INTRODUCTION

INTER-SPECIFIC transplantation of mammalian eggs offer one possible way of studying the significance of intrinsic and external factors in embryonic development and their mutual interactions. The variety of problems which can be attacked by this method is dependent, of course, on the length of time during which development can proceed with a given combination of donor and recipient. The development of an egg in a specifically foreign animal can be adversely affected from the very beginning, though this is more likely to occur from the time of implantation onwards when all morphological, physiological, and immunological differences between the embryo and the mother come to action. The available information, though very scanty, supports the view that during early development up to the formation of the blastocyst, the egg is highly tolerant of a foreign environment.

Cleavage and blastocyst formation of sheep eggs was observed in the rabbit (Averill, Adams, & Rowson, 1955) and the lack of any deleterious effect was proved by their normal development after re-transplantation to another sheep. Sheep and goat eggs, after reciprocal transfers, develop even further, as living sheep embryos aged 30, 40, and 45 days were reported by Warwick & Berry (1949), and the birth of a dead but already fully coated individual was described by Lopyrin, Loginova, & Karpov (1951). According to the former authors the opposite combination, i.e. goat eggs transferred to sheep, is less successful as the embryos die about the 22nd day of gestation.

Briones & Beatty (1954) transferred eggs between mouse, rat, guinea-pig, and rabbit (in 9 out of the 12 possible combinations). These authors were interested only in finding whether development was possible at all. Accordingly, the experiments were terminated after 1, or sometimes 2, days and so did not cover the whole of pre-implantation development. Types of development recorded were cleavage, change from morula to blastocyst, change of form and size of

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blastocyst. The very low per cent. of developing eggs and lack of success in three combinations (rat → mouse, rat → rabbit, guinea-pig → mouse) seem to be due to inadequacies in the technical approach.

The author of this paper has found that 2-cell and 8-cell eggs of field vole develop into blastocysts when transferred to the oviduct of the mouse or the bank vole (unpublished data).

The present work was undertaken to investigate the early development of rat eggs in the mouse and vice versa, the process of implantation, and the ultimate limit of embryonic development.

MATERIAL AND METHODS

The animals used throughout the experiments were L.A.B. Grey mice and Wistar rats from random-bred colonies. Recipients of both species were mated with vasectomized males. The day when the vaginal plug was found was denoted as day 1 (1st day of pseudopregnancy or pregnancy). In the majority of experiments the eggs were transferred to the oviduct on the 1st day by the technique previously described (Tarkowski, 1959a). In a few cases rat eggs were transplanted to the uterus of a mouse on the 3rd day, using, slightly modified, the technique of McLaren & Michie (1956). The same method was used in one series of experiments with rat recipients to which rat eggs were re-transferred after a few days of incubation in the mouse. In this series an innovation in technique was introduced, namely, the eggs were transferred to the mouse oviduct on the 3rd or later days of pseudopregnancy and collected after a few days from the same part of the genital tract. This innovation was based on the observation (unpublished) that when mouse eggs are transplanted to the oviduct from the 3rd day onwards they develop into blastocysts but do not pass to the uterus.

The rat eggs used for transplantation were from the 2nd (2-cell), 3rd (2-, 3-, 4-cell), 4th (8-cell), and 5th day (blastocysts). The mouse eggs were from the 2nd (2-cell) and 3rd day (8-cell). For manipulations and transfers serum of either species diluted 1:1 with normal saline was used. The eggs were recovered from the oviduct or uterus before the normal time of implantation in a number of experiments to check how development was proceeding. After being examined in the living state they were fixed, stained with Ehrlich haematoxylin, and mounted in toto. For the study of implantation and the fate of embryos thereafter the recipient mice were killed from the 5th to 11th day (majority on the 5th, 6th, or 7th) and recipient rats from the 7th to 13th day (majority on the 7th or 8th day). The uteri were fixed in Bouin or Suza fixatives and the decidual swellings sectioned serially at 10 μ and stained with Ehrlich’s haematoxylin and eosin.
RESULTS

A. Development of rat eggs in the mouse

Development before implantation

Fifty-eight eggs, ranging from 2-cell to 8-cell stages, were transferred on the 1st day of pseudopregnancy (Table 1). In addition, 129 eggs were transplanted on later days, most of them being re-transferred subsequently to recipient rats (see section D). Nearly all eggs transplanted on the 1st day developed into blastocysts. Most of them looked perfectly healthy. Some were in process of degenerating and must have started to do so after attaining the stage of an early blastocyst. The mortality among the eggs transferred on later days was very high among ova from the 3rd day (2- to 4-cell stage) and nil among ova 1 day older (8-cell stage). In this series most of the eggs were from hormonally induced ovulation, and it may be that many of them, though they began to cleave, were abnormal. These eggs would not have reached the 8-cell stage and were not used for transplantation. In the description which follows all blastocysts are considered together as the same phenomena have been observed in both series.

<table>
<thead>
<tr>
<th>Age of transferred eggs (days)</th>
<th>Total no. of experiments</th>
<th>Day of transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Transferred</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>31</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>27</strong></td>
<td><strong>58</strong></td>
</tr>
</tbody>
</table>

Up to the stage corresponding to a 4½-day-old blastocyst development proceeds normally, with the exception that this stage is attained with ½ to 1 day delay. This is, however, a non-specific reaction to the transplantation operation (Tarkowski, 1959a). Henceforth the development can proceed along two alternative paths: either the zona pellucida is shed and the blastocyst remains of normal shape and size, or the zona is retained and the blastocoelic cavity increases considerably in volume. This process is accompanied by a decrease of the inner mass as a solid structure. Eventually the blastocyst looks completely 'hollow'—sometimes one hemisphere being darker than the other. As all the blastocysts which have not yet begun this 'growth' phase possess normally formed inner masses, the process must be a secondary one. Though it is difficult to catch these rearrangements in statu nascendi the 'disappearance' of the inner mass is
most probably achieved by gradual spreading of its cells. This leads firstly to a continuous one-cell layer in the embryonic hemisphere (Plate 1, fig. A) and afterwards, though perhaps not always, to a complete dispersion (Plate 1, fig. B). The picture is complicated by the fact that the endodermal cells, which in normal development appear before or just at the time of implantation, are most probably also represented in such 'hollow' blastocysts. The internal layer has thus a double source of origin, but two kinds of cells are no longer discernible (at least when ordinary histological techniques are used).

