PRELIMINARY CONSERVATION OF THE GONARDS OF BIRDS BEFORE TRANSPLANTATION INTO A RECIPIENT

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One of the main causes of failure to transplantates to take is known to be delay in their vascularization, leading to tissue necrosis in the grafted organ. In order to overcome this during transplantation of gonads in birds it is almost impossible to utilize such surgical procedures as anastomosis of vessels (because of the small size of the material) or one-stage parabiosis (on account of the deep situation of these organs within the cavity).

In this connection it is essential to devise biological methods which would stimulate cell division in the tissues of the graft and the bed, or would bring about the maintenance of an isotonic state of the transplanted glands until a blood supply was established.

EXPERIMENTAL METHOD

The effectiveness of biological stimulation of transplantates, according to V. P. Filatov, has been widely tested on a variety of objects. When making use of this method of treatment of transplanted gonads in birds, we slightly modified it, and as the medium in which the grafts were preserved we selected allantoic and amniotic fluids from embryos.

The choice of allantoid embryonic fluid was made because it contains products of protein decomposition such as ammonia, urea and allantoin, which are used for stimulation of regenerative processes in wounds. In this particular fluid the components listed are present in association with each other, and it might be expected that without requiring any special preparation, this sterile fluid would be effective as a general stimulator. Further, as shown by research in B. P. Tokin's laboratory, the allantoic fluid of the embryo posseses bactericidal properties.

In other series of experiments we treated the grafts with amniotic fluid from embryos in order to maintain the transplanted gonads in an isotonic state until the establishment of a blood supply. The amniotic fluid of the embryo, being a natural isotonic medium adapted for the existence and development of the young growing tissues of the embryo, might promote the existence and growth of the regenerating tissues of the graft; in addition the bactericidal properties of the amniotic fluid of the embryo were demonstrated in B. P. Tokin's laboratory in birds.

Some of the grafts were preserved in blood serum and in the fluid which bathes the lens of the eye, or simply kept in cold storage without the use of any of these biological media.

For the experiments we used fowls of the Leghorn, Australorp and New Hampshire breeds. Homoplastic transplantation was carried out on them. The recipient birds were subjected to bilateral castration, after which one gonad of the same sex was implanted. Both male and female gonads were transplanted.

Most of the birds underwent the operation at the age of one month. In isolated cases their ages varied from 15 days to $2\frac{1}{2}$ months, but the comparable series of experiments were set up to provide analogy in this and other features (sex, breed).

The gonads were extracted through an incision between the two last ribs, by means of iris forceps, and were transplanted to the recipient through a corresponding incision, into the depression between the lung and adrenal,

so that survival of the graft could be distinguished from regeneration of the recipient's own gonads. In this particular bed the grafts could be fixed without suturing (which interferes with the survival of small grafts), since in this situation the immobility of the transplanted gonads is not disturbed by peristalsis in the internal organs of the recipient.

For the preliminary treatment of the grafts with the fluids mentioned above, we used the following method.

Treatment of the grafts with allantoic and amniotic fluids. The hatched egg, as soon as it was taken from the brood-hen, was disinfected with alcohol. Using sharp scissors with fine, pointed ends, a small piece of the shell was cut away from the egg, near the obtuse end. At the same time an opening was made in the outer shell membrane, after which the allantois settled to the bottom. With a sterile needle the allantoic membrane was perforated; through the orifice thus formed the allantoic fluid was poured into a sterile tube and kept in the refrigerator at a temperature of +3°C. After this the amnion was pierced and the amniotic fluid poured into another sterile tube which also was placed in a refrigerator at a temperature of +3°C. The grafts were immersed in these tubes of fluid and stored in the refrigerator for 2-3 days.

For the purpose of obtaining these fluids we used 17-19 day embryos, for at this period the antibiotic properties of the amniotic fluid increase because of the using up of the albuminous membrane.

Treatment of the grafts with blood serum. Serum was prepared in the usual manner. Fresh blood was collected in a clean, sterile tube, allowed to stand for a few minutes and then centrifuged. The serum was kept in a refrigerator at a temperature of +3°C. The grafts were kept in the serum until the moment of grafting to the recipient (for 2-3 days).

Treatment of the grafts with the fluid bathing the lens of the eye. The eyes were removed from a fowl dying at operation and placed in clean tubes in the refrigerator at +3°C. Grafts were inserted through a small incision in the membranes of these eyes in the region of the pupil and kept there for about 3 days.

In some cases the grafts were preserved in the cold without treatment with any of these fluids mentioned and were placed directly in a sterile tube in the refrigerator at a temperature of +3°C.

TABLE 1

Survival Rate of Gonads not Subjected to Preliminary Treatment

Total number of experiments	Number of experiments with indeterminate results		Number of experiments in which absorption of the grafts took place	Number of survivals	
	result not known	result indefinite		Number of cases	%
36	2	3	27	4	12.9

Survival of the grafts was judged on the basis of such visual findings as the firm adherence of the graft to the bed, the absence of exudation and of encapsulation of the transplanted organ, and also by the healthy outward appearance of the graft (normal color, normal or increased size).

