THE structure of the mammalian blastocyst has generally been studied on whole mounts in fluid, or on sections of embedded preparations. If the blastocyst of the rabbit is split open and mounted flat, however, the intact cells in their correct topographical relationships may be examined under the high powers of the microscope. This technique, which is quick and simple, may be employed in cytological and cytochemical studies of the large blastocyst cells, in study of mitotic patterns in the blastocyst wall, and in evaluation of the effects of culture media and of various experimental techniques applied to the blastocysts themselves or to the mother.

MATERIAL AND METHODS

The pregnant animals (6–6½ days’ post-coitum) were handled according to the methods previously described (Lutwak-Mann, 1954). In most cases the doe was anaesthetized by intravenous injection of nembutal, and the uterus removed and slit open. The blastocysts were picked out with a small stainless steel spoon and then quickly rinsed in physiological saline and fixed in absolute methanol for 1 hour or longer. The saline rinse is essential, since if it is omitted the acellular outer membrane curls after the vesicle is torn and thus prevents mounting; apparently some substance that shrinks strongly on drying is rinsed off. Ethanol, Bouin’s fluid, and Mayer’s picro-nitric fixative with added osmic acid, which Hartmann (1916) used for opossum blastocysts, all caused crumpling of the trophoblast. Even in methanol the trophoblast occasionally crumpled, rendering the preparation useless.

In mounting, one fixed blastocyst is placed with the embryonic shield downwards on a cover-slip in a flat dish containing just enough methanol to submerge the vesicle. The abembryonic pole is punctured with fine steel needles, and the
trophoblast, together with the covering zona pellucida, is torn into strips by slits that are carried to the edge of the shield. The fluid is drained off, and the star-shaped preparation allowed to dry on its cover-slip. Though a certain amount of wrinkling is inevitable, expanses of smoothly flattened trophoblast and adherent endoderm are nevertheless obtained (Plate, fig. 1). Generally the entire blastocyst is mounted, but, if desired, as, for example, for a series of histochemical tests, a single blastocyst may be readily torn into 5 or 6 longitudinal strips which are each mounted separately. Seven-day blastocysts may also be prepared as flat mounts, but the technique is somewhat more difficult because the trophoblast, having a tendency to separate from the zona pellucida at this stage, is quite fragile.

For staining, the cover slip bearing the blastocyst is dropped into the stain solution. Most often we have used Mayer's acid hemalum, but Delafield's hematoxylin is also satisfactory if it is acidified to minimize staining of the zona pellucida. In either case, the stain is applied for 20–40 minutes, after which the preparation is washed with tap-water. Other stains which we used were the Feulgen (6 minutes' hydrolysis in \( \text{N} \) \( \text{HCl} \) at 60° C, followed by 1 hour in the Schiff reagent) and the Unna-Pappenheim or 0.2 per cent. pyronine alone (both prepared in a pH 4-8 acetate buffer and applied for 2 hours). None of these nucleic acid stains coloured the zona pellucida. After staining, the blastocysts were dehydrated and mounted in a neutral medium.

THE NORMAL BLASTOCYST

In almost all our preparations the trophoblast and endoderm are underlain by zona pellucida and associated covering materials (cf. Boving, 1954). Staining of these non-cellular layers could not be entirely suppressed when hematoxylin is used, and consequently the whole preparation generally took on a pale purplish hue. Usually it was not possible to distinguish zona directly from trophoblast, but sometimes the two layers slipped apart at the torn edges, and it was then possible to see that the cytoplasm, particularly in the abembryonic region, is tenuous and almost uncoloured. The zona, on the other hand, appeared equally, though lightly, stained from the shield to the opposite pole. The shield cells themselves stained intensely, and an abrupt transition may be seen from these small dark cells to the large pale cells of the extra-embryonic area. The shield itself was in most cases featureless at the stage we used, but occasionally showed the first signs of primitive streak formation. In the abembryonic region, to which the endoderm has not extended at 6½ days, cell-walls sometimes stand out (Plate, fig. 2), but they are not otherwise seen. Within any given area the cells appear to be of approximately equal size, but irregularly we noted in the abembryonic hemisphere small cells arranged in clusters which might be the precursors of the trophoblastic knobs (Schoenfeld, 1903). In the endoderm the nuclei are smaller and darker, thus appearing more condensed, than those of the trophoderm (Plate, fig. 3). During prophase, however, the
endodermal nuclei seem to expand, so that it is not possible to distinguish endo-
dermal from trophoblastic mitoses in the area where both layers occur.

