STUDIES ON TYPHUS FEVER

XII. THE PASSIVE IMMUNIZATION OF GUINEA PIGS, INFECTED WITH EUROPEAN VIRUS, WITH SERUM OF A HORSE TREATED WITH KILLED RICKETTIAE OF THE MEXICAN TYPE*

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In the preceding paper of this series (No. XI) (1), we reported that we had succeeded in preventing the development of typhus fever in guinea pigs inoculated with virus of the Mexican type by subcutaneously administering serum of a horse immunized with homologous killed Rickettsiae. Protection was obtained even when the subcutaneous serum injections were made as late as 72 hours after intraperitoneal administration of the virus. The prophylactic possibilities of this serum were suggested by experiments which showed that complete or partial prevention of subsequent infection could be attained when virus was administered 1, 7 and 13 days after serum injections. Prophylactic effects had, however, completely disappeared on the 18th day under the conditions and dosage employed at that time. This phase of our work has, since then, been confirmed by Varela (2) who, using serum sent to him by us, and employing a somewhat larger dosage (i.e. 2 cc. subcutaneously), found that guinea pigs so treated were protected for 15 but not for 30 days. The implications of these experiments for practical prophylactic purposes are obvious if one considers that infected lice die within 10 to 12 days.1

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1 The therapeutic possibilities of the serum in man cannot, of course, be appraised by the guinea pig experiment. However, we have treated, with encouraging results, two severe cases in Boston in which the serum was given only from a sense of obligation because of the severity of the condition; and a Mexican govern-
At the time when the experiments referred to above were carried out, the serum of the *Rickettsia*-immune horse agglutinated the Weigl louse vaccines in dilutions of 1–640, and gave Weil-Felix reactions in dilutions of 1–320. The agglutinative values of the serum for our own Mexican vaccines could not be determined, since both inoculum and subsequent *Rickettsia* suspensions contained small amounts of rat protein, a circumstance which would have rendered agglutinations valueless. It is of interest to note that long and intensive treatment was necessary before the horse serum attained such values. We attribute this to the relatively small amounts of specific antigen which our earlier crude methods of vaccine production yielded. With the present X-ray rat method, which, incidentally, has been successfully continued for over a year, we believe that horse immunization will be less difficult.\(^2\)

In spite of the potent agglutinating power of our horse serum for the *Rickettsiae* of the European disease, we were not able, at the time of our last report, to obtain any corresponding passive protection against the European type of virus. Such failure was difficult to interpret because of the many experimental facts which, in earlier work, had indicated the close antigenic overlapping of the two types of virus. Moreover, the agglutinating values mentioned above constituted valuable direct confirmation of our views of the close relationship between the two responsible organisms.

Believing that the failures of passive protection against the European type might be attributable to purely quantitative difficulties, we decided to continue treatment of the horse throughout the summer, in the hope of increasing the potency of the serum. This was done, and bleedings taken in the early autumn showed that the Weil-Felix

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\(^2\) The X-ray method seems to have given trouble to a number of investigators who have tried to repeat it. This we believe is due to insufficient radiation in most cases. Moreover, a certain amount of clinical experience with rats is necessary for judging the best time for autopsy of the animals. Properly carried out, the method yields large amounts of *Rickettsiae* with considerable regularity.
reaction had then attained about double its previous potency; that is, there was complete agglutination in dilutions of 1–640. We were unable to obtain a further supply of Weigl vaccines at this time, and could not therefore, determine whether reactions with the louse Rickettsiae had likewise increased.

In carrying out experiments with this somewhat more potent serum, we took into consideration the following circumstances: When one wishes to inoculate considerable doses of European virus, this is best accomplished by injecting mixtures of defibrinated blood and brain suspension taken from animals with active typhus on the 10th to the 14th days after inoculation. In such materials, Rickettsiae cannot be found, and are probably scarce and entirely intracellular. And since the infected cells are homologous, one is probably setting up, within the peritoneum, a temporary tissue culture from which any considerable discharge of Rickettsiae takes place only 4 or 5 days after injection when some of the infected cells have died. By that time, serum injected together with inoculum may have been, in part, eliminated. In carrying out our protection experiments, we therefore allowed from 2 to 3 days to elapse between the intraperitoneal virus injection and the subcutaneous administration of the horse serum.

EXPERIMENTAL

Experiment I.—In a preliminary experiment, 4 guinea pigs received large intraperitoneal doses of European virus. After 4 days, 2 of them were given, subcutaneously, 2 cc. of the immune horse serum, and a further 0.5 cc. was given on the 5th day. The results were encouraging, since the 2 controls developed typical and severe typhus, while the treated animals showed nothing more than slight, temporary rise in temperature. No normal horse serum controls were done, since this experiment was carried out for purposes of orientation.

In the following three experiments, untreated controls and controls with normal horse serum were employed.

Experiment II.—All the 13 guinea pigs were intraperitoneally inoculated with European typhus virus in the form of brain emulsion mixed with defibrinated blood, the materials being taken at the height of the disease. 72, 96 and 120 hours after infection, 8 of these animals were each given subcutaneous injections of 1 cc.
of immune horse serum. 3 controls were given normal horse serum at the same intervals, and in the same manner. 2 were left untreated. We have charted these experiments by a simple method which takes up little space, without sacrificing clearness. The caption under the chart is fully explanatory.