**Table 2**

**Presence of zona pellucida on rat blastocysts developing in the mouse**

<table>
<thead>
<tr>
<th>Day of transfer</th>
<th>Blastocysts recovered from</th>
<th>6th</th>
<th>7th</th>
<th>8th</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1st</td>
<td>Oviduct</td>
<td>18</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Uterus</td>
<td>6</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>3rd or later</td>
<td>Oviduct</td>
<td>—</td>
<td>14</td>
<td>34</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>24</td>
<td>16</td>
<td>39</td>
</tr>
</tbody>
</table>

- Zona pellucida present. — Zona pellucida absent.

The delay in shedding the zona pellucida may be responsible for this phenomenon. When 4-cell and 8-cell eggs are transferred to the oviduct they attain the blastocyst stage in this part of the genital tract and the process of 'disappearance' of the inner cell mass is completed before they enter the uterus. In the uterus the zona is eventually shed off but it is not possible to say to what extent the uterine environment, and to what the blastocysts themselves (by rupture due to internal pressure) are responsible. The uterine environment is not, however, indispensable for this purpose as the blastocysts can shed the zona in the oviduct though usually with a considerable delay (Table 2). It is specially evident in the series of transfers on the 3rd or later days when the uterine environment is completely excluded. It can be seen from Table 2 that it is only on the 8th day that nearly all blastocysts are naked.

**Implantation**

Oviduct transfers checked after the normal time of implantation are summarized in Table 3. The word 'implantation' denotes decidual swelling regardless of the presence or absence of a living blastocyst.

To exclude the possibility that failure of rat blastocysts to develop after implantation is due to inadequate technique or to an improper endocrinological state of the recipient, 7 mice received rat eggs together with two mouse eggs in each case. The results are shown in Table 4. When at autopsy 2 mouse embryos were found, the other implantations could be considered to be of rat origin. In
other cases at least some resorptions were of rat-egg origin. No difference in
the development of rat blastocysts between this and previous series was noticed.
A comparison of the level of development of mouse and rat embryos shows
clearly that the retardation in the development of rat embryos and their early
death (see below) was by no means caused by improper treatment of the eggs
nor by a general unreceptiveness of the uterus.

**Table 3**

*Implantation of rat eggs in the mouse*

<table>
<thead>
<tr>
<th>Age of transferred eggs (days)</th>
<th>No. of experiments</th>
<th>No. of transferred eggs</th>
<th>No. of implantations</th>
<th>No. of successful experiments</th>
<th>Day of autopsy in successful experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>8</td>
<td>40</td>
<td>10</td>
<td>5</td>
<td>6, 7, 7, 8, 9</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>47</td>
<td>19</td>
<td>5</td>
<td>5, 5, 5, 6, 7</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>28</td>
<td>3</td>
<td>2</td>
<td>6, 7</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>120</td>
<td>32</td>
<td>12</td>
<td>—</td>
</tr>
</tbody>
</table>

**Table 4**

*‘Mixed’ transfers of rat and mouse eggs to the mouse*

<table>
<thead>
<tr>
<th>Age of rat eggs (days)</th>
<th>No. of experiments</th>
<th>No. of rat eggs transferred</th>
<th>No. of mouse eggs transferred</th>
<th>Total no. of implantations</th>
<th>No. of living mouse embryos</th>
<th>No. of rat blastocysts or resorptions</th>
<th>No. of experiments in which implantation took place</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>4</td>
<td>24</td>
<td>8</td>
<td>15</td>
<td>3</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>15</td>
<td>6</td>
<td>9</td>
<td>4</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
<td>39</td>
<td>14</td>
<td>24</td>
<td>7</td>
<td>17</td>
<td>6</td>
</tr>
</tbody>
</table>

In addition to the main series 18 rat blastocysts were transferred to the uteri
of 5 mice on the 3rd day but none had implanted when autopsies were performed
on the 5th or 6th day.

Six blastocysts, already naked, from the 5th day were transplanted to one
mouse on the 4th day and two resorptions were found on the 11th day.

**Histological observations**

All together 42 decidual swellings were available for examination (32 from
oviduct transfers, 2 from uterine transfers, and 8 from these mixed transfers
when both mouse eggs ‘took’). The latest time at which the remnants of
the blastocyst could still be found was the 7th day.

After entering the mouse uterus the rat blastocysts are spaced out and each
evokes a typical decidual reaction. In each case a crypt is formed with a blasto-
cyst attached to its wall. On the 5th and 6th day nearly all blastocysts are still
alive (Table 5), and it is possible, therefore, to determine beyond any doubt the existence of inner cell masses. Among 21 blastocysts available for this purpose, 13 were without inner masses (Plate 1, fig. C) and 8 with inner masses (Plate 1, fig. D). Lack of an inner mass was already noticed in some blastocysts before implantation. The ‘implantation’ stages show that this process is not reversible and that the affected blastocysts implant as hollow vesicles without any compact embryonic structure. As in the unimplanted blastocysts already described there is an internal layer of cells. At this stage, however, some of these cells undoubtedly represent endodermal cells (more basophilic cytoplasm), though a precise numerical segregation of both kinds of cells is not possible after haematoxylin staining.

**Table 5**

*Survival of rat blastocysts after implantation in the mouse*

<table>
<thead>
<tr>
<th>Day of pregnancy</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of implantations</td>
<td>13</td>
<td>16</td>
<td>9</td>
<td>1</td>
<td>1</td>
<td>—</td>
<td>2</td>
<td>42</td>
</tr>
<tr>
<td>No. of alive or still discernible blastocysts or embryos</td>
<td>13</td>
<td>12</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>—</td>
<td>0</td>
<td>31</td>
</tr>
</tbody>
</table>

However, the character of the crypt and the intensity of the decidual reaction are by no means impaired by the lack of the inner mass in the implanting blastocysts.

