TRANSLANTATION OF FERTILIZED RABBIT EGGS TO THE ANTERIOR CHAMBER OF THE EYE

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(Received 21st April 1961)

Summary. A new and reliable technique for transplanting tubal rabbit eggs to the anterior chamber of the eye is described. The growth of the intra-ocular eggs has been studied for up to 120 hr after mating. From the 2- or 4-cell stage, when the eggs were transplanted, to the morula stage, the intra-ocular eggs grew normally. After that stage, the transplanted eggs showed retardation of growth and degenerative changes.

INTRODUCTION

During the last decade, there has been an extensive search for effective anti-fertility agents, and a great number of drugs has been tried on different points of the complicated process of reproduction. In this search, many drugs have been tested that are supposed to act on the egg after ovulation but before nidation. Among these compounds, some are deleterious to the fertilized rabbit egg even in minute doses, e.g. estrogenic hormones (Burdick & Pincus, 1935; Pincus & Kirsch, 1936). Recently a non-steroid compound, mer-25 (1-(p-2-diethylaminoethoxyphenyl)-1-phenyl-2-p-anisyl-ethanol), has been found to produce severe damage to fertilized rat and rabbit eggs (Segal & Nelson, 1958; Chang, 1959).

When a drug has been found to affect eggs during their passage through the tube, it is — especially if the drug be a hormone — very difficult to differentiate between two possible effector mechanisms: a direct effect on the eggs, and an indirect effect via changed conditions within the tubes. This is a problem of theoretical as well as practical significance and it has therefore been extensively discussed (Pincus & Kirsch, 1936; Csapo, 1955; Greenwald, 1957). The problem, however, remains unsolved.

A suitable method for distinguishing between a direct and an indirect effect of a drug on fertilized eggs should be to transplant them from the tubes to some other situation where their development can proceed and where the drug to be studied may pass unimpeded. The anterior chamber of the eye would seem to fulfil these criteria and successful transplantation of mouse eggs to this site has been reported by Runner (1947) and Fawcett, Wislocki & Waldo (1947). Allen & Priest (1932) failed to transplant rabbit eggs to this site. Shapiro & Harvey (1957) were successful but in only five out of fifty-three experiments
(9.4%) and the eggs were left in the eye for a long period of time (75 to 148 days). Various kinds of tissue developed: bone, cartilage, kidney, hair and fat. The early intra-ocular development of the eggs was not studied.

We have developed a technique for transplanting fertilized rabbit eggs to the anterior chamber of the eye with 90% successful transplantation.

METHODS

Rabbits of mixed breeds, but mainly of Dutch stock, were used. Twenty-four hours after mating or, in most cases, artificial insemination with diluted semen from fertile males and intravenous injection of 33 i.u. of chorionic gonadotrophin to induce ovulation, the eggs were flushed out of the Fallopian tubes.

