

ORGAN GROWTH REGULATION IN EMBRYOGENESIS
COMMUNICATION IV. THE INFLUENCE ON THE GROWTH OF CHICK
EMBRYO ORGANS OF A SUBSTANCE FROM SPLENIC CELLS WHICH
PASSES THROUGH MEMBRANOUS FILTERS

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At the present time although there is no doubt that transplantation of tissues stimulates the corresponding embryo organs, there is disagreement concerning the mechanism.

Some authors [5, 7-11] maintain that stimulation of the organs of the recipient embryo is due to a reaction of the transplanted cells which on establishment in the host become capable of taking part in immunological reactions against the embryo antigens. Other investigators [1-4, 6] speak of a protein (antigenic) influence of the transplant on the chick embryo organs. The results available at the present time are not sufficient to prove either point of view.

The object of the present work has been to produce further evidence that the increase in the size of the organs of the recipient is not necessarily due to the establishment in it of cells of the transplant.

EXPERIMENTAL METHODS

As recipients of splenic cells we used 10-day chick embryos. Adult chicks were the donors.

The spleen was obtained from a freshly-killed cockerel; it was carefully washed in physiological saline and penicillin, freed from the membrane and blood vessels, and transferred into an equal volume of Tyrode's solution containing penicillin. The tissue was first triturated by means of pipettes, and then it was passed repeatedly through syringes without a needle, and then through syringes fitted with a chick needle. The suspension prepared in this way was preserved at 2-4° in a refrigerator until transplantation. Transplantation was carried out not later than 1-2 h after the suspension had been prepared.

Before the suspension was transplanted, it was placed in a small perspex chamber (see figure). The base of the chamber was formed from a fine filter No. 3 (diameter of the pores 0.7μ) fixed in position with collodion; other filters were No. 4 (0.9μ), No. 5 (1.2μ), and No. 6 (1.5μ). After the suspension had been introduced into the chamber, the apertures in the groove were filled with melted paraffin. The completed chamber was introduced with forceps at the point of branching of the chorioallantoic vessels. Other features of the method have been described in the first communication of this series [1].

EXPERIMENTAL RESULTS

As can be seen from the table, when the suspension of splenic cells of adult chicks was introduced into the chamber having a membranous filter No. 3 (pore size 0.7μ), an increase in the weight of the recipient spleen amounting to 83.3% (above the control) was observed. The heart and liver showed no appreciable changes. When the transplantation was made without the chamber, the stimulation of splenic growth was very great (333.3%), but liver growth was less stimulated (25.3%). In this experiment there was also some tendency towards an increased growth of the embryo heart (16.8%, $P = 0.39$).

When transplantation was made in chambers with a filter membrane No. 4 (pore size 0.9μ) the weight of the spleen of the recipient embryos increased to 183.3% of the weight of the control. The heart and liver showed no appreciable changes. When a suspension of adult chick spleen cells was transplanted without chambers, an increase in weight of 200% for the spleen, 14.8% for the heart, and 16.4% for the liver was observed.

Action of a Suspension of Adult Chick Splenic Cells

Method of transplantation of splenic cells	Relative weight of organs of embryos					
	spleen		heart		liver	
	weight of organ as percentage of embryo	as percentage of control	weight of organ as percentage of embryo	as percentage of control	weight of organ as percentage of embryo	as percentage of control
In chamber with filter No. 3	0.11	183.3*	1.06	98.8	2.43	98.5
Without chamber	0.26	433.2*	1.25	116.8	3.07	125.3*
Control†	0.06	100	1.07	100	2.45	100
In chamber with filter No. 4	0.17	283.3*	1.19	110.1	2.85	120.3
Without chamber	0.18	300*	1.24	114.8*	2.76	116*
Control	0.06	100	1.08	100	2.37	100
In chamber with filter No. 5	0.17	242.9*	1.22	114	3.05	120.1
Without chamber	0.21	300*	1.36	127*	3.21	126.4*
Control	0.7	100	1.07	100	2.54	100
In chamber with filter No. 6	0.17	200*	1.26	120	3.20	123.6
Without chamber	0.28	329.4*	1.46	139*	3.60	139*
Control	0.085	100	1.05	100	2.59	100

* Statistically significant change in the weight of the organ.