CHART 1. All of the 13 guinea pigs intraperitoneally inoculated with European typhus fever. The 8 animals whose temperatures are charted in solid lines were given 1 cc. of the immune serum subcutaneously 72, 96 and 120 hours after infection. Those charted in broken lines in Graphs a, b and c received normal serum in the manner and in the quantities in which the solid line animals received immune serum. The guinea pigs charted in broken lines in Graphs d and e were untreated controls. Horizontally, all the graphs represent daily temperatures for 15 days. The heavy line marking 104°F. is at the level above which we regard a guinea pig's temperature as indicating fever.

This experiment indicates that in the 8 treated guinea pigs, European typhus infection was prevented by subcutaneous immune serum injections which were not begun until 3 days after the virus had been administered. The normal serum controls prove that this effect was not non-specific.
Experiment III.—The experiment shown in Chart 2 was done in exactly the same manner as the preceding one. The caption under the chart is sufficiently explanatory.

We were quite convinced that the results obtained in the two preceding experiments could not have been due to coincidence, since fortuitous failure to obtain typical reactions with this European strain has occurred on only rare occasions during the last 2 years of constant observation. However, in a fourth experiment of the same kind we had confusing results, which were readily explained by intercurrent disease. 6 animals were used in this experiment, all of them, owing to difficulties of animal stock, small and in poor condition. One of them died, but was not autopsied. 3 of the other 5 were killed when quite sick and on being autopsied showed pneumonias. Although there was ample reason, therefore, to exclude the atypical temperature reactions obtained in this experiment from any bearing on the effec-

![Chart 2](image-url)

**Chart 2.** All 12 guinea pigs were intraperitoneally infected with European typhus virus on the same day. The solid lines in every case represent the temperature curves of infected guinea pigs which each received 1 cc. of antityphus horse serum 72, 96 and 120 hours after infection. The broken lines in Graphs a, b, c and d represent normal serum controls, and in Graphs e and f untreated controls. These graphs are constructed on the same plan as the two preceding ones. Horizontally the graphs represent daily temperatures for 15 days.
tiveness of the serum, we decided to carry out one further experiment in order to make sure that we had not been the victims of coincidence in the preceding ones.

CHART 3. Again the solid lines represent the treated animals, the broken lines the controls. In Graph a, the treated animal received 2 cc. of immune serum subcutaneously on the 2nd day and 1.5 cc. on the 4th day after infection. The control received normal serum in the same amounts at the same intervals. In Graph b, the solid line animal was treated as above, the other was untreated. In Graph c, the treated animal received 2 cc. of serum subcutaneously on the 3rd day and 1.5 cc. on the 5th day. The control received normal serum at the same intervals and in the same manner. In Graph d, the treated animal received serum exactly as did the analogous one in Graph c. The control was untreated. Sw. indicates that the animals developed scrotal swelling—see comments in text. Horizontally, the graphs represent daily temperatures for 15 days.

Experiment IV.—This last experiment is presented in Chart 3.

These results differ from those of Experiments II and III only in the fact that the serum was given in somewhat larger quantities, but was started later; that is, 3 days after infection. The results are entirely
clear and unambiguous, and together with those shown in Chart 2 seem to leave no room for doubt that the serum produced in a horse by treatment with Mexican *Rickettsiae* has exerted definite protective effect against the European typhus.

It is interesting to note that in Chart 3, Experiment IV, the 2 controls that received normal serum subcutaneously developed scrotal swelling. The increasing frequency with which the European strain is producing scrotal swelling is of considerable significance for the problem of the relationship between the two types of typhus fever. These two instances of swelling in animals receiving normal horse serum are noteworthy in view of Varela’s (3) recent studies on the induction of scrotal swelling in animals infected with the Tunisian strain by the intraperitoneal injection of fresh sterile blood.

**DISCUSSION**

The experiments above recorded demonstrate that under suitable experimental conditions guinea pigs can be protected from infection with the European type of virus by the serum of a horse immunized with killed *Rickettsiae* of the Mexican type.

Apart from any practical implications which obviously encourage therapeutic test, these results seem to us of considerable bearing on the differences of opinion that exist concerning the closeness of the relationship between the two types of virus. It is our opinion that the differences are much less fundamental than they have been supposed to be, and that it is not impossible that they depend largely upon minor modifications (adaptations, possibly reversible) sustained in the course of the passage of the Mexican, so called New World virus through rats and rat fleas. A summary of the evidence in favor of such a view will be published at a later date.

The results of these experiments—showing beyond question that the serum of a horse treated entirely with Mexican *Rickettsiae* acquires a Weil-Felix reaction, agglutinates the European louse vaccines of Weigl and protects against the European virus—strongly support the view which closely relates these two infectious agents.

From the point of view of developing protective serum for prophylactic and therapeutic application to the European disease, these results are of importance because, so far, it has not been possible to obtain,
with the European virus, anything like the large accumulations of \textit{Rickettsiae} which we have been able to produce with the Mexican strain by our X-ray method.

\textbf{CONCLUSION}

Guinea pigs infected with European typhus virus can be passively protected with the serum of a horse that has been treated with killed Mexican \textit{Rickettsiae}.

\textbf{REFERENCES}