

THE ACTION OF IMMUNE SERA ON THE GROWTH OF THE HEART AND CRYSTALLINE LENS OF CHICK EMBRYOS

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Since 1952 we have put forward from our laboratory the view that immunological relationships play a part in the formative processes in the embryo [1-3]. In this connection we may note with interest the results of experiments in which the action of immune organ antisera on the growth and development of the corresponding organs was studied in the course of embryogenesis. In 1939 Weiss [8] reported that antisera against the liver, kidneys and muscles of the chick, injected into developing hens' eggs, caused a sharp increase in the rate of growth of the corresponding organs in the chick embryos. On the basis of these findings, Weiss [9] put forward the hypothesis that antiorgan antibodies act as a matrix on which is formed the specific proteins of the corresponding organ and thereby stimulate the growth of this organ. Pomerat [7], however, did not confirm Weiss's results. Ebert [4] showed that brain, heart and spleen antisera of chicks depress and do not stimulate the growth of tissues of chick embryos cultivated in vitro. Finally, Flickinger, Levi and Smith [5] were unable to find any specific action of antilens serum on the development of the crystalline lens in chick embryos, both in vitro and in vivo.

The findings concerning the action of immune sera on the growth of the organs of embryos are thus very conflicting and do not permit the drawing of any definite conclusions.

In order to solve this problem, in 1956 we carried out an experimental study of the action of antilens and antiheart sera, injected in various doses into developing eggs, on the growth of the heart and crystalline lens of chick embryos.

EXPERIMENTAL METHOD

We obtained the sera by means of immunization of rabbits with tissue suspensions of cardiac muscle of an adult fowl and crystalline lenses of an adult duck. The sera reacted with homologous antigens in the complement fixation test in a dilution of 1:320 and in the precipitation test in a dilution of 1:10,000. The eggs to receive the injections were from chicks of the "Russian White" breed incubated for five days. The sera were injected into the air sac of the egg, since it was shown in a paper by Freericks [6], that solutions of a number of substances injected into the air sac rapidly penetrate the embryo itself. For this purpose we perforated the egg-shell over the air sac and injected the serum through this orifice with a syringe. After the injection the orifice was sealed with paraffin wax and the egg returned to the incubator. Antiheart serum was injected in doses of 0.4, 0.1 and 0.05 cm³, and antilens serum — 0.4 and 0.1 cm³. As a control we injected normal rabbit serum in doses of 0.1 and 0.05 cm³. Some of the eggs were incubated without receiving injections of sera and these were used later for determining the normal weight of the organs. On the 8th day after injection, i.e. on the 13th day of incubation, the eggs were opened up and in each series we determined the weight of each embryo, the weight of the heart, the crystalline lenses and the liver. After weighing, the organs were fixed in Helly's fluid for histological examination. Altogether 104 eggs were used in the experiment.

TABLE 1

Average Weight (in mg) of the Organs of Chick Embryos (13 days of incubation) After Injection of Immune Sera into the Eggs

Name and dose (in cm ³) of serum	Total weight of embryo	Weight of organs		
		heart	crystalline lens	liver
Antiheart 0,4	3 920	46,6	3,0	84,7
» 0,1	5 350	58,1	3,0	102,6
» 0,05	5 055	65,2	3,0	97,5
Antilens 0,4	5 298	76,2	2,5	98,1
» 0,1	6 430	77,6	3,3	144,1
Normal 0,1	5 880	70,4	3,0	114,6
» 0,05	5 715	66,0	3,1	103,9
Control (no serum injected) . .	5 500	67,6	3,0	103,3

TABLE 2

Differences in the Average Weight of the Organs of Chick Embryos (13 days of incubation) After Injection of Immune Sera into the Eggs, and that of Control Embryos of the Same Age (as a percentage of the control weight)*

Name and dose (in cm ³) of serum	Change in weight			
	whole embryo	heart	crystalline lens	liver
Antiheart 0,4	<u>-28,7</u>	<u>-31,1</u>	0	-18,0
» 0,1	-4,5	-14,0	0	-0,7
» 0,05	-9,1	-3,6	0	-5,6
Antilens 0,4	-3,7	+12,7	<u>-16,1</u>	-5,0
» 0,1	<u>+16,9</u>	<u>+14,8</u>	<u>+3,5</u>	<u>+39,5</u>
Normal 0,1	+6,9	+4,2	0	+10,9
» 0,05	+3,9	-2,4	0	+6,6

Note: The figures underlined indicate the statistical significance of the change in weight.

