinea pigs were inoculated with this material. This procedure was carried out four times and in each case at least one of the guinea pigs inoculated from the rat showed a marked scrotal reaction with complete fixation of the testes in the scrotal sac. Whether this phenomenon depends on increased dosage or a true change in virulence of the organism is not clear but the following observations indicate that the latter is more probable.

On numerous occasions when a copious exudate with many *Rickettsiae* was found in the scrotal sac in the Wolbach European strain, large amounts of this exudate were injected intraperitoneally into normal guinea pigs. The percentage of strongly positive scrotal

reactions obtained by this method was no greater than in the routine transfers with heart's blood This seems to indicate that the periodic variation in the scrotal reaction in this strain does not depend on increased dosage but rather upon some unknown factor.

It has been interesting to study the incubation periods of the disease in guinea pigs in the various strains after inoculation by various routes. In the Wolbach strain, routine inoculations of 4 cc. of blood on the 3rd or 4th day of fever resulted quite regularly in an incubation period of 9 or 10 days (rarely and somewhat periodically shortening to 8 days or lengthening to 12 days). In those cases which showed a marked scrotal reaction after blood inoculation, the incubation period was usually 6 or 7 days. By inoculating with scrotal sac exudate, the incubation period was quite regularly 5 or 6 days irrespective of whether a scrotal reaction occurred. In other words, inoculation with a heavy dose of *Rickettsiae* (a generous quantity of scrotal sac exudate) decreased the incubation period markedly but did not appear to increase the intensity of the scrotal reaction.

In the Breinl strain, when transfers were made with blood (4 cc. intraperitoneally) the incubation period ranged from 5 to 7 days. On two occasions when scrotal sac exudate containing visible *Rickettsiae* in moderate numbers was used for inoculation, the incubation period was 5 days and the type of reaction did not differ from that regularly seen after intraperitoneal inoculation with blood.

In the Mexican or North Carolina strains, the incubation period when blood was used for transfer showed great variations (5 to 18 days) and a negative result was obtained much more often than with the Wolbach strain. This was undoubtedly due to the fact that the local reaction in the scrotal sac often results in partial or complete sterilization of the blood stream. When scrotal sac exudate was used for the transfer, the incubation period varied from 24 hours to 16 days, and the period could be fairly accurately predicted by making smears of the exudate and noting the number of *Rickettsia*-filled cells present. When infected cells were present in every low power field, the incubation was never less than 4 days. When a long search was necessary the incubation period was never less than 7 days and when *Rickettsiae* were not found at all, it was only on one occasion less than 10 days.

The evidence indicates that the incubation period is dependent on the number of *Rickettsiae* injected rather than on virulence factors.

SUMMARY

Study of two strains of epidemic (European) typhus and two strains of endemic (American) typhus in laboratory animals has shown their essential identity.

The characteristic typhus brain lesions can be shown to occur in all four strains after subcutaneous inoculation and *Rickettsiae*-filled cells can be found in the scrotal sac in all four strains after intraperitoneal inoculations.

A strain of European typhus, which for 8 years has yielded no obvious scrotal reaction in guinea pigs, afterwards gave rise to a periodic scrotal reaction of variable severity. At present it occupies an intermediate position in this respect between the recently isolated European strain and the American strains.

Variations in the scrotal reaction in guinea pigs appear to be correlated with strain variations in virulence in the human host.

The incubation period in guinea pigs after intraperitoneal inoculation depends largely on the number of *Rickettsiae* injected.

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