INTRODUCTION

Despite recent successes with the cultivation of mouse and rabbit eggs (references in Austin, 1961, pp. 144–7) techniques for the cultivation of post-implantation mammalian embryos have not hitherto advanced beyond those devised in the 1930's. Jolly & Lieure (1938) obtained development of rat and guinea-pig embryos explanted into homologous serum at stages between primitive streak and a few somites. They report that of their explanted rat embryos 37 per cent. developed an embryonic axis with a rhythmically beating heart, but only 9 per cent. a functioning circulation. None formed limb buds or a functioning allantoic circulation. Nicholas & Rudnick (1934, 1938) appear to have had a similar degree of success with rat embryos explanted into heparinized rat plasma and embryo extract. Waddington & Waterman (1933) explanted rabbit blastodiscs of primitive streak to 3-somite stages on to plasma clots; in the most successful cultures a 6–9 somite embryo was obtained with neural tube and beating heart, but without any blood circulation.

These results are much inferior to those obtained with chick embryos in vitro, and have only proved of very limited value for mammalian embryology. We therefore attempted to devise an improved technique. Preliminary trials with a variety of methods suggested that mouse embryos explanted on to plasma clots and grown in 5 per cent. CO₂ were particularly promising. This method has now been tested thoroughly and found to give extensive development in a high proportion of explanted embryos of both the mouse and the rat.

The following account describes the basic technique, a few modifications, and the percentages of explanted embryos that develop to different stages.

MATERIALS AND METHODS

Plasma clots in watch glasses were prepared according to the standard method of Fell & Robison (1929). Each clot consisted of 15 drops of fowl plasma and 5 drops of embryo extract. This provided a clot of convenient size for culturing

---

1 Authors' address: Strangeways Research Laboratory, Wort's Causeway, Cambridge, U.K.
four or five embryos. For growing mouse embryos the embryo extract was made either from 13-day chick embryos or from the entire uterus and contents of 17- to 19-day pregnant mice; the tissue was ground to a thick paste, diluted with an equal volume of Tyrode saline containing 1 per cent. glucose, and centrifuged. For some of the cultures of rat embryos, rat embryo extract and rat plasma were used.

Mouse embryos were obtained from C3H, hybrid and other genetic strains. The rats used were Wistar strain. The adult males and females were caged together for 12 hr.; those females then found to have vaginal plugs were removed and later examined for embryos. Just before removing the embryos the pregnant females were killed by breaking the neck.

Mouse embryos were explanted 7–10 days, and rat embryos 9–10½ days, after mating. By this time the embryos are implanted in the uterine wall and each has become completely surrounded by decidual tissue which has proliferated to such an extent that it blocks the uterine lumen and stretches the uterine muscle. The stages in explantation are shown in Text-fig. 1. Each uterine horn was transferred to a cavity slide containing Tyrode saline; the uterine wall was torn open.
with forceps and the pear-shaped decidual swellings dissected out. The most difficult part of the explantation was the removal of the embryo and its membranes from the decidua. We found that the best method was to make a meridional incision in the broad end of the decidual ‘pear’ (arrow in Text-fig. 1b), preferably cutting along the groove representing the remains of the uterine lumen, and then to tear the decidua into two equal halves. The embryo and its membranes were usually left intact and adhering to one of the halves (Text-fig. 1c), from which they could easily be dissected.

Surrounding the embryo was Reichert’s membrane with its adherent endoderm and trophoblast cells, and the next operation was to remove it. Mammalian eggs are known to be damaged by protein-free salines (Austin, 1961, p. 103) and some workers have considered that later embryos are also affected (Jolly & Lieure, 1938). For this reason we always transferred the embryos to dilute embryo extract (prepared by adding 2–3 parts of 1 per cent. glucose in Tyrode to 1 part of ground tissue, and centrifuging) before removing Reichert’s membrane. Dissecting away the membrane was not difficult if it was first torn open with very fine watchmaker’s forceps.

Finally, therefore, each explant consisted of a sphere of yolk sac with the embryo at one pole and the ectoplacental cone at the other (Text-fig. 2, Stage 0). It was transferred to the clot in a drop of the dilute embryo extract which was spread until its surface was just level with the top of the explant (Text-fig. 1d). The explant was arranged with the ectoplacental cone to one side.

The cultures were housed in gas chambers in an atmosphere of 4–5 per cent. CO₂ in air, maintained by means of the buffer solution devised by Pardee (1949). For some of the cultures the O₂ was raised to 60 per cent. The incubation temperature was 37–38°C.

