

**A STUDY OF THE MECHANISM BY WHICH THE GROWTH OF ORGANS
IS REGULATED IN EMBRYOGENESIS**

**REPORT I. THE EFFECT OF TRANSPLANTED SPLENIC FRAGMENTS FROM ADULT AND
EMBRYONIC CHICKENS ON THE SPLEEN OF EMBRYO RECIPIENTS**

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In the course of many years, investigators working on the problem of growth regulation and tissue development have become interested in the phenomenon of growth stimulation caused by splenic tissue from adult chickens being transplanted to the chorioallantois of chick embryos. Thus, Murphy [3], V. Danchakoff [1], Willer [5], Pomerat [4], Ebert [2] and others showed that splenic fragments from adult chickens transplanted to the chorioallantois of chick embryos previously incubated for 9-10 days stimulate the growth of the spleen in the embryo recipients. The power of the transplanted chicken spleen to cause the stimulation of splenic growth in the embryo recipient increases in direct proportion to the age of the donor [2].

Thus, in its own right the phenomenon of growth stimulation of embryonic organs secondary to the influence of transplants of the corresponding tissues can hardly be doubted at the present time.

However, it remains unknown in what manner the transplanted splenic fragment from the adult chickens acts on the spleen of the embryo recipients, and what mechanisms are involved in this effect.

In order to approach the resolution of this question we carried out an investigation in which we attempted to clarify whether or not there exists a relationship between the nature of the growth stimulation effect and the age of the transplant donors and the embryo recipients.

As the recipients in the work we used chick embryos at 4, 6, 8 and 10 days of incubation. Adult roosters and chick embryos at 14 and 17 days of incubation served as donors.

The experiments were performed in the following manner. The eggs were initially transilluminated by means of an ovoscope, and a site noted where there was satisfactory branching of the chorioallantoic vessels. In the corresponding area of the shell we sawed out a little window, approximately 1 cm². Subsequent operations were carried out in a box, under sterile conditions. The shell was carefully removed with fine forceps, so as not to injure the membrane under the shell. Then one drop of physiological saline containing penicillin was applied to the membrane, and it was carefully removed with the fine forceps. A small fragment of spleen, 1-1.5 mm³ in size, was placed on the vascular field thus exposed. The little window was covered with the shell, which was fixed in place with melted paraffin. It was impossible to transplant tissue to the chorioallantois of embryos in the 4th and 6th days of incubation, so the transplantation was performed to the branching of the vessels in the yolk sac.

TABLE 1

The Action of Splenic Transplants from Adult Chickens on the Growth of Organs in Chick Embryos

Embryo recipient groups	No. of embryos	Relationship of wt. of organs to total wt. of embryos					
		spleen		heart		liver	
		in absolute numbers	in relation to control, %	in absolute numbers	in relation to control, %	in absolute numbers	in relation to control, %
4-Day test group . .	31	0,00063	128,7	0,101	101	0,0151	98
Control . .	16	0,00049	100	0,0100	100	0,0154	100
6-Day test group . .	21	0,00083	105,0	0,011	91,7	0,021	100
Control . .	25	0,00079	100	0,012	100	0,021	100
8-Day test group . .	38	0,0011	122,2	0,0112	99,1	0,023	100
Control . .	26	0,0009	100	0,0113	100	0,023	100
10-Day test group . .	38	0,0026	288,9	0,010	120,5	0,032	139
Control . .	20	0,0009	100	0,0083	100	0,023	100

Note: In Tables 1 and 2 the figures reflecting statistically significant changes in the weight of an organ are set in boldface type.

TABLE 2

The Action of Splenic Transplants from Chick Embryos on the Growth of Organs in Chick Embryos

Embryo donor and embryo recipient groups	No. of embryos	Relationship of wt. of organs to total wt. of embryos					
		spleen		heart		liver	
		in absolute numbers	in relation to control, %	in absolute numbers	in relation to control, %	in absolute numbers	in relation to control, %
Test { donor 17 days recipient 4 days	14	0,0005	125	0,0118	104,4	0,0173	108,1
Control	12	0,0004	100	0,0113	100	0,0160	100
Test { donor 17 days recipient 10 days	14	0,00075	131,6	0,0099	111,2	0,0256	113,8
Control	14	0,00057	100	0,0089	100	0,0225	100
Test { donor 14 days recipient 10 days	28	0,0009	112,5	0,0082	97,6	0,023	104,5
Control	31	0,0008	100	0,0084	100	0,022	100

For the control we used embryos of the same incubation age as those in the experiment. We performed the same manipulations on them as we did on the experimental embryos, with the exception of the transplantation. The material for the transplantation was sterilely removed from a freshly killed rooster or from fresh embryos. The isolated organs were placed in Petri dishes containing physiological saline. Then the necessary tissue was removed with a scalpel, and divided into fragments. Up to the moment of transplantation (in the course of 1-2 hr) the finely divided tissue was kept in a refrigerator at 2-4°.

