PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

ESTIMATION OF THE DOSE OF ANTIGEN REQUIRED TO PRODUCE ANTIBODIES UPON INJECTION INTO THE CAROTID SINUS

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In 1945 we showed [1] that the injection of an antigen into the isolated carotid sinus leads to the formation of agglutinins. This observation was confirmed by other workers and by our own research. At the same time investigations have been reported from which it follows that antibody formation takes place only in cases when the antigen is absorbed [3]. According to other workers, in general it is impossible to isolate the carotid sinus and the formation of antibodies in response to injection of antigen in the isolated carotid sinus depends on the absorption of the antigen [4].

Without stopping to criticize the investigations and in view of the fact that these workers were unable to isolate the carotid sinus, we would like to point out that success in this operation depends on the method of isolation used and on the skill of the experimenter. Experiments in our laboratory have shown that isolation of the carotid sinus is perfectly possible and easily performed [2].

In this paper we try to reach a decision about the reflex production of antibodies by using a different approach. If the authors mentioned above are right in assuming that antibody production takes place only through resorption of antigen injected into the carotid sinus, then two questions need to be answered: 1) what is the minimum quantity of antigen which, injected intravenously, will produce antibodies and 2) what quantity of antigen injected into the isolated carotid sinus will have the same effect. From a comparison of these two values we shall be able to decide what part is played by resorption in the development of this process and does it exclude a reflex mechanism even if resorption does take place when the experiments are performed in this way.

EXPERIMENTAL METHOD

The experiments were carried out on dogs. Altogether 36 experiments were performed on 36 dogs. The weight of the dogs varied from 8 to 12 kg. In some dogs the carotid sinus was isolated by a method previously described by us. A vaccine of killed dysentery bacilli, containing a varying number of organisms, was injected into the isolated carotid sinus. After the vaccine had been in contact with the receptors of the carotid sinus for five minutes additional ligatures were applied parallel to the first ligatures, the intervening ligatures were divided and the carotid sinus, filled with vaccine, was removed. Before injection of the vaccine into the sinus blood was taken and the titer of antibodies to dysentery and paratyphoid bacilli in this serum was determined. Determination of the agglutinin titer to the same organisms was repeated after 7 days. We established the smallest dose capable of causing the formation of agglutinins when injected into the sinus and then investigated the possibility of formation of agglutinins by intravenous injection of the same dose or of a part of it.

EXPERIMENTAL RESULTS

Blood was taken from 6 dogs for determination of the agglutination titer of their serum. Next the carotid sinus was isolated in each dog. Into the isolated sinus was injected 0.25 ml of a vaccine containing 100 million dysentery bacilli in 1 ml. The isolated sinus, containing the vaccine, was removed after 5 minutes. Blood was

taken from all the dogs 7 days later and the agglutinin titer of the serum determined (Table 1).

TABLE 1

Changes in the Agglutinin Titer After Injection of Microorganisms into the Isolated Carotid Sinus

№	Titer			
	initial		after 7 days	
	antigen			
	dysentery bacillus	paratyphoid B bacillus	dysentery bacillus	paratyphoid B bacillus
	25 million organisms			
1	1 : 20+	1 : 20+	1 : 320+	1 : 20+
2	1 : 20—	1 : 20	1 : 160+	1 : 20+
3	1 : 20	1 : 40	1 : 160+	1 : 40+
4	1 : 40	1 : 20	1 : 320	1 : 80
5	1 : 20—	1 : 80	1 : 320	1 : 80
6	1 : 20	1 : 20	1 : 160	1 : 40
	100,000 organisms			
1	1 : 20	1 : 20	1 : 160	1 : 40
2	1 : 10	1 : 20	1 : 160	1 : 20
3	1 : 80	1 : 40	1 : 320	1 : 160
4	1 : 40	1 : 20	1 : 640	1 : 20
5	1 : 20	1 : 20	1 : 160	1 : 40
6	1 : 40	1 : 20	1 : 80	1 : 20
	25,000 organisms			
1	1 : 20	1 : 20+	1 : 40	1 : 20
2	1 : 40	1 : 20	1 : 640	1 : 20
3	1 : 20	1 : 20	1 : 40	1 : 20
4	1 : 20	1 : 20	1 : 160	1 : 20
5	1 : 40	1 : 20	1 : 320	1 : 20
6	1 : 20	1 : 40	1 : 80	1 : 40
	2 500 organisms			
1	1 : 20	1 : 20	1 : 20	1 : 20
2	1 : 20	1 : 20	1 : 20	1 : 20
3	1 : 40	1 : 20	1 : 20	1 : 20
4	1 : 20	1 : 20	1 : 20	1 : 20
5	1 : 40	1 : 40	1 : 40	1 : 20
6	1 : 80	1 : 20	1 : 80	1 : 20

The titer of agglutinins to the dysentery bacillus in the serum of the experimental dogs before the injection of vaccine varied between limits of 1 : 20 to 1 : 40 and that to the paratyphoid B bacillus between limits of 1 : 20 to 1 : 80.

