SEROLOGICAL REACTIVITY OF COLD-BLOODED VERTEBRATES

SPEED WITH WHICH PARENTERALLY ADMINISTERED FOREIGN PROTEIN DISAPPEARS FROM FROG (RANA RIDIBUNDA) BLOOD AT VARIOUS BODY TEMPERATURES

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Parenteral assimilation of foreign protein has a direct relationship to immunity, in particular, to the production of antibodies. However, the essence of these relationships is still unclear. In earlier communications [4, 5] the effect of body temperature changes on the hemolytic activity of sera and on the production of antiviral antibodies in cold-blooded vertebrates was shown and the fact that antigen – vaccinia virus – is preserved for a long time in the blood of reptiles at a low temperature [4] was noted. The present work is devoted to the little-studied problem of the parenteral assimilation of foreign protein by poikilothermic vertebrates at various body temperatures.

EXPERIMENTAL METHOD

Large lake frogs (Rana ridibunda) were injected intraperitoneally by means of a thin needle with 0.5 ml of undiluted horse serum. After this, one group of experimental animals was kept at 5-6°, the other at 28-30°. After various lengths of time frogs from each group were bled completely dry and the presence of horse protein was determined in their sera by means of the most sensitive method – the anaphylactic reaction on guinea pigs.

The guinea pigs were sensitized by subcutaneous administration of 0.1 ml of horse serum no less than 20 days prior to the basic experiments, so that by the time these tests were made, the guinea pigs responded by anaphylactic shock to the administration of less than 0.000005 g of horse serum.

The frog serum under examination was heated at 57° for 30 minutes in order to inactivate its hemotoxic properties [5], then it was diluted doubly by physiological solution and 0.6 ml was administered intravenously to sensitized guinea pigs. Administration of such a dose of heated serum from fresh frogs did not cause a visible reaction in the sensitized guinea pigs. But if the frog serum contained even a minimal amount of horse protein, they developed an anaphylactic reaction of varying intensity.

In cases of nonfatal anaphylactic reaction, the guinea pigs were tested for desensitization by the intravenous administration of 0.5 ml of horse serum diluted (1:2) with physiological solution. If the guinea pigs did not react to the administration of horse serum, it meant that they were desensitized to it, i. e., that the previous reaction really was a consequence of the presence of horse serum protein in frog serum. It is essential to observe that a single administration of a small amount of protein usually does not desensitize the guinea pigs completely, but the intensity of the anaphylactic reaction is sharply decreased. The guinea pigs which died of shock were autopsied.

EXPERIMENTAL RESULTS

The data from the experiments are presented in the Table. It is obvious from them that while horse protein could not be found by means of the anaphylactic reaction in the blood of frogs kept at 28-30° by the fifth day, horse protein could be found in the blood of frogs kept at a temperature of 4-6° even after 20 days and only on the 34th day did the anaphylactic reaction cease. In other words, parenteral assimilation of foreign protein, intensive at 28-30°, is sharply lowered at a temperature of 4-6°.

Results of Horse Serum Protein Content Determination in Frog Blood

Guinea pig number	Administration of frog serum into guinea pigs			Reaction of guinea pigs to frog serum administration	Test of the specificity of the reaction by administration of horse serum
	frog No.	frog's maintenance temperature	day serum was taken after administration of horse protein		
1	72	4-6°	5th	Fatal shock. Lungs slightly inflated	–
2	73	4-6°	10th	The same	–
6	74	4-6°	20th	Shivering, coughing, convulsions. Involuntary excretion of urine and feces. Guinea pig survived	No reaction
7	75	4-6°	34th	Slight shivering. Guinea pig survived	Typical anaphylactic shock. Death
2	69	28-30°	5th	The same	The same
4	70	28-30°	10th	The same	The same

