The development of embryos in the uteri of mice treated with actinomycin D before implantation

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**SUMMARY**

This investigation follows the development of embryos in the uteri of mice in which the differentiation of the implantation chamber has been retarded by the administration of actinomycin D before implantation.

For the first 48 h after the induction of implantation both embryonic and extra-embryonic parts of the blastocyst develop, but after 72 h the embryonic parts cease to grow and die. The giant cells and other extra-embryonic tissues, on the other hand, continue to develop for a much longer period, up to 288 h.

It is suggested that the actinomycin D, by delaying the formation of the implantation chamber in the endometrium, interferes with the formation of a proper functional connection between the trophoblast and endometrium with the result that the embryo receives insufficient nutrition for its development.

**INTRODUCTION**

A single dose of the antibiotic actinomycin D given to intact pregnant mice before implantation prevents pregnancy (Finn & Martin, 1972). More detailed examination of the effect of the drug on implantation, using ovariectomized pregnant mice in which nidation had been induced and maintained with exogenous hormones, demonstrated that the early stages of the uterine response to implantation were not prevented but decidualization and the normal embedding of the blastocyst into the wall of the uterus were delayed. Provided progestin treatment was maintained, nidation took place eventually. Stimulation of the blastocyst to continue its development occurred at the normal time, with the result that the development of the blastocyst became out of phase with the changes taking place in the wall of the uterus.

In the previous paper (Finn & Bredl, 1973) we showed that, 48 h after the induction of implantation by oestradiol and actinomycin D, well-developed blastocysts were surrounded by a mass of epithelial cells with little decidualization in the stroma. By contrast, in control animals receiving only oestradiol, the epithelium had completely disappeared and the blastocysts were surrounded by a mass of decidual cells.

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In the present work we have followed the fate of these blastocysts in the hope that this would yield information about the inter-relationship between the developing embryo and the uterus during pregnancy.

ANIMALS AND METHODS

Randomly bred albino mice were kept under conditions of controlled heating and lighting with an 8 h dark period ending at 12.00 noon. Females were examined for vaginal plugs at the end of the dark period and approximately 72 h later were ovariectomized under tribromoethanol anaesthesia. One mg provera was injected subcutaneously and this was repeated every 4 days (where appropriate). On the day following ovariectomy 15 µg actinomycin D (AMD) or water was administered and implantation induced 1½ h later by a single injection of 20 µg oestradiol 17β. Animals were killed 72–336 h after the nida-tory oestrogen by perfusion with glutaraldehyde under tribromoethanol anaesthesia (Finn & Lawn, 1967). The implantation areas which were readily visible were embedded in Araldite and 1–2 µm sections cut and stained with azur II in borax.

RESULTS

72 h after induction of implantation

All seven of the treated animals showed decidual cell transformation in the stroma, although it was less than that seen at 48 h in animals given only oestradiol (Finn & Bredl, 1973). The three control animals killed at 72 h showed a much greater decidual cell transformation with the formation of large maternal blood spaces, a feature lacking in the experimental group. The AMD-treated animals had uterine epithelial cells persisting, forming a thick partly degenerating layer surrounding the blastocyst and giant cells. The embryonic parts of the treated blastocyst, which had increased in size between 24 and 48 h, showed little increase in size at 72 h. In two of the seven animals areas of inner cell mass derivatives could no longer be distinguished, although all had trophoblastic giant cells. In places these had passed between the endometrial epithelial cells to contact the stroma, but only in small areas and with no penetration of the decidua.

96 h after induction of implantation

Five treated and five control animals were examined at this time. All the uteri showed good decidual swellings although in the treated animals they were obviously much smaller than those in the control animals. In the latter the decidual cells were densely packed with islands of cells separated by large blood sinuses, the beginnings of which were at this time appearing in the AMD-treated animals. Trophoblastic giant cells were visible in all animals of
Fig. 1. High-power picture of the area in Fig. 2 indicated by the square, showing foetal membranes, giant cells and decidual cells.

