The Influence of the Uterine Environment on the Development of Mouse Eggs

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WITH TWO PLATES

INTRODUCTION

From the literature and the results of some preliminary experiments by the author (Kirby, 1960), it appeared that the ability of mouse eggs to develop into embryos in an extra-uterine site was at least partially dependent on the stage of the egg when transplanted. In most previous studies on the extra-uterine development of mouse eggs only tubal eggs have been transplanted. Fawcett, Wislocki, & Waldo (1947) transferred 8–10-cell-stage eggs to the anterior chamber of the eye, 3 or 4 eggs being injected in each case. In another series of experiments reported in the same paper the oviducts were transected, thereby causing the descending eggs to pass into the abdominal cavity. Fawcett (1950) transplanted 2–10-cell-stage eggs to a position beneath the kidney capsule, and, to compensate for possible loss, several eggs were usually introduced. Jones (1951) transplanted 2–10-cell-stage eggs to the eye and beneath the kidney capsule.

Thus all these workers transplanted pre-uterine segmenting eggs to various host organs. The results have one finding in common—in no case do they report the production of an embryo. The grafts contained only trophoblast, some extra-embryonic membranes, and much extravasated blood.

Runner (1947), on the other hand, transplanted cleavage stages, morulae and blastocysts, recovered from the oviducts and uteri to the anterior chamber of the eye. Several, sometimes as many as 10, eggs were transplanted in each case. He reports that ‘younger embryos were capable of developing and implanting in the eye, but did so less frequently than did blastocysts’. His data show that one cleavage stage gave rise to ‘intra-embryonic derivatives’. This isolated case is the only one, either in the literature or the results presented in this paper, in which a pre-uterine egg has so developed. The results of his blastocyst transplants, many of which were pre-treated with carcinogen or injected together with tumour cells, show that the inner cell mass had undergone some differentiation. Thus only the blastocysts gave rise to inner cell-mass derivatives (the

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embryo proper) when transplanted to an extra-uterine site. With the one exception mentioned above all the earlier stages did not do so. This paper presents the results of experiments to determine the relationship between the developmental capacity of the mouse egg and the stage at which it was extracted from the genital tract. It is not intended to give a full analysis of all the tissues derived from the transplanted eggs. This will be the subject of a separate publication.

Since the object of the histological investigation was primarily that of determining whether an embryo had developed or not, the times of autopsy, &c., are not given. All grafts were examined in the period when, if the embryo had developed, it would be most easily recognizable. In the majority of cases recognition presented no difficulty whatsoever, as the embryos were morphologically normal. Others were deformed but still easily recognizable. In two cases a cytological study of the tissues was necessary to establish that they were embryonic rather than extra-embryonic.

MATERIALS AND METHODS

Most of the work was carried out on a heterogeneous population of mice. However, some transplants were made between mice of a genetically pure line, until it was determined that no benefit was bestowed upon the transplanted ova by so doing—see Kirby (1960). Males and pregnant and non-pregnant females were used as hosts.

Eggs up to and including the morula stage were extracted from the oviduct up to 2½ days after mating. The blastocysts, 3½ days post-copulation, were obtained from the uterus. The eggs were kept in sterile Pannett/Compton solution at room temperature until transferred beneath the kidney capsule of the host mouse. Only one egg was transplanted to any one kidney. This was considered preferable to multiple egg transplants for several reasons. Firstly, with a known number of eggs transplanted, the proportion that develop could be determined accurately. Secondly, development of more than one egg in the confined space beneath the kidney capsule complicates subsequent histological interpretation of the graft. Thirdly, single-egg transplants preclude any complications that may have arisen due to more than one embryo competing for space and metabolic requirements.

After the required period of time had elapsed from the transplantation of the egg, usually 4–8 days, the host was killed by dislocating the neck. The host organ was removed, fixed in Zenker's fluid, cut into 8-μ sections, and stained with Mayer's acid haemalum. No counter stain was used.

