FERTILIZATION OF THE MOUSE EGG AND THE EFFECT OF DELAYED COITUS AND OF HOT-SHOCK TREATMENT

By A. W. H. Braden* and C. R. Austin†

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Summary

A total of 329 mated adult mice was used in the investigations.

The mean number of eggs per mouse recovered from 171 mice killed 3-15 hr after ovulation was 8·10 (S.D. 1·94).

In mice maintained under conditions of controlled illumination (light from 3 to 5 a.m., dark from 5 a.m. to 3 p.m.) ovulation began about 11 a.m. and was virtually complete by 4 p.m. The average interval between the ovulation and sperm penetration of an egg was about 5 hr. The sperms spent a mean time of about 35 min in the perivitelline space before entering the vitellus; after a further mean interval of about 2% hr the early pronuclei were seen. The mean delay between the beginning of ovulation and the beginning of sperm penetration, in any one mouse, was 2½-3 hr. The mean time required for the majority (≥ %) of the eggs in any one mouse to be penetrated by sperms, once penetration had begun, was 3½-4 hr. A mean number of 18·6 sperms per tube was found at the site of fertilization shortly after ovulation.

Mice kept under natural lighting conditions were not permitted coitus until 8-8.45 a.m. Sperm penetration of the eggs began between 9 and 9.30 a.m. Once penetration had begun, the average period required for the penetration by sperms of at least three-quarters of the eggs in any one mouse was about 2 hr. The mean number of sperms at the site of fertilization at 9, 9.30, and 10 a.m. was 6·3, 7·9, and 20·6 sperms per tube, respectively. These findings are discussed in relation to the concept that sperms need to spend a period in the female tract to capacitate them for the penetration of the eggs.

The incidence of polyspermy in mouse eggs was not increased by delaying coitus until 3-7 hr after ovulation.

Hot-shock treatment applied to the eggs of mice 3 hr after mating increased the incidence of polyspermy (dispermy) from 0·3 to 3·8 per cent. and of eggs exhibiting suppression of the second polar body from 0·5 to 12·4 per cent. Several other forms of abnormal fertilization were also observed. It is considered that the triploidy reported in mouse embryos following hot-shock treatment of the eggs probably arose in most instances through suppression of second polar body formation, and only occasionally through polyspermic fertilization.

I. INTRODUCTION

Fischberg and Beatty (1952) subjected mouse eggs to an elevated temperature for a short period at about the time of sperm penetration and found that many of the resultant embryos had a triploid number of chromosomes. They postulated that the hot-shock treatment had inhibited the formation of

† Present address: National Institute for Medical Research, Mill Hill, London.
the second polar body, without preventing the completion of the second maturation division. Consequently, the embryos were thought to have a diploid number of maternal chromosomes and a haploid number of paternal chromosomes. In reaching this conclusion, Fischberg and Beatty were guided by results obtained on the eggs of newts by Fankhauser and Godwin (1948) and Fischberg (1948), who showed that hot-shock treatment induced triploidy by inhibiting the formation of the second polar body.

Similar treatment of rat eggs, however, did not support Fischberg and Beatty's hypothesis, for, in this species, the extrusion of the second polar body usually took place (Austin and Braden 1954a). Moreover, the increased incidence of triploidy could be adequately explained by the relatively high incidence of polyspermy (dispermy) in the treated eggs, for there is strong evidence (Austin and Braden 1953a) that dispermy leads to triploidy in the embryo. These triploid embryos would differ from those resulting from suppression of the second polar body, for they would have one maternal and two paternal sets of chromosomes. However, mouse and rat eggs may well differ in their reactions to hot-shock treatment. To test the validity of Fischberg and Beatty's hypothesis it was therefore necessary to examine mouse eggs in the pronuclear stages after such treatment. The results of such an examination are reported in the present paper; in the main, their conclusion is supported. As a preliminary step, a study of the time relations of ovulation and sperm penetration seemed advisable, because little information on this subject was available in the literature.

If, in rats and rabbits, coitus were not permitted until the time of ovulation, or later, a considerable increase in the incidence of polyspermy resulted (Austin and Braden 1953a, 1953b). Delayed mating thus offered a convenient means for increasing the incidence of triploid embryos. The effect of this procedure has also been studied in the mouse, and the results are reported here.

