15539

Effect of Concentrated Hyperimmune Rabbit Serum on Circulating Agent in Louse Borne Typhus.

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Immune serum has been used by a number of workers in the treatment of rickettsial diseases. A recent report by Yeomans, Snyder and Gilliam reviewed the previous work and reported upon the use of a concentrated hyperimmune rabbit serum in the treatment of 25 cases of epidemic typhus fever studied in Cairo, Egypt.[1] These authors concluded that hyperimmune antityphus rabbit serum had a favorable therapeutic effect on the course of typhus if treatment was started in the first 3 days of the disease. Although serum treatment seemed to have reduced the mortality in the group of patients who received serum on the 4th, 5th, or 6th day of illness, the value of serum therapy for late cases could not be determined.

Of the 25 cases studied by Yeomans, Snyder and Gilliam, strains of epidemic typhus were isolated in guinea pigs by the authors in 20 instances. In 11 of these cases, attempt at strain isolation was made immediately before and again at variable times after the administration of the serum. Since the method employed for strain isolation was identical in each instance, it was possible to ascertain the effect of the serum upon the circulating agent. Furthermore, the nature of this effect could be determined, within certain limits, by comparing the period of incubation in the inoculated guinea pigs in the pre- and postserum isolations.

Method. Thirty cc of blood were withdrawn from the patient. The blood was kept in ice and usually was inoculated into guinea pigs within 2 hours after withdrawal. After coagulation, the serum was removed and the clot was ground up in a mortar with alundum. This material was then suspended in 10 cc of physiological saline. After the alundum sedimented, 5 cc of the supernatant fluid were inoculated intraperitoneally into each of 2 male guinea pigs. The temperatures of the guinea pigs were taken for several days prior to inoculation in order to obtain the normal level and for 21 days after. A temperature of 104°F or higher was considered as fever.

Table I records the pertinent data in this study. These data indicate that in 11 cases of epidemic typhus fever treated with hyperimmune rabbit serum, it was possible to isolate the agent in guinea pigs immediately before and again at variable periods after the administration of serum. This was true when the postserum isolations were made immediately after and up to 114 hours after the administration of serum. Even though circulating agent was still present after the administration of serum, some deleterious effect of the serum upon the agent was evident as judged by the prolongation of the period of incubation in the postserum isolations. There were 2 exceptions. In Case 24, blood was drawn for postserum isolation 3 minutes after the administration of serum. The incubation period in the inoculated guinea pigs was reduced 2 days. In Case 20, in an isolation made 21 hours after the administration of serum the period of incubation was prolonged by 3 days, while with the isolation made after 114 hours, this period was reduced 2 days. It should be noted that in 6 cases one guinea pig each in the postserum isolations failed to react when observed over a 21-day period. This failure to react may have been due to an inapparent infection which is characterized by no evident symptoms of disease but is followed by the appearance of specific antibodies, or by a missed infection characterized by no symptoms, or the appearance of specific antibodies.[2] These possibilities may occur normally after the inoculation of

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1 Yeomans, A., Snyder, J. C., and Gilliam, A. G., *J. A. M. A.*, 1945, **129**, 19.

2 Plotz, H., Wertman, K., and Bennett, B. L., PROC. SOC. EXP. BIOL. AND MED., 1946, **41**, 76.

TABLE I.

Case No.	Day of disease when treatment begun	Avg incubation preserum isolation, days	Amt of serum administered between isolations	Interval between isolations	Avg incubation postserum isolation, days	Difference between pre- and post-serum incubation	Total amt serum administered (cc)
6	3	11½	39 iv—20 im	14 hr	14	Prolonged 2½ days	239
11	4	11½	58 iv	15 "	14*	" 2½ "	58
13	4	11½	51 iv	19 "	20*	" 8½ "	51
14	4	10	20 iv—37.5 im	16½ hr	12	" 2 "	218
15	4	13	126 iv	5 min	17*	" 4 "	512
16	4	10	85 iv	11 hr	12*	" 2 "	491
19	5	7	40 iv—40 im	Immediately after	11*	" 4 "	120
20	5	12	78 iv	1st isol. 21 hr 2nd isol. 114 hr	15 10	" 3 " Reduced 2 "	78
22	5	10½	30 iv—60 im	7 hr	16*	Prolonged 5½ "	260
24	6	11½	59 iv	3 min	9½	Reduced 2 "	59
25	6	12	40 iv—38 im	7 hr	14½	Prolonged 2½ "	238

