Development of Mouse Ova in Explanted Oviducts: Fertilization, Cultivation, and Transplantation

Abstract. *The development of fertilized ova in explanted parts of oviducts was studied from 12 to 36 hours after fertilization, and from 36 to 84 hours after fertilization. No egg mortality during cultivation, nor any slowing of development could be detected in either experiment. It may be presumed that the mortality of mouse ova during the period before implantation has no essential effect on the resulting fertility. During cultivation of oviducts for 144 hours, there occurred in most blastocysts herniation of their walls resulting in a formation similar to the blastocyst, and a migration of a disorganized mass of cells, some of which resembled the giant trophoblastic cells. After transplantation of ova fertilized in vitro to recipients, in four cases there occurred nidation and subsequent normal development of the embryo.*

Brinster and Biggers (*1*) first fertilized mouse ova in vitro by introducing semen into the explanted ampular parts of oviducts containing eggs, or by putting the oviducts in a suspension of sperm for 10 to 30 minutes. Fertilization and division of the eggs took place in organ culture as previously described (*2*). With partially modified methods, we achieved fertility of ova which was nearly identical to that of control ova fertilized in vivo (*3*).

We have studied the further development of ova fertilized in vitro and have tried to verify their mortality rate in the stage preceding implantation and to find out whether they are capable of further development after their transplantation to recipients. Parts of the oviducts were left in culture up to 144 hours.

Ovulation in experimental animals was induced hormonally with 3 to 4 international units of pregnant mare's serum (Bioveta) and 15 international units of human chorionic gonadotrophin (Praedyn-Spofa). The fertilization of ova was carried out in principle according to Brinster and Biggers' method (*1*). Instead of chemically defined medium BGJb for organ cultures (*1*), we used medium TC-199 supplemented with glucose (500 mg/100 ml) and inactivated homologous serum (approximately 5 percent). The *p*H was adjusted to 7.8 with an isotonic solution of $NaHCO_3$. The explanted oviducts with unfertilized ova were left for 10 minutes in a sperm suspension and then cultivated on agar sheets (*4*) in petri dishes with the previously described medium. Cultivation was carried out in a gaseous environment containing 5 percent CO_2 and 95 percent oxygen, or 5 percent CO_2 and 95 percent air, at a humidity of about 90 percent and at 37.5°C. After 36 hours of cultivation, two-thirds of the cultivation medium was exchanged. After a fixed period of cultivation, the oviducts were flushed with cultivating medium, and the ova were evaluated by means of fluorescent microscopy (*5*). The morulae and blastulas were evaluated under an inverted microscope. The method of McLaren and Michie (*6*) was used in transplanting the morulae and blastulas into the uterine horns.

The quantitative evaluation of ova fertilized in vitro was carried out in two experiments. In the first, one oviduct, inseminated in vitro, from each female was cultivated 12 to 13 hours; the other oviduct of the same female was cultivated for 36 hours after insemination. The mice were progeny of strain C57 B1/10 Sc Sn females and A males. From a total of 16 oviducts cultivated 12 hours, 126 ova were isolated of which 12 had two or exceptionally three pronuclei, and of which 20 were in syngamy. In all, 73 percent were fertilized. From a total of 16 oviducts cultivated 36 hours, 132 ova were isolated of which 11 had two blastomeres and 90 had three or four blastomeres. Differences between the percentage of ova with two or three pronuclei and the percentage with two to four blastomeres were not significant ($P < .25$).

In carrying out the seecond experiment, one oviduct of each female was cultivated 36 hours, the second one 84 hours. Two strains of mice (H and C57 B1/10 Sc Sn) were used. From a total of 15 oviducts (eight oviducts of strain H and seven oviducts of strain C57 B1/10 Sc Sn) cultivated 36 hours, 160 ova were isolated of which 22 had two blastomeres and 68 had three or four blastomeres. In all, 56.2 percent had two to four blastomeres. From a total of 15 oviducts (eight oviducts of strain H and seven oviducts of strain C57 B1/10 Sc Sn) cultivated 84 hours, 153 ova were isolated of which 13 were morulae and 75 were blastulas. In all, 57.5 percent were in

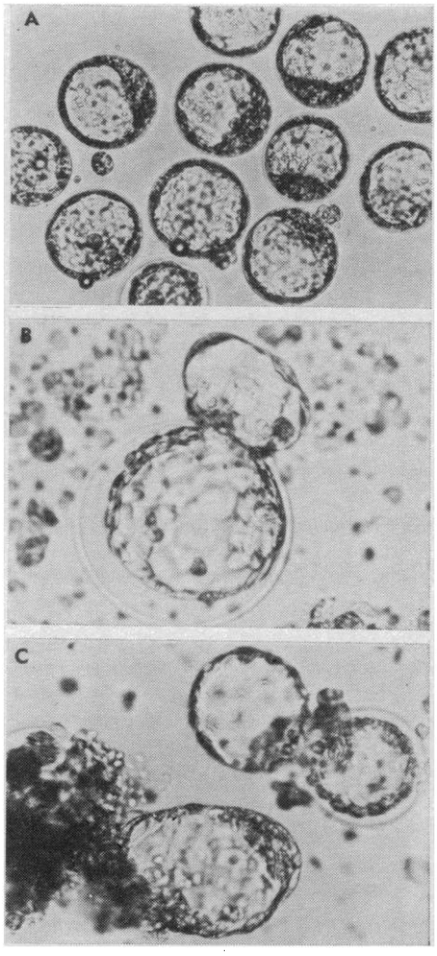

Fig. 1. Mouse ova, fertilized and cultivated in explanted ampular parts of oviduct; (A) 84 hours after fertilization; (B) 120 hours after fertilization; (C) 144 hours after fertilization.