The most striking feature of the implantation process is that the appearance of a decidual reaction and the formation of a crypt take place without, or at least before, the destruction of the uterine epithelium. Though on the 5th and 6th days the blastocysts are still alive and the trophoblast adheres closely to the epithelium, the latter remains either completely intact (Plate 1, fig. C) or becomes broken down over very small areas (Plate 1, figs. E, F). There are usually only a few trophoblastic cells involved in such contact areas. Though the number of blastocysts which succeeded in establishing such a contact increased with age (2 out of 13, 6 out of 12, and all 9 on 5th, 6th, and 7th day respectively) the whole process is considerably delayed and abnormal. The denudation of the mucosa, which at the very beginning of implantation can be attributed to the action of the individual trophoblastic cells, later on is due to spontaneous degeneration of the epithelium caused by oedematous changes in the mucosa. This degeneration begins at the bottom of a crypt and proceeds mesometrically. The character of the epithelium can be clearly correlated with the changes in the underlying mucosa (Plate 1, fig. G). In normal development this does not play any part, as by the time the wave of degeneration reaches the embryo, the epithelium around it has been already completely destroyed by the trophoblast.

The abnormal character of the implantation of rat blastocysts becomes even more plain if one remembers that the mouse embryo on the 6th day has already

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5584.4  

11
quite a big egg-cylinder and its trophoblast is in intimate contact with the mucosa over its whole surface. In the case of rat blastocysts it is evident that the lack of established normal contact with the mucosa (as the small areas cannot be considered normal), prevents both growth and differentiation in the inner mass.

The number of embryos which could potentially undergo some further differentiation is reduced because more than 50 per cent. of blastocysts starting implantation lack the inner cell mass. Among the remaining blastocysts there are some, however, which exhibit slight signs of differentiation. On the 6th day among 12 blastocysts still alive (5 of them with an inner mass) 1 embryo with a small egg-cylinder was found. On the 7th day there were 5 of these though the total number of living blastocysts was 9 only. These proportions show that the formation of an egg cylinder is considerably delayed and that if a blastocyst survives the initial period there is a high probability of its being able to begin to differentiate. It seems that the denudation of the mucosa around the blastocyst, due to spontaneous degeneration of the epithelium, is indispensable for the initiation of these changes. Establishment of contact with the mucosa and the initiation of differentiation afterwards, probably begins too late, however, to ensure any further development and longer survival. It must be emphasized that all egg cylinders encountered were rather small and some were evidently abnormal (Plate 2, figs. H, I, and J). All of them looked unhealthy and would not have survived much longer.

In some decidual swellings there were separate cells which might be considered to be trophoblastic cells which had survived after the death and disintegration of the whole blastocyst. Since in one resorption opened on the 11th day there were groups of giant cells (Plate 2, fig. K) and no other remnants of the embryo, there is some support for this assumption.

B. Development of mouse eggs in the rat

Thirty-three 2-cell and eight 8-cell mouse eggs were transferred to the oviducts of 3 rats. The recipients were autopsied on the 5th day. Among 23 eggs recovered 15 developed into blastocysts, the remainder had degenerated during cleavage or only reached the morula stage. Four out of 15 blastocysts still possessed zonae pellucidae though they were in their 6th day of development. Judging by the presence of mitosis in nearly all blastocysts, they were alive at the time of autopsy.

Nine recipients were killed from the 7th to 13th day. The results are summarized in Table 6. Most animals were autopsied on the 7th day as this day was found to be most convenient for histological examination. At this time the decidual swellings are clearly visible, the lumen of the implantation crypt is not yet obliterated, the presence of even a dead blastocyst can still be determined, and finally the attachment of the blastocyst to the uterine wall can be studied.
RAT AND MOUSE EGGS

Table 6

Implantation of mouse eggs in the rat

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Age of transferred eggs</th>
<th>Day of autopsy</th>
<th>No. of transferred eggs</th>
<th>No. of implantations</th>
<th>No. of alive blastocysts</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2-cell</td>
<td>7</td>
<td>5</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>7</td>
<td>9</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>&quot;</td>
<td>7</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>&quot;</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>8-cell</td>
<td>7</td>
<td>10</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>2-cell</td>
<td>8</td>
<td>6</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>&quot;</td>
<td>9</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>&quot;</td>
<td>12</td>
<td>10</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>&quot;</td>
<td>13</td>
<td>6</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>60</td>
<td>27</td>
<td>5</td>
</tr>
</tbody>
</table>

In 11 out of 16 decidual swellings from the 7th and 8th day the blastocysts were already dead. Death must have occurred earlier as the blastocysts were completely compressed, without even any traces of the blastocoelic cavity, and with a majority of the cells pycnotic (Plate 2, figs. L, M). It is, however, usually possible to see a ‘mould’ in the walls of a crypt (Plate 2, fig. L), suggesting that at the time of crypt formation these blastocysts were still vesicular. In the depression occupied by a dead blastocyst the uterine epithelium was, as a rule, only slightly flattened, but completely intact (Plate 2, figs. L, M). The decidual reaction and the formation of the crypt were, however, quite normal.

The remaining 5 blastocysts were still alive or just beginning to degenerate. In spite of a longer survival and of the close adhesion of the trophoblast to it, the epithelium either remains completely intact over the whole surface of the crypt (Plate 2, figs. N, O) or has broken down over very small scattered areas only (Plate 3, fig. P). In one extreme case the mesometrial half of the blastocyst is in nearly continuous contact with the mucosa (Plate 3, fig. R). Even the smallest contact is, however, decisive in prolonging the survival of the blastocyst and must provide some materials for its growth. The three blastocysts which have succeeded in establishing such a contact were extremely big, occupying 10, 12, and 14 sections each 10 μ thick. The increase in size expresses itself as an increase in the blastocoelic cavity and, possibly, in the multiplication of the trophoblastic cells. The inner cell masses are small and do not exhibit any signs of differentiation which would lead to the formation of an egg cylinder (Plate 3, fig. Q). It can be added that in two of these blastocysts the inner masses are not placed mesometrially but parallel to the axis of the crypt (Plate 3, fig. Q) and in the third one there are two aggregations of inner mass cells separated from each other.

It is quite plain, however, that even in these three most successful cases, the blastocysts had no chance of surviving and would inevitably have died.

