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A Procedure for Explanting the 72-Hour Chick Embryo.* (32350)

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In an attempt to understand the mechanism of early mortality produced by ionizing radiations, we focused our studies on the circulatory system. The 72-hour chick embryo has a well developed circulatory system but *in ovo* is relatively inaccessible to microscopic study. Previously described techniques for explanting and culturing embryos for experimental manipulation and detailed microscopic studies were limited to embryos of less than 48 hours incubation(1-5). In the 72-hour embryo, the vascular area is too large and the embryo is too heavy to allow the required manipulation necessary for explantation without trauma to the embryo and the microcirculation. We have developed a technique for explanting the 72-hour embryo and the entire area vasculosa onto a solid agar-albumen medium. Such explanted embryos were cultured in a heated microscope chamber and detailed studies of the circulation in the transparent extraembryonic membrane were carried out. With this technique, we were able to maintain explanted embryos on an agar-albumen medium for periods up to 24 hours and in some cases as long as 30 hours.

Method and results. For our experiments, fertile eggs were incubated (Stage 18)(6) at a temperature of 38°C and 60% humidity. Equipment was sterilized and general aseptic procedures were employed.

The embryos were cultured on an agar-albumen medium that contained: 100 cc Howard-Ringer solution(7), 2 g agar, 1 g glucose, and about 50 cc thin albumen from unincubated eggs (about 6 eggs). The agar was added to the Howard-Ringer solution and the mixture was heated until the agar was completely in solution. The glucose was then added and the solution was cooled to about 50°C; the thin albumen was added and the solution was swirled gently until completely mixed. About 2 cc of this medium, enough to form a thin layer, was poured into small petri dishes (about 40 mm diameter) and the dishes were set aside to allow the medium to gel.

The shell and the shell membrane were cut off the blunt end of the egg, the albumen was removed, and the embryo and yolk were placed in a shallow finger bowl containing prewarmed Howard-Ringer solution (38°C). The yolk sac was cut at the equator and a modified spatula with a plastic ring (Fig. 1A) was inserted into the yolk (Fig. 1B). The ring was centered under the embryo, then the petri dish containing the medium was inverted over the embryo (Fig. 1C) and pressed down over the ring so that the vitelline membrane

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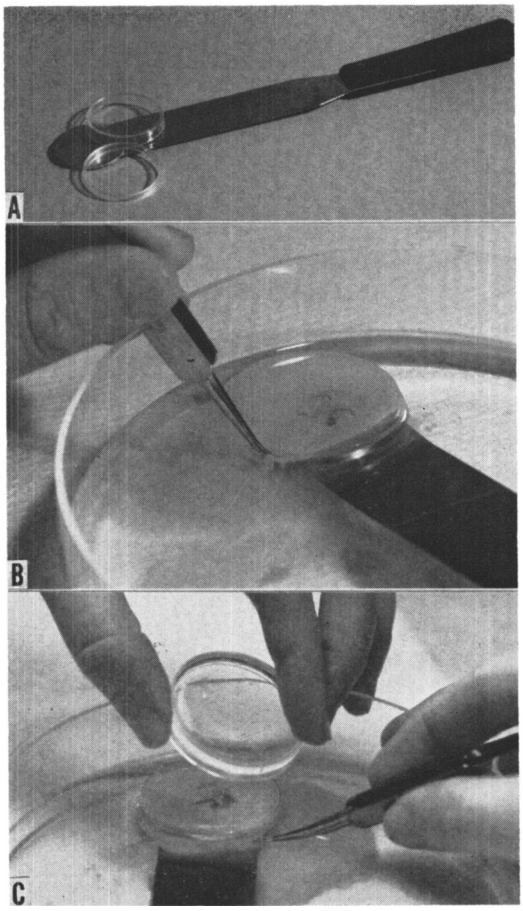

FIG. 1. (A) Spatula, modified with a solid stainless steel disc welded to the end, supports the plastic ring and dish. (B) Modified spatula with fitted ring is inserted into yolk sac. (C) The ring is centered under the embryo before placing petri dish (containing medium) over embryo.

was held firmly between the sides of the petri dish and the ring. The excess vitelline membrane was cut away. The dish and the embryo were lifted from the spatula in the Howard-Ringer solution and manipulated gently to dislodge adhering yolk particles. The embryo and membrane were then covered by a thin film of sterile surgical mineral oil (Blandol, a light mineral oil specially prepared to be free of toxic contaminants, made by Witco Chemical Co.†) to prevent evaporation but permit sufficient exchange of gases. The explanted embryo was placed in a heated chamber (Fig. 2A) and was checked microscopically for injury to the circulation. Culture of the explanted embryo was carried out at 38°C in an atmosphere of 40% O_2, 5% CO_2, and 55% N_2.