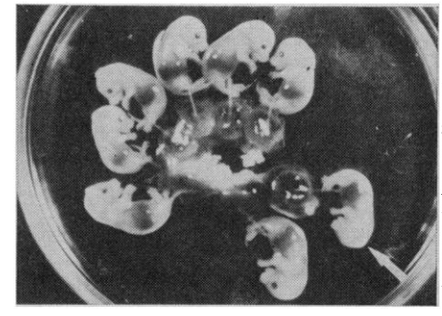

Fig. 2. Seventeen-day-old embryos of the recipient female C57/BL with seven own and one transplanted embryo (arrow) which developed from an egg fertilized in vitro.

morula or in blastula stage. Differences between the percentage of ova with two to four blastomeres and the percentage of morulae and blastulas are not significant ($P < .25$), as in the first experiment.

The lower percentage of developing ova in the second experiment can be accounted for by the lower fertility of superovulated ova of the H strain. From the total of 76 ova from mice of the H strain isolated after a 36-hour cultivation, only 46 percent developed to two- to four-blastomere stage, and after an 84-hour cultivation only 49.2 percent developed to the morula and blastula stage from 67 isolated ova of the same animals.

Even though our experiment has not provided evidence for the dying of developing fertilized ova in the period before implantation, nor for their slowing in development, the relatively small number of experiments does not allow us to generalize these results. The disturbances in development of ova in the period before implantation may be manifested in some other inbred strains. Differences in the occurrence of the lethal gene t^{12} (*7*) may serve as example.

Part of the strain-H ova fertilized in vitro was left in organ culture for 144 hours. After 120 hours we observed in some blastocysts a herniation of their walls from one opening of the zona pellucida (Fig. 1B). Later this formation resembled a blastocyst (Fig. 1C). It was also possible to observe a migration of cells in the form of an unorganized cell mass, a part of which reminded one of the giant trophoblastic cells. An analogous development of blastocysts in vitro has been observed elsewhere (*8*). Dickson (*9*) observed the structural changes of blastocysts after their passage into the uterus, but he did not find similar forms.

The last test of the viability of oocytes fertilized in vitro and cultivated 72 to 84 hours was their transplantation to recipient animals. Eighty-three morulae and blastulas were transplanted to 19 female recipients that had been mated with fertile males. From nine pregnant females there were, however, only three with four developed transplanted embryos. Genetic control was assured by the use of pigmented recipients (C57/BL) and albinotic donors (H) (Fig. 2). Although only about 5 percent of transplanted morulae and blastulas implanted, the result may be regarded as being successful.

The relatively high fertility and normal development of the ova fertilized in vitro is a further advance on results of Brinster and Biggers (*1*) and made it possible to obtain, in some cases, normal embryos after transplantation.

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Serum Alpha Globulin Fraction: Survival-and-Recovery Effect in Irradiated Mice

Abstract. *An alpha macroglobulin fraction (19S) was isolated from the serum of rats and BC_3F_1 mice by zonal ultracentrifugation. Both the isologous and heterologous macroglobulin fractions increased survival among BC_3F^1 mice x-irradiated with 750 roentgens. The mouse macroglobulin fraction also enhanced radiation recovery of hematopoietic tissue as measured by colony-forming assay and iron-59 incorporation into erythropoietic cells. The overall difference in hematopoietic activity in the irradiated (400 roentgens) mice treated with the macroglobulin fraction, in comparison with this activity in the controls, was three- to fivefold in the bone marrow and nine- to tenfold in the spleen between days 4 and 7 after irradiation. This effect was not obtained with the isologous serum protein fraction containing proteins of smaller molecular weight.*

During the course of immunologic studies on mice injected with isologous and horse macroglobulin labeled with ^{125}I, radiation damage from the isotope and subsequent spleen recovery was observed. On day 7 after injection of isologous ^{125}I-macroglobulin with high specific activity, a two- to threefold increase in spleen weight was measured. Histologically, there was a marked hyperplasia of hematopoietic cells in the spleens of these mice. Normal animals injected either with horse macroglobulin labeled with ^{125}I of a comparable specific activity or with unlabeled isologous macroglobulin did not show these changes. Therefore, we investigated the effects of various macroglobulin preparations on irradiated mice, and found that isologous and rat macroglobulin isolated by zonal ultracentrifugation significantly enhanced radiation survival and accelerated the recovery rate of hematopoietic tissue.

Macroglobulins were prepared by centrifuging 25 to 30 ml of whole serum on a 1000-ml sucrose-density gradient in the B-IV zonal ultracentrifuge (*1*). The separation and characterization of the rat macroglobulin has been described (*2*). For separation of mouse and horse macroglobulins, the ammonium sulfate step cited in the procedure for rat macroglobulin was omitted. The gradient was monitored at 260 and 280 mμ as it passed through a 0.2-cm Oak Ridge flow cell; a Beckman B spectrophotometer, modified as described by Anderson (*3*) was used. The separation showed two peaks. The gradient was collected in 40-ml fractions. After four to six runs were made, the material in the second peak was pooled and concentrated. Analytical ultracentrifugation showed that it contained approximately 50 percent of 19*S* protein (about 750,000 molecular weight). An ultracentrifugally homogeneous 19*S* fraction was obtained by a second centrifugal separation in the B-IV zonal rotor. The 19*S* fractions were characterized as the alpha complex by acetate-strip electrophoresis in 0.05*M* barbital buffer at *p*H 8.8 (Fig. 1). Protein concentrations were estimated by the method of Lowry *et al.* (*4*).

Initially, we studied survival of mice after whole-body irradiation. Random-