From the 9th day onwards the lumen of the crypt becomes obliterated and no traces of blastocysts can be found.
C. Development of mouse and rat eggs after reciprocal transfers under the kidney capsule

Early death of mouse and rat blastocysts after reciprocal transfers to the genital tract is due most probably to their inability to establish a normal contact with the mucosa. The observations showing that when such a contact is established, even over a very small area, the survival of a blastocyst is prolonged and some growth or differentiation occurs, suggest that we are not dealing with a complete inability of the embryo to grow on a specifically foreign tissue.

In order to clear up this question reciprocal transfers of blastocysts to beneath the kidney capsule were carried out. It has been shown that the surface of a rat and mouse kidney provides a favourable environment for the development of their own eggs (Nicholas, 1942; Fawcett, 1950; Kirby, 1960). While in the rat Nicholas found disorganized growth of embryonic tissues accompanied by regional differentiation, Fawcett in the mouse encountered mainly trophoblastic tissue giving rise to giant cells and only occasionally abortive yolk sacs. The results of Kirby's work on the mouse show that when segmenting ova are transplanted, trophoblastic tissue develops, but normal egg cylinders or even embryos can be formed sometimes, if blastocysts are used for transplantation. Inter-specific transfers of eggs under the kidney capsule have not yet been performed.

In the present work mature males, both rats and mice, were used as recipients. The eggs were fully grown blastocysts taken from the uteri of mice on the 4th day and from those of rats on the 5th day. Three rats received 3, 5, and 7 blastocysts and three mice 4, 8, and 9 blastocysts respectively. The day of autopsy referred to denotes the age of the embryo, including the period spent in the mother before transplantation.

Two rats were killed on the 12th day but macroscopically no changes could be seen in the kidney. In the third animal autopsied on the 14th day two nodules were present under the capsule. Sectioning revealed two cavities filled with blood and containing a network of degenerating giant cells (Plate 4, fig. S). Nephric tubules have degenerated around the cavities, giving rise to a zone composed of small, closely packed cells. There is an infiltration of leucocytes, a few of them being visible in some of the giant cells which have survived.

Recipient mice were killed on the 11th, 14th, and 16th day and in all of them red swellings were present on the kidney. In the first case the swelling proved to contain a huge bulk of a non-differentiated embryonic tissue (Plate 4, fig. T) and rather scanty giant cells of small size around the periphery. The overwhelming majority of cells seems to be of embryonic, and not of trophoblastic, origin. On the periphery of this mass of non-differentiated tissue an organized roundish structure, of unknown origin was present (Plate 4, fig. T). Leucocytes, though scanty, were already present, being scattered in free spaces filled with blood and around the edge of the invading tissue.
The enormous swelling found in the second animal contained a network of degenerating giant cells and strips of small, presumably embryonic, cells, embedded in extravasated blood. There was an intensive leucocytosis.

On the 16th day necrosis was complete and no traces of living giant cells could be found at all.

**D. Re-transfer of rat eggs to rat recipients after ‘incubation’ in the mouse**

The experiments described below were mainly aimed at finding out to what structure would the hollow rat blastocysts, developed in the mouse, give rise, when transferred again to recipients of their own species.

A number of eggs from the 3rd and 4th day of development were transferred to the oviducts of 9 mice on the 3rd, 4th, and 5th day of pseudopregnancy (Table 1). The eggs were recovered after 3 or 4 days. From among those which developed into blastocysts 51 were afterwards transferred to 6 rats—3 of them received one set of eggs into each horn and 3 were operated unilaterally. With one exception the blastocysts were transplanted on the 4th day of pseudopregnancy. Due to a prolonged stay in the mouse oviduct and 1 day extra in the rat uterus, the blastocysts were at the time of implantation in their 7th (5 specimens), 8th (38 specimens), or 9th day of development (8 specimens). In the short time available at operation it was found to be difficult to select blastocysts according to the existence and size of their inner masses, and so all recovered blastocysts, representing a whole range from normally built to completely hollow ones, were used for transplantation.

In only 2 of the 19 decidual swellings which resulted were normal embryos encountered. In one female, killed on the 12th day, apart from a normal embryo, there were three purely trophoblastic vesicles deprived of any embryonic structures. The approximate diameters of these vesicles were 360, 500, and 1,270 μ respectively. Though of different sizes they all had a similar structure, represented by the biggest one shown in Plate 4, fig. V. Because of the opening of the decidual swelling the giant cells were removed with the uterine tissue leaving the Reichert membrane naked on the lateral and antimesometrial sides. The internal surface of the membrane is covered by a single layer of cells, which in some places forms structureless aggregations growing into the lumen. There are cells in the cavity also, some separate and some in small clumps or empty follicles. In the two smaller vesicles the trophoblast is represented exclusively by giant cells, which cover the Reichert membrane on all sides, including the mesometrial side. In the biggest vesicle on the mesometrial side there is a narrow layer of trophoblastic cells, not of giant type, covered on the outside by numerous typical giant cells.

A vesicle found in a series of ‘rat to rat’ transfers (unpublished) could be considered as an intermediate link between these vesicles and hollow blastocysts. After a development in the oviduct prolonged for 3 days the vesicle has developed, which on the 10th day had the structure represented in Plate 4, fig. U.
Here again, there is no egg cylinder and the Reichert membrane is embedded in a network of giant cells.

Taking all these facts into consideration the origin of such vesicles from hollow blastocysts can be assumed on the following grounds. (1) On the mesometrial side there is no aggregation of cells, which could correspond to the broken down egg cylinder. (2) Cells inside the vesicle are non-differentiated and the clumps have the character of secondary aggregations. (3) Reichert membrane is continuous all the way round. (4) All, or nearly all, cells of the ectoplacental cone have changed into giant cells.

It can be concluded that despite the absence of an embryo, the trophoblastic vesicle can survive for a long time and undergo considerable increase in size and some differentiation.