* One guinea pig of each of these groups did not react when observed 21 days.
The case numbers employed here correspond to those used by Yeomans, Snyder and Gilliam.[1]
Strain isolation for Case 6 and 13 is incorrectly recorded by Yeomans, Snyder and Gilliam.[1]

infectious material into guinea pigs. Finally, there may be a rickettsiostatic effect of the serum upon the circulating agent. The precise reason is not known for antibody studies were not made on these 5 specimens of guinea pig convalescent serum.

Seven of the cases (6, 14, 15, 16, 19, 22 and 25) received further doses of serum after the second isolation of the agent and hence, it is impossible to determine the possible effect of the early administration of serum upon the clinical course of the disease. However, Cases 11, 13, 20 and 24 were of particular interest since the total amount of serum administered occurred between the 2 strain isolations.

Cases 11 and 13 were recorded[1] as being of moderate severity, showing slight prostration, central nervous system involvement, cardiovascular changes or mild complications. The only effect observed after the administration of 58 cc and 51 cc of serum, respectively, was to prolong the incubation period in the guinea pigs when isolations were made 15 and 19 hours after serum treatment. Case 24, who received 59 cc of serum, was characterized as being a severe typhus with definite prostration, central nervous system involvement, cardiovascular changes or serious complications (nitrogen retention). When an isolation was attempted 3 minutes after the administration of serum, the period of incubation was reduced from what was found for the preserum isolation. In Case 20, which was characterized as being of such severe illness that a fatal outcome was expected at some point in the clinical course, the administration of 78 cc of serum had a slight effect upon the agent when isolation was made after 21 hours and none after 114 hours.

Since a favorable effect of the serum on the course of the illness was obvious from the clinical observations, the question arises as to what this effect may be attributed. It is evident that the serum did not have a rickettsiocidal effect upon the agent, for in 11 instances we were able to isolate the agent after the administration of serum. The serum may have had a rickettsiostatic effect. In favor of this hypothesis would be the prolonged incubation period in the guinea pigs in the postserum isolations. The final and

most likely hypothesis is that the serum exerted beneficial clinical effect because of an antitoxic effect. It has been shown that a "toxic factor" is associated with the epidemic and murine strains of typhus rickettsiae cultivated in yolk sac cultures and that this "toxic factor" can be neutralized specifically by convalescent serum.[3-6] This may explain why such relatively small amounts of serum as were employed in Cases 11, 13, 20 and 24 exerted a beneficial clinical effect. The hyperimmune rabbit serum used in these cases was prepared with infected yolk sac cultures.

Conclusion. 1. In 11 cases of epidemic typhus treated with hyperimmune rabbit serum, strains were isolated immediately before and again at variable periods after the administration of serum. 2. The only effect observed was that in the postserum isolations the period of incubation in the inoculated guinea pigs was usually, but not always, prolonged. 3. While the effect of the serum upon the agent may be rickettsiostatic, it is believed that a more likely explanation of the favorable clinical effect was due to its antitoxic effect.

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15540

Propagation of Hog Cholera Virus in Rabbits.

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The literature in regard to negative results in attempts to transmit hog cholera virus to hosts other than swine, is too voluminous to be cited. The only evidence of actual transmission of the virus in other hosts has been furnished by Zichis[1] who was able to propagate the virus for 10 passages in domestic sheep. Cultivation of the virus in hog tissue outside the body of the animal has been reported by Hecke[2] and TenBroeck.[3]

Even though all evidence indicated that hog cholera would be a difficult virus to adapt to other hosts, it seemed that in view of the knowledge gained with other viruses—such as: poliomyelitis,[4] dengue fever,[5] Colorado tick fever[6,7] and rinderpest,[8-11] the problem should be reinvestigated.

For some time attempts have been made in this laboratory to adapt hog cholera virus to some host other than swine. In accordance with the experience of most previous investigators most of our attempts ended in failure. Recently, however, by using an alternating passage method similar to that reported by Baker[10] in his work with rinderpest virus in rabbits, we have obtained results indicating that hog cholera virus may be successfully passed for a number of generations in rabbits.

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