Transplantation apparatus (see Plate 1, fig. A)

This merits at least a short description, since its use greatly facilitates operations of this type. With practice it was possible to transplant single mouse eggs beneath the kidney capsule with the greatest ease and certainty.
The apparatus consisted of a 'Singer' microdissector and 'Agla' micrometer outfit (Burroughs & Wellcome, London). The microsyringe was modified by spring-loading the plunger, so that when pressure was released by unscrewing the micrometer head the plunger withdrew. Thus donor material could be taken up as well as ejected by the action of the micrometer. The needle of the microsyringe was connected to a glass micropipette by fine-bore polythene tubing. This micropipette (internal diameter 100 μ) was held in the microdissector as shown. Liquid paraffin was used as a hydraulic medium to fill the pipette, polythene tubing, and syringe.

The egg was taken up in the micropipette between two small air bubbles, which acted as markers. The release of both air bubbles beneath the kidney capsule indicated that the egg had been ejected from the micropipette.

EXPERIMENTS AND RESULTS

Pre-uterine segmenting eggs and uterine blastocysts

Thirty-six pre-uterine eggs of various stages of development and 50 uterine blastocysts were transplanted beneath the kidney capsule (see Table 1). The batches of host mice used for each of the 5 developmental stages were composed of a similar proportion of males and pregnant and non-pregnant females. From the results of the experiments, however, it was realized that the sex, or condition of pregnancy, had no discernible effect on the development of the transplanted egg. No distinction will therefore be made between the host animals.

<table>
<thead>
<tr>
<th>Stage of egg</th>
<th>No. of experiments</th>
<th>No. of 'implants'</th>
<th>No. of embryos</th>
<th>No. of trophoblast &amp; E.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 cell</td>
<td>10</td>
<td>8</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>2 cell</td>
<td>8</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>4 cell</td>
<td>8</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Morulae</td>
<td>10</td>
<td>7</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Blastocysts</td>
<td>50</td>
<td>41</td>
<td>16</td>
<td>25</td>
</tr>
</tbody>
</table>

No. of 'implants' = implants is used here and elsewhere in this paper to mean the attachment and subsequent development of the transplanted egg on the host organ. It is not strictly analogous to the process in utero.

1 Embryos = No. of experiments showing embryonic shield derivatives.

2 Trophoblast and E.E.M. = No. of experiments showing extra-embryonic membranes and trophoblast only.

Each ovum developing beneath the kidney capsule causes the production of a characteristic haemorrhagic vesicle or nodule (see Fawcett, 1950). The time of appearance and rate of development of this mass was approximately the same for all the stages of eggs transplanted.
Subsequent histological examination revealed, however, that the contents of the grafts differed. All the pre-uterine stages of eggs that implanted gave rise to trophoblast and extra-embryonic membranes only (see Plate 1, fig. B).

No differences could be detected between the grafts resulting from the different stages of these pre-uterine ova. In no graft was there any tissue resembling embryonic shield derivatives.

Of the 50 uterine blastocysts that were transplanted, nearly a third gave rise to tissues of the embryo proper, of which several were of normal form (see Plate 1, figs. C, D). The differences in the number of embryos developing from uterine blastocysts and pre-uterine eggs is highly significant ($p < 0.01$).

**Uterine blastocysts and tube-locked blastocysts**

About 12 hours after mating the left oviduct of the donor mouse was ligated with fine silk below the position of the fertilized eggs, thus preventing their descent into the uterus. The right oviduct was left patent as a control.

Three days later, i.e. $3\frac{1}{2}$ days after mating, the left oviduct was removed and the eggs extracted. These tube-locked eggs had developed into blastocysts, which appeared indistinguishable from the control blastocysts extracted from the right uterine horn (see Plate 2, figs. E, F).