II. Methods

Adult mice of mixed stock from our colony were used throughout the investigations. The arrival of eggs in the fallopian tube was taken to be synchronous with ovulation. The eggs were recovered by dissection of the tubes under normal saline solution, and were examined in the fresh state by phase-contrast microscopy in the manner described by Austin and Smiles (1948).

Mice used for the study of the normal time relationships of ovulation and sperm penetration were kept in a cabinet, in which the period of illumination could be accurately controlled. The same cabinet had been used previously for establishing these time relationships in rats (Austin and Braden 1954b). The virgin females were kept in the cabinet for 3 wk before the males were introduced; thereafter the males were allowed to remain in the cages continuously.

In the hot-shock experiments, the mice were kept under natural lighting conditions and were allowed to mate between 8 and 9 a.m. Between 11 a.m.
# Table 1

**NUMBERS OF MICE WITH EGGS AND MEAN NUMBERS OF EGGS; MICE KILLED 6-24 HR AFTER THE ONSET OF DARKNESS**

<table>
<thead>
<tr>
<th>Hour of Killing</th>
<th>Number of Mice</th>
<th>Mean Number of Eggs per Mouse</th>
<th>Number of Fallopian Tubes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Killed</td>
<td>With Eggs</td>
<td>With Penetrated Eggs</td>
</tr>
<tr>
<td>11 a.m.</td>
<td>20</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Noon</td>
<td>20</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>1 p.m.</td>
<td>20</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>2 p.m.</td>
<td>20</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>3 p.m.</td>
<td>20</td>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td>4 p.m.</td>
<td>20</td>
<td>18</td>
<td>13</td>
</tr>
<tr>
<td>5 p.m.</td>
<td>20</td>
<td>19</td>
<td>16</td>
</tr>
<tr>
<td>7 p.m.</td>
<td>20</td>
<td>19</td>
<td>15</td>
</tr>
<tr>
<td>10 p.m.</td>
<td>20</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td>Next day</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>
and noon the animals were anaesthetized by subcutaneous administration of "Nembutal" and incisions were made in the body wall adjacent to the ovaries. The fallopian tubes and ovaries were brought to the exterior and the animal was suspended over a water-bath so that the tubes were completely immersed. The water-bath was maintained at a temperature between 43.5 and 45.0°C and treatment was applied for 5-10 min.

![Graph](image)

Fig. 1.—Frequency distribution of the number of eggs per mouse recovered from 171 mice.

### III. Observations

(a) Number of Eggs Ovulated

Records were kept of the number of eggs found in the fallopian tubes of 171 mice killed 3-15 hr after ovulation. Eggs recovered during this period were usually embedded in the cumulus oophorus, so that it was not difficult to ensure removal of all the eggs present. The frequency distribution of the number of eggs per mouse is shown in Figure 1. The total number of eggs recovered was 1385, and the mean number per mouse 8.10 (S.D. 1.94; range 2-14).

(b) Time Relations of Ovulation and Sperm Penetration

The mice were kept in a cabinet in which the "day" lasted from 3 p.m. till 5 a.m. (solar time) and the "night" from 5 a.m. till 3 p.m. After the males had been introduced, the females were examined at hourly intervals from 10.30 a.m. to 2.30 p.m., and also at 4.30 p.m. In about half of the mice mating occurred before 10.30 a.m.; in most of the remainder it occurred between 10.30 a.m. and 1.30 p.m. In only a small number of mice did mating occur after 1.30 p.m. Twenty mice were killed each hour from 11 a.m. to 5 p.m. and also at 7 p.m., 10 p.m., and during the morning of the following day. The completeness of coitus in each mouse was confirmed by examination of the uterine fluid; if sperms were not found the animal was discarded.