When the Unna-Pappenheim procedure is used the cytoplasm in the em-
bryonic hemisphere is found to be rich in brilliant red granules. Such granules
are also present in the abembryonic area in the previously mentioned clustered
small cells, but since the granules are otherwise completely lacking from tropho-
blast that has not been underlain by endoderm, they probably are present in the
endoderm. The resting nuclei are very pale. When pyronin is used alone the
nucleoli of the trophoblastic cells stain, whereas in the endoderm the entire
nucleus is reddened. Since the tendency to stain with pyronin is abolished from
both nuclear and cytoplasmic sites by extraction with cold 10 per cent. per-
chloric acid, it appears that ribonucleic acid is present in all red-staining sites.
After Feulgen treatment all resting nuclei present a fine purple-stained network
with usually a single mass (probably a sex chromosome) lying against the
nuclear membrane.

**RATE AND DISTRIBUTION OF MITOTIC ACTIVITY**

Mitotic figures are easy to recognize in the large, intact nuclei of the blasto-
cyst wall and may be readily counted at 440 x magnification. For counting
mitoses we used a line cut in a thin glass plate inserted in the microscope ocular.
One ‘arm’ of a preparation was chosen, and a narrow strip of it at the ab-
embryonic end moved across the field with all cells transected by the line at
each random turn of the mechanical stage screw being counted; in effect this is
a simple adaptation of the method of Chalkley (1943) for measuring a cellular
component. After one strip was counted a neighbouring strip was brought into

**Table 1**

*Mitotic activity in different areas of the blastocyst wall. (For description of
areas, see text)*

<table>
<thead>
<tr>
<th>Area</th>
<th>No. of cells counted</th>
<th>No. of cells in mitosis</th>
<th>Percentage of cells in mitosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abembryonic trophoblast</td>
<td>5,444</td>
<td>644</td>
<td>11.8</td>
</tr>
<tr>
<td>Outer endoderm+trophoblast</td>
<td>8,550</td>
<td>982</td>
<td>11.5</td>
</tr>
<tr>
<td>Inner endoderm+trophoblast</td>
<td>7,422</td>
<td>798</td>
<td>10.7</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>21,416</strong></td>
<td><strong>2,424</strong></td>
<td><strong>11.2</strong></td>
</tr>
</tbody>
</table>

place and the process repeated until the entire ‘arm’ from abembryonic pole to
shield margin had been examined. The data were recorded for trophoblast only
(roughly the abembryonic third of the system), the outer endoderm plus over-
lying trophoderm (equatorial third), and the inner endoderm plus overlying
trophoderm (the remainder, not including the shield). Counts of 12 ‘arms’ in
6 preparations from 3 litters gave the results shown in Table 1. There is no
significant difference in proportion of cells that are dividing at a given time in different parts of the system.