For the transplantation, the animal was anaesthetized with about 2 ml Nembutal (Abbott) 6% intravenously. The abdomen was opened through a flank incision and the number of recently ruptured follicles of the ovary of that side counted. A corresponding number of eggs — usually five or six — should be obtained from the tube. (Both tubes can of course be removed and flushed for eggs; in this series, however, one tube was always left to permit comparison between tubal and intra-ocular eggs at the end of the experiment.) Together with about 1 cm of the uterus, the tube was carefully removed. The separation was made close to the tube to get it as free from fat and blood as possible. Fat beads and blood might make it impossible to see the eggs later in the flushing fluid. Close to the ovary, the tube makes a sharp bend which was carefully followed. After removal of the tube, it was carefully rolled on a sheet of soft paper to get rid of fat and blood on its surface. As a flushing medium, Ringer’s solution (NaCl 9·0 g, KCl 0·42 g, CaCl₂ 0·24 g, MgCl₂ 0·05 g, in 1,000 ml water) was used, to which was added 10% freshly prepared rabbit serum. M. C. Chang (personal communication, 1960) has shown that this addition increases the survival rate of the eggs. Before flushing, the fimbriae of the ovarian end of the tube were cut off in order to prevent the eggs from adhering to them. By means of a glass pipette with a small rubber balloon, the tube was then flushed from the uterine end, the small piece of uterus serving as a handle. About 1 ml was flushed through the tube into a watch-glass. Most of the eggs were obtained in this first portion. A second and third millilitre were flushed down into separate watch-glasses. The eggs sedimented to the centre of the watch-glasses. Under a dissecting microscope (×30), the eggs were sucked into a special syringe-micropipette (Text-fig. 1), similar to the instrument used by Falck (1959) for transplanting rat ovary cells to the anterior chamber of the eye. The instrument was filled with Ringer’s solution with added rabbit serum. After the topical application of Xylocain 2% drops (whereby less Nembutal could be used), the bulb of the eye was grasped with dressing forceps at the insertion of one of the external ocular muscles, and the bulb was lifted out of the socket. With a corneal knife, the cornea was incised near the limbus, the incision being Z-shaped to establish a valve closure postoperatively, as described by Dyster-Aas & Krakau (1956). By this means, loss of aqueous humour and transplanted eggs was prevented. After withdrawal of the knife, a dissecting microscope was placed over the eye, and the entire transplantation was
Transfer of eggs to anterior chamber performed under visual control. The microscope lamp was placed opposite the operator and somewhat below the horizontal level so that the light passed through the centre of the cornea. With this arrangement, the eggs were clearly visible in the capillary and also when they passed from the capillary into the anterior chamber. After insertion of the capillary through the corneal incision, its tip was scratched against the posterior surface of the cornea until a fine fibrous network was formed. The eggs were expelled from the pipette by means of the micrometer (managed by an assistant under the direction of the operator) and were safely deposited in the fibrous network where they stayed when the pipette was withdrawn.

When the eggs were to be recovered from the eye, the animal was killed by air embolism, the eye was enucleated and carefully cleared of fat, blood and muscle tissue. The bulb was then opened at the limbus and the lens together with the vitreous body in situ were flushed with Ringer's solution. The anterior chamber was flushed separately and all the flushing fluid was searched for eggs. If the expected number of eggs were not recovered, the surfaces of the cornea and of the iris were examined where eggs still attached to the fibrous network could be found. The eggs were transferred to a slide, mounted in toto, fixed and stained according to Chang (1955). In some cases, the eggs were so firmly attached to the cornea or to the iris as to necessitate preparation of the tissue with the attached egg for fixation in Bouin's solution, serial sectioning and staining with haematoxylin and eosin.

RESULTS

With the technique described, twenty-nine rabbits were submitted to transplantation of ova from one tube to the anterior chamber of one or both eyes. In three animals, no egg could be recovered from the eye (i.e. successful recovery was achieved in 90% of the experimental animals). Altogether 128 eggs were transplanted and seventy-four (58%) were recovered.

![Text-fig. 1. Syringe-micropipette, (a) glass capillary, (b) polyethylene tube, (c) needle, (d) 2-ml syringe, (e) spring, (f) micrometer, (g) base frame, with adjustable part (h), ending in rubber pad (i).](image_url)
The comparative study of the tubal and intra-ocular eggs showed the same development up to 72 hr after mating, when they had reached the morula stage. Later (at 96 and 120 hr after mating) all eggs had reached the blastula stage, but the intra-ocular eggs showed retardation of growth and degenerative changes. These changes, which will be described in detail in a subsequent paper, were not influenced by the number of eggs transplanted to the same eye.

DISCUSSION

The technique described represents a reliable method of transplanting tubal rabbit eggs to the anterior chamber of the eye where they have been studied for up to 120 hr after mating. This method may facilitate the study of many problems concerning fertilized rabbit eggs, including the role of extragenital factors in their growth and the direct effect of hormones and antifertility compounds.

ACKNOWLEDGMENT

This investigation was supported by a grant from the Medical Faculty, Lund.

REFERENCES