As coitus and ovulation proceeded concurrently in the mice as a group, those killed at the earlier hours could not have been a representative sample
### Table 2

**Numbers of Mice with Penetrated Eggs and Numbers of Eggs; Mice killed 1-8 hr after mating at 8 a.m.**

<table>
<thead>
<tr>
<th>Hour of Killing</th>
<th>Number of Mice</th>
<th>Number of Eggs per Mouse</th>
<th>Number of Fallopian Tubes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Killed</td>
<td>With Penetrated Eggs</td>
<td>With &gt;½ of Eggs Penetrated</td>
</tr>
<tr>
<td>9 a.m.</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9.30 a.m.</td>
<td>10</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>10 a.m.</td>
<td>10</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>11 a.m.</td>
<td>10</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Noon</td>
<td>10</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>1 p.m.</td>
<td>10</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>4.30 p.m.</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
</tbody>
</table>
of the whole population. For instance, those killed at 11 a.m. of necessity only comprised mice that had mated before 10.30 a.m. If a correlation existed between the time of mating and the time of ovulation in each mouse, then those killed at 11 a.m. would have been "early ovulaters," and the result reported for that hour would be relatively high. The number of mice that mated before 10.30 a.m. was a little less than half the number that were killed at later hours, so that the figures obtained for the hours subsequent to 12 noon may be slightly low.

The results are given in Table 1 and illustrated graphically in Figures 2 and 3. Ovulation began in the mice as a group just before 11 a.m. and was virtually completed by 4 p.m. One of the mice killed at 5 p.m. and one at 7 p.m. had no eggs in the tubes, but it is not certain that they would have ovulated that day. Lewis and Wright (1935) noted that, in mice, mating occasionally occurred in the absence of ovulation. Curve 1 (Fig. 2) illustrates the progress of ovulation in the mice as a group. Penetration of the eggs by sperms began about midday and reached completion soon after 10 p.m. The progressive increase in the mean number of penetrated eggs per mouse is illustrated in curve 2 (Fig. 2). The average interval between curves 1 and 2 corresponds to about 5 hr, or, in other words, the mean period that elapsed between the ovulation and the penetration of an egg was about 5 hr.

The presence or absence of pronuclei was also noted in penetrated eggs. Pronuclei were first seen at 2 p.m. and the incidence increased progressively up to 10 p.m.; this is illustrated in curve 3 (Fig. 2). The average distance be-
between curves 2 and 3 represents about 3½ hr, indicating that a mean period of about 3-3½ hr elapsed between sperm penetration of the zona pellucida and formation of pronuclei.

The data (Table 1) can also be used to estimate these time intervals as mean values for any one mouse. This was done by constructing curves relating time with the number of mice containing eggs (or penetrated eggs) as a percentage of the total number of mice killed at each hour. Curve A (Fig. 3)

![Graph showing increase in the percentage of mice with eggs (curve A), percentage of mice with one or more penetrated eggs (curve B), and percentage of mice that had at least three-quarters of the eggs penetrated by sperms (curve C).](image)

represents the percentage of mice found to have eggs in the tubes, and curve B the percentage of mice containing one or more penetrated eggs. The average distance between them corresponds to about 2½ hr, so that the mean interval, for any one mouse, between the beginning of ovulation and the beginning of penetration may be taken as 2½-3 hr. Curve C represents the percentage of mice in which at least 75 per cent. of the eggs had been penetrated. The figure of 75 per cent. was chosen because, whereas 7 out of 20 mice killed about 24 hr after mating had less than 100 per cent. of the eggs penetrated, in only one mouse were less than three-quarters of the eggs penetrated. The average distance between curves B and C corresponds to the average period required, in any one mouse, for the majority of the eggs to be penetrated by sperms, once penetration had begun. It was found to be about 3½ hr.

In a similar way, the data given in Table 1 can be used to show that the average period required for the penetration of at least two-thirds of the eggs in any one fallopian tube, once penetration had begun, was about 2 hr. The
value of two-thirds was taken because in only one out of 40 tubes from mice killed 24 hr after mating were less than two-thirds of the eggs penetrated.