Seven days after the operation the embryo on whose chorioallantois we noted good growth of the transplants were removed. Both the whole embryos and their individual organs were weighed (spleen, heart, liver).

In order to evaluate the results both in the test group and in the control we calculated the relationship between the weight of the given organs and the weight of the intact embryo. The data obtained was subjected to statistical analysis, following the method of Fisher-Student. The results were considered significant when P did not exceed 0.02.

RESULTS

In Table 1 we present the results obtained in the experiments dealing with the action of splenic transplants from adult chickens on the growth of organs in chick embryos in the 4th, 6th, 8th and 10th days of incubation.

As can be seen from Table 1, under the influence of fragments from the spleens of adult chickens growing in the vascular field of the yolk sac in embryos at the four day incubation stage, an enlargement occurred in the spleen of 28.7%, while the heart and liver did not show changes.

Splenic fragments from adult chickens transplanted to the yolk sac of chick embryos at the six-day incubation stage did not demonstrate any actual effect on the growth of the organs in the recipients.

Under the influence of splenic fragments from adult chickens transplanted to the chorioallantois of 8-day chick embryos, growth stimulation of the spleen occurred in the recipients, equal to 22.2%; growth of the heart and liver did not change. Under the influence of splenic fragments from adult chickens transplanted to the chorioallantois of 10-day chick embryos, growth stimulation occurred in the spleen (by 188.9%) and in the liver (by 39%).

Thus, under the influence of transplanted splenic fragments from adult chickens, growth stimulation took place in our experiments on the spleen of almost all the embryo recipients. The exception was the six-day embryos, in which growth stimulation of the organs was not observed. In addition, it may be noted that during transplantation of the spleen from adult chickens to the 4- and 8-day embryos there took place an increase in the growth rate of the spleen only, while with transplantation to the 10-day embryos the growth rate of both the spleen and the liver increased.

In Table 2 we present the results of experiments dealing with the action of splenic transplants from chick embryos of the 14- and 17-day incubation stages on the growth of organs in embryos of four and ten days incubation.

As can be seen from Table 2, growth stimulation of the 4-day chick embryos occurred in the spleen (by 25%) under the influence of transplanted splenic fragments from 17-day chick embryos, at the same time that the growth of the other organs (heart and liver) did not show essential changes.

However, under the influence of transplanted spleen from the 17-day embryos to the 10-day chick embryos, there occurred growth stimulation both in the spleen (by 31.6%) as well as in the heart (by 11.2%) and in the liver (by 13.8%).

Growth stimulation only occurred in the spleen (by 12.5%) under the influence of transplanted spleen from the 14-day chick embryos to the 10-day embryos.

Thus, under the influence of transplanted spleen from the chick embryos of the 17- and 14-day incubation stages, there occurred an increase in the growth rate of the spleen in the embryo recipients of all stages investigated. In addition, with transplantation of the spleen from the 17-day embryos to the 10-day chick embryos, an increase in the growth rate took place not only in the spleen, but in the heart and liver as well.

Hence, the results of the experiments presented above showed that transplantation of splenic fragments from adult chickens exerts an influence on the growth rate of organs in chick embryo recipients at the various incubation stages studied. An exception is noted in the 6-day embryo recipients.

However, the nature of this influence depends on the age of the embryo recipients. At some stages of development (4 and 8 days of incubation) a specific effect took place on the growth of the spleen. At the same time, at other stages (10 days) transplantation of splenic fragments from adult chickens led to nonspecific growth stimulation of the spleen, liver, and heart.

Splenic fragments from the chick embryos (14 and 17 days of incubation) exerted an influence on the growth rate of the organs in the chick embryo recipients. However, this influence differed in its character from the influence which was demonstrated by the splenic fragments from the adult chickens.

The spleen from the 17-day embryos transplanted to the 4-day embryos, and the splenic tissue from the 14-day embryos transplanted to the 10-day embryos, demonstrated a specific influence on the growth of the spleen. Nonspecific influence on the growth rate of the spleen, heart and liver in the 10-day embryos was exerted by the tissue from the 17-day embryos. These data show that the character of the changes in the growth

of organs of the embryo recipients depends on the age of the transplanted tissue donor as well.

Thus, the data presented in this work show that the character of the changes in the growth of embryonal organs in response to transplantation of the corresponding tissue to the embryo depends both on the stage of development of the recipients and on the stage of development of the donors from which the transplants were taken.

SUMMARY

Spleen fragments obtained from chickens and chick embryos (after 4, 6, 8 and 10 days of incubation) were grafted on membranes of chick embryos. The transplants exerted a definite effect on the growth of the organs in the recipients (specific and nonspecific growth stimulation). Changes occurring in the growth of the embryonic organs in response to transplantation of corresponding tissues depended on the stage of the recipient's and donor's development.

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