

THE REFLEX PRODUCTION OF ANTIBODIES CAUSED BY THE INJECTION OF ANTIGENS INTO THE ISOLATED SPLEEN

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In the work which G. A. Srazhin conducted in our laboratory, it was shown that the injection of an antigen into a spleen isolated from circulation and then removed, with the antigen, five minutes later causes the production of antibodies [2]. This data is confirmed by the studies of V. A. Tyehinin and E. A. Tatarinov [3,4]. Recently, K. A. Pozdeev [1] concluded that isolating a spleen does not prevent an antigen injected into it from being resorbed, and that antibody production only occurs when the antigen is found in the blood.

In order to prove that antibodies are produced when an isolated spleen is injected with an antigen, which is not resorbed from the place of its injection, we decided to develop a method of isolating the spleen and, using this method, to prove that the action of the antigen on the spleen receptors causes the reflex production of antibodies.

EXPERIMENTAL METHODS

In sterile conditions, operating on dogs under anesthesia, we opened the coeliac cavity, brought the spleen to the surface, separated the splenic nerve and applied a ligature. A probe was inserted between the nerves and vessels of the spleen, from its hilum to its convex part. Two ligatures were applied along the probe — one tying completely all the vessels in the upper portion of the spleen, the other tying completely all the vessels in the lower part of the spleen; these two ligatures effected the complete isolation of the spleen from the circulatory system; the only remaining connection between the spleen and the body was the intact nerve.

We verified the complete isolation from circulation of the spleen by injecting radioactive phosphorus into the spleen in a dose of 200 microcuries and then determining it in the blood by counting the impulses with a Type B dosimeter. For this purpose, blood was taken before the P^{32} injection, five minutes after the injection and simultaneously with the removal of the spleen. The number of impulses was computed several times on each batch of blood. The number of impulses did not change much and remained within the limits of normal fluctuation. This indicated that the radioactive phosphorus injected into the spleen was not absorbed and that the isolation of the spleen from general circulation was complete.

As well as radioactive phosphorus, typhoid fever vaccine was injected into the spleen, isolated by the method described above. Five minutes after the antigen injection, the spleen was removed. Blood was taken seven to eight days after the immunization, and the titer of antibodies to the typhoid bacillus and to the enteric bacillus was determined from the serum obtained from this blood.

EXPERIMENTAL RESULTS

In the control series of experiments, an analogous injection of a physiological solution into the subsequently removed spleen did not change the antibody content, although the original titer was rather large (Fig. 1, b). The

determinations showed that the titer of typhoid bacillus antibodies increased, but that the titer of enteric bacillus antibodies did not change, if the increase observed in some cases of antibodies by one dilution, which is shown in Fig. 1a is discounted.

From the data obtained, we concluded that the typhoid fever bacillus, when injected into a spleen isolated from circulation, stimulates the spleen receptors and causes the reflex production of specific antibodies. There were no antibodies to the related group produced in this case.

In the second series of experiments, a dog's spleen was isolated by the method described above, but additional ligatures were also applied to the vascular plexi, and the plexus containing vessels and cellular tissue was transected between the first and second ligatures. In this case, therefore, the spleen was left on one nerve (Fig. 2).

The typhoid and radioactive vaccines were injected into the spleen connected to the body by only one nerve. The spleen was removed after five minutes, and the wound was sutured under sterile conditions. Seven days after this operation, the titer of antibodies in the animal's blood was determined. A corresponding determination of radioactive phosphorus in the blood of these animals showed that the activity of the blood had changed only very slightly, within the margin for error. In seven days, the titer of antibodies to the typhoid and enteric bacilli in the blood of these animals was determined. The experiments showed that the titer of antibodies to the typhoid bacillus had increased, while the titer of antibodies to the enteric bacillus was practically unchanged (Fig. 3a). Therefore, when an antigen is injected into a spleen connected by one nerve to the body, specific antibodies form in approximately the same quantity as after ligation of the vessels alone. The titer of antibodies to the enteric bacillus changed slightly, which was an indication of the slight, nonspecific change in the antibodies which is caused by the action of any antigen.

