uents of the mixtures being the same as described above. The results are expressed in Table II.

TABLE II.

Amount of substrate	% sugar 24 hr.	% sugar 72 hr.
20 mg.	.135	.144
30 mg.	.202	.234
40 mg.	.270	.340

8243

Protective Action of Concentrated Antityphus Serum (Murine Type) Against European Typhus Virus.

HANS ZINSSER, M. R. CASTANEDA, AND F. D. HAGER.

From the Department of Bacteriology and Immunology, Harvard University Medical School, and the Massachusetts Antitoxin and Vaccine Laboratory.

Two of the writers[1] have described a method of producing concentrated suspensions of Rickettsiae from peritoneal washings of X-ray radiated rats infected with typhus virus of the murine (Mexican-American) type. Such vaccines have since been used for human vaccination and for the production of antityphus serum by horse immunization. The reactions in the horse, the Weil-Felix reactions and the prophylactic and experimental therapeutic action of the immune horse sera have been elsewhere described.[2] Therapeutic test in man, which is giving encouraging results in Mexico, was justified by preliminary experiments in guinea pigs. In these experiments, moderate doses of the serum appeared to confer complete protection against the murine virus upon guinea pigs infected after, together with, or even 3 or 4 days before the administration of the serum. In the case of similar protection experiments in which the classical European virus was employed,[3] protection was not absolute, but sufficiently definite to encourage further efforts. Our observations of the close antigenic overlapping of the 2 principal varieties of human typhus Rickettsiae led us to believe that our difficulties, in regard to protecting against the European "humanized"

1 Zinsser, H., and Castaneda, M. R., *J. Exp. Med.*, 1930, **52**, 649; and PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 840.

2 Zinsser, H., and Castaneda, M. R., *J. Exp. Med.*, 1933, **57**, 391.

3 Zinsser, H., and Castaneda, M. R., *J. Exp. Med.*, 1934, **59**, 471.

virus, might be quantitative and could be overcome by increased potency of the antityphus horse serum. The present note reports work by which we believe the difficulty has been overcome.

With the cooperation of the Massachusetts Antitoxin and Vaccine Laboratory, where our horse serum is produced, a method of concentration has been developed which has furnished a product which in moderate dosage protects guinea pigs against powerful injections of the European virus.

The method of concentration is, briefly, as follows: Typhus horse serum, produced as previously described (Weil-Felix test complete usually at about 1-320), is centrifuged in a Sharples centrifuge. It is then dialyzed in cellophane bags in running water to precipitate all insoluble protein (3 to 5 days). The precipitated protein is collected in a Sharples centrifuge and dissolved, so as to have a final content of NaCl 1%, phenol 0.2%, total solids about 12% to 13%. This solution is then clarified in the Sharples centrifuge and filtered through an N Berkefeld candle.

Preliminary observations showed that the Weil-Felix antibodies were concentrated in the sediment of such dialyzed serum, and subsequent protection tests have demonstrated that protective antibodies are similarly precipitated. Complete analysis of the results of concentration will be published more fully elsewhere, but in a number of cases, from an original volume of somewhat less than 5 liters of a weak serum having a Weil-Felix titre of 1-160, 700 cc. of concentrate has been obtained, which gave a powerful Weil-Felix in a dilution of 1-1280 and a partial Weil-Felix in a dilution of 1-2560. Two series of protective tests with such concentrated serum against the European virus have been carried out as follows:

In each set, the European virus—in the form of 2 cc. of fresh blood, in the first experiment; and 2 cc. of a defibrinated blood-brain mixture, in the second experiment—was injected intraperitoneally into a series of 7 guinea pigs. One animal in each case was kept as a virus control. In the first experiment, 3 hours after intraperitoneal inoculation with virus, 3 animals received, subcutaneously, 1 cc. of antityphus serum concentrate. Three others received, by the same route, 1 cc. of a similarly concentrated antipneumococcus serum. In the second experiment, the serum was given 24 hours after virus injection, in similar amounts and again subcutaneously. The controls, with a concentrate of known specific immune serum, were made in order to insure against accidental phenomena due possibly to non-specific protein factors or other unfavorable effects.

The consolidated charts show the results.