When the eggs were being examined, account was taken of the number of eggs that had a sperm in the perivitelline space, but not in the vitellus. The number of such eggs and the proportion they represented of the total number of eggs recovered at each hour were: noon, 2 eggs (3.6 per cent.); 1 p.m., 2 eggs (2.1 per cent.); 2 p.m., 0 eggs; 3 p.m., 4 eggs (2.8 per cent.); 4 p.m., 7 eggs (4.3 per cent.); 5 p.m., 13 eggs (8.3 per cent.); 7 p.m., 11 eggs (7.3 per cent.); 10 p.m., 9 eggs (5.7 per cent.). As all the penetrated eggs recovered 24 hr after mating contained a sperm in the vitellus, it may be assumed that the eggs seen earlier with sperms only in the perivitelline space would eventually each have a sperm in the vitellus. From curve 2 (Fig. 2) it can be seen that the rate of increase in the number of penetrated eggs was approximately constant over the period 2 to 7 p.m., so that the frequency with which eggs were seen with sperms in the perivitelline space, but not in the vitellus, should be proportional, in this period, to the average time a sperm spent at that site before entering the vitellus. The mean interval was calculated from the foregoing data by the method previously used for the rat (Austin and Braden 1954b) and it was found to be approx. 35 min.

Two of the penetrated eggs recovered were abnormal. The first was obviously degenerate; there were nine sperms in the vitellus and eight of them were very active, causing considerable disturbance of the cytoplasmic granules. The heads of the sperms did not exhibit any loss of optical density, such as normally occurs after entry into the vitellus. The second egg contained one male and two female pronuclei. After the egg had been fixed it was seen that from the vicinity of each of the two female pronuclei spindle fibres passed to two polar bodies. The "central bodies" of the second meiotic spindles (Braden and Austin 1954a) were also readily distinguishable. The evidence thus seems to establish conclusively that this egg had been a binuclear oocyte.

(c) Effect of Delaying Coitus

In three groups of mice that had been kept under natural lighting conditions, coitus was not permitted until 8, 9.30, or 11.30 a.m. Males were placed in the cages at those times and removed about 45 min later. Mating did not readily occur at 11.30 a.m. Seventy-five mice that had mated between 8 and 8.45 a.m. were killed at 9, 9.30, 10, 11 a.m., noon, 1 p.m., or 4.30 p.m. (Table 2). Sperm penetration of the eggs in these mice began between 9 and 9.30 a.m. and reached completion in about 6 hr (curve 1, Fig. 4). The mean period required for the penetration of the majority (≥%) of the eggs in any one mouse was determined by the method employed in the previous section. It was found to be about 1½ hr; the mean period necessary for the penetration of at least two-thirds of the eggs in any one tube was about 1 hr.

Pronuclei were first seen at midday. The mean delay between sperm penetration of the zona pellucida and formation of the pronuclei was about 2½ hr (curve 2, Fig. 4).
No egg was found with more than one sperm in the vitellus. Three eggs recovered at 4.30 p.m. were abnormal; they contained only one pronucleus although a sperm mid-piece was present in the vitellus.

Twenty mice that had mated between 9.30 and 10.15 a.m. were killed at about 4.30 p.m. the same day. Again, no instance of polyspermy was seen, but there were two eggs that had one large (presumably male) pronucleus and two small (presumably female) pronuclei. Neither egg showed evidence of having extruded the second polar body.

Sixteen mice that had mated between 11.30 a.m. and 12.15 p.m. were killed at about 5 p.m. the same day. One egg was found to be undergoing polyspermic fertilization: two sperm mid-pieces were present in the vitellus, but the pronuclei had not yet formed.

The frequency distribution of eggs containing various numbers of sperms following delayed matings is given in Table 3. Apart from the fact that there were more unfertilized eggs after 11.30 a.m. mating, the distributions do not differ significantly from that reported earlier for eggs recovered after mating at the normal time (Braden, Austin, and David 1954).

(d) Number of Sperms at the Site of Fertilization

In some of the mice kept under conditions of controlled illumination, the number of sperms at the site of fertilization was counted, using Austin's (1952) method. Estimates were made on six mice killed at midday, one at 1 p.m., one at 2 p.m., five at 3 p.m., three at 4 p.m., and three at 5 p.m. Although
the mean number of sperms per ampulla was higher at the later times, the increase was not statistically significant. The mean number of sperms for all the mice was 18.6 and the standard error of the mean was 2.43 (range 1-51).

