THE SURVIVAL OF VARIETIES OF TYPHUS VIRUS IN MOUSE PASSAGE, WITH PARTICULAR REFERENCE TO THE VIRUS OF BRILL'S DISEASE

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No differences between types of typhus infection were known until 1928, when Mooser (1) recognized the significance of the scrotal swellings observed by Neill (2) and found *Rickettsiae* in the tunica vaginalis of guinea pigs intraperitoneally inoculated with the Mexican variety of virus. There is no need, for our present purposes, to review the extensive literature dealing with this problem which has accumulated since the publication of Mooser's paper. At the present time, differentiation between the Mexican-American and the classical European varieties by their respective behavior in guinea pigs offers little difficulty to investigators familiar with typhus experimentation. Differences of opinion have, however, arisen within the last few years regarding the relationship between the two types. The point at issue has been whether the observed discrepancies represented temporary modifications of a single type, comparable to the reversible dissociations of bacteria and dependent upon passage through different animal and insect hosts, or whether each variety had become irreversibly fixed in the biological sense. At the Harvard laboratory, at the present time, the view is held that the two varieties are of originally common stock, but that the European type has become stabilized in its present form by continuous passage through man. For this reason it has seemed logical, in agreement with Nicolle and Laigret (3), to speak of the Mexican-American as the murine and of the European as the human virus. This problem has been discussed in greater detail by Zinsser (4) in a paper on Brill's disease which is in press.

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In the study of this problem, much attention has been given to the behavior of the infectious agents in rodents other than guinea pigs, particularly in rats and in mice. Nicolle (5) was the first to determine the survival of typhus virus, in an inapparent form, in both of these species. That the Mexican-American strain possesses a higher degree of virulence for rats than does the European has been generally recognized since Maxcy (6) showed that definite fever curves resulted from the inoculation of these animals with the first named virus. In a recent study, Laigret and Jadin (7) have reported further differences of considerable importance between the two types in regard to their respective survivals in mouse passage. They carried out mouse passages in series at 9 and 10 day intervals. In one set of experiments they injected brain into peritoneum; in the other, brain into brain. The results of these experiments permitted the conclusions that, by both methods of inoculation, the Mexican virus could be carried from mouse to mouse for at least sixteen passage generations; whereas the human, Tunisian virus was lost after the second passage in the intraperitoneal method, and after the third in brain to brain transmission. These observations are of great interest since, if accurate, they clearly indicate that the observed differences between the two types of virus are not easily reversible dissociations, but are more or less fixed. Incidentally, the method offered an opportunity for further investigation of the virus strains obtained by Zinsser and Castaneda from three cases of Brill's disease.

In 1933, Zinsser and Castaneda (8) reported upon a strain of virus isolated from a typical case of Brill's disease in Boston. In all its attributes in guinea pigs, this virus resembled the European or human variety. Kept alive by guinea pig passage in the Harvard laboratories for 14 months, it has maintained its original character, in spite of repeated experimental efforts to modify it in the direction of the Mexican type. Since the isolation of the first strain, in May, 1933, the same investigators have obtained two further strains from cases of Brill's disease—one in December, 1933; the other in January, 1934. Both of these, like Boston No. 1, have, from the beginning, exhibited the characteristics of the European or human type.

The experiments which are herein reported consisted of a series of mouse passages carried out as follows: The original mouse inoculation