When the phases of mitosis are considered separately (Table 2), a deficiency in percentage of anaphases in the pure trophoderm suggested a possible difference in rate for this phase between endoderm and trophoderm, or perhaps

### Table 2

Percentage of mitotic figures in the phases of mitosis in different areas of the blastocyst wall

<table>
<thead>
<tr>
<th>Area</th>
<th>Percentage of dividing cells in</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prophase</td>
<td>Metaphase</td>
<td>Anaphase</td>
<td>Telophase</td>
</tr>
<tr>
<td>Abembryonic trophoblast</td>
<td>55.7</td>
<td>19.5</td>
<td>8.4</td>
<td>16.4</td>
</tr>
<tr>
<td>Outer endoderm+trophoblast</td>
<td>55.3</td>
<td>18.5</td>
<td>11.4</td>
<td>14.8</td>
</tr>
<tr>
<td>Inner endoderm+trophoblast</td>
<td>58.4</td>
<td>20.3</td>
<td>11.3</td>
<td>10.0</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>56.3</td>
<td>19.4</td>
<td>10.6</td>
<td>13.7</td>
</tr>
</tbody>
</table>

### Table 3

Relative proportions of metaphases and anaphases in the embryonic and abembryonic hemispheres of the blastocyst wall

<table>
<thead>
<tr>
<th>Hemisphere</th>
<th>No. of metaphases</th>
<th>No. of anaphases</th>
<th>M/A</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryonic</td>
<td>978</td>
<td>697</td>
<td>1.40±0.11*</td>
<td>0.01</td>
</tr>
<tr>
<td>Abembryonic</td>
<td>551</td>
<td>212</td>
<td>2.59±0.24</td>
<td></td>
</tr>
</tbody>
</table>

* Standard error of the mean.

between embryonic and abembryonic trophoderm. To investigate this possibility further, without the labour of complete mitotic counts, we adopted the practice of counting all metaphases and anaphases, but not other stages, in single ‘arms’. This procedure, which the large size of the blastocyst cells makes very facile, is justified by the relatively constant proportions of metaphases in different regions. When these results were scored for the three areas, the ratio of metaphases to anaphases was significantly higher in the abembryonic third than in the embryonic (mean difference, 0.85; standard error of mean, 0.05; \( P < 0.01 \)). The ratio in the equatorial third was intermediate between the other two, but, because of the greater variability in the equatorial strip, the difference between this and either of the others was not significant. A striking difference is, however, obtained if the results are scored by hemispheres, as Table 3 shows. A similar result is also obtained if the data are compared as M/A ratios for the two halves of single ‘arms’, instead of being pooled. Since the abembryonic half or third is predominantly or solely trophoblastic, these results apparently indicate that the
time spent in anaphase is relatively less in trophoblast than in endoderm. The results may also indicate a gradient in anaphase rate in one or both layers, but the similar appearance of endodermal and trophoblastic nuclei in mitosis makes this possibility difficult to evaluate.

EXPERIMENTAL OBSERVATIONS

It has long been known that the fully expanded free blastocyst is not amenable to maintenance in culture (Pincus, 1936). The technique described in this paper offers a simple means of assaying the effects of various treatments and media, for we have observed that the nuclei, particularly in the abembryonic area, are swiftly affected by exposure to extra-uterine agencies. A slight deficiency in number of anaphases, indicating metaphase arrest, is the first sign of impairment; in many preparations, the metaphase figures appeared perfectly normal, even when the anaphases were greatly reduced in number. A more severe effect is signalized by clumping of the metaphase chromatin, and the most seriously damaged blastocysts also show marked nuclear abnormality, including condensation of chromatin and appearance of vacuoles in and around the nuclei (Plate, fig. 4). The effects are always most striking in the abembryonic hemisphere, and sometimes only a small area around the pole is affected. Under the treatments we have used, nuclear condensation and vacuolization were rarely seen in the embryonic hemisphere.