In the mice kept under natural lighting conditions and mated between 8 and 8.45 a.m., the number of sperms present at the site of fertilization was determined at 9, 9.30, and 10 a.m. The mean values with their standard errors were: 9 a.m. (10 mice) 6.3 ± 2.2, 9.30 a.m. (8 mice) 7.9 ± 2.4, and 10 a.m. (5 mice) 20.6 ± 6.0. No sperms were found in 10 out of the 20 tubes from mice killed at 9 a.m., and in one tube from those killed at 9.30 a.m.

*(e) Effect of Hot-shock Treatment*

The eggs of 18 mice which had been kept under natural lighting conditions and mated between 8 and 9 a.m. were subjected to hot-shock treatment for 5-10 min between 11 a.m. and noon. The animals were killed 4-5 hr later and a total of 132 eggs was recovered. Of these, 27 eggs were unpenetrated and 37 others contained sperms but were fragmented. Twelve of the 27 unpenetrated eggs contained a large single nucleus resembling a normal pronucleus; this phenomenon has been reported elsewhere (Braden and Austin 1954a). Of the remaining 68 penetrated eggs, only 37 could be classified as normal: they each contained two apparently normal pronuclei and one sperm mid-piece in the vitellus; in addition, one well-formed and one more or less disintegrated polar body were visible in most eggs. The abnormalities of fertilization seen among the remaining 31 eggs included:

1. Four eggs that were polyspermic (dispermic): they each had one small and two large pronuclei and two sperm mid-pieces in the vitellus.
2. Thirteen eggs that each contained one large and two small pronuclei and one sperm mid-piece. The two small pronuclei were usually identical in size and form. Only one small, rather degenerate, polar body was seen in most of these eggs; in the remainder no polar bodies could be detected.
3. Four eggs in which there was one normal pronucleus and one to three subnuclei.
4. Five eggs in which there was only a single large pronucleus. In three of the eggs, the pronucleus, because of its proximity to the sperm mid-piece, seemed to be the male. In the other two eggs the male element appeared to be represented by a small vacuole lying between the mid-piece and acrosome. These two types of abnormality may possibly represent early phases of androgenesis and gynogenesis, respectively.
5. In one egg, four sperm mid-pieces and four acrosomes were visible in the vitellus, but only a single pronucleus had formed. The sperm nuclei appeared as small spherical vacuoles, a stage which appears to occur in normal eggs just prior to pronucleus formation.
6. Four 2-cell eggs; two of the eggs had one mononucleate and one binucleate blastomere similar to that illustrated in an earlier paper (Austin
and Braden 1954c). The other two eggs closely resembled normal 2-cell eggs. One sperm mid-piece was present in each of the four eggs, but well-formed polar bodies were not seen. The most logical explanation of the mode of origin of these eggs, found in the 2-cell stage so soon after sperm penetration, is that they were formed by a process similar to that which apparently occurs in heat-treated unfertilized eggs and which has been referred to as "immediate cleavage" (Braden and Austin 1954a). One of the two nuclei in the binucleate blastomeres could therefore have been a male pronucleus.

**Table 3**

FREQUENCY DISTRIBUTION OF EGGS CONTAINING VARIOUS NUMBERS OF SPHERMS; EGGS FROM MICE KEPT UNDER NORMAL LIVING CONDITIONS

<table>
<thead>
<tr>
<th>Time of Mating</th>
<th>Number of Mice</th>
<th>Total Eggs</th>
<th>Unpenetrated Eggs</th>
<th>Number of Eggs Penetrated by 1 Sperms</th>
<th>Total Penetrated Eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal*</td>
<td>20</td>
<td>178</td>
<td>9</td>
<td>138 27 2 1 0 1 0</td>
<td>169</td>
</tr>
<tr>
<td>8 a.m.†</td>
<td>15</td>
<td>104</td>
<td>2</td>
<td>84 11 4 2 1 0 0</td>
<td>102</td>
</tr>
<tr>
<td>9.30 a.m.</td>
<td>20</td>
<td>170</td>
<td>3</td>
<td>126 29 5 6 1 0 0</td>
<td>167</td>
</tr>
<tr>
<td>11.30 a.m.</td>
<td>16</td>
<td>130</td>
<td>24</td>
<td>77 27 2 0 0 0 0</td>
<td>106</td>
</tr>
<tr>
<td>8 a.m.—Hot</td>
<td>18</td>
<td>135</td>
<td>26</td>
<td>66 28 3 3 2 3 4</td>
<td>109</td>
</tr>
</tbody>
</table>

* These figures have been reported previously (Braden, Austin, and David 1954).
† Mice killed at 4.30 p.m. only.