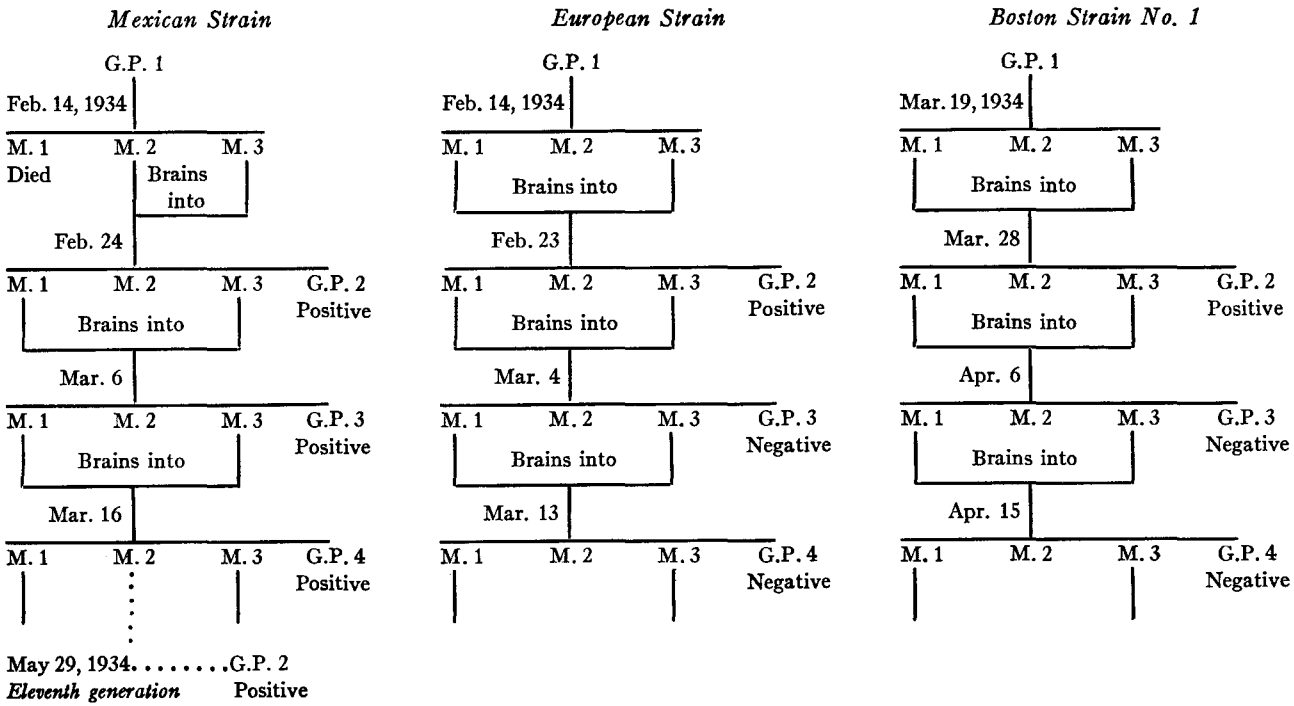

CHART 1. This chart illustrates the manner in which mouse passage was carried out with three strains of typhus virus, each generation being controlled by guinea pig inoculation.

with each strain was carried out intraperitoneally. Three mice were used in each generation, to allow for accidental death and possible irregularities of behavior in individual mice. At the end of 10 days, the mice were killed, the brains pooled, ground in a mortar, and injected intraperitoneally into three, second generation mice and into a guinea pig. This procedure was repeated every 10 days, and the guinea pigs carefully observed by daily temperature and, when appropriate, by *Rickettsia* examination of the tunica vaginalis. When the guinea pig controls of any given generation showed any irregularities of temperature, they were retained and later tested for immunity against the particular strain used for the experiment in which they were controls. The chart shows in graphic form the simple method followed in the experiments.

In this manner, we carried out mouse passages with the Mexican strain originally obtained from Mooser, which is now being used in the Harvard laboratories for vaccine and serum production, and which has been maintained uninterruptedly for about 4 years; with a European strain (Breinl) similarly maintained for about 3 years in this laboratory and, before that, in the National Institute of Health at Washington; and with the three Boston Brill disease strains mentioned above. All of the passage experiments were done in duplicate except in the cases of the Mexican strain and the Boston No. 3.

EXPERIMENTAL RESULTS

Mexican Murine Strain.—The mouse series was begun on Feb. 14, 1934, by the intraperitoneal injection of tunica material into three mice. Passages were carried out every 10 days from Feb. 14 to May 29, inclusive (the last passage was allowed to go 12 days). The strain is now in its eleventh mouse passage, and the guinea pig controls of every passage, including the last, have reacted typically. It is noticeable, moreover, that the strain has not lost virulence as a result of mouse passage. In the second generation, the incubation time in the control guinea pig was 8 days; by the fourth generation, this had dropped to 7 days; in the fifth and sixth generation, it was 6 days; and in subsequent generations, was 7 days.

While we are continuing to carry on this strain through mice, it seems logical to assume that a virus which can pass through eleven mouse passages without material loss of guinea pig virulence, is not undergoing either attenuation or any other form of modification.

It is apparent, therefore, that our experiments agree with those of Laigret and Jadin in showing that the Mexican virus can be maintained by mouse passage without attenuation and for at least eleven generations—and possibly indefinitely.

European Strain.—Two experiments, in all particulars like the preceding, were carried out with the European strain. The first began on Jan. 15, 1934; the other on Feb. 14. In the first experiment, no guinea pig control was set up for the first generation. In the second experiment, where this was done, the guinea pig reacted positively after an incubation time of 13 days. In both experiments, the guinea pig controls of the third generations were negative, and subsequent tests of these guinea pigs with European virus showed that they had not been immunized by the preceding injection of the mouse brains of the third passage.

This, again, is in agreement with the experiments of Laigret and Jadin, who found that the Tunisian strain of human virus died out after one peritoneal and two cerebral passages.

Brill's Disease Strains.—Experiments like the above were carried out with the three Boston strains, in the case of No. 1 and No. 2 in duplicate.

Boston Strain No. 1.—The second generation guinea pig control was positive in one series after an incubation time of 16 days. In the other experiment, the third generation guinea pig control showed a short lived temperature, but was found to be susceptible on subsequent test. The temperature was, therefore, due to some cause other than typhus infection. In both experiments, the virus had thus died out in the third mouse passage. Passages were nevertheless continued in both series to the fourth and, in one case, to the fifth generation, to insure against possible errors of observation. In neither case did any of the guinea pigs subsequent to the second generation show either typhus reaction or immunity.

Boston Strain No. 2.—The results with this strain were entirely analogous to the ones obtained with Boston No. 1.

Boston Strain No. 3.—Experiments were exactly like the preceding except that, in this case, the reaction in the second generation guinea pig was unusually slight and short lived, so that it could not have been called a typical typhus reaction. This guinea pig was not tested for immunity. Again, however, in the third and fourth generations, the virus died out completely.

CONCLUSIONS

The experiments above described have confirmed the observations of Laigret and Jadin that the European human typhus virus cannot be maintained for more than two generations in mice by brain-peritoneum passage; whereas the murine Mexican variety can be carried

on by this method in mice through at least eleven passage generations. The fact that within eleven passages there is no attenuation of the murine virus renders it likely that this agent can continue in mice, in an inapparent form, without material modification.

Brill's disease virus from three different isolations has behaved like the European type, a fact which strengthens the opinion previously expressed from this laboratory that Brill's disease represents an imported European strain of the classical European infection.

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