Results and conclusions. The developmental stage at which embryos were explanted is shown in Fig. 3A. Of 169 explanted control embryos, we were able to maintain 125 for 24 hours and 60 for more than 30 hours. However, maintenance on agar-albumen-glucose medium was limited, due to accumulation of toxic products of metabolism as well as to lack of balanced nutrients. The stage of development usually observed after 24 hours in culture is shown in Fig. 3B. Although the blastoderm was explanted onto a solid medium, the area vasculosa was able to expand laterally over the surface of the vitelline

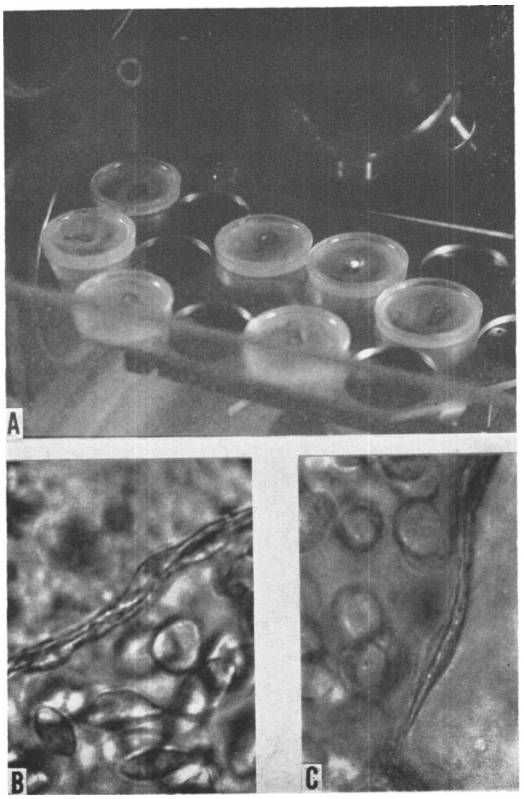

FIG. 2. (A) Explanted embryos, ventral side up, are cultured on microscope stage in a heated chamber where they may be observed microscopically. (B) Cellular details of the vascular endothelium immediately after explantation as visualized through the 50X objective. Magnification 475 ×. (C) Cellular details of the vascular endothelium 24 hr after explantation. Magnification 475 ×.

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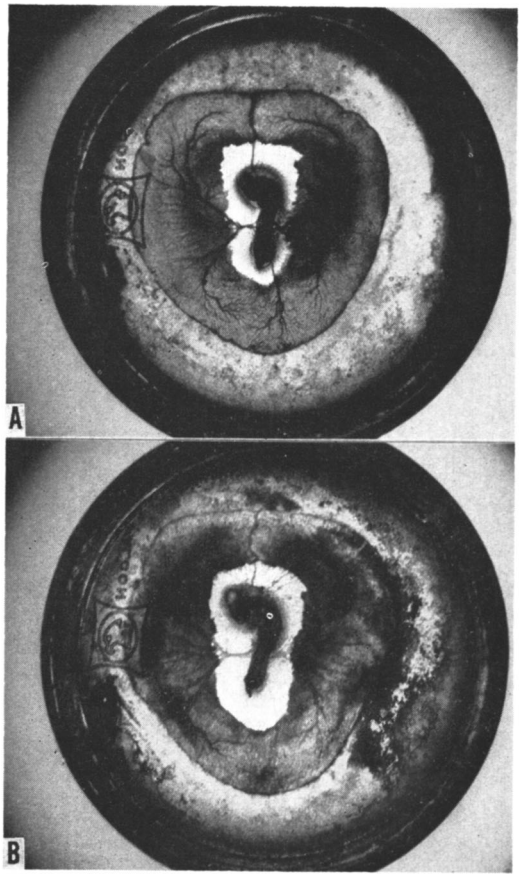

FIG. 3. (A) Developmental stage at which embryos are explanted. Magnification 1.5 ×. (B) Stage of development observed after 24 hr in culture. Magnification 1.5 ×.

membrane and folds did not form as development of the membrane progressed. Considerable expansion of the area vasculosa was observed in explants maintained for 24 hours, as well as growth and increase in flexion and torsion of embryos.

We were primarily interested in maintaining embryos *in vitro* for cinemicrographic studies of the microcirculation. Observations were made with a Leitz Ortholux microscope using objectives up to 50X. Cellular details that were visualized under these conditions include nuclei and thin cytoplasmic extensions of the endothelial cells, as well as cytoplasmic vacuoles and other inclusions (Fig. 2B and C). During 24 hours of observation, normal circulation and cellular details of the vascular endothelium were observed (Compare Fig. 2B and C). In our studies of the effects of ionizing radiations, we have found that the survival rate of X-irradiated explanted embryos and embryos irradiated *in ovo* were comparable. The effects of X-irradiation on the microcirculation will be described elsewhere.

Summary. We have developed a technique for explanting the 72-hour embryo and the entire area vasculosa onto a solid albumen-agar medium. With this technique, we were able to maintain such explanted embryos for periods up to 24 hours and longer. Explanted embryos were used for detailed studies of radiation effects on the microcirculation. Considerable expansion of the area vasculosa was observed in explants maintained for 24 hours.

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