† In the control experiments no transplantation was performed.

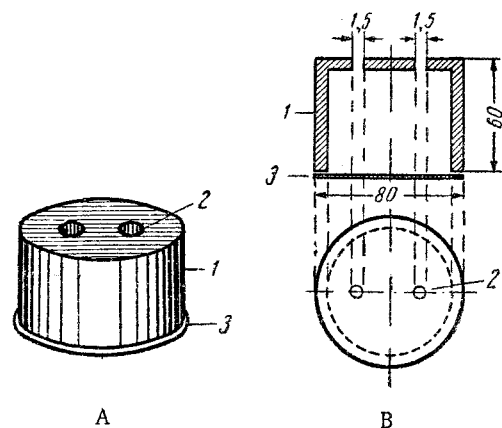
With transplantation into chambers having filter membrane No. 5 (pore size 1.2μ) the weight of the spleen of the recipient embryo increased by 142.9%. There was some tendency for the liver also to increase in size (20.1%, $P = 0.04$). The heart showed no significant change. When the transplantation was made without the chambers, spleen growth was stimulated by 200%, the heart increased by 27%, and the liver by 26.4%.

When the transplantation was made in chambers having filter membrane No. 6 (pore size 1.5μ) the weight of the spleen of the recipient embryos increased by 100%. In this experiment the heart and liver showed some increase in weight (heart 20%, $P = 0.045$; liver 23.6%, $P = 0.035$). When a cell suspension was transplanted without chambers, the spleen increased in size by 229.4%, and the heart and liver by 39%.

Thus under the influence of adult chick spleen cells transplanted on to the chorioallantois and placed in chambers through which whole cells cannot pass (smallest diameter of the spleen cells 14.5μ), a considerable stimulation of the growth of the spleen of the recipient embryos was observed. A great increase in size was also observed when a suspension of spleen cells was transplanted in chambers having filter membranes Nos. 4, 5, and 6. Statistical treatment shows that in these three cases the stimulation was uniform. When the splenic cell suspension was transplanted in chambers fitted with filter membrane No. 3, there was a smaller increase in size of the spleen of the recipient embryos.

A comparison of the influence of the splenic cell suspension in chambers having filter membranes, and suspensions introduced without chambers has convinced us (after statistical treatment) that transplantation of splenic

cell suspensions in chambers having filter membranes Nos. 3 and 6 produced less splenic growth than did transplantation without chambers. However, when the transplantation was carried out with filters Nos. 4 and 5 and again without chambers, statistical treatment showed that the increase was the same in both cases.



Chamber for transplantation of a suspension of adult chick splenic cells onto the chorioallantois of chick embryos. A) General view; B) diagram of chamber. 1) Cylinder; 2) aperture for introduction of a cell suspension; 3) membranous filter stuck on with collodion.

We may also note that when the transplantation was carried out without chambers there was a nonspecific stimulation of growth of the organs in the recipients. With increase in diameter of the membrane pores there was a reduction of organ-specificity in the action of the splenic cell produced substances which passed through the membranous filters.

Our results therefore show that the noncellular substances which pass through the pores of the membrane filters exert a stimulating influence on chick embryo spleen growth.

Consequently, in the stimulation of organ growth in the recipient an important part is played by proteins which pass from the transplant into the corresponding organ of the recipient.

SUMMARY

Cellular suspensions of adult chicken spleens were placed in chambers fitted with membrane filters through which whole cells could not pass; they were introduced into the chorioallantois of 10-day chick embryos. Noncellular substances passing through the pores of the membrane filters exerted a stimulating effect on splenic growth.

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