**Table 2**

Results of transplanting tube-locked and uterine blastocysts beneath the kidney capsule

<table>
<thead>
<tr>
<th>Type of egg transplanted</th>
<th>No. of experiments</th>
<th>No. of 'implants'</th>
<th>No. of embryos$^1$</th>
<th>No. of trophoblast &amp; E.E.M.$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tube-locked blastocysts</td>
<td>32</td>
<td>20</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Uterine blastocysts</td>
<td>21</td>
<td>15</td>
<td>4</td>
<td>11</td>
</tr>
</tbody>
</table>

(Key as for Table 1.)

The tube-locked blastocysts were transplanted beneath the kidney capsule. Control experiments, using the uterine blastocysts from the right uterine horns, were also carried out. Table 2 shows the results.

More than a quarter of the uterine blastocysts that implanted gave rise to embryonic shield derivatives. The tube-locked blastocysts, on the other hand, produced only trophoblast and extra-embryonic membranes, similar to those obtained by transplanting pre-uterine segmenting eggs. The difference in the number of embryos that developed from tube-locked and uterine blastocysts is significant ($p < 0.05$).

**Tube-locked blastocysts → uterus**

Tube-locked blastocysts, obtained by the method described above, were extracted, $3\frac{1}{2}$ days after mating, from the left oviduct and injected into the animal's
own left uterine lumen. The eggs in the right-hand genital tract were allowed to pursue their normal descent to the uterus unimpeded.

Table 3 shows that more than a third of the 3½-day-old tube-locked blastocysts, when transferred to the uterus, developed into normal embryos, similar in all respects to the control embryos in the contra-lateral horn.

<table>
<thead>
<tr>
<th>Age of T/L blastocyst when transferred</th>
<th>Number transferred</th>
<th>No. of deciduomata</th>
<th>No. of normal embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>3½ days</td>
<td>15</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>4½ days</td>
<td>6</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Eggs retained in the oviduct for 4½ days, although they appeared normal, failed to develop when transferred to the uterus (see Plate 2, fig. G). In one mouse a small decidua was formed in the experimental horn, but on examination was found to be lacking embryo or trophoblast.

**Uterine pre-blastocyst-stage ova**

These were obtained in two ways—one natural, one experimental. The rate at which developing mouse eggs pass down the oviduct is very variable. Consequently the age at which they pass into the uterus varies from 2½ to 4 days (Snell, 1941). Those eggs which descend quickly enter the uterus in the morula stage. They could be extracted 2½ to 3 days after mating before they developed into blastocysts.

Pre-blastocyst uterine eggs could be obtained experimentally in the following way. uncleaved fertilized eggs were extracted from the oviduct soon after mating. They were then injected into the animal's own uterine horn. Two days later this horn was removed and the eggs, in the 8-cell stage, were recovered. The proportion of eggs recovered was, however, very low, and only 4 were obtained from 6 mice so treated.

<table>
<thead>
<tr>
<th>Type of egg transplanted</th>
<th>No. of experiments</th>
<th>No. of ‘implants’</th>
<th>No. of embryos¹</th>
<th>No. of trophoblasts &amp; E.E.M.²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine morulae</td>
<td>30</td>
<td>25</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Uterine 8-cell stage</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

(Key as for Table 1.)

The uterine morulae and the uterine 8-cell-stage eggs were then transplanted beneath the kidney capsule of host mice. A third of these mice were females.
that mated at the same time as the donors, and were thus supporting intra-uterine eggs of the same developmental stage as those introduced to their kidneys. All the eggs transplanted, both uterine morulae and 8-cell stage, failed to develop into embryos (see Table 4).

**DISCUSSION**

The results presented show that eggs of the 1-, 2-, and 4-cell stages, and morulae, extracted from the oviduct, did not give rise to embryos when transplanted to the kidney. These eggs, during their development on the host organ, form blastocysts that are histologically and morphologically normal. These observations confirm those of Fawcett, Wislocki, & Waldo (1947) and Runner (1947) who transplanted early stage eggs to the anterior chamber of the eye. In all cases, although these blastocysts appeared normal, subsequent development showed they could only give rise to extra-embryonic tissues.

