Induction of the
trophoblastic giant-cell transformation after
ovariectomy in the mouse

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INTRODUCTION

The trophoblastic giant-cell transformation of mouse blastocysts (Duval, 1891; Dickson, 1963) is blocked by ovariectomy carried out on the fourth day of gestation (Dickson, 1966b). Such blocked blastocysts have a greater overall length and a shorter inner cell mass, and hence a larger blastocoele, than untransformed blastocysts from mice that have not been ovariectomized (Dickson, 1966b, c). Since implantation can be induced in lactating and ovariectomized mice by the injection of ovarian hormones (Bloch, 1958, 1965; Smithberg & Runner, 1960), it may be assumed that the giant-cell transformation is brought about by such treatment, for this differentiation of the ovum seems to be an integral part of the implantation process (Snell, 1956). It is nonetheless well worth demonstrating that the transformation can be induced by the injection of ovarian hormones, for the morphological changes then become valuable as indicators of response to experimentation which are detectable before implantation sites can be identified visually. The initiation of development in the blastocyst may then be amenable to investigation separately from implantation, which is an interaction between egg and uterus. With this end in view, ovariectomized mice have been treated with progesterone and the morphology of the blastocysts observed.

MATERIALS AND METHODS

One hundred and twenty Swiss Webster albino female mice, derived from a colony kept under artificial light with a 10 h night centred on midnight, were used. All were virgins at least 8 weeks old and 25 g in weight. Ovariectomy was carried out under Nembutal anaesthesia between 9.30 a.m. and noon on the fourth day of gestation, counting as the first day that on which vaginal plugs were found in an examination carried out at 9 a.m. Each ovary, approached through a separate lumbar incision, was removed with most of the oviduct after

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A cotton ligature had been placed round the utero-tubal junction and the ovarian vessels. Removal of the oviduct simplified the operation, in that it was easy to ensure that no fragment of ovary was left behind. It has already been shown (Dickson, 1966b) that the anaesthetic and operative procedures, carried out at this time, do not themselves significantly interfere with implantation. The ovaries of each mouse were examined, to ensure that corpora lutea of pseudopregnancy were present.

The mice were divided into four groups of equal size. The females of the first group received a subcutaneous injection of 2.5 mg progesterone in 0.1 ml corn oil early in the afternoon of the fourth day. Those of the second group received 0.1 ml corn oil at the same time, while those of the third group received no injection. The mice of the fourth group were given 2.5 mg progesterone on the sixth, seventh and eighth days of gestation.

The mice of the first three groups were sacrificed by dislocation of the neck early in the afternoon of the fifth day, while sacrifice of the fourth group took place on the ninth day of gestation. The uterine horns were dissected from each mouse and the contents flushed into a watch-glass with physiological saline: 124 blastocysts were recovered from the first group, 145 from the second, 110 from the third and 127 from the fourth. All were inspected for the presence of giant-cell transformation (Dickson, 1966a). The overall length in the embryonic-abembryonic axis and the length of the inner cell mass in the same axis were measured for 95 blastocysts recovered from 23 mice of the first group and for 89 blastocysts from 23 mice in the third.

\section*{RESULTS}

Table 1 shows the number of mice in each group from which transformed or untransformed blastocysts were recovered, as well as the number containing abnormal or no blastocysts. By the $\chi^2$-square test, there are significant differences ($P < 0.001$) in respect of the numbers of mice with transformed blastocysts, between group 1, which received progesterone on the fourth day, and the other three groups.

\begin{table}[h]
\centering
\caption{The numbers of ovariectomized mice in each group with blastocysts in the conditions indicated are given}
\begin{tabular}{|l|l|l|l|}
\hline
Group & Treatment & Transformed & Untransformed or none \\
\hline
1 & Progesterone, 4th day & 27* & 2 & 1 \\
2 & Corn oil, 4th day & 2 & 27 & 1 \\
3 & No injection & 3 & 23 & 4 \\
4 & Progesterone, 6th–8th day & 1 & 26 & 3 \\
\hline
\end{tabular}
\end{table}

* Six had definite and thirteen had possible implantation sites.
Transformation after ovariectomy

Table 2 gives the mean overall and inner cell mass lengths in microns, with standard deviation in each case, of the blastocysts measured for groups 1 and 3. Each mean was calculated from the average blastocyst or inner cell mass length for each mouse. When the means are compared by Student's t test, after taking a pooled estimate of variance and a best estimate of the standard error of the difference of the means, there is, with 44 degrees of freedom, a highly significant difference ($P < 0.001$) in each case.