DISCUSSION

The experiments described in this paper show that the early development of mouse and rat eggs is not much affected by reciprocal transfer to the genital tract of the other species. These results are not in full accord with the observations by Briones & Beatty (1954) who found no development after ‘rat to mouse’ transfers and only slight developmental changes when the eggs were transplanted in the opposite direction. It must be kept in mind, however, that the experiments of these authors were short term and covered only 1–2-day periods. It is known (Tarkowski, 1959a) that a check in development due to transplantation shock lasts from 12 to 24 hours and therefore, the delay in, or complete lack of, visible developmental changes, observed after a short period cannot be considered as a proof that the environment is unsuitable or harmful. Briones & Beatty also were inclined to consider the observed delay in development as a shock effect. In view of the present work the complete lack of success of these authors with ‘rat to mouse’ transfers must be due to technical inadequacies. On the basis of the work by Averill et al. (1955) on the development of sheep eggs in the rabbit, it seems that even taxonomically very remote species can be employed for in vivo incubation.

A very striking feature in the development of rat eggs in the mouse results from the delay in shedding the zona pellucida. This causes spreading of the inner mass cells beneath the trophoblast leading eventually to the disappearance of the inner mass. The process does not affect all blastocysts uniformly, some individuals responding differently, even in one group of eggs developing in the same oviduct. It must be emphasized that these abnormal rearrangements take place in the oviduct and are completed before the blastocysts reach the uterus. The whole phenomenon should be attributed to the abnormal environment of the oviduct for this particular stage, rather than to a foreign environment in general.

It is not quite clear yet what part the uterine environment and what part the blastocyst itself plays in shedding the zona pellucida. In normal development
most likely these two factors contribute jointly. The uterine environment is not, however, indispensable as the blastocysts liberate themselves from their zonae when transferred to extra-uterine places, such as for instance the kidney surface (Nicholas, 1942; Fawcett, 1950; Kirby, 1960). The present author has performed a number of intra-specific transfers of mouse and rat eggs to the oviduct followed by a short-term examination (unpublished). It has been found that in this part of the genital tract the shedding of the zona pellucida is always considerably delayed, presumably until the time when the blastocysts attain such a size that the zona is ruptured by the abnormally increased internal pressure. Under normal conditions the blastocysts do not increase to such a volume and the uterine environment must undoubtedly facilitate the process of releasing them from their zonae pellucidae.

The embryological effect of the spreading of the inner mass cells is striking, as a hollow blastocyst, without a compact embryonic structure, is formed from the whole egg. The cells of inner mass origin, though undoubtedly present beneath the trophoblast, are usually no longer distinguishable from primitive endodermal cells, at least when ordinary histological techniques are used, and do not tend to aggregate again even at the time of implantation, when the zona is no longer present.

Huber (1915) describes, among normal rat blastocysts, a hollow one built from two layers and considers the inner layer as endoderm, distinguishing yolk endoderm and parietal endoderm. Huber is struck by the fact that the supposed endoderm is arranged in a continuous layer while in normal blastocysts at the corresponding stage the endodermal cells are loosely scattered. It seems quite probable that this is a spontaneous example of a similar process to that described in this work. There is no reason, however, to expect such forms to be represented often in normal development.

Apparently similar structures have been encountered among experimentally produced blastocysts developed from 'half' and 'quarter' blastomeres of the rabbit (Seidel, 1956, 1960) and 'half' blastomeres of the mouse (Tarkowski, 1959b, 1959c). This phenomenon may be due to intrinsic factors and may be explained in terms of the internal organization of an egg. Lack of an hypothetical centre in certain isolated blastomeres according to the first author, and lack of cytoplasm from the dorsal zone of the egg destined in normal development to form inner mass cells according to the second, is considered to be responsible for the formation of blastocysts lacking their inner masses.

For the development of hollow rat blastocysts originating from whole eggs, special conditions must exist, i.e. a prolonged sojourn in the oviduct. Though the whole phenomenon was described after inter-specific transfers, there are some hints that it may occur occasionally after 'rat to rat' transfers to the oviduct also (Tarkowski, unpublished). These secondary rearrangements, however, are unlikely to influence the development of isolated blastomeres, as the time relations of the transfers in such work do not require a prolonged stay in
The hollow rat blastocysts do not differ from normal ones in their ability to elicit typical decidual reactions. The ‘rat to mouse’ transfers do not provide, however, any information concerning the further fate of such structures. The three trophoblastic vesicles encountered among implantations, which resulted from re-transfer experiments, show that the lack of an inner mass at the time of implantation need not cause necessarily the immediate death of a hollow blastocyst. These are able to survive for quite a long time and even to increase considerably in size. Moreover, they undergo some differentiation as a whole and a new structure, i.e. Reichert’s membrane, is formed, as in normal development.

The inter-specific transfer of eggs can be of some use for studying the implantation process. When examining the decidual swellings resulting from ‘mouse to rat’ and ‘rat to mouse’ transfers it becomes quite evident that the decidual reaction and the formation of a crypt, on the one hand, and the degeneration of the uterine epithelium on the other, represent two unconnected phenomena. As in normal development these two processes occur nearly simultaneously, they were sometimes thought to be causally linked. In the rat, however, it has been already decisively shown by Blandau (1949) that the decidual reaction starts before any damage in the epithelium can be detected. In the mouse, though the sequence of events is probably similar, it has not been so conclusively shown, and the conclusion reached by Boyd & Hamilton (1952) and Amoroso (1952) is that the decidual changes start at the time when degeneration of the epithelium becomes visible. The inability of the foreign trophoblast to destroy quickly the epithelium, as described in the present experiments, makes it possible to separate in time these two processes in both species.

Alden (1948) has demonstrated that in the rat the primary trophoblastic giant cells actively remove the epithelium by their undermining, cytolytic, and phagocytic action. A similar conclusion can be drawn indirectly from the fact that when ‘artificial ova’, i.e. glass and paraffin beads, implant in the rat uterus (Blandau, 1949), the epithelium is retained for a longer time than in normal implantations. After complete formation of a crypt the epithelium, though thinned out, remains intact for some time, except on a small area in the vicinity of a bead.

Blandau concludes that the eventual disintegration of the epithelium in artificially induced deciduoma is due to oedema, and possibly also ischemia, in the underlying mucosa.