Further evidence for the incomplete developmental potentiality of blastocysts that have formed outside the genital tract is furnished by Whitten (1956). He cultured tubal mouse eggs of the 8-cell stage *in vitro*. They developed into blastocysts. To test their viability they were inserted under the kidney capsule and produced 'characteristic growth', which according to a later publication (Whitten, 1958) consisted of trophoblastic giant cells and abortive yolk sac.

Mouse eggs which have been denied entrance to the uterus will develop into blastocysts in the oviduct. Although about half of such tube-locked eggs were abnormal (see Plate 2, fig. 1), those used for transplanting purposes were morphologically normal (see Plate 2, figs. E, G).

Alden (1942) prevented rat eggs from leaving the oviduct by a ligature at the tubo-uterine junction. The blastocysts produced 'compared favourably' with control blastocysts found in the uterus. For further experiments on tube-locked eggs in the mouse the reader is referred to Burdick, Whitney, & Pincus (1937) and Burdick, Emerson, & Whitney (1940). All authors are agreed that in the extra-uterine sites nothing necessary for blastocyst formation is lacking. However, in the present series of experiments we have seen that these blastocysts are not functionally normal since when transferred to the kidney they do not develop into embryos as do the uterine ones.

The lost developmental potentiality of these tube-locked blastocysts is not irrevocable. Complete developmental potentiality may be restored by transferring them to the uterine lumen. In this situation they developed on a par with control embryos in the contralateral horn. The results of some experiments performed by McLaren & Biggers (1958) supports this conclusion. They extracted tubal mouse eggs in the 8–16-cell stage and cultured them *in vitro* to the blastocyst stage. These cultured blastocysts, when transferred to the uteri of recipient females, gave rise to normal living young. The yield of embryos from the cultured blastocysts (20.4 per cent.) did not differ significantly from the
control yield (21.7 per cent.) obtained by transplanting uterine blastocysts to the uteri of recipient females.

Thus normality of the blastocyst may be achieved in a stepwise manner. Firstly, the morphologically normal but functionally abnormal blastocyst may be obtained, and secondly functional normality may be acquired. However, not all tube-locked blastocysts may be thus restored to the totipotent condition. Table 3 shows that eggs retained in the oviduct for more than 3½ days lose this capacity. Although only a small number of experiments were carried out, and the results are not statistically significant, they do suggest an inability of the 4½-day-old blastocyst to develop when transferred to the uterus.

These experiments suggest the possible existence of a ‘uterine factor’, the denial of which renders mouse eggs incapable of developing into embryos—extra-embryonic tissues only being produced. Further, its action seems to be specific to 3½-day-old blastocysts. This may mean that the egg has a limited period during which it is competent to respond to the stimulus. It is not surprising that the period of reactiveness should be confined to the 3½-day-old blastocyst, which normally is free in the uterine lumen. The 4½-day-old blastocyst, which does not respond, is normally in the process of implanting in the endometrium (Plate 2, fig. H).

Similarly, Table 4 shows that pre-blastocyst-stage eggs do not derive any benefit from the uterine environment to which they were subjected prior to transplantation to the kidney. Normally these eggs would still be in the oviduct and hence competence to respond to the ‘uterine factor’ would be of no avail.

The effect of the ‘uterine factor’ is localized to the lumen of the uterus, for its action cannot be mediated by the blood-stream. The action of the ‘uterine factor’ may then be compared with that of embryonic organizer substances such as those of the chorda-mesoderm or the optic cup, inasmuch as these also only evoke a response in nearby competent tissues.

Mention should be made of a further experiment which was attempted. It consisted of transferring 3½-day-old tube-locked blastocysts to the uterus, from whence, after 12 hours, they were transplanted to the kidney. Although the results of this experiment would furnish valuable evidence about the action of the ‘uterine factor’ it was found to be too difficult to perform satisfactorily. This was mainly due to the fact that when transferred from the oviduct the zona pellucida of the egg was ‘sticky’, and subsequent extraction from the uterine wall was very difficult to carry out without damage to the egg. The results were therefore discounted. It is intended to subject 3½-day-old tube-locked blastocysts in vitro to an extract of the uterus to see if they can thus be rendered totipotent.