Table 2. Mean blastocyst and inner cell mass lengths

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of mice</th>
<th>No. of blastocysts</th>
<th>Blastocyst</th>
<th>Inner cell mass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean length ($\mu$)</td>
<td>Mean length ($\mu$)</td>
</tr>
<tr>
<td>1</td>
<td>23</td>
<td>95</td>
<td>161.7</td>
<td>42.2</td>
</tr>
<tr>
<td>3</td>
<td>23</td>
<td>89</td>
<td>109.3</td>
<td>32.0</td>
</tr>
</tbody>
</table>

DISCUSSION

It is clear from the observations described above that blastocysts recovered from females ovariectomized and injected with progesterone on the fourth day of gestation undergo the giant-cell transformation. That progesterone is involved is indicated by the statistically highly significant differences between the number of mice having transformed blastocysts after progesterone is given and the numbers having ova in this state after either no injection or corn oil is given on the fourth day.

There is a statistically significant difference between the mean blastocyst lengths in uninjected females and females injected with progesterone on the fourth day. It has been demonstrated (Dickson, 1966a) that, during the transformation in normal mice, the length increases to 185.9 $\mu$ (s.d. 28.7). The mean length found here (161.7 $\mu$) for transforming blastocysts is less than this, but blastocyst length is time-dependent (Dickson, 1966a) and the blastocysts recovered in this study were collected and measured before they would be expected to have attained their maximum length.

The mean length of the 89 blocked blastocysts recovered from 23 uninjected mice of group 3 (109.3 $\mu$, s.d. 9.3) on the fifth day of gestation in this study is not significantly different from that found earlier (Dickson, 1966a) for normal, untransformed blastocysts (108.1 $\mu$, s.d. 17.4). It is, however, significantly less ($t = 6.49$, d.f. = 53, $P < 0.001$) than that found for 118 blastocysts (132.4 $\mu$, s.d. 14.8) collected from 32 ovariectomized mice on the seventh to eleventh days of gestation (Dickson, 1966b). It may be concluded that, on the day following ovariectomy, the blastocyst expansion seen later has not yet occurred.

Just as the mean blastocyst length increased after progesterone injection in
ovariectomized mice on the fourth day, so also did the inner cell mass length increase. The increase was about 10 μ, which is of about the same magnitude as that found in normal blastocysts as they undergo the giant-cell transformation (Dickson, 1966c).

When progesterone is given on the sixth, seventh and eighth days of gestation, following ovariectomy on the fourth day, the giant-cell transformation was not found to occur (except in one mouse), which contrasts with the finding that when progesterone is given on the day of operation, it does occur. This clearly indicates that progesterone is not the only factor involved in control of the transformation. It may be that oestrogen, which, if the mouse has a pre-implantation oestrogen surge like the rat (Mayer, 1963; Shelesnyak, 1963), is presumably present in the circulation at the time of operation on the fourth day, is also essential. It has been shown that for implantation to occur in ovariectomized mice untreated for several days after the operation, both oestrogen and progesterone are necessary (Smithberg & Runner, 1960; Bloch, 1965). The result of administering oestrogen with progesterone on the sixth, seventh and eighth days is being investigated.

The mechanism of induction of the giant-cell transformation needs further elucidation. Further, it may be possible to correlate morphological changes with metabolic alterations of the type indicated by Weitlauf & Greenwald (1965). By ovariectomy and artificial induction of the giant-cell transformation, the mouse ovum comes to resemble that of certain marsupials, in which development after diapause can be studied as a process separate from implantation (Tyndale-Biscoe, 1963).

**SUMMARY**

1. Mice were ovariectomized on the fourth day of gestation and the trophoblastic giant-cell transformation sought in blastocysts recovered from them after progesterone or corn oil had been injected or no treatment given.

2. The giant-cell transformation does not occur in blastocysts of untreated mice. It does take place after progesterone but not after corn oil injection on the fourth day.

3. The transformation does not occur after progesterone on the sixth, seventh and eighth days.

4. Progesterone injection on the fourth day is associated with growth of the blastocyst as a whole and also of its inner cell mass.

**RÉSUMÉ**

*Induction de la transformation en cellules géantes trophoblastiques après ovariectomie chez la souris*

1. Des souris ont été ovariectomisées au quatrième jour de la gestation et la transformation en cellules géantes trophoblastiques a été recherchée dans les
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blastocystes prélevés chez ces animaux, après injection de progèstérone ou d'huile de céréale ou en l'absence de traitement.

2. La transformation en cellules géantes ne se produit pas dans les blastocystes de souris non traitées. Elle apparaît après traitement à la progèstérone mais non après injection d'huile de céréale au quatrième jour.

3. La transformation ne se produit pas après traitement à la progèstérone aux sixième, septième et huitième jours.

4. L'injection de progèstérone au quatrième jour est associée à une croissance de l'ensemble du blastocyste et également de sa masse cellulaire interne.

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REFERENCES


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