1. Influence of medium

Routinely we tested the effect of exposing blastocysts to culture media for 20 minutes at 37° C. before fixing; 2 ml. of medium were used for each 4–6 blastocysts. Representative results, expressed as M/A ratios for entire ‘arms’, are given in Table 4. These results, and others we have obtained, suggest that Tyrode solution is a relative favourable medium for short-term maintenance; but a different conclusion emerges if we examine the effect on the two hemispheres separately of the litter D90 blastocysts (Table 5, part A). The sensitive abembryonic hemisphere clearly shows mitotic arrest. Moreover, not all litters gave such a favourable response to Tyrode as did D90. Some suffered mild or strong mitotic inhibition in both hemispheres, as is shown in the pooled data from a large number of counts (Table 5, part B). The variation in response is to be attributed to litter more than to individual differences, for we found that, with occasional exceptions, the members of a single litter tend to be affected uniformly by a given treatment. For this reason it is essential to have some littermate controls in Tyrode (or other medium) alone, whenever the effect of any chemical substance added to (or subtracted from) the medium is tested.

Fluid aspirated from blastocysts seemed to alleviate the deleterious effects of Ringer-phosphate (Table 4, litter C76). When used in combination with Tyrode, 8-day fluid gave us the most favourable results we ever obtained, for the blasto-
cysts kept in this solution showed only a slight increase in M/A ratio, even in the abembryonic region (Table 4; Table 5, part C), with no sign of metaphase clumping or other nuclear damage. Clotting of the 8-day fluid around the test

**Table 4**

Relative proportions of metaphases and anaphases in blastocysts exposed to various media for 20 minutes. Controls were litter-mates fixed immediately after rinsing

<table>
<thead>
<tr>
<th>Litter</th>
<th>Medium</th>
<th>No. blastocysts</th>
<th>No. metaphases</th>
<th>No. anaphases</th>
<th>M/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>E 59</td>
<td>Control</td>
<td>3</td>
<td>206</td>
<td>153</td>
<td>1.53</td>
</tr>
<tr>
<td></td>
<td>Ringer-bicarbonate*</td>
<td>3</td>
<td>255</td>
<td>56</td>
<td>4.55</td>
</tr>
<tr>
<td>D 90</td>
<td>Control</td>
<td>4</td>
<td>417</td>
<td>197</td>
<td>2.12</td>
</tr>
<tr>
<td></td>
<td>Tyrode</td>
<td>4</td>
<td>567</td>
<td>225</td>
<td>2.52</td>
</tr>
<tr>
<td>C 76</td>
<td>Ringer-phosphate*</td>
<td>3</td>
<td>184</td>
<td>5</td>
<td>36.8</td>
</tr>
<tr>
<td></td>
<td>Ringer-phosphate+rabbit serum (3:1)</td>
<td>2</td>
<td>165</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ringer-phosphate+8-day blastocyst fluid (3:1)</td>
<td>3</td>
<td>354</td>
<td>137</td>
<td>2.58</td>
</tr>
<tr>
<td></td>
<td>Ringer-phosphate+7-day</td>
<td>1</td>
<td>123</td>
<td>26</td>
<td>4.70</td>
</tr>
<tr>
<td>D 73</td>
<td>Control</td>
<td>3</td>
<td>239</td>
<td>129</td>
<td>1.85</td>
</tr>
<tr>
<td></td>
<td>Tyrode+8-day blastocyst fluid (3:1)</td>
<td>4</td>
<td>435</td>
<td>179</td>
<td>2.43</td>
</tr>
</tbody>
</table>

* Blastocysts in this group showed incipient pycnosis.

**Table 5**

Relative proportions of metaphases and anaphases in the embryonic and abembryonic hemispheres of blastocysts maintained in Tyrode or Tyrode with 8-day blastocyst fluid for 20 minutes. Controls as in Table 4