The fate of eggs, of different ages and stages of development, when transplanted to the kidney varied considerably. Although this is so, their ability to ‘implant’ on the host organ seems unimpaired to any great extent.
Table 5 shows that the ability of the uterine blastocyst to implant was not significantly higher than the other stages of eggs, which failed to develop into embryos. Also the ability of the trophoblast to develop and flourish was independent of the stage of development of the egg. Indeed, the trophoblast was often larger and more vigorous in grafts not containing embryos.

**Table 5**

*Number of eggs that 'implant' expressed as a percentage of the number of eggs transplanted*

<table>
<thead>
<tr>
<th>Stage of egg transplanted</th>
<th>Percentage that 'implant'</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 cell</td>
<td>80</td>
</tr>
<tr>
<td>2 cell</td>
<td>75</td>
</tr>
<tr>
<td>4 cell</td>
<td>75</td>
</tr>
<tr>
<td>Morula</td>
<td>70</td>
</tr>
<tr>
<td>54-day-old tube-locked blastocyst</td>
<td>62</td>
</tr>
<tr>
<td>Uterine 8 cell</td>
<td>60</td>
</tr>
<tr>
<td>Uterine morula</td>
<td>83</td>
</tr>
<tr>
<td>Uterine blastocyst</td>
<td>79</td>
</tr>
</tbody>
</table>

The stage of development reached by the egg, or the position in the genital tract from which it was transferred to an extra-uterine site, is not as critical for embryogenesis in all mammals as it appears to be in the mouse. The wealth of examples of human tubal pregnancies demonstrate that man is an exception. However, in many such cases there is no proof that the egg did not reach the uterus, and thence migrate back to the oviduct. This is, of course, unlikely, and there exists evidence in at least one case (Streeter, 1925) that the developing egg was prevented from entering the uterus. It implanted and developed into a well-formed embryo. The so-called primary abdominal pregnancies in man suggest that the fertilized egg can develop to term, with the production of a living child, without the egg having been in the genital tract at all (Werquin, 1943; Baldwin, 1954; Bright & Maser, 1961).

Nicholas (1942) reported that rat eggs transplanted in the 2–4-cell stage to the kidney gave rise to gut, nerve elements, skin, and muscle of the embryo. These formed a completely disorganized mass. He also found that the development of the trophoblast was suppressed; that the extra-embryonic membranes failed to differentiate; and there was no invasion by the graft of the host tissue. These results, of course, are in direct contradiction to those obtained by all the workers using a similar stage of mouse egg. Fawcett (1950) comments thus: ‘Although similar experiments were done in very closely related species, our observations are at variance with those of Nicholas on several points. It is impossible to state at present whether the divergent results of these two investigations are due to species differences, to differences in the stage of development of the ova at the time of transplantation or to variations in technique.’
In an attempt to resolve the somewhat unexpected results of the rat experiments the present author repeated them, following as closely as possible Nicholas's techniques. Thus 120 rat eggs in the 2–4-cell stage were transplanted to the kidney. Some of the results of these experiments are reported elsewhere (Kirby, 1962).

About 75 per cent. of these eggs implanted but did not confirm the results obtained by Nicholas; in fact they developed similarly to the 2- and 4-cell-stage mouse eggs—the embryonic shield did not differentiate, whereas the trophoblast flourished and actively invaded the host tissue (Plate 2, fig. J). The explanation of these irreconcilable results is not at present forthcoming.