The explantation of each embryo took 5–10 min. Usually the time between killing the mouse or rat and transferring the embryos to the incubator was 1½–1 hr.; but on several occasions the time was extended to 1½–2 hr. without apparent effect on the results, and a few embryos that were kept for 2 hr. at 6°C. also developed well.

With reasonable care all the embryos in a uterus can be transferred undamaged to the culture vessels.

RESULTS

Mouse embryos with 1–7 somites at explantation

Text-fig. 2 summarizes the results obtained from 199 embryos explanted under several different conditions of culture—mouse embryo extract or chick embryo extract in the plasma clots, 20 per cent. O₂ or 60 per cent. O₂ in the culture chambers. For convenience of description, development in culture has been divided into five stages, Stage 1 representing about 12 hr. of development in vivo and each succeeding stage a further 6 hr.
Seventy-two per cent. of the embryos reached Stage 2. By this time the yolk sac had greatly enlarged, blood islands had formed in it, and a network of blood vessels had appeared over its entire surface. The embryonic heart, which had

started to beat several hours earlier, was now circulating blood through the embryonic and yolk sac vessels. The enlarging yolk sac had digested a cavity in the clot and had sunk into it, thus bringing the circulating blood into close contact with the clot over a wide area (Text-fig. 1e). However, despite this close contact,
the yolk sac had not formed any firm cellular adhesion with the clot. The allantois had extended from the embryo and fused with the ectoplacental cone forming an allantoic 'placenta'; cells from the ectoplacental cone had become firmly attached to the clot and migrated for a short distance into and over it.

Fifty-nine per cent. of the embryos reached Stage 3. Histological examination showed normal development of optic and auditory vesicles, pharyngeal arches, nephric tubules, liver and other organs. Many of the embryos now had blood circulating through the allantois. A common abnormality was a failure of the embryo to turn from the dorsally concave to the ventrally concave position. This change should be complete in embryos with about 12 somites (Snell, 1956), but of 117 of our embryos that attained 16–24 somites, only 43 had turned, and many of these only partially.

Thirty-three per cent. of the embryos developed prominent anterior limb buds (Stage 4), and a few acquired posterior limb buds and a tail bud (Stage 5). None developed further. In a few cultures some circulation of blood continued for 3 days and a rhythmical beating of the heart for even longer. But the maximum amount of embryonic development obtained was equivalent to about 40 hr. growth in vivo.

As long as the embryos had a good blood circulation they developed at about the same rate in vitro as in vivo, forming about 8 extra somites every 12 hr.

**Mouse embryos of different stages at explantation**

Most of the embryos that were explanted 8–9 days after mating had 1–7 somites, but some were at the head-fold stage or earlier, and a few had 8–12 somites. In Table 1 the embryos have been classified into four groups according to the number of somites present at explantation, and the amount of development obtained in culture is shown for each group. The 'stages' are numbered to correspond with Text-fig. 2.

### Table 1

<table>
<thead>
<tr>
<th>Embryos explanted</th>
<th>Number explanted</th>
<th>1 (8–16 somites)</th>
<th>2 (blood circulation)</th>
<th>3 (about 20 somites)</th>
<th>4 (about 24 somites)</th>
<th>5 (about 28 somites)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-somite</td>
<td>(65)</td>
<td>60</td>
<td>26</td>
<td>11</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>1–4 somites</td>
<td>(113)</td>
<td>94</td>
<td>66</td>
<td>53</td>
<td>26</td>
<td>8</td>
</tr>
<tr>
<td>5–7 somites</td>
<td>(86)</td>
<td>98</td>
<td>79</td>
<td>66</td>
<td>42</td>
<td>19</td>
</tr>
<tr>
<td>8–12 somites</td>
<td>(19)</td>
<td>100</td>
<td>89</td>
<td>89</td>
<td>84</td>
<td>68</td>
</tr>
</tbody>
</table>
The table shows that usually the embryos that were more advanced at explantation developed furthest in culture. Thus Stage 3 was attained by nearly all the 8–12 somite group, by about half the 1–4 somite group, and by only a few of the pre-somite group. The table also suggests that the majority of embryos in the 8–12 somite group developed for a longer time in culture, e.g. two-thirds of the 5–7 somite group formed 14 somites in culture, whereas two-thirds of the 8–12 somite group formed 18 somites, representing about 6 hr. more development. The other groups did not show obvious differences in duration of development after explantation.

Thirty-six embryos were explanted 7–8 days after mating. Most of these were at the primitive streak stage. The yolk sacs enlarged and the embryos developed for about 20 hr. in culture, reaching early somite stages, but none developed a blood circulation.