It is interesting and at the same time puzzling that in a similar experiment carried out by Alden & Smith (1959) no decidual reaction and crypt formation
resulted after transfer of unfertilized fixed sea-urchin eggs, and unsegmented or 2-cell mouse eggs (living or fixed). In sections the eggs were found lying in small recesses of uterine epithelium, which suggests mechanical pressure. Regardless of this no changes whatever could be detected in the epithelium and neighbouring mucosa. Disagreement between results of this and of Blandau's work is obscure, and the problem deserves further investigation.

In the mouse the degeneration of the epithelium during the course of normal implantation has been attributed to the action of trophoblast and/or to oedematous changes in the underlying mucosa (see Amoroso, 1952; Boyd & Hamilton, 1952, for review). The abnormal course of implantation of rat blastocysts in the mouse helps to show that these two processes act independently. In normal development, when the blastocyst attaches itself some distance from the very bottom of a crypt, the local denudation of mucosa around the embryo is established well before the wave of spontaneously degenerating epithelium reaches this region. When the blastocyst itself is not able to secure such a contact (as in the case of rat eggs) the spontaneous degeneration may eventually, though with considerable delay, contribute to denuding the mucosa around the blastocyst. This, however, is accomplished too late to ensure further normal development.

In the present experiments the spontaneous degeneration of the epithelium in the crypts was more evident in the mouse than in the rat. When mouse blastocysts in the rat fail to establish a contact with the mucosa, the epithelium remains intact, even in the vicinity of the egg, up to at least the 7th day. In the mouse these degenerative changes in the epithelium begin earlier and are more clearly visible as they proceed mesometrially. Moreover, it is possible to correlate the state of the epithelium with the character of the cells in the underlying mucosa. It is evident that there must be an incompatibility of some kind between the trophoblast and the uterine epithelium of a foreign species. Why is the activity of trophoblastic cells impaired after reciprocal transfers? No reasonable explanation can be offered, especially as this incompatibility seems to be confined to the system 'trophoblast-uterine epithelium' only. This is not a species incompatibility as on the kidney surface the trophoblastic elements proliferate freely, invading the host tissue and causing intensive haemorrhage. Among the blastocysts implanted in the uterus, those which succeeded in establishing some contact with the mucosa grow a little, but the total increase in size of inner mass derivatives is usually subnormal. However, this may be due to the fact that the contact is established too late, at the time when the blastocysts are beginning to degenerate already. The observation reported in this work that the separate trophoblastic cells can survive in the regressing decidual swellings and change into giant cells proves that the substratum (uterine mucosa) is suitable for the growth of specifically foreign foetal tissue.

Both aspects of development—differentiation and growth—have been detected in the implanted blastocysts. Mouse blastocysts in the rat can sometimes exhibit considerable growth, though this does not seem to include the inner mass. In
rat blastocysts implanted in the mouse, both initial differentiation (i.e. formation of an egg cylinder) and growth have been observed. It is very unlikely, however, that these are species differences, and one is rather inclined to expect that with the increasing number of experiments the results would become more uniform. Observations on rat blastocysts in the mouse allow for one generalization: there are no developmental changes in the inner cell mass until some contact with the mucosa is established. This seems indispensable for initiation of changes leading toward formation of an egg cylinder.

The survival of mouse and rat blastocysts after implantation is not of the same duration. The majority of rat blastocysts are still alive on the 6th day. In the rat, on the 7th day, which would correspond to the 6th day of pregnancy in the mouse, most mouse blastocysts are already dead. Very advanced degeneration of these eggs suggests that the conditions in the rat uterus are already unsuitable for mouse eggs at the moment of implantation or even some time before. On the contrary, rat eggs seem to tolerate the environment of the mouse uterus for a longer time.

The species chosen in the present work as donors and recipients are taxonomically fairly closely related, yet, the development of eggs stops at or just after implantation. Averill (1956) describes an experiment in which sheep eggs were left inside the rabbit uterus till the 10th or 13th day. The recovered blastocysts were degenerate, shrunken, and still with an intact zona pellucida. As it is known that sheep eggs can be kept alive until they are 8- or 9-day-old blastocysts (Averill, Adams, & Rowson, 1955), death of eggs in the above experiment must have occurred after completing the whole pre-implantation development. In this case the species differences in environmental requirements must be too great even to ensure the initiation of implantation. Perhaps the uterine environment of the rabbit becomes detrimental for sheep eggs even before they can liberate themselves from their zonae pellucidae.

In the present work no special effort was made to check whether the development of eggs in a foreign species has any effect on their further development after re-transplantation to a recipient of their own species. The experiments described in section D, though they had this character, were aimed mainly at defining the ultimate fate of hollow blastocysts. They cannot be considered as adequate controls, because the rat eggs after attaining the blastocyst stage were kept in the mouse oviduct for an extra period, most of them being at the time of implantation in their 8th day of development (7½ days old). A very high mortality rate after re-transplantation to the rat recipients suggests that the ‘incubation’ had some deleterious effect on the blastocysts. What is the cause of such a mortality? Dickmann & Noyes (1960) in their work on egg-transfer in the rat describe one series of experiments in which the eggs were transplanted after previous additional incubation in another female. Because of this procedure the eggs were in their 6th and 8th day of development at the normal time of implantation (i.e. on the 5th day of pregnancy) and have undergone two
transfer operations. After transferring 27 7½-day-old blastocysts 8 embryos (in fact 9 but 2 were identical twins) and 6 resorptions were found at autopsy on the 18th day. From another work by these authors (Noyes & Dickmann, 1961) on oviduct transfers in the rat it is evident that the eggs can still develop normally into embryos, being at the time of implantation as old as 8½ days (9th day of development). The comparison of the above data with those described in the present paper show that the age of the blastocysts cannot itself be responsible for the high mortality in the re-transfer series and that it must be caused by prolonged development in the mouse oviduct. It may be, perhaps, of some importance that in the ‘rat to mouse’ oviduct transfers the lowest proportion of implanting blastocysts was observed with the oldest eggs used, i.e. 8-cell eggs. These data suggest that the environment of the genital tract of the mouse while tolerable at first for the developing rat egg, becomes unsuitable when the egg attains the blastocyst stage and remains there in a ‘dormant’ state.