The frequency distribution of eggs containing various numbers of sperms is given in Table 3. Excluding the unpenetrated eggs, the proportion of eggs that contained more than one sperm was significantly greater ($P < 0.001$) in the heat-treated mice than in the untreated mice that had been mated at the same time (8 a.m.). This indicates that the normal reaction of the zona pellucida to sperm penetration (Braden, Austin, and David 1954) had been partially inhibited by the hot-shock treatment.

**IV. Discussion**

It has been reported elsewhere (Austin and Braden 1954b) that when rats were kept under natural lighting conditions (14-hr day), ovulation began in the rats as a group about midnight (i.e. 5-6 hr after sunset), and was complete by 4 a.m. When the times of light and dark were artificially reversed, the time of ovulation was also changed: it still began about 6 hr after the onset of darkness and reached completion in about 4 hr. The available evidence indicates that, in mice also, ovulation is regulated by the times of light and dark. Snell et al. (1940) and Snell, Hummel, and Abelmann (1944) showed that oestrous began in mice 3-6 hr after the onset of darkness, irrespective of the
solar time, and ovulation followed 1-4 hr later. When the period of darkness began at 6.15 a.m. each day ovulation commenced in the mice as a group about 5 hr later, and reached completion in about 5% hr. These findings are in close agreement with those reported in the present paper.

In mice and rats subjected to identical diurnal light cycles the time relationships of ovulation and sperm penetration were found to be very similar. As already noted, the interval between the onset of darkness and the beginning of ovulation in the animals as a group was 5-6 hr for both species. For other time relationships, the mean values obtained for rats (Austin and Braden 1954b) were a little shorter than those found for mice. For instance, the period required for the completion of ovulation in the animals as a group was about 4 hr for rats and about 5 hr for mice, and the average interval between ovulation and the penetration of an egg in rats was 3 hr and in mice 5 hr. These differences appear to be referable, in part at least, to greater variability between animals in the mice.

In rats mated after the time of ovulation it has been found that sperm penetration does not begin until 2-4 hr after coitus, even though sperms are present about the eggs at 1 hr (Austin 1952; Austin and Braden 1954b; Braden and Austin 1954b). This was taken as support for the concept that a sperm must spend a certain period in the female genital tract for "capacitation" before it is able to penetrate the egg (Austin 1951; Chang 1951; Austin and Braden 1952). The present data indicate that, if this is true in mice also, the period required for "capacitation" must be much shorter than in rats, for, when mating occurred between 8 and 8.45 a.m., sperm penetration began at about 9.30 a.m. The lower limit of the capacitation period, which in rats is 2 hr, is therefore possibly about 1 hr in mice. As the mean number of sperms at the site of fertilization was small at 9 a.m. and had increased considerably by 10 a.m., the delay of about 1 hr between coitus and the beginning of sperm penetration of the mouse eggs may represent simply the period required for sperms to ascend the genital tract and to meet the eggs. It does not seem possible to decide between these two alternative explanations on the data available at present.