Twenty-four embryos were explanted 9–10 days after mating. These had 15–20 somites, circulating blood, and were concave ventrally. Thirteen cultured as described above showed little development. But 11 explanted on to the clot in sufficient of the dilute embryo extract to cover the whole clot to a depth of about 2 mm., and placed in 60 per cent. O₂, developed to about 30 somites; anterior and posterior limb buds were formed and the blood circulation continued to function in culture for over 24 hr.

**Rat embryos**

Eighty-five rat embryos were explanted 9–10 days after mating; nearly all were at head-process or head-fold stages. In culture all but a few developed enlarged yolk sacs and 6–12 somite embryos with beating hearts, but only four formed a blood circulation. Various combinations of rat or chick embryo extract, rat or chick plasma, 20 per cent. O₂ or 60 per cent. O₂ were tried, but the results remained the same.

Twenty-two embryos explanted 9½–10½ days after mating developed well. At the time of explantation they had 6–12 somites and the yolk sacs were 2·5 mm. diameter. After 48 hr. in culture nearly all had prominent anterior limb buds and 25–30 somites; the yolk sacs were 5 mm. diameter and in several the blood was still circulating. These cultures were grown in 60 per cent. O₂, and the clots were made of rat embryo extract and chick plasma or rat embryo extract and rat plasma; both combinations gave the same results.

**Different culture conditions**

The mouse embryos described above were all grown in an atmosphere containing 4–5 per cent. CO₂, but the oxygen level (20 or 60 per cent.) and the embryo extract (mouse or chick) varied in different experiments. To obtain more precise information about the effect of these variables the following experiments were made. To ensure that the embryos were initially as similar as possible each litter was halved and one half was used for each treatment.
PLATE

FIG. A. Mouse embryos as explanted. Amnion, yolk sac and most of ectoplacental cone left intact, but Reicher's membrane removed. ×20.

FIG. B. Mouse embryo explanted at head fold stage and grown in culture until 18 somites developed. Section through brain, optic and otic vesicles, stomodaeum, pharynx with visceral pouches, amnion, yolk sac and allantois. ×58.

FIG. C. Mouse embryo explanted at 6-somite stage and grown in culture for 48 hr. Photographed in its membranes. Twenty-eight somites, limb buds, tail bud and allantoic 'placenta'. ×20.

FIG. D. Rat embryo explanted at 9-somite stage and grown in culture for 48 hr. Twenty-seven somites and anterior limb buds. (Membranes removed.) ×20.

D. A. T. NEW and K. F. STEIN

(Facing page 106)
Cultivation of mouse and rat embryos

Mouse embryo extract compared with chick embryo extract, as a constituent of the clot and of the liquid medium

Mouse embryo extract was prepared from the embryos, placentae and uterus of 17–18-day pregnant mice. Chick embryo extract was made from 13-day chick embryos. Table 2 shows that both gave very similar results. (The slightly greater development attained with mouse embryo extract could be accounted for by the slightly more advanced stage of these embryos at explantation.)

Air compared with air + 5 per cent $CO_2$

The cultures were housed in two similar gas-tight chambers. A dish of Pardee buffer solution was placed in one of the chambers to raise the $CO_2$ to 4–5 per cent. (Pardee, 1949). The $CO_2$ concentration was checked by measuring the pH of a bicarbonate solution. The results are given in Table 3. Though many of the embryos in both chambers survived for a considerable time, those in the chamber containing extra $CO_2$ showed appreciably more development. The difference in numbers attaining Stage 3 is highly significant ($p = 0.004$).

Air containing 5 per cent. $CO_2$ and 60 per cent. $O_2$ compared with air containing 5 per cent. $CO_2$ but no additional $O_2$

The cultures were housed in two similar gas-tight chambers, both containing a dish of Pardee buffer solution to maintain the $CO_2$ level at 4–5 per cent. Oxygen from a cylinder was run into one of the chambers until the $O_2$ concentration was 60–65 per cent. Samples of the gas were taken at intervals and analysed for $O_2$ by a pyrogallol absorption method. The results are shown in Table 4. In general the

### Table 2

<table>
<thead>
<tr>
<th>Embryos explanted</th>
<th>Number explanted</th>
<th>1 (8–16 somites)</th>
<th>2 (blood circulation)</th>
<th>3 (about 20 somites)</th>
<th>4 (about 24 somites)</th>
<th>5 (about 28 somites)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chick embryo extract</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-somite</td>
<td>10</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1–4 somites</td>
<td>13</td>
<td>12</td>
<td>7</td>
<td>7</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5–7 somites</td>
<td>9</td>
<td>9</td>
<td>8</td>
<td>8</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>8–12 somites</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>27</td>
<td>16</td>
<td>15</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Mouse embryo extract</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-somite</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1–4 somites</td>
<td>14</td>
<td>14</td>
<td>12</td>
<td>10</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>5–7 somites</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>7</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>8–12 somites</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>25</td>
<td>21</td>
<td>19</td>
<td>9</td>
<td>5</td>
</tr>
</tbody>
</table>
Table 3