The inter-specific transfer of eggs might become a useful method of research if normal development of a foreign embryo could be extended for at least some time after implantation. So far, this has been achieved only in reciprocal transfers between sheep and goats (Warwick & Berry, 1949; Lopyrin, Loginova, & Karpov, 1951). Considering the question from this point of view, the results of the present work are rather disappointing. It seems justifiable to suppose that only the use of very closely related species can lead to better success.

**SUMMARY**

1. Mouse 2- and 8-cell and rat 2-, 4-, and 8-cell eggs develop into blastocysts when reciprocally transferred to the oviduct of the other species.

2. Some rat eggs, after attaining the blastocyst stage in the mouse oviduct, do not shed the zona pellucida at the normal time and continue to increase in volume beyond the normal. This is accompanied by spreading of cells of the inner mass beneath the trophoblast, leaving the blastocyst without any compact embryonic structure. Other eggs develop into normal blastocysts with an inner cell mass present. Mouse eggs in the rat give rise to normally built blastocysts.

3. Both kinds of rat blastocysts—hollow and normal—and mouse blastocysts evoke a typical decidual reaction in the uterus of the foreign species. Normal crypts are formed with the blastocysts attached to the epithelium. These processes begin well before any changes in the uterine epithelium become visible.

4. The blastocysts of neither species are able to secure a normal contact with the mucosa. Degeneration of the epithelium under the influence of the trophoblast is delayed, irregular, and only small areas are involved. In the mouse, the eventual denudation of the mucosa around the blastocyst is thought to be largely an effect of spontaneous degeneration of the epithelium due to oedematous changes in the underlying mucosa.

5. The survival of mouse and rat blastocysts after implantation is very limited, the upper time limit being, in the mouse, the 7th day and, in the rat, the 8th day.
Those rat blastocysts which have succeeded in establishing a contact with the mucosa undergo some differentiation, i.e. form small egg cylinders. This is accompanied by growth which in some cases is, however, very slight. Mouse blastocysts in the most successful cases exhibit immense increase in size but neither differentiation nor growth have been observed in their inner cell masses.

6. The kidney surface of both species provides a favourable environment for the development of rat and mouse blastocysts reciprocally transferred. The trophoblast gives rise to numerous giant cells. The cells presumably of 'inner mass' origin are also visible, but organized embryonic development has not been observed.

7. A number of rat eggs which developed into blastocysts in the mouse were re-transferred to rat recipients. The subsequent mortality rate was very high. Three purely trophoblastic vesicles were encountered and it is believed that they have developed from hollow blastocysts.

8. These findings are discussed in the light of embryology and reproductive physiology.

RÉSUMÉ

Transferts interspécifiques d'œufs entre le Rat et la Souris

1. Des œufs de souris aux stades 2 et 8 blastomères, et de rat aux stades 2, 4 et 8 blastomères, se développent en blastocystes quand on les transfère réciproquement dans l'oviducte de l'autre espèce.

2. Après avoir atteint le stade du blastocyste dans l'oviducte de souris, quelques œufs de rat n'éliminent pas leur zone pellucide en temps normal et leur volume continue à s'accroître au-delà de la normale. Ceci s'accompagne d'un étallement des cellules de l'amas embryogène au-dessous du trophoblaste, laissant le blastocyste sans structure embryonnaire compacte. D'autres œufs se développent en blastocystes normaux avec l'amas embryogène. Les œufs de souris, chez la ratte, donnent naissance à des blastocystes de structure normale.

3. Les deux sortes de blastocystes de rat, — creux et normaux —, et les blastocystes de souris, provoquent une réaction déciduale typique dans l'utérus de l'espèce étrangère. Il se forme des cryptes normales, où les blastocystes s'attachent à l'épithélium. Ces processus commencent bien avant que deviennent visibles des modifications de l'épithélium utérin.

4. Les blastocystes ne peuvent, pour aucune des deux espèces, établir de contacts normaux avec la muqueuse. La dégénérescence de l'épithélium sous l'influence du trophoblaste est retardée, irrégulière, et n'intéresse que des surfaces restreintes. Chez la souris, on pense que la dénudation éventuelle de la muqueuse autour du blastocyste est essentiellement un effet de dégénérescence spontanée de l'épithélium due à des modifications œdémateuses dans la muqueuse sous-jacente.

5. La survie des blastocystes de souris et de rat après leur implantation est très limitée, la limite maximale étant, chez la souris, le 7e jour et, chez le rat,
le 8e jour. Ceux des blastocystes de rat qui ont réussi à établir un contact avec la muqueuse subissent une certaine différenciation, c'est-à-dire forment de petits cylindres œufs. Ceci s'accompagne de croissance, néanmoins très faible dans quelques cas. Les blastocystes de souris, dans les cas les plus favorables, montrent un énorme accroissement de taille, mais on n'a observé ni différenciation ni croissance dans leur amas embryogène.

6. La surface rénale des deux espèces constitue un milieu favorable au développement des blastocystes de rat et de souris transplantés réciproquement. Le trophoblaste donne naissance à de nombreuses cellules géantes. On voit aussi des cellules provenant probablement de l'amas embryogène', mais on n'a pas observé de développement embryonnaire organisé.

7. Un certain nombre d'œufs de ratte qui s'étaient développés en blastocystes chez la souris ont été transférés de nouveau sur des ratten. Le taux de mortalité ultérieur a été très élevé. On a trouvé trois vésicules purement trophoblastiques, et on pense qu'elles se sont développées à partir de blastocystes creux.

8. Ces résultats sont discutés à la lumière de l'embryologie et de la physiologie de la reproduction.

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REFERENCES


EXPLANATION OF PLATES

PLATE 1

Fig. A. 64-day-old rat blastocyst developed in a mouse oviduct after transplantation of a 2-cell egg. The inner mass cells have spread into a 1-cell layer at the embryonic hemisphere. The recovered blastocyst was introduced into another oviduct, to be subsequently fixed and sectioned. × 400.

Fig. B. 64-day-old rat blastocyst developed in a mouse oviduct from a 2-cell egg. There is neither an inner cell mass nor a continuous layer of cells in one hemisphere (as in the blastocyst on fig. A) and the cells are scattered beneath the whole inner surface of the trophoblast. × 400.