**SUMMARY**

1. Oviducal segmented eggs of the mouse, when transferred beneath the kidney capsule, give rise to trophoblast and extra-embryonic membranes only.
2. Uterine blastocysts transferred to a similar position develop into embryos.
3. Mouse eggs prevented from entering the uterus develop into blastocysts that are morphologically normal. These tube-locked blastocysts, however, that have been denied the uterine environment, do not develop into embryos when transferred to the kidney.
4. 3½-day-old tube-locked blastocysts develop into normal embryos if restored to the uterus.
5. Eggs retained in the oviduct for 4½ days, although they appear normal, do not subsequently develop when transferred to the uterus.
6. Oviducal, pre-blastocyst-stage eggs, transferred for a time to the uterus prior to transplantation to the kidney, develop into trophoblast and extra-embryonic membranes only.
7. It is concluded that a blastocyst, to realize totipotency, must be subjected to the uterine environment 3½ to 4½ days after copulation. The substance producing this effect is termed the 'uterine factor'.
8. The action of the 'uterine factor' and the egg's limited period of competence to react to it are discussed.
9. A comparison between the potency of tubal mouse eggs and the tubal eggs of other mammals is made.

**RÉSUMÉ**

*L'influence du milieu utérin sur le développement des œufs de Souris*

1. Des œufs de souris, prélevés dans les oviductes et transplantés sous la capsule rénale, ne donnent naissance qu'à un trophoblaste et à des membranes extra-embryonnaires.
2. Des blastocystes utérins, transplantés dans une position similaire, se développent en blastocystes morphologiquement normaux. Néanmoins, ces blastocystes
bloqués dans la région tubaire, qui ont été privés du milieu utérin, ne se développent pas en embryons quand on les transplante dans le rein.

4. De tels blastocystes bloqués de 3 jours ½ se développent en embryons normaux si on les remet dans l’utérus.

5. Des œufs retenus dans l’oviducte pendant 4 jours ½, quoique d’apparence normale, ne se développent plus ensuite quand on les transfère dans l’utérus.


7. On conclut qu’un blastocyste, pour être totipotent, doit être soumis à l’influence du milieu utérin 3 jours ½ après l’accouplement. La substance responsable de cet effet est nommée ‘facteur utérin’.

8. On discute de l’action du ‘facteur utérin’, et de la période limitée de compétence que présente l’œuf pour réagir à cette action.


ACKNOWLEDGEMENTS

I wish to express my thanks for the advice of Professors M. Fischberg and A. Blackler, and Mr. H. K. Pusey. Thanks are also due to Mr. John Haywood for expert technical assistance. The work was carried out during the tenure of a Ford Foundation Fellowship.

REFERENCES


EXPLANATION OF PLATES

PLATE 1

Fig. A. Apparatus used for transplanting the eggs. The use of the etherizing table, shown on the microscope stage, was later discontinued in favour of injected anaesthetic.

Fig. B. The result of transplanting an egg, other than a uterine blastocyst, beneath the kidney capsule. It consists of a crescent-shaped abortive Reichert’s membrane, surrounded by the trophoblast. To the upper left is a nucleus of a placental giant cell, whilst the lower right consists of junctional zone trophoblast.

Fig. C (i). Textbook diagram of 6½-day-old mouse embryo seen in sagittal section. (Reproduced from The Biology of the Laboratory Mouse, ed. G. D. Snell, by permission of the publishers.)

Fig. C (ii). Embryo developing beneath the kidney capsule of an adult male mouse. One uterine blastocyst injected 4 days prior to autopsy.

Fig. D. An embryo, easily recognizable as such although it is somewhat abnormal in structure in that it has a double layer of embryonic ectoderm. One blastocyst injected 4 days prior to autopsy.

PLATE 2

Fig. E. A 3½-day-old tube-locked blastocyst.

Fig. F. For comparison, a blastocyst in the lumen of the contra-lateral (control) horn of the uterus.

Fig. G. A 4½-day-old tube-locked blastocyst.

Fig. H. A 4½-day-old blastocyst implanting in the contra-lateral (control) uterine horn.

Fig. I. A grossly abnormal 3½-day-old tube-locked blastocyst. These were easily recognizable under the dissecting microscope.

Fig. J. The trophoblast (left) invading the kidney tissue in the rat. The graft did not contain any embryonic shield derivatives. One 4-cell-stage egg transplanted beneath the kidney capsule 4 days prior to autopsy.

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Plate 1
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Plate 2