Development of mouse embryos in air + 5 per cent. CO₂ compared with their development in air

<table>
<thead>
<tr>
<th>Embryos explanted</th>
<th>Number explained</th>
<th>1 (8–16 somites)</th>
<th>2 (blood circulation)</th>
<th>3 (about 20 somites)</th>
<th>4 (about 24 somites)</th>
<th>5 (about 28 somites)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-somite</td>
<td>12</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1–4 somites</td>
<td>17</td>
<td>15</td>
<td>9</td>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>5–7 somites</td>
<td>14</td>
<td>12</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>8–12 somites</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>34</td>
<td>23</td>
<td>11</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Air with 5% CO₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-somite</td>
<td>10</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>1–4 somites</td>
<td>16</td>
<td>14</td>
<td>12</td>
<td>9</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>5–7 somites</td>
<td>14</td>
<td>14</td>
<td>11</td>
<td>10</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>8–12 somites</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>37</td>
<td>29</td>
<td>24</td>
<td>12</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 4

Development of mouse embryos in 60 per cent. O₂ compared with their development in 20 per cent. O₂

<table>
<thead>
<tr>
<th>Embryos explanted</th>
<th>Number explained</th>
<th>1 (8–16 somites)</th>
<th>2 (blood circulation)</th>
<th>3 (about 20 somites)</th>
<th>4 (about 24 somites)</th>
<th>5 (about 28 somites)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20% O₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-somite</td>
<td>16</td>
<td>11</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1–4 somites</td>
<td>16</td>
<td>15</td>
<td>9</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5–7 somites</td>
<td>10</td>
<td>10</td>
<td>6</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8–12 somites</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>39</td>
<td>21</td>
<td>12</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>60% O₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-somite</td>
<td>14</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1–4 somites</td>
<td>18</td>
<td>16</td>
<td>12</td>
<td>12</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>5–7 somites</td>
<td>12</td>
<td>12</td>
<td>11</td>
<td>10</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>8–12 somites</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>33</td>
<td>26</td>
<td>25</td>
<td>15</td>
<td>7</td>
</tr>
</tbody>
</table>

Higher O₂ atmosphere is clearly beneficial; the numbers attaining Stages 3, 4 and 5 are significantly higher (p = 0.02) in 60 per cent. O₂. The pre-somite group, however, did better at the lower O₂ level.

Discussion

The results indicate that mouse and rat embryos can be grown in vitro with much greater success than has hitherto been considered possible. The percentage of explanted embryos that develop, and the extent of their development, suggest
that cultivation in vitro is now a practicable technique for many studies in mammalian embryology. Embryos at the early somite stages are particularly successful in culture; they develop as well as chick embryos, and the technique of explantation is neither more difficult nor more time-consuming than for the chick.

A few details of the technique require comment. Previous workers (Jolly & Lieure, 1938; Nicholas & Rudnick, 1938) advise that Reichert's membrane be removed when the embryos are explanted, and we have followed this recommendation. A small number of embryos that we explanted with the membrane intact showed some development, a few even acquiring a good blood circulation, but they were much inferior to those explanted without the membrane. In vivo Reichert's membrane expands at the same rate as the yolk sac, but in culture it shows little or no expansion and prevents the growth of the yolk sac.

Addition of extra CO₂ to the air in the culture vessels was found to be beneficial. This may be because the saline (Tyrode) used to dilute the embryo extract contains bicarbonate and loses CO₂, with rapid rise of pH, if exposed to air. In 5 per cent. CO₂ its pH remains at about 7·3. If a non-bicarbonate saline were used the extra CO₂ might be unnecessary.

The tendency for the older embryos to sink into the clot means that only part of the yolk sac capillary network is able to carry out respiratory exchanges with the gas in the culture vessel. These embryos are therefore likely to be short of O₂ and it is not surprising that they benefit from a raised O₂ level. It is interesting, however, that the youngest embryos apparently do better at the lower O₂ concentrations.