When interpreting the results, we took into considration not only prolonged and stable survivals but also primary "takes," in which the graft was fixed for a short time after transplantation, since both were equally exposed to the influence of the treatment of the grafts on their condition in the period immediately after grafting. (Subsequent absorption of already surviving grafts is largely due to immune relationships between donor and recipient).

Operations were performed on 152 birds.

The place at which the experiments were carried out and the birds kept was the premises of the Department of Darwinism and Genetics of the Kiev State University and the "Severinovka" collective farm in the Makarov district of Kiev region.

TABLE 2

Survival Rate of Gonads in Relation to the Method of Preliminary Treatment Before Transplantation to the Recipient

Storage of grafts before transplantation	Total number of experiments	Number of experiments with indeterminate results		Number of experiments in which absorption of the graft took place	Number of survivals	
		result not known	result indefinite		Number of cases	%
No preliminary treatment carried out	20	1	–	15	4	21.1
Preserved in the cold without the use of isotonic solutions	19	–	1	12	6	31.6
Preserved in the cold in fluid from the eye	20	–	2	13	5	27.8
Preserved in the cold in blood serum	17	–	1	11	5	31.3
Preserved in the cold in amniotic fluid from embryos	18	1	1	9	7	43.8
Preserved in the cold in allantoic fluid from embryos	22	3	2	9	8	47.1

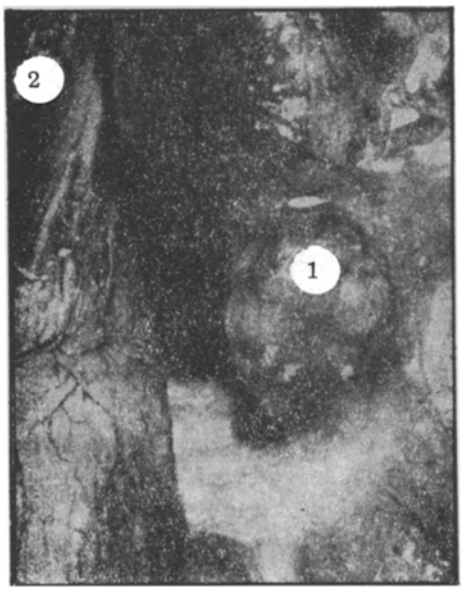

Fig. 1. The left oviduct of chick No. 089, displaced to the right side of the body. Its infundibulum (2) is found below the right lung, near a surviving grafted ovary (1) on the right lobe of the liver.

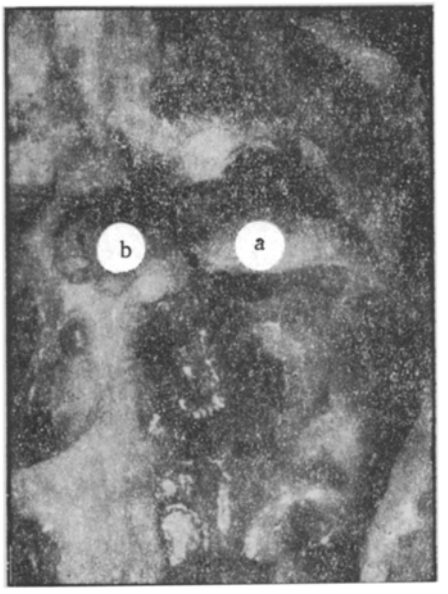

Fig. 2. Development of a transplanted testicle (a) in the same area as a regenerated ovary (b).

EXPERIMENTAL RESULTS

The results of our experiments may be judged from the figures given in Tables 1 and 2.

It can be seen from Tables 1 and 2 that in the experiments where no preservation of the grafts was carried out, their survival rate was comparatively low. On comparing the number of survivals in the different series of experiments it is evident that preservation of the grafts in allantoic and amniotic fluids of embryos had a favorable effect on their survival as compared with those preserved in fluids such as the fluid from the eye, which evidently possesses no property for promoting survival of grafts other than that of isotonicity. Even perservation of the grafts in blood serum, not only a natural isotonic solution but also an excellent nutrient medium for dividing cells, gave a lower survival rate than similar treatment of the grafts with amniotic and allantoic fluids from embryos. Healthy survival of grafts, as obtained in several experiments using the latter method, may be illustrated by Figs. 1 and 2.

SUMMARY

The transplants of the gonads of chicks aged from 18 days to 2.5 months were preserved in the allantoic and the amniotic fluids previous to transplantation. It was demonstrated that the percentage of take of these transplants is much greater than that of the transplants preserved in the aqueous humor, blood serum, or at low temperatures without any biological media.

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