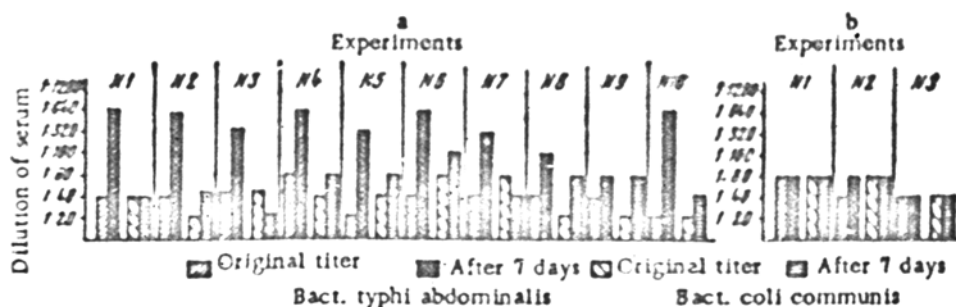


Fig. 1. Changes in the titer of agglutinins in the blood due to typhoid vaccine injection (a) and to physiological solution injection (b - control) into an isolated spleen, followed by splenectomy.

In the third series of experiments, we examined the effect of preliminarily cutting the splenic nerve on the reflex production of antibodies. For this purpose, we isolated the spleen in the experimental dogs by the above-described method and cut the splenic nerve in addition. The typhoid vaccine was injected into the denervated spleen, and additional ligatures were applied to the vessels after 5 minutes; then the vessels were cut between the ligatures, and the spleen was removed. After 7 days, the titer of antibodies in the dogs' blood was determined. In three animals, neither the titer of agglutinins to the typhoid bacillus nor that to the enteric bacillus changed at all; in two animals, it was raised by one dilution (Fig. 3b).

These data make it clear that the antigen acts on the receptors and causes the reflex production of antibodies. Cutting the splenic nerve disturbs the reflex arc from the spleen receptors and also the reflex production of antibodies.

One may assume from the data obtained that this proposed method of splenic isolation makes it possible

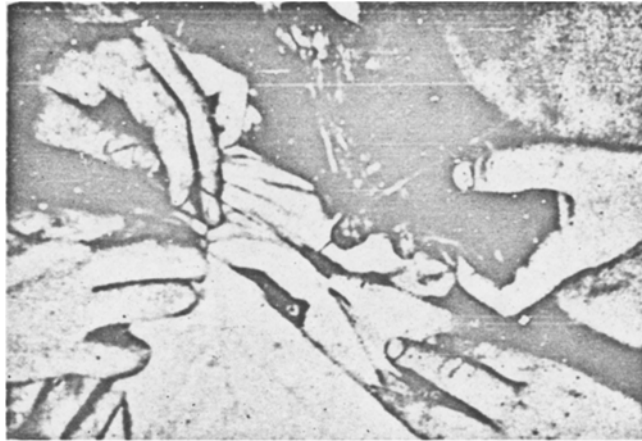


Fig. 2. Spleen, with preserved nerve connection and ligated, transected vessels.

to isolate the spleen from circulation and leave it connected with the central nervous system by the intact nerve. Antigen injected into such a spleen, when the spleen is then removed 5 minutes after the injection, causes the production of specific antibodies and does not much change the titer of nonspecific antibodies. Preliminary sectioning of the splenic nerve sharply lowers the production of antibodies to the antigen injected into the isolated spleen. The extirpation operation of the spleen and the manipulations of it associated with the antigen injection do not change the titer of antibodies.

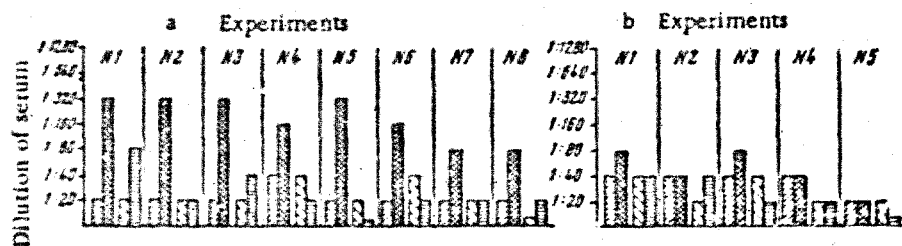


Fig. 3. Change in titer of antibodies caused by injection of typhoid antigen: in isolated spleen with ligated and transected vessels and intact nerves (a) and in denervated spleen (b) with subsequent splenectomy.

SUMMARY

Various antigens were injected into a spleen which had been isolated from circulation. Isolation was effected by separating the splenic nerve and tying all the underlying blood vessels and tissues. The degree of splenic isolation was tested by introducing P^{32} into the spleen and subsequently determining its presence in the blood. When the isolated spleen was removed five minutes after being injected with the antigen, the antigen injection brought about the formation of specific antibodies, although no resorption occurred. When no antigen was injected into the spleen, surgical intervention did not change the antibody titer.

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