Fig. C. Hollow rat blastocyst implanted in the mouse. There is no inner cell mass and on the inner surface of the trophoblast there are only loosely scattered cells. Trophoblast adheres to the uterine epithelium, but at no place has the denudation of mucosa been achieved. Degeneration of the epithelium is more advanced below the blastocyst, at the bottom of the crypt. Late evening of the 5th day. × 400.

Figs. D and E. Two sections of a rat blastocyst with the inner cell mass present, implanted in the mouse. No differentiation in the inner mass has begun yet. In the fig. D the uterine epithelium is still intact. Fig. E shows the only place in the crypt where the epithelium was removed. The trophoblastic cells are here in intimate contact with the mucosa. Note that the nuclei of these cells are enlarged. 6th day. × 400.

Fig. F. Rat blastocyst with a small inner cell mass, implanted in a mouse. The epithelium is missing on one side but intact on the other. 6th day. × 400.

Fig. G. An implantation crypt in the mouse uterus containing at the bottom a rat blastocyst. The degenerative changes in the epithelium have progressed far mesometrially and correspond to decidual changes in the underlying mucosa. A boundary between changed and unchanged mucosal tissue can be distinguished most clearly by comparing the sizes of the nuclei, which are considerably greater in the area affected by the decidual reaction. Late evening of the 5th day. × 200.

PLATE 2

Fig. H. Rat embryo with a regularly shaped egg cylinder, implanted in the mouse. There is a continuous contact of the trophoblast with the mucosa. Very few cells contribute to the embryonic part of the egg cylinder. 6th day. × 400.

Fig. I. A well-formed egg cylinder of a rat embryo, implanted in the mouse. Some degenerative changes can be seen already in the extra-embryonic part of the egg cylinder. 7th day. × 400.

Fig. J. An abnormal egg cylinder of a rat embryo implanted in the mouse. The cylinder is nearly hollow with most of the cells forming its wall of only 1 cell in thickness. The lumen in the cylinder is not continuous and the existence of two parts, corresponding to embryonic and extra-embryonic parts of a normal egg cylinder, can be assumed. 7th day. × 400.

Fig. K. Giant cells found on the 11th day in the regressing decidual swelling of the mouse. These cells are supposed to develop from trophoblastic cells which have survived after death and disintegration of the transplanted rat blastocyst. × 200.
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Plate 1
A. K. TARKOWSKI

Plate 2
FIG. L. A dead and completely flattened mouse egg (blastocyst) in the implantation crypt of a rat. Around the egg a 'mould' in the walls of the crypt is visible. The uterine epithelium, though slightly flattened, is completely intact. 7th day. \( \times 400 \).

FIG. M. A degenerated and flattened mouse blastocyst in the rat. Judging by its size the blastocyst must have grown a little before death. The epithelial cells, though these have been loosened and flattened, still form a continuous layer. 8th day. \( \times 400 \).

FIG. N. Mouse blastocyst in the implantation crypt of the rat, presumably already degenerating. Regardless of the expansion of the blastocyst and its close adhesion to the epithelium, the latter, though flattened, remains intact. 7th day. \( \times 400 \).

FIG. O. Living mouse blastocyst implanted in the rat. The uterine epithelial cells are considerably flattened and the lining at some places is attenuated to form thin protoplasmic bridges. On the left side there is only one possible penetration of the trophoblastic cytoplasm between the epithelial cells. 7th day. \( \times 400 \).

PLATE 3

FIG. P. A living mouse blastocyst implanted in the rat. It has grown to an enormous size (compare with blastocysts shown on figs. M, N, and O, reproduced in the same scale). Over a small area the trophoblast has established a contact with the mucosa but only a few cells are involved. The section reproduced goes through the margin of the inner cell mass (see fig. Q). 7th day. \( \times 400 \).

FIG. Q. The same blastocyst as in fig. P, but another section. The inner cell mass is situated in an abnormal position—parallel to the axis of the crypt—and shows no differentiation. In this section the epithelium remains intact. \( \times 400 \).

FIG. R. A living mouse blastocyst implanted in the rat. The mesometrial half of the blastocyst is in nearly continuous contact with the mucosa. The undifferentiated inner cell mass, not visible in this section, is situated laterally as in the blastocyst shown in fig. Q. \( \times 400 \).

In figs. P, Q, and R the mesometrial side of the crypt is directed to the left.

PLATE 4

FIG. S. A group of mouse giant cells developed in the rat kidney. They are embedded in extravasated blood and represent the only elements which survived till the 11th day after transfer of several 3½-day-old blastocysts. Leucocytes are abundant, some of them being present inside giant cells. \( \times 150 \).

FIG. T. A mass of undifferentiated rat embryonic tissue growing under the kidney capsule of a mouse. Scanty and still small giant cells are found only around the periphery. An organized rounded structure can be seen at the left side, on the top. Leucocytes are already present in cavities filled with blood and on the boundary of invasion of the renal tissue. 11th day of development (7th day after transplantation of several 4½-day-old blastocysts). \( \times 150 \).

FIG. U. Rat trophoblastic vesicle completely deprived of embryo and/or of any other derivatives of the inner cell mass. The vesicle is surrounded by a loose network of giant cells and embedded in extravasated blood. It was encountered among normal 9½-day-old embryos developed after oviduct transfer of 8-cell eggs on the 1st day of pseudopregnancy. \( \times 100 \).

FIG. V. A trophoblastic vesicle encountered in the rat after transfer of rat blastocysts which had developed to this stage in a mouse oviduct. Because of the opening of the decidual swelling before fixation the giant cells were removed from the antimesometrial and lateral sides of the vesicle, leaving Reichert membrane naked. The membrane is covered on the inside with a 1-cell layer. There are also many cells lying free in the lumen, some separate and some in small aggregations. Trophoblastic cells on the mesometrial side also have changed into giant cells, apart from those in a thin zone adjacent to the vesicle. There are no signs of a broken down egg cylinder and the Reichert membrane is formed on the mesometrial side (which is not the case in normal development). \( \times 50 \).

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