On the orientation of the implanting blastocyst

By D. R. S. KIRBY¹, D. M. POTTS² & I. B. WILSON³

From the Department of Zoology, University of Oxford

INTRODUCTION

In viviparous mammals the implanting blastocyst is positioned according to a number of parameters which are species specific. First, in polytocous animals the blastocysts become spaced out along the length of the uterus, secondly they take up a particular relationship to the circumference of the endometrium, and lastly the embryonic pole always assumes a constant orientation to the mesometrial–antimesometrial (vertical) axis of the uterus.

The first two factors have been reviewed by Blandau (1961) and are generally agreed to be uterine-dependent. Wilson (1963) has shown that even melanoma cells introduced into the uterine lumen of the pseudopregnant mouse become segmented into clumps along the length of the uterus and are always positioned in the antimesometrial segment of the lumen as are blastocysts during normal development.

This paper is limited to discussing the third parameter only—the orientation of the inner cell mass of the blastocyst with regard to the underlying maternal tissue. The evidence is taken from studies of implantation in the mouse in which the normal blastocyst invariably implants with its inner cell mass directed towards the mesenteric attachment of the uterus.

HYPOTHESES

When the blastocysts are spaced along the length of the uterus and have reached the antimesometrial extremity of the uterine lumen there seem to us to be six ways a priori in which the orientation of the blastocyst could be achieved (Text. fig. 1). Each hypothesis, with the exception of the first, suggests that there is a mechanism for moving the blastocyst in response to a directional stimulus.

The hypotheses are:

(1) The blastocyst develops at its prospective implantation site with the position of the inner cell mass determined in response to the underlying maternal tissue.

(2) While the blastocyst is being manoeuvred by the myometrial contrac-
tions towards the antimesometrial side of the uterus its correct orientation is established as a result of differential compressibility of the abembryonic pole and the inner cell mass regions.

Text-fig. 1. Diagrams representing the six mechanisms by which the blastocyst could be correctly orientated. See text.

PLATE 1

All figures are orientated with the mesometrium to the top of the plate. The tissue in figs. A and B was fixed in Clarke’s fluid and stained with Heidenhain’s iron haematoxylin; the space around the blastocysts is an artifact. The tissue in fig. C was fixed by freeze-substitution and stained with toluidine blue, that in fig. D by osmium tetroxide. Fig. A: the zona pellucida (z) is still present around this blastocyst which is almost ‘upside down’ in the implantation chamber. Gland openings (g) are also shown. (Approximately 90 h post coitum.)

Fig. B. The zona pellucida has gone but the blastocyst is still ‘upside down’ though clearly it is firmly attached to the uterine epithelium, particularly to the side of the inner cell mass. There is an inclusion (i) of a primary invasive cell at the free border of the inner cell mass. (Approximately 90–92 h post coitum.)

Fig. C. The blastocyst is now correctly orientated with the inner cell mass at its mesometrial pole. (Approximately 98 h post coitum, though this stage is reached usually by about 92 h post coitum.)

Fig. D. Electron micrograph of implanting blastocyst. The trophoblast (t) is intimately attached to the underlying uterine epithelium (e). The inner cell mass (icm) is loosely related to the trophoblast shell. A discontinuous extracellular deposit (arrows) is present between the inner cell mass and trophoblast.
(3) Uterine epithelial cells have a capacity for movement independent of the underlying stromal tissue. The movement of the uterine epithelial cells rotates the blastocyst lying upon them into the correct orientation.

(4) The initial attachment between the blastocyst and the uterine epithelium is random. Thereafter the trophoblast cells which differentiate from the wall of the blastocyst anchor it to the underlying epithelium by cytoplasmic processes. As a result of selective shortening of these processes the blastocyst is rotated into its correct orientation.

(5) Before implantation the blastocyst is subject to rotary movements by myometrial contractions. There is a morphogenetic field across the vertical axis of the uterus. When the inner cell mass, as a result of random movements, arrives in the correct orientation attachment is initiated in all, or in a previously determined part of the trophoblast wall.

(6) The surface of the blastocyst has a uniform potential for attaching to the uterine epithelium and attachment occurs at random immediately after the loss of the zona pellucida. The inner cell mass is free to move round the inside of the trophoblast shell. The final position of the inner cell mass is determined either by a morphogenetic gradient across the vertical axis of the uterus or by changes in the trophoblast associated with its attachment to the underlying tissues, or by both.

OBSERVATIONS

In arriving at a conclusion the following observations of both normal and experimental material must be considered.

(a) Shortly before 86 h post coitum, in a randomly bred colony of mice, the blastocysts are distributed along the length of the uterine horn randomly positioned in the vertical axis of the uterine lumen. Rather abruptly, at about 86 h, the uterine lumen closes up and the blastocysts appear to be forced down to the antimesometral limit of the lumen (which generally lies along the central axis of the uterus). At approximately 90 h the blastocysts may be found occupying the presumptive implantation sites. The zona pellucida is still intact but the inner cell mass has no constant orientation (Plate 1, fig. A). The zona pellucida then disappears and there is a short interval during which specimens can be

PLATE 2

Fig. E. Blastocyst firmly anchored to the cornea, which has become fragmented during histological preparation. The blastocyst was transplanted into the anterior chamber of the eye 48 h before autopsy. Note that the inner cell mass is directed away from the point of attachment to the host tissue.