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of litters</th>
<th>No. blastocysts</th>
<th>Embryonic hemisphere</th>
<th>Abembryonic hemisphere</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Control</td>
<td>1 (D 90)</td>
<td>4</td>
<td>265</td>
<td>149</td>
</tr>
<tr>
<td>Tyrode</td>
<td>1</td>
<td>4</td>
<td>347</td>
<td>209</td>
</tr>
<tr>
<td>B. Control</td>
<td>4</td>
<td>11</td>
<td>768</td>
<td>486</td>
</tr>
<tr>
<td>Tyrode</td>
<td>9</td>
<td>30</td>
<td>2,259</td>
<td>905</td>
</tr>
<tr>
<td>C. Control</td>
<td>1 (D 73)</td>
<td>3</td>
<td>151</td>
<td>87</td>
</tr>
<tr>
<td>Tyrode+8-day blastocyst fluid (3:1)</td>
<td>1</td>
<td>4</td>
<td>280</td>
<td>117</td>
</tr>
</tbody>
</table>

blastocysts made them difficult to handle, but may have contributed to their good survival. An experiment in which five blastocysts were kept in Tyrode with 7-day fluid unfortunately gave equivocal results, for the controls maintained in Tyrode alone had an abnormally low proportion of anaphases; the experimentals showed no improvement as compared with their controls.
Although we made no detailed counts of mitoses in the embryonic shield, we nevertheless noted that the mitotic pattern appeared normal even when the abembryonic trophoblast showed severe damage. This finding is not unexpected, since the shield will develop to a limited extent in culture when separated from the rest of the system (Waddington & Waterman, 1933).

2. Age

Comparison of treated 6- and 6½-day blastocysts indicated no difference in ability to survive 20 minutes' exposure to Tyrode or Ringer-bicarbonate solution.

3. Temperature, anaerobiosis, and anaesthesia

It was thought that temperature shock or oxygen lack or the nembutal administered to the mother might be responsible for the inability of the blastocysts to continue normal mitotic activity in culture. The first possibility was eliminated by placing the freshly excised uterus in a chamber maintained at 37° C. in which all subsequent operations up to fixation were carried out. Exposure of blastocysts so handled to Tyrode solution gave results that were not better than in experiments in which the blastocysts were removed at room temperature. To check on the possibility that oxygen lack has an inhibitory effect, we left one uterine horn with its contained blastocysts intact for 30 minutes after the death of the mother, while the blastocysts of the other horn were removed as quickly as possible, rinsed, and fixed at once. The preparations of the two groups (four in each group) could not be distinguished from each other by their appearance. There was no significant difference, either in the embryonic or the abembryonic hemispheres, between their M/A ratios; and prophases, which are most sensitive to oxygen deprivation (Bullough & Johnson, 1951), were abundant in both groups. The effect of nembutal was examined by administration to a pregnant animal of 1·5 ml. of a solution containing 60 mg. of the anaesthetic per kilogramme. The treated doe was killed by a blow on the head 30 minutes later. Six blastocysts were then removed and fixed after brief rinsing. They did not differ, either in general appearance or in M/A ratio, from those of litters removed promptly after nembutal injection.

4. Superovulation

Because of the desirability of having adequate numbers of controls within a single litter, we tested the effect of superovulation induced by a gonadotrophin preparation according to the procedure of Chang (1948). Of two does thus treated we obtained 41 blastocysts from one and 46 from the other. The first group were definitely ellipsoidal and apparently near implantation; they are difficult to spread at this stage and were discarded. Of the larger batch, eight were selected at random and mounted. Of these, one, which stained only lightly, showed no mitotic figures, but the others were indistinguishable from blastocysts of normal-sized litters.
5. **Stilboestrol**

Since stilboestrol, like other oestrogens, is effective in preventing nidation, we looked into the possibility that this substance acts directly on the blastocysts. Five days after mating, two young does were given 5 mg. of stilboestrol by subcutaneous injection; 32 hours later they were sacrificed and their blastocysts (2 from one, 6 from the other) were fixed after rinsing. One blastocyst of the larger litter had almost no cells in mitosis, but the other seven appeared quite normal.