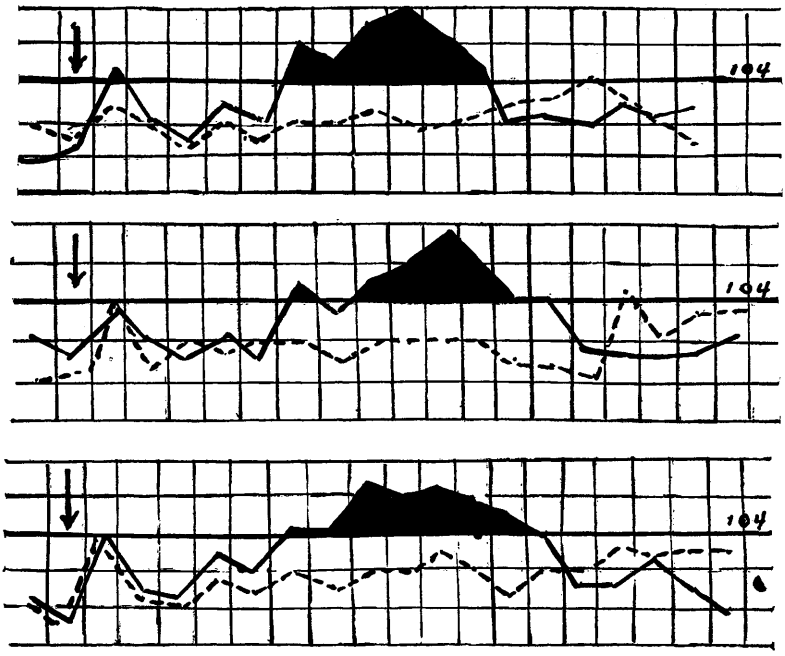

FIG. 1.

Charts of Guinea Pig Temperatures of Second Experiment.

Control without serum is omitted, but ran the typical course of European guinea pig typhus. All the 6 animals charted received on the first day an intraperitoneal injection of 2 cc. of defibrinated blood and brain emulsion mixtures of a passage animal, the materials being taken on the tenth day of the disease at a time when the temperature was almost 105°. 24 hours later, at the points shown by arrows, 3 animals, whose temperature curves are shown by the broken line, received, subcutaneously, 1 cc. of concentrated antityphus serum, as described. The 3 controls, whose temperature curves are shown by the uninterrupted line, received, subcutaneously, 1 cc. of similarly concentrated antipneumococcus serum. Temperatures above 104° are blocked in black.

It will be seen that the serum concentrate, although obtained from a horse immunized entirely with Rickettsiæ of the murine Mexican type, protected satisfactorily against powerful doses of European virus, even when the virus was given a considerable advantage by intraperitoneal injection and a 24-hour start.

In the first experiment, the virus control showed a typical curve of European typhus of ordinary severity. The animals which had received the concentrated antipneumococcus serum all passed through a typical attack of typhus, temperatures rising on the 7th and 8th days and following the usual 7 to 8-day fever characteristic of this disease in guinea pigs. The 3 animals which had received the antityphus concentrates showed no reactions except that in one animal, on the 13th day, the temperature rose to 104.2°, promptly falling again to 103° on the following day. In the second experiment, in which the dosage of virus was much more severe, owing

to the admixture of typhus brain suspension with the defibrinated blood, the result was identical.

In previous experiments it was found that unconcentrated antityphus horse serum, while highly efficient in protecting guinea pigs against the Mexican murine virus, was only partially effective in similar experiments carried out with European humanized virus. The results of these experiments, added to observations of serological overlapping encountered in active immunization and in various types of agglutination tests, indicated that a possible increase of potency might render a serum produced by immunization with the murine Rickettsiae effective against the virus of the classical European disease. The experiments outlined demonstrate that this is the case. Concentration on a practical basis, by a method outlined, has yielded a product of which 1 cc. administered subcutaneously after infection will completely protect guinea pigs against considerable doses of intraperitoneally administered European or humanized virus. The gradual absorption known to take place after subcutaneous injections of serum gave the virus a considerable advantage. The method was used in order to simulate more closely actual therapeutic conditions. The production of sufficient quantities of such serum concentrate for therapeutic test in the classical European typhus is not prohibitive either in time or work for practical application.*

* Since these notes were prepared for publication, an observation has been made in the laboratory which is well worth recording, though not deserving a separate communication. A worker, "M," ran a needle on which was balanced a heavily infected bit of tissue culture, loaded with Rickettsiæ, into his thumb. He had been vaccinated with our murine typhus vaccine four months previously, but had no Weil-Felix reaction. He was given 5 cc. of a concentrated antityphus horse serum on the day of this accident (Weil-Felix of serum, 1-2480) and 10 cc. forty-eight hours later. Our own nervousness accounts for the probably unnecessary high dosage. Since the simple washing with iodine immediately practiced probably had little effect, we feel that we should at least record the fact that outside of mild serum sickness, "M" has remained entirely well. No conclusions can of course be drawn from this, but in connection with the experiences of Bustamante and Varela with prophylactic use of our serum in Mexico, the experience is worth recording and emergency serum injections are recommended to other workers.