Lewis and Wright (1935) found sperms at the site of fertilization 15 min after coitus in one mouse and 40 min after coitus in another. Merton (1939) reported sperms about the eggs in three mice killed 2, 2%, and 3 hr after artificial insemination at the first post-partum oestrus. In three others killed at ½, 1, and 1½ hr after insemination he found no sperms in the ampullae. In the present investigations, sperms were found at the site of fertilization in 10 out of 20 tubes from animals mated between 8 and 8.45 a.m. and killed at 9 a.m. At 9.30 a.m. 15 out of the 16 tubes examined had sperms in the ampullae. The mean number of sperms per ampulla rose from 6·3 at 9 a.m. to 20·6 at 10 a.m. From the foregoing data it may be concluded that, in the mouse, sperms may reach the site of fertilization within 15 min after coitus, but that they are not present at this location in the majority of animals until about 1 hr after coitus; the mean period required for the ascent of sperms to the site of fertilization is possibly about ½ hr.
Several workers have published estimates for the duration of the interval in mice between coitus or artificial insemination and sperm penetration of the eggs. Comparison is difficult, however, because, in three instances, the first post-partum oestrus was used; the time relations of oestrus, ovulation, sperm transport, and the penetration of the eggs may well be different under such circumstances. The estimates given were 6-10 hr (Gerlach 1906), 4-7 hr (Long and Mark 1911), and 3-4 hr (Merton 1939). Mating at the second oestrus post-partum was employed by Sobotta (1895), whereas Lewis and Wright (1935) and the present authors used virgin females. Sobotta's estimate of the interval was 6-10 hr, based on the results from one animal; Lewis and Wright saw sperms in the perivitelline space of the eggs of one mouse as early as 2 hr after coitus. In the present series, the mean interval between ovulation and sperm penetration was approximately 5 hr, and coitus generally took place 1-4 hr prior to ovulation, so that the mean interval between coitus and sperm penetration may be taken as 7-8 hr. The shorter interval reported by Lewis and Wright (1935) occurred in an animal that was not allowed coitus until it was in "mid-oestrus," as judged by the vaginal smear. Ovulation had probably already taken place, and this may explain the short interval, for, in the present series, it was found that, if mating was delayed until 3-8 hr after ovulation, the interval between coitus and sperm penetration was 1-5 hr. It seems that penetration of the eggs by sperms proceeds more quickly when coitus is delayed until after ovulation. A similar phenomenon was noted in the rat (Austin and Braden 1954b) and it was suggested that maturation of the egg membranes after ovulation was necessary before sperm penetration could occur.

Long and Mark (1911) thought that the pronuclei were formed within a few minutes of sperm penetration in the mouse; however, the present observations show that this is not so. Sobotta (1895) concluded that the interval required was of the order of 1 hr, but even this estimate appears to be low. It has now been found that sperms spend, on the average, about ½ hr in the perivitelline space before entering the vitellus, and that a further period of 2-3 hr is required for the formation of the pronuclei. These estimates are similar to those derived for the rat (Austin and Braden 1954b).

The incidence of polyspermy in rat eggs was shown to be greatly increased by delaying coitus until the time of ovulation or later (Austin and Braden 1953a, 1953b), but this has not been found to be so for mice. From 20 mice mated at the normal time two polyspermic eggs were recovered (Braden, Austin, and David 1954), whereas, in the present series, only one instance of the phenomenon was seen amongst the eggs from 51 mice mated 3-12 hr after ovulation. Hot-shock treatment, on the other hand, caused a considerable increase in the incidence of polyspermy in the eggs of both species. Suppression of second polar body formation in penetrated eggs was also seen much more commonly after hot-shock treatment than in untreated animals. In rat eggs the effect was transient; the initial disturbance of the correlation in the development of male and female elements was soon adjusted and fertilization then proceeded normally (Austin and Braden 1954a). In mouse eggs, however, it appears that the second meiotic division was completed, but both groups of chromosomes re-
mained within the egg and formed pronuclei, so that one male and two female pronuclei were formed. Two female pronuclei have been shown to originate in this manner in Triturus eggs, following hot-shock treatment (Fankhauser and Godwin 1948).

Hot-shock treatment of mouse eggs at about the time of sperm penetration has been shown by Fischberg and Beatty (1952) to result in a quite high incidence of triploidy in 3½-day embryos. They thought it very likely that the condition arose by suppression of second polar body formation, the embryos thus having one paternal and two maternal sets of chromosomes. The present findings clearly support this conclusion. In some (about 25 per cent.) of the mouse embryos, however, the triploidy may well have arisen from polyspermic (dispermic) fertilization, and this would have resulted in embryos with one maternal and two paternal sets of chromosomes.

V. References

SOBOTTA, J. (1895).—Arch. mikr. Anat. 45: 15.