* Injection of normal serum stimulated growth of the embryos, hence comparison of the results obtained with the weight of the embryos injected with normal serum led to a greater difference between the experimental and control figures.

EXPERIMENTAL RESULTS

Injection of the sera into the eggs sharply increased the mortality rate among the embryos. Normally this was 14.3% but after injection of the sera the total mortality was 29.7%. The highest mortality rate (68.2%) was observed after the injection of 0.4³ of antiheart serum.

The gravimetric results are shown in Tables 1 and 2. We subjected the numerical results to statistical treatment by the Fisher-Student method. Differences were considered to be significant if P did not exceed 0.01.

It will be seen from Table 2 that after injection of the antiheart serum in doses of 0.4 and 0.1 cm³ growth of the heart was sharply retarded. Injection of 0.4 cm³ of antiheart serum resulted also in inhibition of growth of the embryo. Antilens serum, in a dose of 0.4 cm³, caused a reduction in the weight of the crystalline lens but had no appreciable effect on the weight of the other organs. It is interesting that antilens serum, in a dose of 0.1

TABLE 3

Mitotic Activity of the Cardiac Muscle of Chick Embryos (13 days of incubation) After Injection of Antiheart Serum into the Eggs

Dose of serum injected, cm ³	Mitotic activity		
	No. of cells in interphase	number of mitoses	mitotic coefficient, %
0.4	2 900	32	1.1
0.1	3 150	103	3.27
0.05	3 100	128	4.13
Control (no serum injected)	3 150	137	4.35

Note. The number of mitoses was counted in 50 fields of vision in 3 embryos of each series.

cm³, had an obviously stimulating action on the growth of the whole embryo, the heart and liver. At the same time the weight of the crystalline lens was unchanged.

A nonspecific stimulating action on the growth of the embryos was also found on injection of 0.1 cm³ of normal rabbit serum. Antiheart and normal serum, in a dose of 0.05 cm³, caused no perceptible changes in the weights of the organs of the embryos. The weight of the liver in both experimental and control embryos varied so greatly that in our opinion it is not a suitable object for studying the action of antisera on the growth of the organs of chick embryos. Histological study of the crystalline lens showed no structural abnormalities after injection of the antilens sera. On the other hand, the heart muscle of the embryos subjected to the action of a large dose of antiheart serum (0.4 cm³) showed gross damage. Examination of the hearts of this series of embryos showed that they were considerably smaller than normal. Sections showed areas of necrosis side by side with areas of normal muscle. In these places loss of the transverse striation of the muscle fibers is seen; the fibers are friable and broken up into separate fragments. In areas of necrosis can be seen many degenerating cells with pyknotic nuclei.

A count of the mitoses in different portions of the heart showed (Table 3) that antiheart serum depresses mitosis in cardiac muscle. The fall in the number of mitoses is greater the larger the dose of antiheart serum. Injection of 0.05 cm³ of antiserum did not alter the number of mitoses. It is important to point out that after injection of 0.4 cm³ of antiheart serum, side by side with large areas in the heart where mitoses were encountered extremely rarely other areas were observed in which the number of mitoses was even higher than normal; here there may be almost five times as many mitoses as normally: in these areas there was one mitosis on the average to 4-5 nuclei in the stage of interphase. It is not yet possible to account for the increase in mitotic activity in the individual areas of the cardiac muscle in embryos treated with antiheart serum. Further research will undoubtedly be required to shed light on this interesting phenomenon.

SUMMARY

Thus the injection of antiheart and antilens sera, in a dose of 0.4 cm³, resulted in our experiments in a considerable loss of weight of the corresponding organs of chick embryos. Antiheart serum, in this dosage, also caused retardation of growth of the whole embryo. Histological examination revealed necrotic areas in the cardiac muscle and a sharp fall in mitotic activity in this tissue. Injection of 0.1 cm³ of antiheart serum led to loss of weight of the heart but did not affect the growth of the other organs. Antilens serum, in a dose of 0.1 cm³, stimulated growth of the embryo and the individual organs, with the exception of the lens. A nonspecific stimulating action on the growth of the embryos was also shown by injection of 0.1 cm³ of normal rabbit serum.

The results of this investigation show that organ-specific antibodies are capable of a specific action on the growth of the corresponding organs.

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