The question arose: is foreign protein really assimilated or is it excreted? In order to decide this question, 0.5 ml of horse serum was administered intraperotoneally with a very thin needle into two frogs. So that the administered material could not be excreted through the opening made by the needle, the skin was first pierced and moved, then the muscles of abdominal wall were pierced. After the needle was removed, the skin was moved, preventing leakage of the material. The frogs were washed thoroughly in running water and placed in jars with 100 ml of water. Penicillin was regularly added to the water in order to suppress sensitizing flora. The jars were covered with three layers of gauze; the animals were kept at a temperature of 28-30°. On the 6th and 12th day the frogs were bled. At the same time the water in which they were found was taken. The water was centrifuged, brought to the concentration of the physiological solution of warm-blooded animals with table salt (0.9%) and filtered through a Seitz filter. Guinea pigs were sensitized with the filtrate. In this experiment the most sensitive arrangement of the anaphylactic reaction for the determination of foreign protein was used, since the dose necessary for sensitization can be many times smaller than the precipitating one. The guinea pigs received 6 ml of filtrate subcutaneously twice with an interval of 24 hours. Simultaneously, 2 guinea pigs received 6 ml horse serum each at the same interval, diluted 1:100,000 with physiological solution. Along with the guinea pigs sensitized with the water in which the frogs had been, two guinea pigs were sensitized with the blood serum of these frogs. 21-22 days after the sensitization, each guinea pig received 0.5 ml of horse serum, diluted twice with physiological solution intravenously. From the results of this administration, we judged the presence of horse protein in the material used for sensitizing. In both control guinea pigs, which were sensitized with horse serum diluted 1:100,000, the characteristic signs of anaphylaxis were observed, neither of the experimental guinea pigs showed any signs of anaphylaxis. Thus, traces of horse protein could be found neither in the serum of the frogs nor in the surrounding water by means of the anaphylactic reaction. The possibility that the protein is destroyed in the water prior to its possible discovery by the anaphylactic reaction is not excluded, although the probability of this, taking into account the great sensitivity of the anaphylactic reaction, is, apparently, not great. Anaphylaxis in the control guinea pigs indicates that horse protein diluted 1:100,000 is easily

detected in the experiment as set up. It is very probable that the horse serum protein was not excreted in these experiments, but destroyed in the frog's system. In any case, the above experiments indicate that parenterally administered foreign protein quickly disappears from the blood of frogs when they are kept at a high temperature and circulates in it for a long time at a low temperature.

In accord with contemporary opinion regarding the formation of antibodies [6, 7, 8, 9, and others], which considers that a globulin molecule is transformed into an antibody molecule under the direct influence of the antigen, the decomposition of antigen by the system is not necessary. Moreover, the complete destruction of antigen molecules makes the formation of further antibodies impossible from this point of view. However, there is a definite relationship between the assimilation of antigen and antibody production. I. I. Mechnikov [2] already observed that turtles (Emys orbicularis) do not produce tetanus antitoxin at a low nor at a high temperature and tetanus toxin administered to them circulates in their blood for months. Alligators (Alligator mississipiens) produce tetanus antitoxin in high titres – tetanus toxin administered to them quickly disappears from their blood. Both for alligators and turtles, tetanus toxin is a completely nontoxic antigen. Similar occurrences are known in warm-blooded animals also. In all animals, when antibodies against a given antigen can be developed, the latter disappears from the blood fairly quickly.

These facts and the existence of anamniotic antibody formation a long period of time after the administration of antigen contradicts the more widespread theories of the antibody formation mechanism and tend to favor the assumption, first formulated by Barnet [1], that antigen does not change globulins directly at the time they are formed, but the enzymes which are involved in the synthesis of globulins. Although almost nothing is known regarding the synthesis of specific proteins, by analogy with the synthesis of polypeptides, for which the reversibility of the action of enzymes or groups of enzymes during the synthesis of specific serum proteins can be imagined. It seems highly probable to us that in response to the parenteral administration of foreign protein, groups of enzymes adapt themselves to its destruction, basically to the destruction of the specific protein structure, its determining groups. This adaptation leads to the specific change of certain enzyme groups and such changed enzymes later do not synthesize the usual globulin, but the antibody. All of this agrees well with the supposition [3] that the ability to produce antibodies arises in the course of evolution as a side function of protein metabolism under warm-blooded conditions.

In any case, when antigen is not assimilated by the system in one form or another, there is no antibody formation. The cycle of work we carried out [4, 5] and the present communication serve to confirm this stand.

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