

ON THE NON APPEARANCE OF THE NEGATIVE PHASE IN TREATMENT WITH HETEROPHILE ANTIGEN BY MOUTH.

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In 1893 Brieger and Ehrlich¹ showed the sequence of events on injecting a previously immunized animal with a bacterial protein. In experiments upon goats injected with tetanus toxin they notice marked drops in antitoxic titres following successive injections and plotted curves showing these depressions. In later studies of A. E. Wright upon the opsonin content in human blood these changes in potency were referred to as phases, an increase being called a positive phase while a decrease was designated as a negative phase. It is largely through the careful work of Wright that the attention of immunologists has been called to these fluctuations in antibody in the course of artificial immunization.

Since the time of these pioneer studies, it has become common knowledge that more or less of a "negative phase" may be noted on injecting a previously immunized animal, and almost everyone engaged in measuring the potency of serums from experimental animals, and human serums in opsonin titrations has encountered this sudden drop in titre after injection, later to be probably followed by an increase in antibody or a "positive phase". Particularly in the case of man this negative phase or depression of immunity has been a cause for apprehension on the part of those carrying on artificial immunization. As justification of this idea or not no attempt will be made here to try to state what relation may exist between potency of demonstrable antibody in general and resistance of the host. The object of this paper is to report the results of some experiments in treating rabbits by mouth with heterophile antigen as compared to the usual parenteral treatment with special reference to the negative phase. Brief reference has been made in a former article² to publications dealing with the principal properties of heterophile antigen and antibody.

Experimental. In hemolytic tests used here a unit of red blood corpuscles consisted of 0.1 cc. of a 1 in 4 dilution (in terms of whole blood concentration) of washed corpuscles. The dose of complement was 0.1 cc. of a 1 in 5 dilution of fresh guinea pig serum. Hemolytic tests were carried out at 37° C. for one hour, and readings were in terms of complete hemolysis.

In the following experiments six rabbits were used, five previously immunized with heterophile antigen from different sources being tested for the development of a negative phase following a treatment with heterophile antigen in the shape of boiled sheep cells by mouth,

¹ Brieger and Ehrlich. Beiträge zur Kenntniss der Milch immunisirter Thiere. Zeit. f. Hyg., vol. 13, 1893, pp. 336-346.

² Powell, H. M. Immunization with heterophile antigen when given by mouth. Amer. Jour. Hyg., vol. 5, 1925, pp. 228-229.

while one control rabbit having a high natural anti-sheep titre was tested by an intraperitoneal injection of boiled sheep cells before immunization and then again after immunization. Of the five test rabbits used, one was immunized with boiled sheep corpuscles by mouth, three with the intravenous injection of bacteria containing heterophile antigen (rabbit septicemia culture RD3) and one with the intraperitoneal injection of horse kidney.

(1) A rabbit of 3,000 grams weight with a natural anti-sheep hemolysin titre of 100 was given 5 cc. of boiled sheep red corpuscles intraperitoneally. One hour later the titre was unchanged; two hours later it was 75, five hours later it was 60 and 24 hours later it had dropped to 50. This decrease in titre or negative following injection therefore is observed in connection with a primary injection into animals having a sufficiently high natural antibody titre.

The same rabbit on receiving three more similar injections at three or four day intervals showed an anti-sheep hemolysin titre of 4,000 three days after the last injection. The titre at this time was either stationary or at least not changing rapidly as indicated by a titre measurement of 4,000 made three hours after the first. The rabbit now received 5 cc. of boiled sheep corpuscles intraperitoneally. Two hours later the titre was 3,500, four hours later 2,500, 24 hours later 3,000, and three days later the titre was 5,000.

The above titrations illustrate what may be expected, namely a drop in titre or a negative phase, on giving heterophile antigen parenterally and testing the potency of the serum at intervals during a period of time following, and is characteristic of the course of injections of many common antigens.

(2) A second rabbit weighing 2,500 grams having a natural anti-sheep hemolysin titre < 2 received three 5 cc. doses of boiled sheep corpuscles by mouth at intervals of one or two days. Two days after the last treatment the anti-sheep hemolysin titer was 500. A 5 cc. dose of boiled sheep corpuscles was then given by mouth. One hour and 12 hours later the titre had not changed, 19 hours later the titre was 600, and three days later it was 500. Another similar dose was given by mouth. Two hours later the titre was unchanged, and 12 and 18 hours later was also unchanged. The maximum titre seemed in this case to be about 500. The titrations indicate that treatment with heterophile antigen by mouth elicits no negative phase.

(3) (4) Two rabbits each of 2,800 grams weight and each having a natural anti-sheep hemolytic titre < 5 received three intravenous injections of heat killed (60° C. $\frac{1}{2}$ hour) rabbit septicemia RD3 culture (this culture contains heterophile antigen). Four days after the last injection the anti-sheep hemolysin titre of one was 10,000 while that of the other was 12,000.

After three more days the titres were 5,000 and 8,000 respectively. At this time each rabbit received 5 cc. of boiled sheep corpuscles by mouth. After 3, 6, 12, and 21 hours these titers had neither decreased or increased. After 48 hours the titers were 6,000 and 10,000 respectively, and after 72 hours they were the same.

(5) A rabbit of 2,200 grams weight having a natural anti-sheep hemolysin titre < 2 was given four intravenous injections of heat-killed rabbit septicemia RD3 culture (containing heterophile antigen) at three day intervals. Four days after the last injection the antisheep hemolysin titer was 10,000. Two months and two days after the last injection this rabbit was used in a test for a negative phase. At this time the hemolytic titer was 2,000. 5 cc. boiled sheep red corpuscles were given by mouth. At 1, 3, 6, 12, 24, and 30 hours after treatment specimens of serum tested just 2,000. No drop in titer took place. Forty eight hours after treatment the titer was 2,500, and after two more days the titer was 2,000 again.

Two weeks after testing for a negative phase the titer had dropped to 1,000. On the day following, the titer also was 1,000 at which time 5 cc. boiled sheep red corpuscles were given by mouth to test again for the development of a negative phase. After 2, 6 and 12 hours the titer remained 1,000. After 24 hours it was 1,300, and after 72 hours it still remained 1,300. These two treatments with heterophile antigen by mouth produced no negative phase.

(6) A rabbit of 3,000 grams weight having a natural antisheep hemolysin titre < 2 was given two intraperitoneal injections of one gram of finely ground horse kidney, the second dose being two days after the first. Eight days after the second injection the antisheep hemolysin titer was 16,000. After an interval of four months the titer had dropped to 2,000 at which point it was very nearly stationary as shown by a second titration of 2,000 made seven days after the first. At this time the animal was used for a negative phase test, and 5 cc. of boiled sheep red corpuscles were given by mouth. After 1, 3, 6, 12 and 24 hours the titer had not changed. At 30 hours after treatment the titer had increased slightly and after 48 hours the titer was 2,500. Treatment of mouth with heterophile antigen produced no negative phase in this rabbit's hemolytic antibody just as such treatment had not done before. Two weeks after boiled sheep corpuscles were given by mouth the rabbit's serum had a titer of 1,000. On the day following, another test showed a titer of just 1,000. A second treatment of 5 cc. boiled sheep corpuscles by mouth was given and the serum drawn and tested at intervals thereafter. After 2, 6 and 12 hours the titre remained 1,000, After 24 hours and 72 hours the titer was just 1,300. No depression or negative phase in hemolytic antibody resulted.

Conclusion. In the treatment of rabbits with heterophile antigen by mouth negative phases of any significance do not occur.