Fig. 2. Cross-section through the centre of an implantation swelling from an animal treated with actinomycin D. Implantation induced 168 h before autopsy.
both groups. Spaces between the giant cells in the controls contained a few erythrocytes. The inner cell mass area in the control animals had increased considerably in size whilst in the treated animals, although clearly surrounded by Reichert's membrane, it was noticeably smaller than at 72 h. The implantation areas in the experimental animals were congested with blood cells, embryonic cells including giant cells, and degenerating epithelial cells rich in lipid.

120 h after induction of implantation

Four treated animals were examined at this time and three controls. The difference in size of the implantation swellings between the treated and control animals was even more obvious at this time. Compared with the 96 h group the decidual cells and the trophoblastic giant cells of the treated animals were considerably larger than before. The maternal blood spaces had developed considerably, possibly reflecting the sudden increase in the size of the giant cells which surrounded the yolk sac and Reichert's membrane. Little else of inner cell mass derivatives could be made out except for a few regressing remnants. In the control group well-developed embryos were present, attached to normal placentae with well developed giant cells.

168 h after induction of implantation

Three actinomycin D-treated animals were examined. They all had large maternal blood sinuses surrounding islands of decidual tissue. These were closely associated with the trophoblast giant cells. In some implantation sites only giant cells could be found whilst in others Reichert's and extra-embryonic membranes persisted surrounding hollow implantation chambers (Figs. 1, 2). Control animals at this time maintained healthy placental and embryonic growth.

240 h after induction of implantation

Four out of five experimental animals killed at this time showed further placental development. Externally the implantation sites varied considerably in size, but were always smaller than those in the control groups. In many cases the areas formerly occupied by embryonic remnants were now filled with blood cells, which had not been dislodged during the perfusion procedure. Trophoblastic giant cells could be found surrounding the former implantation chamber and Reichert's membrane with attached yolk sac was still present.

The embryos and placentae of the control animals continued to develop normally and it was noticeable that the giant cells were now absent from most antimesometrial areas of the implantation chamber and were concentrated around the placental site.
Animals killed 288–336 h

The control animals continued to yield normal embryos and well formed placentae. The placentae of the AMD-treated animals, however, were in various stages of degeneration and resorption and the whole area of the conceptus was very much smaller than in the previous group. A few giant cells could be identified in those killed at 288 h but by 336 h none were distinguishable. A prominent feature of these animals was re-establishment of the epithelium around the periphery of the implantation area.

DISCUSSION

Actinomycin D, when administered as a single injection to mated mice prior to implantation, does not prevent the attachment of the trophoblast to the uterine epithelium or the activation and early development of the blastocyst (Finn & Bredl, 1973; Pollard, Bredl & Finn, 1973). The later development of the embryo is however profoundly affected.

The embryonic part of the blastocyst which had increased in size and differentiated when examined 48 h after treatment (Finn & Bredl, 1973), showed no further development at 72 h and definite signs of regression at 96 h. By 120 h very little of the embryo remained. The extra-embryonic parts of the conceptus, however, fared much better, especially the giant cells which were still present at 288 h.

The failure of embryonic development is presumably associated with the delay in the morphogenesis of the implantation chamber in the wall of the uterus. This would prevent the establishment of an adequate connexion between the trophoblast and maternal tissue at a time when the nutritive requirements of the embryo are increasing rapidly. In control animals rapid development of the embryo takes place between 48 and 72 h after the induction of implantation, accompanied by considerable vascular development in the uterus. A direct effect of the drug on the embryo is unlikely in view of the earlier experiments in which blastocysts were transferred from treated to untreated mice and developed normally (Finn & Bredl, 1973).

The finding that the growth and development of the giant cells is much less affected is not surprising in view of Kirby’s experiments in which blastocysts were transferred to the spleen (Kirby, 1963). Under these conditions the vast majority of the embryos did not develop very far but trophoblastic giant cell formation continued unimpeded, with invasion of host tissue.

These results thus highlight the importance of the temporal relationship between the differentiation of the implantation chamber with its associated decidual cell reaction and vascular sinuses (Krehbiel, 1937) and the development of the embryo.

We would like to thank Mr J. Lewin and Miss G. Blair for technical assistance. We also acknowledge the financial support of the Wellcome Trust.
REFERENCES


(Received 26 February 1975, revised 21 April 1975)