6. **Aminopterin and citrovorum factor (leucovorin)**

The folic acid antagonist aminopterin arrests mitosis in metaphase within 15 minutes when applied at concentrations as low as 1:20,000 to chick embryo tissues in culture (Jacobson, 1954). We maintained blastocysts for 20 minutes in Tyrode solution containing from 0.025 to 0.2 mg. of aminopterin per ml.; there was no sign of metaphase abnormality, and the M/A ratios did not differ significantly from those of litter-mates maintained in Tyrode only. In one experiment in which the aminopterin concentration was raised to 0.5 mg. per ml., there was an almost complete absence of anaphase figures from the five treated specimens; but the controls of this litter themselves showed signs of metaphase inhibition, with abnormally high M/A ratios. The failure of aminopterin to exert any effect suggested that the zona pellucida might have inactivated the inhibitor or prevented its passage into the blastocyst itself. To check this point we tried slitting the vesicles open before culturing them in order to permit the aminopterin to reach the inside of the system; but this treatment invariably resulted in crumpling of the trophoderm, which could not subsequently be spread. Citrovorum factor (leucovorin), used at 0.25 mg. per ml., had no clear-cut effect, either alone or in combination with aminopterin.

**DISCUSSION**

The fully expanded rabbit blastocyst, at the time when it is almost ready to be implanted, has long been known to be in a critically sensitive condition, whether in utero or in culture. Beyond this stage, the embryonic disk, at least, develops readily in vitro. Earlier, morulae and young blastulae can be raised in aerated serum up to the condition of the 6-day blastocyst (Pincus & Werthessen, 1938). When the blastocyst of 5–6 days after coitus is placed in culture, however, it generally collapses in a relatively short time (Brachet, 1913; Waterman, 1933), although Chang (1950) has shown that 6-day blastocysts isolated for as long as 45 minutes may develop normally if transplanted to a pseudopregnant uterus. That the behaviour of the blastocyst in the sensitive period is under external control has been accepted since Corner (1928) showed that removal of the ovaries or corpora lutea shortly after mating terminates the pregnancy at the blastocyst stage. Although it is clear that progesterone plays an essential role,
the nature of the uterine conditions that carry the blastocysts through the critical period is not yet understood.

The findings we have presented in this paper suggest that internal factors are also of importance, for the mitotic mechanisms of the 6-day blastocyst seem to be extraordinarily susceptible to the influence of external agents. This susceptibility is not a general property of the rabbit egg, for cleavages go on readily in culture (cf. Pincus, 1936). It might be thought that the sensitivity of the blastocyst nuclei is merely a secondary result of the thinning of the external coverings, particularly in the abembryonic region, prior to implantation. If this were the correct explanation, however, one would expect 6-day blastocysts to be more resistant to mitotic inhibition than those of 6½ days, but this is not so. Moreover, although we had no means of estimating the exact thickness of the covering coats, our flat mounts do in all cases show a firm, dense zona pellucida underlying the delicate trophoblast in abembryonic as well as embryonic areas. Boving (1954) has shown in addition that Tyrode solution does not tend to dissolve the gloriolemma.

Whether the mitotic suppression that we have observed in culture is due to the presence of an inhibitor, or lack of an essential nutrient, cannot be decided from our data; both effects may be involved. The severe inhibition caused by Ringer's solution with phosphate is interesting in light of the fact that at this stage the blastocyst fluid contains very little phosphate (Lutwak-Mann, 1954). The protective influence exerted against the phosphate medium by 8-day blastocyst fluid should be examined further. Possibly the fluid binds the phosphate; or it might contain some substance required for the mitotic process. The clotting of the 8-day fluid does not seem to be the critical factor, for the blastocysts remain firmly distended, and carry on mitotic activity, in fluid that offers no special mechanical support. On the other hand, the clotting might well be a useful adjunct to continued culture, for the best survival of isolated blastocysts has involved embedding them in plasm clots (Brachet, 1913; Waterman, 1933).