Fig. F. A projection drawing of a 20 μ thick section of mouse uterus approximately 100 h post coitum. The blood supply is shown in detail only for the endometrium. Main arteries and all smaller vessels solid black, main veins stippled, lymph vessels, as far as they could be distinguished, are unshaded. Note that the antimesometrial limit of the lumen is more or less central to the body of the uterus and that the capillary supply and the lymph vessels are concentrated in this region.
found with the trophoblast in intimate contact with the epithelium and the inner cell mass haphazardly positioned (Plate 1, fig. B). By 92 h the blastocysts are usually correctly orientated (Plate 1, fig. C). Approximately 6 h later trophoblast giant cells first appear at the abembryonic pole.

(b) Electron-microscopic studies reveal that immediately following the loss of the zona, when the inner cell mass is not always correctly orientated, the uterine epithelium embraces the blastocyst intimately (Plate 1, fig. D), and microvillous processes from the uterine epithelium and trophoblast interlock.

(c) The junction between the inner cell mass and the trophoblast shell is loose (Plate 1, fig. D). There is considerable extracellular space within the inner cell mass (Plate 1, fig. C) and there are no desmosomes uniting the cells.

(d) In a series of previously unpublished experiments, blastocysts in which the inner cell mass had already formed, were transplanted to the anterior chamber of the eye. In this site the blastocysts can rotate freely before implantation. Sections were made of the anterior chamber when the blastocysts had implanted. In all 10 blastocysts which were found of the 16 transferred, the inner cell mass was opposite the point of attachment (Plate 2, fig. E). Judging from the variation in the development of the implanted blastocysts, it seems that attachment does not occur synchronously as it does in the uterus, but that this process is spread over a considerable period. This precludes the possibility of constructing a time schedule for implantation processes in the anterior chamber of the eye.

EVALUATION

It is clear that hypothesis (1) is excluded, by observations (a) (b) and (d). The inner cell mass bears no constant relationship to the uterus until after the zona pellucida is lost, which occurs about 20 h after the inner cell mass becomes delineated. Experiments on the transfer of blastocysts to the eye (observation d) eliminate hypotheses (2) and (3). Electron-microscope observations (b) on the trophoblast-maternal junction and the time sequence of normal development (a) exclude hypotheses (4) and (5). Only hypothesis (6) is compatible with all the observations.

DISCUSSION

Additional arguments are available for hypothesis (6) that the inner cell mass moves round the inside of the trophoblast shell of the blastocyst. Electron-microscope observations of the stage under discussion show a discontinuous extra-cellular deposit on the inside of the trophoblast shell (which for most of its circumference is the precursor of Reichert's membrane). This deposit is always found between the inner cell mass and the trophoblast shell. Small segments of this material are found in other places on the trophectoderm suggesting that these regions were formerly occupied by underlying inner cell mass cells (Plate 1, fig. D). In experiments involving interspecific transfer of rat and mouse eggs, Tarkowski (1962) found that the inner cell-mass cells of rat eggs seem to lose
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adhesion with one another and distribute themselves as a monolayer round the
trophoblast shell. Clearly the cellular arrangement of the blastocyst is labile, as
is further indicated by the work of Mintz on the fusion of mouse eggs (Mintz,
1964a, b). The degree to which the trophoblast and inner cell mass cell are
morphogenetically independent of one another is shown by the experiments of
Munland (1965) in which isolated mouse blastomeres, cultured in vitro, could
give rise to empty trophoblast vesicles.

The nature of the stimulus responsible for the final orientation of the inner
cell mass is unknown. Change in oxygen tension might set up a gradient. The
uterine epithelium underlying the abembryonic pole of the blastocyst has a rich
capillary blood supply (Plate 2, fig. F) and the inner cell mass might move away
from the area of highest oxygen tension. Such a mechanism could also explain
observation (d). Cowell (see Kirby, 1966) has shown that a mouse blastocyst will
implant in an area mechanically denuded of epithelium even though this area is
located on the mesometrial side of the uterus. In this case the inner cell mass
migrates towards the antimesometrial aspect of the blastocyst, and orientation
is upside-down with reference to the vertical axis of the uterus. However, these
studies were carried out on ovariectomized mice, in which the vasculature of the
uterus is unlikely to be the same as that of pregnant mice. Indeed the oxygen
tension may be highest near the hyperaemic area beneath the damaged epithelium.

At the time when the orientation of the inner cell mass is taking place the
circumference of the trophoblast shell is uniform in structure. When, a few hours
later, the giant cells appear they always have a constant relationship to the inner
cell mass. It is not known how this relationship is mediated except that it is
independent of any uterine influence. It is also found when blastocysts are trans-
planted into the mesenchyme of organs other than the uterus, for example the
spleen (Kirby, 1963).

The mechanism controlling the orientation of the inner cell mass may well
apply to species other than the mouse.

SUMMARY

1. The sequence of events in the orientation of the blastocyst is described.
2. Six a priori mechanisms for orientating the blastocyst are presented.
3. It is concluded that the only mechanism fulfilling the available morpho-
   logical and experimental observations is one in which the inner cell mass is free
to travel round the inside of the trophoblast shell once the latter has become fixed
to the uterine epithelium.

RÉSUMÉ

Sur l'orientation du blastocyste en implantation

1. On décrit la série d'événements intervenant au cours de l'orientation du
   blastocyste.
2. On présente six mécanismes pouvant a priori réaliser cette orientation.
3. On conclut que le seul mécanisme compatible avec les observations morphologiques et expérimentales est celui dans lequel la masse cellulaire interne est libre de se déplacer autour de l'intérieur de l'enveloppe trophoblastique, une fois que cette dernière s’est fixée à l’œpithélium utérin.

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