At present it is only possible to guess the factors which ultimately cause the death of the embryos in culture. Blockage of capillaries with partial failure of the blood circulation is often the first sign of deterioration to be observed, but this may only be because it is the most obvious. As the embryos are in a static medium, shortage of nutrients, respiratory difficulties, and accumulation of waste products may all have harmful effects. If more information were available as to the physiological requirements of rodent embryos of this stage, their life in culture could probably be prolonged. Work along these lines is being continued.

**SUMMARY**

1. Mouse and rat embryos of primitive streak to early somite stages have been grown on clots of plasma and embryo extract.
2. Of nineteen mouse embryos explanted at 8–12 somites, seventeen acquired a blood circulation and thirteen developed to limb bud stages with 24–32 somites.
3. Of the mouse embryos explanted at 1–7 somites, 72 per cent. developed a blood circulation and 33 per cent. went on to limb bud stages.
4. Of the mouse embryos explanted at head fold and head process stages, 60 per cent. developed over 8 somites and 26 per cent. a blood circulation.
5. Mouse embryos explanted at primitive streak stages developed neural folds and a few somites, but not a blood circulation.


7. Eighty-five rat embryos explanted at head process or head fold stages mostly became 6–12 somite embryos with beating hearts. Only four formed a blood circulation.

8. The rate of development in vitro, at least for the first 24 hr., was about the same as in vivo. The maximum amount of development obtained in vitro was equivalent to about 40 hr. in vivo. Often the blood continued to circulate for over 48 hr., and the heart to beat for over 72 hr.

9. Addition of 4–5 per cent. CO₂ to the air in the culture vessels was found to be beneficial. Increasing the O₂ to 60 per cent. improved the development of the older, but not of the younger embryos.

10. It appeared immaterial whether the embryo extract used in the culture media was obtained from chick or mouse.

RÉSUMÉ

Culture d’embryons de souris et de rat après l’implantation sur des coagulum de plasma

1. Des embryons de rat de de souris prélevés au stade du prolongement céphalique ont été cultivés sur des coagulum de plasma et d’extrait embryonnaire.

2. Sur 19 embryons de souris explantés au stade 8–12 somites, 17 ont acquis une circulation sanguine, 13 ont évoluté jusqu’aux stades des bourgeois de membres avec 24–32 somites.

3. Parmi les embryons de souris explantés entre 1 et 7 somites, 72% ont développé une circulation sanguine, 33% ont atteint les stades des bourgeois de membres.

4. Parmi les embryons de souris explantés aux stades du pli céphalique et du prolongement céphalique, 60% ont évoluté au delà de 8 somites et 26% ont développé une circulation sanguine.

5. Des embryons de souris explantés aux stades de la ligne primitive ont différencié des plis neuraux et quelques somites, mais pas de circulation du sang.

6. 22 embryons de rat explantés entre 6 et 12 somites ont différencié des membres antérieurs et 25 à 30 somites.

7. 85 embryons de rat explantés aux stades du prolongement céphalique ou du pli céphalique ont évoluté pour la plupart en embryons de 6–12 somites avec des cœurs animés de pulsations. 4 seulement ont formé une circulation sanguine.

8. La rapidité du développement in vitro, au moins pendant les 24 premières heures, est à peu près la même qu’in vivo. La durée maximum du développement qu’on obtient in vitro correspond à environ 40 heures in vivo. Souvent le sang
Cultivation of mouse and rat embryos

continue à circuler encore plus de 48 heures et le coeur à battre pendant plus de
72 heures.

9. L'addition de 4–5% de CO₂ à l'air des récipients de culture s'est montrée
favorable. Si l'on élève à 60% la teneur en O₂, le développement des embryons
les plus âgés est amélioré, non celui des plus jeunes.

10. Il paraît sans importance que l'extrait d'embryon utilisé dans les milieux
de culture provienne du poulet, de la souris ou du rat.

ACKNOWLEDGEMENTS

We would like to thank Mr L. J. King, Mr W. G. Stebbings, and Mr M. F. Applin for valu-
able technical assistance.

One of us (K. F. S.) was supported by research grant NB-00684-09 of the U.S. National
Institute of Neurological Diseases and Blindness.

Figure A of Plate 1 has previously been used in an article published in Nature, Lond., and
we are grateful to the editor of that Journal for permission to reproduce it.

REFERENCES


Fell, H. B. & Robison, R. (1929). The growth, development, and phosphatase activity of

Anat. micr. 34, 307–74.


Nicholas, J. S. & Rudnick, D. (1938). Development of rat embryos of egg cylinder to head-
fold stages in plasma cultures. J. exp. Zool. 78, 205–32.

Pardee, A. B. (1949). Measurement of oxygen uptake under controlled pressures of carbon

pany.


(Manuscript received 16th August 1963)