Our results as yet do not shed much light on the biochemical mechanisms underlying mitosis in the blastocyst. The ineffectiveness of oxygen deprivation is not surprising, for pre-implantation blastocysts are probably adapted, as are many other organisms in early developmental stages (cf. Bullough, 1952), to very low oxygen tensions. The ineffectiveness of aminopterin might be indicative of some fundamental difference between the mitotic process of the blastocyst and those of many older tissues (Jacobson, 1954); but this matter remains equivocal because the inhibitor may have failed to penetrate the outer coats of the egg, or may have been inactivated by them. The failure of a large dose of stilboestrol to injure the blastocysts or impair their mitotic activity is another indication that oestrogens interfere with implantation in an indirect way (Pincus & Kirsch, 1936).

The greater sensitivity to mitotic inhibition of the abembryonic hemisphere as compared with the embryonic might be an artefact, resulting from greater
permeability in the abembryonic region, but is probably a real phenomenon. The fact that the zona pellucida and associated coverings are not obviously thinned toward the abembryonic pole has already been pointed out. Boving (1954), moreover, has demonstrated a distinct chemical difference between the two hemispheres. Such a difference is reflected in our observations both in the different mitotic rate of the abembryonic trophoblast and also in the weak hematoxylin staining of the nuclei and particularly of the nucleoli of this material. The fact that it is always the pole opposite to the embryonic shield that implants also indicates that the vesicle is chemically non-uniform. The differential sensitivity of the dividing nuclei of different areas of the blastocyst may well be an aspect of this non-uniformity.

SUMMARY

1. A technique is presented for splitting fixed blastocysts open and mounting the entire vesicle on a flat surface. This technique permits close examination of all the cells of the vesicle in a single preparation. The appearance of the extra-embryonic cells when stained with hematoxylin or by nucleic acid techniques is described. Superovulated blastocysts showed no evident differences from those of normal-sized litters.

2. The extra-embryonic layers have a mitotic rate of 11.2 per cent., which appears to be uniform in the endoderm and different areas of the trophoblast. The abembryonic trophoblast, however, seems to be relatively deficient in number of anaphases, and the difference in the ratio of metaphases to anaphases in the embryonic and abembryonic regions is highly significant. Apparently anaphases are completed more quickly in the abembryonic trophoblast than elsewhere.

3. Exposure of blastocysts to various culture media for 20 minutes before fixing frequently resulted in damage to the nuclei in both resting and mitotic states. The abembryonic nuclei are especially sensitive, and metaphase arrest regularly occurs to a greater extent in this hemisphere than in the embryonic. Nuclear integrity appeared to be sustained best in media containing fluid aspirated from 8-day blastocysts.

4. Blastocysts of 6 and 6½ days did not differ in ability to survive 20 minutes' maintenance in fluid culture. Temperature shock, anaerobiosis, and maternal anaesthesia were also ruled out as causal factors in the nuclear damage occurring during culture. Administration of a large dose of stilboestrol to the pregnant doe did not result in any apparent injury to the blastocysts.

ACKNOWLEDGEMENT

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REFERENCES


EXPLANATION OF PLATE

All preparations shown in this plate were stained with Mayer’s acid hemalum. Fig. 1 was photographed at a magnification of 6 ×, the others at 250 ×.

FIG. 1. An entire 6½-day blastocyst spread on a cover-slip. The dark-stained mass at the centre is the embryonic shield.

FIG. 2. Area of abembryonic trophoblast showing cell-walls. In some preparations the cell boundaries are indistinct or cannot be seen at all. Along the cut edge (lower right) there is an area of zona pellucida not covered by trophoblast.

FIG. 3. Area of embryonic trophoblast and endoderm. The edge of the embryonic shield may be seen in the upper left corner. In the extra-embryonic area the endodermal and trophoblastic nuclei may be distinguished by the fact that the former are smaller, darker, and generally have fewer nucleoli than the latter.

FIG. 4. Area of abembryonic trophoblast showing clumping of chromatin and vacuolization of nuclei and cytoplasm. This kind of damage frequently occurs when the blastocysts are exposed to culture media.

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