On the 7th day after the injection of 25 million dysentery bacilli into the sinus the agglutinin titer to these organisms rose to within limits of 1 : 160 to 1 : 320. The titer of agglutinins to the paratyphoid B bacillus in the majority of cases was unchanged and only in one case was it increased from dilutions of 1 : 20 to 1 : 80.

The results of this series of experiments show that injection of 25 million microorganisms into the isolated carotid sinus causes agglutinin formation in dogs.

In another 6 dogs the same procedure was followed as in the foregoing group but an injection of 100,000 dysentery bacilli in a volume of 0.25 ml was given into the carotid sinus (see Table 1). The experiments showed that this dose of antigen also causes antibody formation. The titer of agglutinins to the dysentery bacillus varied before immunization between limits of 1 : 20 to 1 : 80 and after immunization between limits of 1 : 160 to 1 : 640; that to the paratyphoid bacillus was unchanged.

TABLE 2

Agglutinin Titer in Dogs After Intravenous Injection of Antigen

№	Titer			
	initial		after 7 days	
	antigen			
	dysentery bacillus	paratyphoid B bacillus	dysentery bacillus	paratyphoid B bacillus
	2500 organisms			
1	1 : 80	1 : 80	1 : 80	1 : 80
2	1 : 20	1 : 20	1 : 20	1 : 20
3	1 : 20	1 : 80	1 : 20	1 : 40
4	1 : 40	1 : 20	1 : 40	1 : 40
5	1 : 20	1 : 20	1 : 20	1 : 20
6	1 : 20	1 : 20	1 : 20	1 : 40
	25,000 organisms			
1	1 : 20	1 : 20	1 : 20	1 : 20
2	1 : 20	1 : 20	1 : 20	1 : 40
3	1 : 40	1 : 40	1 : 40	1 : 20
4	1 : 20	1 : 40	1 : 20	1 : 20
5	1 : 80	1 : 20	1 : 80	1 : 20
6	1 : 20	1 : 40	1 : 40	1 : 40

In the next group of experiments 25,000 dysentery bacilli were injected into the isolated carotid sinus of 6 dogs under the same conditions. This number of dysentery bacilli, injected into the isolated carotid sinus in a volume of 0.25 ml of fluid, causes a reliable increase in the agglutinin titer in the majority of cases and in a smaller number of cases an unreliable increase.

In the fourth group a dose of 2500 organisms was used. The injection of such a dose into the isolated carotid sinus caused no change in the agglutinin titer. The results of these experiments are shown in Table 1.

These groups of experiments show that the threshold dose of dysentery bacilli capable of causing agglutinin formation on injection into the isolated carotid sinus is 25,000 organisms. In the majority of dogs this dose produces a reliable increase in the agglutinin titer.

Having established the threshold dose and admitting a maximum possible resorption of 10% (10 times higher than that demonstrated by I. A. Oivin and Iu. V. Sergeev [4]), we attempted to find out whether this dose causes an increase in the agglutinin titer when injected into the blood stream. For this purpose 6 dogs were injected with 0.25 ml of physiological saline containing 2500 dysentery bacilli and after 7 days their agglutinin titer was determined. The agglutinin titers to both paratyphoid and dysentery bacilli were found to be unchanged (Table 2). Since injections of 2500 organisms into dogs had not altered their agglutinin titer we increased this dose to 25,000 organisms. Intravenous injection of 25,000 dysentery bacilli likewise caused no change in the agglutinin titer, as can be seen in Table 2.

The results obtained demonstrate that an injection of 25,000 dysentery bacilli into the isolated carotid sinus

is capable of causing agglutinin formation in dogs and that this does not occur if the same number of dysentery bacilli is injected intravenously. These results dispel all doubt that a vaccine, injected into the isolated carotid sinus, acts primarily on its receptors and causes antibody formation in a reflex manner. If resorption takes place as a result of unskillful isolation of the carotid sinus, this may account for the denial of a reflex mechanism of antibody formation.

SUMMARY

The minimal quantity of microbic bodies capable of causing antibody production when in contact with receptors of isolated carotid sinus was determined. It was demonstrated that this antigenic dose is not connected with increase of the titer of specific agglutinins, when injected intravenously to dogs of the same weight. This is an additional confirmation of the possibility of the reflex mechanism of antibody production.

LITERATURE CITED

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* Original Russian pagination. See C. B. translation.

** Name of Journal omitted in Russian original. Editor.